Flexibility in metabolic rate in a
small Afrotropical bird *Zosterops virens*

by

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GENERAL ABSTRACT

The scientific literature contains hundreds of studies on avian basal metabolic rate (BMR), many of which assumed that BMR was fixed for each species. Yet those from the last few decades have shown avian BMR to be a flexible trait, changing temporarily and reversibly in response to numerous environmental variables. Given that birds from lower latitudes are relatively understudied compared with temperate and Holarctic species, and that seasonal trends in BMR of southern hemisphere birds are not well understood, we looked at seasonal variation in BMR of a small Afrotropical bird, the Cape white-eye (*Zosterops virens*), over two years, and found that small birds may reverse the direction and amplitude of seasonal change between years. We also looked at circannual rhythm in avian resting metabolism (RMR), and found that peaks and troughs in resting metabolic rate (RMR) may not necessarily correspond with peaks and troughs in ambient temperature, suggesting that some of the confusion regarding the direction and magnitude of seasonal change in avian BMR may be caused by timing of seasonal measurements.

Since we were using captive birds for my work, and since captivity may have an effect on avian BMR, we compared the BMR of freshly wild-caught birds with that of long-term captives housed in outdoor aviaries. The captive birds had higher BMR, giving weight to the argument that some physiological data of captive birds should not be used as representative of wild conspecifics, however the direction of seasonal change was similar in freshly wild-caught and long-term captive birds. Along the same vein, acclimation to laboratory conditions, experimental procedure, and different thermal environments, may also affect avian BMR, and thus before we started the final experiment, Cape white-eyes were acclimated to two different thermal regimes,

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with no change in RMR over an eight-week period, although there was an increase in body mass over the first three weeks, presumably due to the captive diet being of higher quality than a wild one. These results suggested that in some instances, small birds that are freshly wild-caught may not need to be acclimated in terms of their metabolism, before respirometry trials begin.

Finally, given that anthropogenic climate change is anticipated to eclipse all other threats to biodiversity, and since many current predictive models pay no heed to metabolic flexibility of birds, we investigated the effect of a 4°C increase in housing temperature on resting metabolism of the Cape white-eye. This temperature increase is equivalent to that predicted for the range of this species by 2080, and therefore gives an indication of the effect of a sustained increase in mean surface air temperature. The results showed only a marginal difference in various metabolic parameters, suggesting that these birds may cope with the mean temperature increase predicted for their range in the coming decades.

Together, these results highlight the importance of considering phenotypic flexibility when studying avian resting or basal metabolic rate. This has special implications for seasonal studies that implicitly assume that summer and winter measurements provide snapshots of the maximum and minimum RMR of which birds are capable, and for comparative studies, which may incorporate metabolic data from both wild and captive populations, or from study birds that were acclimated for different periods.

PREFACE

The data described in this thesis were collected in Pietermaritzburg, in the Republic of South Africa, from February 2012 to November 2014. Experimental work was carried out while registered at the School of Life Sciences, University of KwaZulu-Natal, Pietermaritzburg, under the supervision of Professor Colleen T. Downs and co-supervision of Dr. Mark Brown.

This thesis, submitted for the degree of Doctor of Philosophy in the College of Agriculture, Science and Engineering, University of KwaZulu-Natal, Pietermaritzburg campus, represents original work by the author and has not otherwise been submitted in any form for any degree or diploma to any University. Where use has been made of the work of others, it is duly acknowledged in the text.

Lindy Jane Thompson

November 2014

I certify that the above statement is correct and as the candidate's supervisor I have approved this thesis for submission.

Professor Colleen T. Downs

Supervisor

November 2014

COLLEGE OF AGRICULTURE, SCIENCE AND ENGINEERING

DECLARATION 1 - PLAGIARISM

I, Lindy Jane Thompson, declare that

1. The research reported in this thesis, except where otherwise indicated, is my original research.

2. This thesis has not been submitted for any degree or examination at any other university.

3. This thesis does not contain other persons' data, pictures, graphs or other information, unless specifically acknowledged as being sourced from other persons.

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Lindy Jane Thompson

November 2014

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DECLARATION 2 - PUBLICATIONS

Details of contributions to publications that form part of and/or include research presented in this thesis.

Publication 1

Thompson LJ, Brown M and Downs CT (2014)

Factors affecting avian basal metabolic rates.

Author contributions:

LJT conceived paper with MB and CTD. LJT collected and analysed data, and wrote the paper. MB and CTD contributed valuable comments to the manuscript.

Publication 2

Thompson LJ, Brown M and Downs CT (2014)

Seasonal metabolic variation over two years in an Afrotropical Zosteropid bird.

Author contributions:

LJT conceived paper with MB and CTD. LJT collected and analysed data, and wrote the paper. MB and CTD contributed valuable comments to the manuscript.

Publication 3

Thompson LJ, Brown M and Downs CT (2014)

Circannual rhythm in metabolic rate of Cape white-eyes (*Zosterops virens*).

Author contributions:

LJT conceived paper with MB and CTD. LJT collected and analysed data, and wrote the paper.

MB and CTD contributed valuable comments to the manuscript.

Publication 4

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The effects of long-term captivity on the metabolic parameters of a small Afrotropical bird.

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MB and CTD contributed valuable comments to the manuscript.

Publication 5

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Author contributions:

LJT conceived paper with MB and CTD. LJT collected and analysed data, and wrote the paper.

MB and CTD contributed valuable comments to the manuscript.

Publication 6

Thompson LJ, Brown M and Downs CT (2015)

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Author contributions:

LJT conceived paper with MB and CTD. LJT collected and analysed data, and wrote the paper. MB and CTD contributed valuable comments to the manuscript.

Signed: ███████████████████……………

Lindy Jane Thompson

November 2014

DEDICATION

For my parents, Bobbie and Jeff Thompson,

and my sister, Susie Lee Thompson,

who supported me every step of the way.

Thank-you.

ACKNOWLEDGEMENTS

TABLE OF CONTENTS

CHAPTER 1

FACTORS AFFECTING AVIAN BASAL METABOLIC RATES

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Abstract

Reversible, within-individual variation (flexibility) in phenotypic traits may be influenced by natural selection, and may confer costs and benefits on the individual. Specifically, flexibility in avian basal metabolic rate (BMR) seems to be linked to flexibility in the masses of certain organs and in the metabolic intensities of certain tissues, and depends primarily on body mass, but also on various environmental factors, including thermal acclimation and seasonal acclimatisation. Latitude of origin, captivity and aridity can also affect avian BMR, however authors warn against the use of simple dichotomies (e.g. arid vs. mesic) when analysing results, since various factors are interdependent, and this may complicate analyses. Furthermore, the effect of phylogeny on avian BMR continues to be strongly debated, with differences between passerines and non-passerines disappearing when phylogenetically informed analytical techniques are employed.

Although the fast responses of many species to recent climate change allow for some optimism in the face of predicted climate change scenarios, much of the current knowledge of the effects of climate change on birds is restricted to passerines from northern hemisphere

temperate zones, and more work is needed on their southern hemisphere counterparts. Thus the role of phenotypic flexibility relative to changing environmental conditions needs to be evaluated, to more accurately predict how species may respond to global climate change. In addition, although winter acclimatisation is well documented in small passerines and Holarctic birds, more data is needed on seasonal BMR adjustments in larger birds, non-passerines, and tropical and subtropical species. In particular, there are large knowledge gaps regarding the energetics of birds from the African, Asian and South American tropics, even though the tropics have the greatest diversity of species, and Africa is the continent most at risk from climate change.

Key words: acclimation, acclimatisation, avian BMR, phenotypic flexibility.

Introduction

A history of BMR studies in birds

Birds are endotherms; they can adjust their metabolism to maintain a constant body temperature (T_b) over a broad range of ambient temperatures (T_a) (McNab, 2009). Comparative analyses of the metabolic rates of birds date back to at least the 1920s (Benedict and Fox, 1927; Terroine and Trautmann, 1927), with the 1950s seeing the first comparative studies on ecophysiological characteristics of birds in relation to environment-related energetic demands (Piersma et al., 1996; Scholander et al., 1950a; Scholander et al., 1950b; Scholander et al., 1950c). As broadly comparative data on avian metabolism accumulated (Kendeigh et al., 1977; Weathers, 1979), allometric analyses correlated avian standard metabolic rate with taxonomic status and body

mass (Lasiewski, 1964; Lasiewski and Dawson, 1967; Root, 1988; Weathers, 1979). Subsequently, other factors were found to influence avian metabolic rate, including climate (Weathers, 1979), length of time in captivity, and season (Kendeigh et al., 1977). Avian metabolic rate has also been shown to correlate with species behaviour (McNab, 2009), distribution (Root, 1988), life history attributes such as growth and reproduction (Hulbert and Else, 2000; Tieleman et al., 2003a), and even survival (Olson et al., 2010; Root, 1988; Sharbaugh, 2001); all of which have ecological consequences (McNab, 2009).

Basal metabolic rate (BMR) has been described as the 'obligatory cost of living for endotherms' (Barceló et al., 2009); it represents the lowest maintenance metabolism, measured during the inactive period and at thermoneutrality, of a resting, post-absorptive, non-reproductive, adult endotherm (McKechnie et al., 2006; McNab, 1997). BMR is a standardised baseline metabolic parameter (McKechnie, 2008; Olson et al., 2010), defined by criteria which ensure that differences in metabolic rate reflect the species, rather than the conditions under which measurements were made (McNab, 2009).

In addition to BMR, the scientific literature includes a range of metabolic measurements, including field metabolic rate (FMR, the energetic costs of normal daily activities in animals under natural environmental conditions (Swanson, 2010), resting metabolic rate (RMR, the minimum metabolic rate of an organism that is in a non-mobile state and is post-absorptive during its natural rest phase at a particular T_a (Nzama et al., 2010; Wilson et al., 2011), summit/peak metabolic rate (M_{sum}/PMR, the maximum resting thermogenic metabolic rate in response to cold exposure (I.U.P.S. Thermal Commission, 2001; Smit and McKechnie, 2010), and standard metabolic rate (SMR, the minimal metabolic rate of a resting endotherm (which does not need to be fasting), measured during its natural rest phase, at temperatures within

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thermal neutral zone or TNZ (I.U.P.S. Thermal Commission, 2001; Weathers, 1979). The TNZ is the range of T_as at which metabolism is minimal (Williams and Tieleman, 2001), and temperature regulation is achieved simply by control of sensible heat loss, without evaporative heat loss or regulatory changes in metabolic heat production (I.U.P.S. Thermal Commission, 2001; Williams and Tieleman, 2001). Currently, comparisons of avian maximum metabolic rate (MMR, the upper limit for thermogenesis of an endotherm relating to work) (I.U.P.S. Thermal Commission, 2001) are not viable, due to the difficulty of proving that oxygen consumption ($\dot{V}O_2$) during sustained flight has truly reached maximal values (Swanson, 2010).

Studies of BMR have contributed significantly to our understanding of animal energetics (Reynolds and Lee, 1996; Williams and Tieleman, 2000). Although wild animals may only rarely function at basal levels of energy expenditure, BMR correlates with the energy expenditure of free-living animals (Daan et al., 1990; Nagy, 1987). BMR may comprise up to 60% of avian FMR (Swanson, 2010), and may be correlated with MMR, once the effects of body mass and phylogeny are removed (Rezende et al., 2002). BMR has been used to assess the energy costs of thermoregulation (Dawson and O'Connor, 1996); increments in energy expenditure due to activity in the wild (Bryant and Tatner, 1991; Drent and Daan, 1980; Nagy et al., 1999; Ricklefs et al., 1996); limits to maximum physiological performance (Hinds et al., 1993; Peterson et al., 1990); the effects of body size and circadian phase on energy flux (Hayssen and Lacy, 1985; Tieleman and Williams, 2000); and evolutionary adjustments of metabolic rates to specific environments (Piersma et al., 1996; Weathers, 1979; Williams and Tieleman, 2001).

Over the last eight decades, BMR has become one of the most commonly measured physiological variables of endotherms (Bech et al., 1999; Rønning et al., 2007). This long history of empirical measurements and comparative analysis has made it an extremely useful

metric for comparing metabolic power output among avian taxa (Liknes and Swanson, 1996; McKechnie, 2008; McKechnie et al., 2006). Therefore, since BMR is the most commonly used comparative measure of the metabolic rate of endotherms (Barceló et al., 2009; Daan et al., 1990; Piersma et al., 1996; Rezende et al., 2002; White and Kearney, 2012), this review will focus on flexibility in avian metabolic rates in terms of BMR.

Factors affecting avian metabolic rates

BMR varies greatly both within and between endothermic species (Bech et al., 1999), and numerous factors have been shown to affect BMR reversibly (Broggi et al., 2009), many of them covarying with avian BMR independently of the effect of body mass (Seavy and McNab, 2007) and thereby explaining the residual variation of BMR around allometric curves (Bosque et al., 1999). Some of the factors that influence avian BMR include altitude (McNab, 2003, 2005); body composition (Daan et al., 1990); climate (Weathers, 1979); disease (Hayworth et al., 1987); distribution (Merola-Zwartjes and Ligon, 2000); diet (McNab, 1986, 1988c, 2005); foraging style (McNab and Bonaccorso, 1995); habitat type (Hulbert and Dawson, 1974); latitude (Weathers, 1979); moult (Klaassen, 1995; Lindström et al., 1993); plumage colour (Ellis, 1980); season (Seavy and McNab, 2007); temperature; and the intra-annual coefficients of variation of precipitation (White et al., 2007a). For example, an analysis of 533 bird species showed that body mass, climate, diet, habitat, a highland/lowland distribution, the use or not of torpor and of flight, as well as the passerine/non-passerine dichotomy, accounted for 97.7% of the variation in avian BMR (McNab, 2009).

High avian BMR may be linked to an 'energetically expensive' way of life (Kersten and Piersma, 1987), and is characteristic of aquatic species (McNab, 2009), and those with a high level of aerobic activity (Bech et al., 1999), or living in cooler climates or at higher latitudes

(Bryant and Furness, 1995; Gabrielsen et al., 1988). Conversely, a low BMR has been found to be characteristic of some tropical (Hails, 1983; Pettit et al., 1985; Schleucher and Withers, 2002; Wiersma et al., 2007; Williams et al., 2010) and nocturnal birds (Bech et al., 1999), and of those living on islands (McNab, 1994). The latter may have evolved in response to a lack of predators on islands (Noakes et al., 2013) or to help island birds cope with food scarcity, since higher metabolic rates demand more food (Furness, 2003).

It is important to note that although comparative methods take advantage of large, intraspecific variation in analysed traits to increase the power of their statistical analyses, their results are correlative, and may not imply causation (Garland Jr. and Adolph, 1994).   The true causal relationships underlying variation in BMR can only be elucidated through experiments designed to demonstrate the ecological factors underlying the physiological or other changes underlying changes in BMR (Kvist and Lindström, 2001). Furthermore, changes in BMR reflect the net result of (i) various heat sources that are generated by the numerous metabolic reactions essential to an animal's life (Klaassen et al., 1989), and (ii) various ecological factors that often interact, thereby making it difficult to determine the effect of a particular factor on BMR (Kvist and Lindström, 2001).

Despite the challenges associated with interpreting how a factor may affect metabolic rate, studies on flexibility in avian metabolic rate are increasing dramatically (Broggi et al., 2009). Indeed, while many earlier studies regarded avian BMR as representative and fixed for a certain temperature (Wilson et al., 2011), reporting a single BMR value for a species (McKechnie, 2008; Speakman et al., 2004), avian BMR is now known to be a highly flexible phenotypic trait (Piersma, 2002; Vézina et al., 2006). This flexibility offers a solution to the problem of adaptation to spatially or temporally heterogeneous environments (DeWitt, 1998;

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DeWitt et al., 1998; Schlichting and Pigliucci, 1998; Tieleman et al., 2003b; Via et al., 1995). Moreover, laboratory studies have shown that despite its plastic nature, intra-individual variation in BMR is repeatable and heritable (Broggi et al., 2009; Careau et al., 2014; Hõrak et al., 2002; Rønning et al., 2007; Tieleman et al., 2003b; Versteegh et al., 2008), suggesting an intra-individual consistency of BMR (relative to the mass-adjusted population mean), on which natural selection can act (Bech et al., 1999; Konarzewski et al., 2005).

Birds adjust their BMR in response to season (Cooper et al., 2002; Cooper and Swanson, 1994; Petit et al., 2013; Pohl and West, 1973), geographic location (Broggi et al., 2004; Kersten et al., 1998; Kvist and Lindström, 2001; Vézina et al., 2006), and to varying temperatures during acclimation experiments (Tieleman et al., 2003b; Williams and Tieleman, 2000), and so interspecific differences in physiological traits should be interpreted with caution. In addition, the speed of the responses to thermal acclimation may differ, even within species (Barceló et al., 2009; McKechnie et al., 2007), and the magnitude of BMR adjustments varies greatly between species and populations (Lindsay et al., 2009a; Tieleman et al., 2003b; Williams and Tieleman, 2000) with the most pronounced avian metabolic flexibility seen in long-distance migrants and species resident at high latitudes (Lindström and Klaassen, 2003; Piersma et al., 1995; Swanson and Olmstead, 1999), where energetic costs are potentially higher (Drent and Daan, 1980).

There are still uncertainties about the mechanisms that birds use to vary their metabolic rates in response to fluctuating environmental conditions (Carey et al., 1978; Liknes and Swanson, 1996), as well as the ways in which natural selection acts upon these mechanisms (Tieleman et al., 2003b). Nevertheless, studies suggest that there are three major physiological and morphological pathways for the up- or down-regulation of avian metabolic rates: (i) adjustments in transport capacities for oxygen and metabolic substrates (Carey et al., 1978); (ii)

adjustments in the mass-independent metabolic intensities of specific organs (Kvist and Lindström, 2001), and (iii) adjustments in organ and tissue masses (Lindström et al., 1999; McKechnie, 2008; Piersma et al., 1996).

Body mass (and conventional versus phylogenetically independent allometric analyses)

Of all the factors known to affect avian BMR, body mass has the greatest influence (Geiser, 1988; Reynolds and Lee, 1996). In 13 species of birds of paradise, for example, and in five bulbul species (Family: Pycnonotidae), body mass accounted for 91.7% and 97.7% of the variation in BMR respectively (McNab, 2005; Seavy and McNab, 2007).

Since small endotherms have higher surface area to volume ratios than larger endotherms, they also have higher mass-specific energy expenditure rates and thermoregulatory costs (Arens and Cooper, 2005; McNab, 2009; Sharbaugh, 2001). Thus small animals need to make physiological adjustments in metabolic rate or insulation at a rate that equals the rapid rate at which they gain and lose heat from/to their environment (Chaui-Berlinck et al., 2002; Williams and Tieleman, 2001). However, small birds are limited in the amount of insulation they can carry before normal movement is affected and predation risk increased, and so most small temperate and Holarctic birds show behavioural and physiological adjustments to cold winter T_a, acclimatising to winter conditions mainly with higher metabolic rates, which may reflect greater thermogenesis and cold tolerance in winter than in summer (Chaplin, 1974; Dawson and Carey, 1976; Newton, 1969; Pohl and West, 1973; Weathers and Caccamise, 1978).

It has been argued that the significantly smaller body masses of passerines, as well as their lower rate of evolution in body mass and mass-corrected BMR, justifies the need for separate, class-specific, allometric equations for the prediction of avian BMR, and that there are

significant differences in equations derived using conventional and phylogenetic methods (Garland Jr and Ives, 2000). Indeed, conventional allometric studies have shown that passerines tend to have higher metabolic rates than other birds of the same mass (McNab, 2009; Rezende et al., 2002). For example, when body mass was included in the analysis, tropical frugivorous passerines showed a 57% higher whole-animal BMR than frugivorous non-passerines, and this difference was highly significant (McNab, 2005).

An alternative approach is the phylogenetically corrected method, which recognizes that there should be greater similarity between closely related species than between distantly related species, and so species may not represent independent, identically distributed data points; hence statistical methods such as standard least squares regression cannot be used in comparative analyses (Garland Jr and Ives, 2000). Phylogenetically informed methods correct for the relatedness (non-independence) of species by weighting sample data in relation to the level of relationship shown between species (Felsenstein, 1985), and give preference to phylogeny as a possible explanation of the variation in BMR beyond the variation associated with body mass (McNab, 2009). Phylogenetic methods have been used for comparative analyses since the 1990s, and have found no difference in BMR between passerines and non-passerines (Reynolds and Lee, 1996; Rezende et al., 2002); thus allometric curves predicting BMR have similar slopes for passerines and non-passerines (McNab, 2009).

To illustrate this, a conventional least-squares regression using 126 avian species produced the following estimate of BMR (W): $\log BMR = -1.461 + 0.669 \log Mb$, while the corresponding phylogenetically independent regression for the same data set was $\log BMR = -1.581 + 0.677 \log Mb$; the former (conventional) approach indicated that the BMR of passerines was significantly higher than that of nonpasserines, while the latter (phylogenetically

independent) analysis of the same data set showed no significant differences once phylogenetic relatedness was taken into account; that is, the differences were due to phylogenetic relatedness, not adaptive variation (McKechnie and Wolf, 2004a).

Various equations have been produced to describe the relationship between body mass (represented by M in the following equation) and metabolic rate, where: metabolic rate $= aM^b$ (Hudson et al., 2013). Reynolds and Lee (1996) used phylogenetically corrected least-squares regression to give $b = 0.635$ (95% CI: $0.532 - 0.710$), while McNab (2009) found that once phylogenetic and ecological effects had been accounted for, $b = 0.689$ ($0.681 - 0.769$) for avian BMR. Rezende et al. (2002) used independent contrasts to give $b = 0.756$ ($0.516 - 0.997$), while an earlier analysis using independent contrasts found $b = 0.709$ ($0.671 - 0.748$), and argued that phylogenetically informed approaches can have greater statistical power than conventional analyses in determining whether a species or population deviates from expectations (Garland Jr and Ives, 2000).

Assuming that metabolic rate is an evolved trait, phylogenetic correlation is likely to be widespread in such data, and as such, allometric studies should have a phylogenetic perspective (White et al., 2007b), however the scaling of avian basal metabolic rate and body mass may not be governed by a single universal law; instead it may depend on various physical constraints, whose relative influence will be affected by the study animal's metabolic state (Glazier, 2008; McNab, 2009)

Diet

The rate of energy acquisition may limit the rate of energy expenditure (McNab, 1986) and so diet may be important in determining maintenance energy costs (Bosque et al., 1999). One

hypothesis linking mass-independent BMR to diet is the food habit hypothesis, which states that species or populations exploiting food with a lower energy content and/or reduced digestibility may evolve lower mass-independent BMRs, however support for this hypothesis has not been unanimous (Sabat et al., 2009; Sabat et al., 2010)

Folivory may be associated with low BMR: for example, the Grayish Saltator (*Saltator coerulescens*) and the Orinocal Saltator (*S. orenocensis*), two arboreal folivores, both had much lower BMRs than predicted from their body size (Bosque et al., 1999). Similarly, Speckled (*Colius striatus*) and Blue-naped Mousebirds (*Urocolius macrourus*), two partially folivorous African species (Bartholomew and Trost, 1970; Prinzinger, 1988), also had lower than expected BMRs (Bosque et al., 1999). The low BMRs observed in folivorous birds may be linked to their reduced intake as a means of decreasing ingestion of toxic plant secondary metabolites, and detoxification costs could represent a substantial part of their energy budget (Guglielmo et al., 1996). Alternatively, a low BMR may be correlated with the low muscle mass linked to sedentary habits (McNab, 1988b, 1994), since, among folivorous birds, only the more arboreal species seem to have reduced metabolic rates; for example, two ground-dwelling (Tetraoninae) species of temperate latitudes had metabolic rates that were similar to or higher than those expected from body mass alone (McNab, 1988a; Weathers, 1979).

Frugivory has also been associated with lower than expected metabolic rates in passerines (Bartholomew et al., 1983) and non-passerines (McNab, 2001). The evolution of omnivory was associated with an increase in BMR (McNab, 2005). Indeed, a more catholic diet may allow a higher BMR since it reduces periods of food shortage, compared with species that specialise on highly seasonal foods, and which have lower BMR (McNab, 2009). It has been suggested that the effect of diet is second only to that of body mass on the BMRs of birds of paradise (McNab,

2005). However, factors accounting for variation in BMR often interact, (for example, frugivorous species may be more likely to have a tropical distribution), and so it may be difficult to determine the influence of each of these (McNab, 1995, 2001, 2005).

Season

Historically, BMR and RMR were considered specific for a particular temperature, however seasonal flexibility in both has been shown to be widespread in birds (Bech, 1980; Bush et al., 2008a; Cooper and Swanson, 1994; McKechnie, 2008; Piersma et al., 1995), and usually these changes seem to follow seasonal changes in energy expenditure (Cooper and Swanson, 1994; Dawson, 2003; Piersma et al., 1995; Smit et al., 2008). Whereas acclimation refers to adjustments occurring within an organism in response to experimentally induced changes (especially climatic factors such as T_a), acclimatisation refers to responses to natural environments (McKechnie, 2008). Most studies of thermal acclimation in birds investigate changes in thermoregulatory responses after a brief period of cold acclimation (McKechnie et al., 2007; Tieleman et al., 2003b), which, for many species, results in higher BMR and RMR (Klaassen et al., 2004; Lindsay et al., 2009a; Vézina et al., 2006). For example, Laughing Doves (*Streptopelia senegalensis*) increased their BMR during cold-acclimation and then decreased BMR when moved into a warm room (McKechnie et al., 2007).

The higher winter metabolic rates of some species suggest increased thermogenic capacity, and are correlated with improved cold tolerance (Cooper and Swanson, 1994). For example, increased thermogenic capacity, rather than increased insulation, was found to be the main feature of winter acclimatisation in American Goldfinches (*Carduelis tristis*) (Carey et al., 1978; Dawson and Carey, 1976). Summer-acclimatised House Sparrows (*Passer domesticus*) died

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when exposed to $T_a < 0$°C for more than a few days (Davis Jr., 1955), and goldfinches quickly became hypothermic when exposed to severe cold during spring, summer or autumn, however under the same conditions in winter they showed a significant increase in thermoregulation (Carey et al., 1978). Conversely, winter-acclimatised House Sparrows are more tolerant of low temperatures than summer-acclimatised birds (Davis Jr., 1955; Kendeigh, 1949; Nzama et al., 2010). However, metabolic rate does not always increase with cold acclimation (Vézina et al., 2006; Williams and Tieleman, 2000); Rock Kestrels (*Falco rupicolis*) had higher RMRs after acclimation to 25°C than when they were acclimatised to winter temperatures, perhaps since Afrotropical winters are mild, but food is still scarce, and so it makes sense to conserve energy during winter acclimatisation by lowering RMR (Bush et al., 2008b). Similarly, Monk Parakeets (*Myiopsitta monachus*) significantly reduced their fasting metabolic rate in winter, allowing improved maintenance of T_b (Weathers and Caccamise, 1978).

Seasonal acclimatisation may be aided (through reduced energy expenditure and subsequently decreased metabolic rate) by change in substrate metabolism (Marsh and Dawson, 1982; Marsh et al., 1990; Swanson, 1991), vascular oxygen transport (Liknes and Swanson, 1996), thermal conductance (Novoa et al., 1994), altered levels of thyroid hormones (Zheng et al., 2014a) and nocturnal hypothermia or torpor (Chaplin, 1976; Reinertsen, 1983). However, there is a lower limit to T_b, and thus a finite amount of energy that can be conserved by reducing T_b (Sharbaugh, 2001). For example, passerines that employ nocturnal hypothermia successfully do not let their T_b drop below 30°C (Chaplin, 1974; Reinertsen and Haftorn, 1983; Sharbaugh, 2001).

The effects of season on metabolic rate may be pronounced in some bird species (Chapters 2 and 3), yet absent in others (Dawson and O'Connor, 1996; Liknes and Swanson,

1996; Rezende et al., 2002). For example, no significant change in seasonal BMR was recorded in certain populations of House Finches (*Carpodacus mexicanus*) and Black-capped Chickadees (*Poecile atricapillus*), two north-temperate species (O'Connor, 1996; Rising and Hudson, 1974), or in Rufous-collared Sparrows (*Zonotrichia capensis*) and White-browed Scrubwrens (*Sericornis frontalis*), both from the southern subtropics (Ambrose and Bradshaw, 1988; Maldonado et al., 2009; Smit and McKechnie, 2010). However, generally, avian BMR is significantly adjusted between seasons (McKechnie, 2008), with BMR of birds in cooler climates usually higher in winter than in summer, since these birds normally have increased cold tolerance, RMR, SMR, metabolic capacity and peak metabolic rate in winter relative to summer (Dawson and Carey, 1976; Dawson and Marsh, 1989; Downs and Brown, 2002; Hart, 1962; McKechnie et al., 2007; Olson et al., 2010; Pohl and West, 1973; Weathers and Caccamise, 1978; Williams and Tieleman, 2000; Zheng et al., 2014b). It is important to note that species may or may not show differences in RMR between years (Chapter 2), and that the timing of seasonal measurements may underestimate the range of flexibility of which birds are capable in terms of their metabolic rates (Chapter 3). Thus results from seasonal studies should be regarded with caution, unless measurements have first been taken throughout the year, to establish precisely when peaks and lows in BMR and RMR occur (Chapter 3).

The direction and magnitude of seasonal BMR adjustments in birds vary with M_b (Dawson, 2003; Hayworth and Weathers, 1984; McKechnie, 2008; Smit et al., 2008; Swanson and Weinacht, 1997) and there seems to be general agreement that in winter, large birds (> 1000 g) decrease the lower critical limit of their TNZ and down-regulate both RMR and BMR (Bush et al., 2008a; Smit et al., 2008; Weathers and Caccamise, 1978); medium-sized birds (200-1000 g) show either slight decreases or increases, or no seasonal flexibility in BMR (Bush et al.,

2008a; Dawson, 2003; Masman et al., 1988; Smit et al., 2008; Swanson and Weinacht, 1997; Weathers and Caccamise, 1978); and small birds (< 200 g) (often from northern latitudes, where winters are severe) up-regulate their RMR and BMR in winter as a cold defence mechanism (Bush et al., 2008a; Dawson, 2003; Smit and McKechnie, 2010; Weathers and Caccamise, 1978). There are however exceptions, with some small Southern Hemisphere birds down-regulating their metabolic rate in winter (Maddocks and Geiser, 2000b; Smit et al., 2008).

Latitude

Generally, species inhabiting subtropical regions where mean winter $T_a > 0°$ C tend to have lower winter than summer BMR, whereas the opposite is true for species inhabiting colder, high-latitude regions (McNab, 2009; Smit and McKechnie, 2010). Thus latitude may also have an effect on seasonal variation in metabolic rate, with birds inhabiting areas of higher latitude usually having higher metabolic rates than those closer to the equator (Broggi et al., 2004; Hails, 1983; Kersten et al., 1998; Kvist and Lindström, 2001; Tieleman et al., 2003a; Tieleman et al., 2003b; Vézina et al., 2006; Weathers, 1979). For example, chickadees maintain the minimum metabolic machinery necessary to survive winter T_a, such that their BMR is associated with temperature isotherms (Olson et al., 2010), and shorebirds at high latitudes have a higher BMR than those from tropical latitudes (Williams and Tieleman, 2000). Similarly, the rest phase O_2 consumption of a variety of Malaysian birds was 35% lower than that of similar sized temperate species (Hails, 1983). On a global scale, the direction and magnitude of seasonal BMR variation seems to be correlated with severity of winter climate and latitude, representing a trade-off between energy-saving and cold-tolerance; extremely cold winter conditions result in increased

metabolic machinery associated with thermogenesis, but this incurs the cost of higher maintenance energy requirements (Smit and McKechnie, 2010).

Rubner's law states that the heat increment of feeding contributes toward temperature regulation at low T_a, and this heat is therefore not available in the fasting bird (Kendeigh, 1969). Birds inhabiting areas with cold winter climates are almost always exposed to temperatures below the TNZ, resulting in increased thermoregulatory costs which increase the energy demand on the bird (Williams and Tieleman, 2001; Wilson et al., 2011). The 'Energy Demand' hypothesis for short-term or seasonal adjustments in BMR suggests that birds adjust the size of their internal organs relative to food intake, which itself correlates with energy demand (Smit and McKechnie, 2010; Tieleman et al., 2003b; Williams and Tieleman, 2000). As energy demands increase because of lower T_a or greater activity levels, birds ingest more food, and so organs involved in catabolism (stomach, intestine and liver), oxygen transport to tissues (heart and lungs), and elimination of waste (kidneys), hypertrophy
(Williams, 1999; Williams and Tieleman, 2000). These organs all have relatively high metabolic intensities, energy consumption, thermogenic capacity and mass-specific metabolic rates (Bech et al., 1999; Burness et al., 1998; Daan et al., 1990; Hammond et al., 2000; Martin and Fuhrman, 1955; Vézina and Williams, 2003), and so total oxygen demand under basal conditions increases as these structures become larger, and this may be reflected in significantly higher BMR (Kersten and Piersma, 1987; Liknes et al., 2002; Williams and Tieleman, 2000). The resulting increase in thermogenic capacity and higher mass-independent BMR allows wild birds sufficient reserve capacity in terms of heat production to buffer the effect of sudden cold spells on daily energy expenditure (Vézina et al., 2006; Williams and Tieleman, 2000). For example, changes in organ mass (particularly of those organs with high metabolic activity) (Maldonado et al.,

2009) explained some of the variability in BMR between cold- and warm-acclimated Rufous-collared Sparrows (*Zonotrichia capensis*) (Barceló et al., 2009). Similarly, cold-acclimated Hoopoe Larks (*Alaemon alaudipes*) showed greater food intake and increased mass of the digestive organs and a higher BMR (Williams and Tieleman, 2000), and cold-acclimated Red Knots (*Calidris canutus islandica*) showed increased food intake, lean body mass, metabolic intensity, and whole-organism BMR, compared with conspecifics kept at higher temperatures (Vézina et al., 2006).

The intensity of the tissue-specific metabolic rates of various organs may also affect BMR (Tieleman et al., 2003b). For example, cold-acclimated Pigeons (*Columba livia*) have shown structural changes in their pectoral muscles (including increased density of capillaries and mitochondrial volume in aerobic fibres), to meet the increased energetic demands of shivering (Mathieu-Costello et al., 1998; Tieleman et al., 2003b).

In high latitudes, wintering birds often face the interacting problems of cold, short days and lower food supplies, which may force the bird to fast for longer during the rest phase than in summer (Arens and Cooper, 2005), thereby presenting severe physiological challenges to thermoregulation (Carey et al., 1978; Kersten and Piersma, 1987; Liknes and Swanson, 1996; Lima, 1986; Sharbaugh, 2001; Williams and Tieleman, 2000). The resulting increased energy usage by the skeletal muscles requires enlarged abdominal organs for support (Kersten and Piersma, 1987), and since larger organ masses command a high BMR (Kersten and Piersma, 1987; Williams and Tieleman, 2000), avian metabolic rate could be expected to increase with increasing latitude (Weathers, 1979).

Thus, for high latitude resident species, increased winter BMR represents an important component of winter acclimatisation (McKechnie, 2008) and can be seen as an adaptation to

maximal power output level (Kersten and Piersma, 1987; Klaassen et al., 1989), aiding thermogenesis and survival in severe environments (Sharbaugh, 2001). Indeed, increased BMR has been found in most studies of avian winter acclimatisation (Klaassen et al., 2004; McKechnie et al., 2007; Smit and McKechnie, 2010; Tieleman et al., 2003b; Vézina et al., 2006; Williams and Tieleman, 2000). However, not all species fit this trend; some showed no apparent seasonal adjustments in BMR (Hart, 1962; O'Connor, 1995; Williams and Tieleman, 2000) while others reduced their winter metabolic rates (Bech et al., 1999; Sharbaugh, 2001; Williams and Tieleman, 2000).

Relatively few seasonal studies have been conducted on Afrotropical birds, in comparison with birds from the northern hemisphere (Bush et al., 2008a; Chamane and Downs, 2009; Nzama et al., 2010; Seavy and McNab, 2007; Smit et al., 2008), and so patterns of seasonal BMR variation in subtropical species are largely unknown (Smit and McKechnie, 2010). In the Afrotropical region, winters are milder than in Holarctic and temperate regions, with less need for enhanced winter cold tolerance than at higher latitudes (Smit and McKechnie, 2010). Thus the direction and magnitude of seasonal variation in BMR may be expected to differ from the trends seen in high-latitude residents (Maddocks and Geiser, 2000a; Swanson, 1991). In addition, the El Niño Southern Oscillations render the Afrotropical climate less predictable, which in turn results in less predictable rainfall and primary production (Smit et al., 2008), presenting a challenge to many endotherms (Nzama et al., 2010).

Where seasonal differences in T_a are large, increased BMR can aid thermoregulation as BMR is related to the peak metabolic rate associated with heat production (Bush et al., 2008a; Dawson, 2003). However, more energy is expended with a higher BMR and food scarcity during winter demands energy conservation, so if the winter T_a is not too severe, then lowering

metabolic rate in winter would conserve energy (Bush et al., 2008a; Dawson, 2003). Indeed, non-Holarctic species such as Australian Silvereyes (*Zosterops lateralis*) (Maddocks and Geiser, 2000a), White-browed Scrubwrens (*Sericornis frontalis*) (Ambrose and Bradshaw, 1988), Rock Kestrels (Bush et al., 2008a), a coastal population of Southern Red Bishops (*Euplectes orix*) (Van de Ven et al., 2013), and five Kalahari Desert-resident species (Smit and McKechnie, 2010), all had significantly lower metabolic rates in winter than in summer; contrary to what might be expected in similarly-sized Holarctic species (McKechnie, 2008). These seasonal changes in BMR shown by species resident in lower latitudes, may represent adjustments related to saving energy and/or water, rather than to increased thermoregulatory capacity or cold tolerance (Smit and McKechnie, 2010).

Despite these trends in avian BMR with latitude (Hails, 1983; Lasiewski et al., 1970; Piersma et al., 1996; Seavy and McNab, 2007; Weathers, 1979; Wiersma et al., 2012; Williams and Tieleman, 2000), there are exceptions that may complicate our understanding of environmental influences on BMR (Vleck and Vleck, 1979; Williams, 1999; Williams and Tieleman, 2000). The Southern White-faced Scops-owl (*Ptilopsis granti*) showed increased winter RMR (Smit and McKechnie, 2010), the Knysna Turaco (*Tauraco corythaix*) exhibited seasonal thermoregulatory responses representing cold defense, rather than energy conservation (Wilson et al., 2011), and an inland population of the Southern Red Bishop increased winter BMR by 31% (Van de Ven et al., 2013). Meanwhile, other studies recorded no seasonal change (Bush et al., 2008a) or an increased winter BMR (Chamane and Downs, 2009; Nzama et al., 2010; Smit and McKechnie, 2010). These exceptions highlight the importance of continued data collection on the physiology of Afrotropical birds, especially since BMR has been measured for relatively few tropical bird species (Seavy and McNab, 2007).

Thus the magnitude and direction of seasonal changes in BMR vary with latitude, from marked winter increases at high latitudes where winters are extremely cold, to the converse in warmer, subtropical environments, where winter metabolism may be driven mainly by the need for energy and/or water conservation rather than for cold tolerance (Smit and McKechnie, 2010). Indeed, latitudinal differences in BMR have been explained as an adaptation to heat stress, due to selection for a reduced endogenous heat production in tropical regions (Klaassen et al., 1989; Weathers, 1979). The BMR of many species has been shown to vary by 50-100% of predicted values, depending on the latitude the bird came from (Weathers, 1979). Thus predictive equations might have little use outside temperate regions (Hails, 1983).

The relationship of BMR to seasonal acclimatisation is complex and still unclear (Liknes et al., 2002). The current lack of data from the true tropics, and from lower latitudes in the Northern Hemisphere and higher latitudes in the Southern Hemisphere, means that any conclusions regarding correlations with latitude and/or temperature must remain tentative (Smit and McKechnie, 2010). Future studies should further investigate the functional roles of specific environmental variables (e.g. food and water availability, temperature and photoperiod) on seasonal metabolic variation, particularly in sub-tropical and tropical species (Smit and McKechnie, 2010; Swanson et al., 2014).

Altitude

Rosy (*Leucosticte arctoa*) and House Finches (*Carpodacus mexicanus*) both dropped their BMR significantly with decreased altitude (Clemens, 1988), and 99% of the observed variation in BMR 13 species of birds of paradise (Family Paradisaeidae) was due to interspecific variation in body mass, food habits and altitudinal distribution (McNab, 2003, 2005). Birds of paradise

species that were limited to lower altitudes had lower BMRs than those found at higher altitudes, however, food habits were not independent of altitude; committed frugivores were found at higher altitudes (McNab, 2003, 2005), and frugivory is associated with low metabolism in both passerines (Bartholomew et al., 1983) and non-passerines (McNab, 2001).

Amethyst Sunbirds (*Chalcomitra amethystina*) showed significant altitudinal variation in winter BMR, presumably due to altitudinal variation in T_a, such that lower T_as at higher altitudes would necessitate increased metabolic heat production by homeothermic species (Lindsay et al., 2009a; Lindsay et al., 2009b; Soobramoney et al., 2003). These results highlight the need to acknowledge altitudinal differences within species, to make accurate predictions about the thermal physiology of species and their response to changes in T_a (Lindsay et al., 2009a, b).

Climate

Although climate was originally thought not to affect metabolism (Scholander et al., 1950a), and some analyses report no effect of climate on metabolic rate in certain bird populations (McNab and Ellis, 2006), various studies show otherwise (Hails, 1983; Hudson and Kimzey, 1966). Climate may affect birds directly, by influencing energy and water balance, and/or indirectly, by affecting vegetation and food availability (Weathers and van Riper III, 1982).

Aridity gradient

Compared to non-flying endotherms, birds have high mass-specific energy requirements, and the challenge of maintaining energy balance is particularly acute for those inhabiting arid environments (Williams and Tieleman, 2001). Various studies have reported that birds living in arid habitats have significantly lower BMR (as well as RMR and FMR) than their similar-sized mesic counterparts (McKechnie, 2008; Nagy, 1987; Schleucher and Withers, 2002; Smit and

McKechnie, 2010; Trost, 1972; Williams and Tieleman, 2000, 2001), and a study of BMR in 12 species of lark along a gradient of increasing aridity, found that while body mass alone accounted for 53% of the interspecific variation in BMR, including aridity raised the explained variance to 91%, and natural selection might explain the decrease in BMR of larks along an aridity gradient (Tieleman et al., 2003a, b).

In deserts, three factors may select for reduced avian field and basal metabolic rates (Tieleman and Williams, 2000). Firstly, food scarcity may restrict energy intake, thus favouring individuals with low rates of energy expenditure and metabolic rate (Williams and Tieleman, 2001). For example, the BMR of the Scrub-wren (*Sericornis frontalis*) in western Australia was lower in a xeric environment than in a semi-xeric or mesic environment, but only in summer when the rainfall and insect abundance was very low (Ambrose and Bradshaw, 1988). A possible link between increased food availability and higher BMR involves larger digestive organs and/or higher metabolic intensity of maintenance organs (Williams and Tieleman, 2000), although various species held in captivity also showed winter reductions in BMR even when food was provided *ad libitum* (Bush et al., 2008a; Smit and McKechnie, 2010; Weathers and Caccamise, 1978). Secondly, high T_a would reduce thermoregulatory requirements, possibly resulting in decreased food intake with lower energy demand, and a consequent reduction in mass of digestive organs, thereby reducing BMR (Williams, 1999; Williams and Tieleman, 2000). Thirdly, lowering endogenous heat production may reduce the need for evaporative cooling and so prevent further respiratory evaporative water loss in an environment where water is already scarce (Smit and McKechnie, 2010; Tieleman and Williams, 2000; Williams and Tieleman, 2001; Withers and Williams, 1990). Thus, a reduced BMR may have adaptive value for birds living in waterless environments (Weathers and van Riper III, 1982).

Ambient temperature (T_a) and the link between climate change and avian BMR

Fluctuations in T_a are known to affect avian metabolic rate (McKechnie, 2008), with a negative

link between avian BMR and T_a frequently reported at both the population (Broggi et al., 2004;

Merola-Zwartjes and Ligon, 2000) and species levels (White et al., 2007a). For species that

elevate their BMR in response to low T_a during winter-acclimatisation (Cooper and Swanson,

1994; Liknes et al., 2002) and cold-acclimation (Klaassen et al., 2004; McKechnie and Wolf,

2004b; Williams and Tieleman, 2000), the resulting increase in heat production may help to

maintain thermal balance (Dawson and O'Connor, 1996; Dutenhoffer and Swanson, 1996;

Rezende et al., 2002). For example, a study on Black-capped Chickadees revealed an overall

significant correlation between lower T_as and higher metabolic rates (Olson et al., 2010).

Conversely, the T_b of Little Greenbuls (*Andropadus virens*) decreased with decline in T_a, a

strategy widely used by small birds (McKechnie and Lovegrove, 2002; Seavy and McNab, 2007;

Sharbaugh, 2001), and those found in warmer regions, to reduce energy expenditure and

endogenous heat load (McNab and Morrison, 1963). Interestingly, even within a species,

geographically separate populations may differ in the magnitude of seasonal acclimatisation

(O'Connor, 1996), with greater changes in metabolic rate in populations from higher latitudes

with greater seasonal temperature ranges (Weathers, 1979; White et al., 2007a).

How a species responds to climate change (and subsequently altered local conditions)

will depend largely on its ability to colonise new areas, or change its physiology and seasonal

behaviour (Thuiller, 2007). As temperatures rise, any trait that is phenotypically plastic in terms

of temperature will alter (Visser, 2008), and many avian species have already responded to

recent climate change (Crick, 2004). One way in which a species may respond to climate change

is by shifting its range, and indeed the northward movement (Bronson et al., 2003a; Bronson et

al., 2003b) shown by populations of Black-capped and Carolina Chickadees (*Poecile carolinensis*), and their hybrids, coincides with Ohio's warming winter temperatures (Olson et al., 2010), which suggests a lack of the phenotypic flexibility necessary to deal with cooler temperatures, since the northward range shift occurred only after temperatures in those areas increased. Other bird species with low BMRs, including tropical birds, are less likely to have the phenotypic flexibility necessary to adapt to climate change (Şekercioğlu et al., 2012), and birds with a narrower thermal range have been shown to be at greater risk, particularly during extreme heat events (Jiguet et al., 2006), which are to become more frequent with increased global warming (Boyles et al., 2011). A little more optimism may be allowed for those species that do show intra-individual phenotypic flexibility in their metabolic rate, such as the desert larks that inhabit Saudi Arabia, where there is a predicted increase in mean annual T_a of 5°C over the next 100 years (Mitchell and Hulme, 2000 ; Tieleman et al., 2003b). Similarly, various subpopulations of Amethyst Sunbirds exhibited high levels of phenotypic flexibility between them, which allowed them to adapt to changes in T_a (Lindsay et al., 2009b). Furthermore, there was no significant difference in RMR of Cape White-eyes (*Zosterops virens*) (Sundevall, 1850) housed indoors at 4ºC above outdoor Ta, and conspecifics housed at Ta (Chapter 6). Another way in which some avian species have responded to climate change is by advancing their egg laying dates, and smaller species (with relatively higher thermoregulatory costs and which are thus more temperature-sensitive) have advanced their egg laying dates the most (Stevenson and Bryant, 2000).

Endotherms show great variation in their abilities to tolerate high T_b and/or Ta (Boyles et al., 2011), which may lead to different survival predictions for each species, and even each population. Thus one subpopulation should not be used to represent an entire species, and the

location and altitude of experimental subpopulations should be considered when making species predictions in terms of metabolic rate (Lindsay et al., 2009b). Furthermore, the ecological and genetic factors leading to inter- and intraspecific differences in avian metabolic response to shifting environmental conditions need to be identified (Møller et al., 2004; Rønning et al., 2007), in order to better predict responses to future climatic scenarios (Boyles et al., 2011). Finally, much of the current knowledge of the effects of climate change on birds is restricted to passerines from northern hemisphere temperate zones, and so more work is needed on their southern hemisphere counterparts (Møller et al., 2004).

Microhabitat

Microclimate selection is of huge importance to thermal ecology and ecological energetics, since variation in the local physical environment can greatly affect thermoregulatory requirements (Buttemer, 1985).

Solar radiation can have a large impact on avian energy budgets (Lustick et al., 1970; Olson et al., 2010; Wolf and Walsberg, 1996); for example, at low T_as, sunlight absorbed by the feathers reduces the thermal gradient between the skin and the feather surface, decreasing conductive heat loss and reducing energy expenditure (De Jong, 1976; Lustick, 1969; Ohmart and Lasiewski, 1971; Williams and Tieleman, 2001). Meanwhile, high ambient humidity (such as that found in lowland tropical regions) may reduce the dissipation of heat through evaporative cooling (Hails, 1983; Weathers, 1979; Weathers and van Riper III, 1982). Therefore, high heat load and reduced evaporative capacity may act as selective forces favouring lower BMRs in sun-foraging tropical birds (Weathers and van Riper III, 1982). The consequent evolution of lower endogenous heat production (and lower metabolic rate) may have adaptive value in sun-foraging

species, effectively reducing total heat load, and so extending the time birds can be exposed to direct insolation (Hails, 1983; Weathers, 1977; Weathers, 1979). Indeed, the BMRs of sun-foraging tropical species are often lower than those of shade-foraging birds (Weathers, 1977; Weathers, 1979), which are not faced with exogenous thermal loads and so do not need lower BMRs (Weathers and van Riper III, 1982), although both have been shown to be lower than expected (Hails, 1983).

Wind conditions can also have a large influence on the energy that birds expend to keep warm (Buttemer, 1985; Wolf and Walsberg, 1996), and small increases in wind speed may result in elevated metabolic rates in smaller birds (Olson et al., 2010; Walsberg, 1986). For example, at 0°C, a single Red Knot increased its energetic expenditure by 30% when wind speed increased from 0 to 1 $m.s^{-1}$ (Duriez et al., 2004), while Verdins (*Auriparus flaviceps*) can reduce their metabolic rate by up to 50% by moving from a shady, windy position to one protected from the wind and exposed to $1000W/m^2$ of solar radiation (Wolf and Walsberg, 1996). This may be because wind disturbs the layer of air within the feathers, penetrating below the coat surface and causing convective cooling at the coat surface (Williams and Tieleman, 2001). Thus, at T_as below body-surface temperature, birds must increase their metabolic rates with increasing wind speeds in order to maintain a constant T_b (Williams and Tieleman, 2001). Shelter from wind, whether in the form of nocturnal roosts or more sheltered habitats, reduces heat loss and thus the need for a high BMR (Duriez et al., 2004; Walsberg, 1986). For example, Eurasian Woodcocks (*Scolopax rusticola*), nocturnal waders that inhabit forests and meadows, have lower BMRs than waders living in windy, unsheltered habitats (e.g. mudflats and beaches), where heat loss may be markedly increased by wind (Duriez et al., 2004). Appropriate roost site selection, postural adjustments, and various other behavioural responses have been well documented, supporting the

hypothesis that adaptations acting to reduce thermoregulatory stress (e.g. reducing wind penetration into the plumage) should be strongly selected for (Walsberg, 1986).

Flight and migratory cycles

The presence and style of avian flight reflects body composition, and may therefore have an important effect on avian BMR (McNab, 2009; McNab and Salisbury, 1995). For example, flightless rails have lower pectoral muscle mass and lower BMR than their flighted relatives (McNab and Ellis, 2006), and the low BMRs of penguins may similarly be due to their flightless condition (McNab, 2009). Conversely, birds that chase insects on the wing have higher BMRs than those that sit and wait for insect prey (Bonaccorso and McNab, 2003), and raptors that use powered flight to pursue vertebrate prey (*Falco*, *Accipiter*, *Pandion*) have heavier pectoral muscles and higher BMRs than those that search for prey by soaring or by using sedentary hunting techniques (*Buteo*, *Parabuteo*, *Aquila*) (McNab, 2009; Wasser, 1986).

Thus the evolution of avian flight seems to have had an expansive impact on avian energetics, perhaps contributing to the widespread occupation of polar and island environments (McNab, 2009). However, adaptations that maintain a favourable balance of productive energy in birds may also limit their migratory status or distribution (Davis Jr., 1955). For example, migrant Malaysian birds have higher BMR than residents (Hails, 1983). Furthermore, significant phenotypic flexibility in BMR has been revealed over different stages of long-distance migration cycles in a variety of species (Battley et al., 2001; Lindström and Klaassen, 2003; McKechnie, 2008; Piersma et al., 1995).

During the flight and stopover cycles of migration, body mass (and the masses of many organs) may change quickly and drastically (Battley et al., 2000; Piersma, 1998), with large,

rapid concomitant changes in BMR (Lindström et al., 1999), reflecting physiological adaptations to the ecological conditions and demands at each site (Kvist and Lindström, 2001). For various migratory species, BMR is lower in the tropical African wintering grounds than in temperate areas or in the Arctic breeding grounds (Castro et al., 1992; Kersten et al., 1998; Lindström, 1997; Lindström and Klaassen, 2003; McKechnie, 2008). A study of 19 wader species showed an average reduction in BMR of 16% between the Arctic and southern Sweden, possibly because long-distance migration represents an energy-demanding period in the annual cycle, and the deposition of large amounts of fat during migratory stopovers requires a high energy intake, thus requiring support from a large, active digestive machinery, which may generate a high BMR (Kvist and Lindström, 2001). However, since BMR refers to the maintenance metabolism of adults (I.U.P.S. Thermal Commission, 2001), the measurement of BMR during the growing period at stop-over sites should be regarded with caution.

Circadian rhythm and a nocturnal/diurnal way of living

Many bird species show clear circadian rhythms in $\dot{V}O_2$ and T_b in response to the light:dark cycle (Downs and Brown, 2002; Hails, 1983; McKechnie and Lovegrove, 2001; Wijnandts, 1984; Zungu et al., 2013). It may be that these birds may use their metabolic rates to increase their T_bs to active levels prior to photophase (Bush et al., 2008a). Indeed, the condition of measuring BMR during the rest phase was incorporated into the definition of BMR after the observation that thermoneutral rates of avian metabolism were minimal during this period (Aschoff et al., 1970; McNab, 2005; McNab, 2009). However, some avian species such as the Eared Grebe (*Podiceps nigricollis*) (Ellis and Jehl Jr, 2003) and eight rail species (Rallidae) (McNab and Ellis, 2006), do not seem to show such clear circadian rhythms (MacMillen and Trost, 1967;

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McNab, 2005). Whether birds have these circadian rhythms in metabolic rate or not may depend on their size; larger birds (> 100 g) are more likely to show these differences (McNab, 2005; McNab, 2009).

Lower BMRs are found in nocturnal birds, such as the Stone Curlew (*Burhinus oedicnemus)* and Eurasian Woodcock (*Scolopax rusticola*) (Duriez et al., 2004), as well as owls (Weathers et al., 2001), nightjars (McNab and Bonaccorso, 1995), kiwis (Calder III and Dawson, 1978) and frogmouths (Bech and Nicol, 1991). Indeed, lower BMR could be an adaptation to a nocturnal life (Bech et al., 1999; Wijnandts, 1984), whereby the extra heat produced by nocturnal birds during activity and digestion at night (i.e., the heat increment of feeding), may aid in thermoregulation (Bech et al., 1999). However, intermittent feeders (such as frogmouths and eurostopodid nightjars) have lower BMRs than protracted feeders, and so foraging style may contribute to the low BMRs of some nocturnal birds (Bonaccorso and McNab, 2003).

Hybrid-status

Studies have reported evidence of gene flow across hybrid contact zones (Sattler and Braun, 2000), however, most of these zones are maintained by a form of endogenous selection against individuals of mixed ancestry (Olson et al., 2010), and therefore against hybrid reproductive success (Bronson et al., 2003b). This suggests that the genetic incompatibilities of hybrids may have physiological manifestations beyond reproduction, for example, a mismatch of proteins encoded by nuclear and mitochondrial genes, such as may be the case in hybrids, may result in improper functioning of metabolic systems because of misassembled metabolic proteins (Olson et al., 2010). Hybrids of California and Black Capped Chickadees were found to have significantly higher mass-corrected BMR than either parental species, which may be due to

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genetically induced mitochondrial deficiencies (Olson et al., 2010); the mtDNA-nDNA mismatch of hybrids may be due to impaired function of metabolic enzymes encoded in the mtDNA (Breeuwer and Werren, 1995; Ellison and Burton, 2008; Perrot-Minnot et al., 2004; Sackton et al., 2003; Zeyl et al., 2005), and may produce less efficient mitochondrial protein complexes, thus reducing ATP production efficiency, and so increasing $\dot{V}O_2$ rate to meet ATP demands (Olson et al., 2010).

Sex

Very few papers mention the effect of sex on avian metabolic rate. A study on Great Tits (*Parus major*) showed that the interactions between sex and other factors partly explained the variation in whole-animal winter BMR (Bouwhuis et al., 2011), however, in seasonally-acclimatised House Sparrows, there was no significant difference between the sexes for thermal conductance, M_{sum}, metabolised energy, BMR or $\dot{V}O_2$ (Arens and Cooper, 2005; Chappell et al., 1999; Davis Jr., 1955). Nor was the BMR of European Stonechats (*Saxicola torquata rubicola*) (Versteegh et al., 2008) or House Wrens (*Troglodytes aedon*) (Tieleman et al., 2008), the SMR of Eurasian Woodcocks (*Scolopax rusticola*) (Duriez et al., 2004), the minimal metabolic rates of adult Red Junglefowl (*Gallus gallus*) (Chappell et al., 1996), or the $\dot{V}O_2$ of five tropical bird species (Vleck and Vleck, 1979) influenced by sex. Nevertheless, sex should be taken into account when analysing metabolic rate in sexually dimorphic species (Kersten et al., 1998; Kvist and Lindström, 2001); for instance, adult Red Junglefowl showed significant intersexual differences in $\dot{V}O_{2max}$, with males showing 54% higher $\dot{V}O_{2max}$ than females at standardised body masses (Chappell et al., 1996). This may be due to the high metabolic demand during inter-male combat in this species, together with a reduced need for aerobic performance in other contexts (Chappell

et al., 1996).   Although physiological studies pay relatively little attention to intersexual differences (compared with ecological studies), a greater understanding of the effect of sex is important to understand the mechanisms animal use in a rapidly changing environment (Caro, 2012), and so physiological studies should at least state the sex of the animals used.

Age

The metabolic pathway of oxidative phosphorylation is known to generate reactive oxygen species (ROS), which cause cumulative damage to biomolecules, and so metabolic rate may be partly responsible for the deterioration and damage of cells and tissues with age (Broggi et al., 2010). However, there are few studies on the influence of age on avian BMR.

Snow Petrels (*Pagodroma nivea*), very long-lived seabirds, have been shown to maintain their BMR throughout their lives (Moe et al., 2007), just as waders on autumn migration in southern Sweden (Kvist and Lindström, 2001) and a wild population of Leach's Storm Petrels (*Oceanodroma leucorhoa*) (Blackmer et al., 2005) showed no age-related differences in BMR. Indeed, an analysis of 325 species found no evidence for a negative association between metabolic rate and life-span (Trevelyan et al., 1990).

In fact, the trend of decreasing BMR with increasing age, although observed in laboratory animals (Moe et al., 2009) and humans, was not observed in a wild animal population until it was noted that in wild Great Tits (*Parus major*) living under stressful conditions near the northern limit of their distribution in Finland and Sweden, BMR decreased as the birds aged, and was higher during winter than the BMRs of conspecifics living further south (Broggi et al., 2010). A subsequent study on Great Tits in southern England showed that the interactive effects of age and various other factors partly explained the variation in whole-animal winter BMR (Bouwhuis

et al., 2011). Perhaps the harsher northerly conditions required greater energy expenditure and thus higher BMR, leading to decreased survival and breeding success, and the observed 'functional senescence', or decline in BMR with age, which may reflect a trade-off between the need for a high work rate, and investment in somatic maintenance and repair, to counteract the action of radical oxygen species (Broggi et al., 2010).

Origin and thermal history

While some avian comparative studies assume that the BMR of captive-raised birds represents that of wild individuals (McKechnie et al., 2006; Schleucher and Withers, 2002), others have paid great attention to whether study birds are captive-bred or wild-caught, and how this affects BMR (Lindsay et al., 2009b; McKechnie, 2008; McKechnie et al., 2007; Weathers et al., 1983). Captivity may induce changes in avian metabolic rate (Rezende et al., 2002; Thompson et al., 2015); for example, Merlins (*Falco columbarius*) held in captivity for seven months to three years had higher BMRs than freshly caught birds (Warkentin and West, 1990), while two subspecies of captive Red Knots (Piersma et al., 1996) showed significantly lower BMR than their wild counterparts, possibly due to smaller pectoral muscle oxidative capacity (Saarela and Hohtola, 2003). However, other studies found that captivity does not affect BMR (Kendeigh, 1944; McKechnie et al., 2006; Piersma et al., 1996; Weathers et al., 1983). The degree to which captivity affects BMR may depend on how closely the captive environment mimics the natural one.

Avian metabolic adjustments to artificial environments have been found to vary with M_b and consequently there may be differences in metabolic scaling between birds from wild-caught and captive-raised populations (McKechnie, 2008). In general, small species have higher BMR

in captivity, while large species (> 200 g) have lower BMR (McKechnie et al., 2006), possibly because limited exercise in small cages may reduce energy expenditure, leading to lower mass and/or oxidative capacity of pectoral muscles (which have relatively high metabolic activity), so that energy is used predominantly for maintaining existence (Davis Jr., 1955; McKechnie, 2008; McKechnie et al., 2007; Saarela and Hohtola, 2003). Captive birds are also likely to experience a smaller temperature range and more moderate minimum temperatures than wild populations, which may result in reduced BMR (McKechnie et al., 2006; Tieleman et al., 2003b). Conversely, food may be of higher quality and/or more freely available in captivity, which may lead to higher mass of metabolically active organs such as the intestines, liver and stomach, and therefore to elevated BMR (Dekinga et al., 2001; Mueller and Diamond, 2001; Starck, 1999). In addition, the possibility that handling and capture stress may induce higher BMRs in freshly-caught birds (Duriez et al., 2004; Hails, 1983; Speakman et al., 1993) has lead to intraspecific comparisons of BMR of captive and wild-caught birds. For example, Papuan Boobook (*Ninox theomacha*) had slightly lower BMR in captivity, while Loria's Birds-of-paradise (*Cnemophilus loriae*) and Apapanes (*Himatione sanguinea*) showed no significant difference in BMR between captive and field-caught birds, and yet captive Amakihis (*Hemignathus virens*) had higher BMRs than their wild-caught conspecifics (McNab, 2009). Thus the effect of various factors associated with captivity on BMR may be complex and species-specific (McNab, 2009; Weathers et al., 1983), making it difficult to make specific predictions concerning the direction of differences in BMR between wild-caught and captive-raised birds.

Thus, to avoid misleading results, the physiological parameters of captive-bred populations should not be compared to those of wild-caught populations (Lovegrove et al., 2011), nor should the physiological trends of captive-bred individuals be linked to evolutionary

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adaptations of wild populations, unless the study includes a proper validation of the results using wild-caught individuals (Caro, 2012). Furthermore, the apparent M_b-dependence of metabolic responses to captivity should be considered when testing hypotheses concerning metabolic adaptation (McKechnie, 2008; McKechnie et al., 2006).

When measurements are made on wild-caught birds, the conditions that the animals were in immediately prior to metabolic measurements being taken (that is, their thermal history) should be considered, since environmental temperature may affect the magnitude of a flexible response of avian BMR to thermal acclimation (Barceló et al., 2009; Lindsay et al., 2009a; Lindsay et al., 2009b; Sharbaugh, 2001; Swanson and Olmstead, 1999). Consistent with this hypothesis, various studies have found significant, negative correlations between mean minimum winter temperature and winter metabolic rate of Black-capped Chickadees (Cooper and Swanson, 1994; Dutenhoffer and Swanson, 1996; Olson et al., 2010; Swanson and Olmstead, 1999). Furthermore, studies have shown that birds exposed to fluctuating low temperatures show higher SMR than birds exposed to constant low temperature (Brooks, 1968).

Consequently, one study allowed birds a six-week acclimation period, after which individuals from each of the different altitudinal subpopulations were expected to react similarly to the range of T_as of the respirometry trials, and thus differently from pre-acclimation trials, thereby displaying similar phenotypic flexibility (Lindsay et al., 2009b). However, altitudinal subpopulations still showed differences in metabolic rate post-acclimation, indicating that captive-bred populations of birds would not represent the entire species as well as might be assumed, unless the altitudinal origin of the original population were known, and laboratory populations represented subpopulations over the entire altitudinal gradient occupied by the

species (Lindsay et al., 2009b). Therefore, studies on avian metabolic rate should specify the origin of study individuals.

Future research

Although seasonal acclimatisation is well documented in small passerines and Holarctic birds, more data are needed on seasonal BMR adjustments in larger birds, non-passerines, and tropical and subtropical species (Bush et al., 2008a; Liknes and Swanson, 1996; McKechnie, 2008; Smit et al., 2008). In particular, there are relatively few data on the energetics of birds from the African, Asian and South American tropics, even though the tropics have the greatest diversity of species (McNab, 2009). Similarly, although studies on individual physiological variation are increasing (Bech et al., 1999; Hayes and Jenkins, 1997; Pough, 1989), data on the effects of captivity on avian metabolic parameters within species are lacking (McKechnie et al., 2006), and although BMR is presumed to have adaptive importance, few studies have investigated its ecological and evolutionary constraints (Rezende et al., 2002). Thus, despite the high correlation between BMR, body mass, and other factors, there is still a considerable inability to explain the variation in avian BMR (McNab, 2009).

In addition, given the realities of anthropogenic climate change and its influence on adult survival (Thomas et al., 2004), avian phenology (Both et al., 2004; Coppack and Both, 2002; Crick, 2004; Jonzén et al., 2006; Knudsen et al., 2007; Stenseth and Mysterud, 2002; Visser et al., 2009), body size (Gardner et al., 2014; Yom-Tov et al., 2006), distribution (Princé and Zuckerberg, 2014; Thomas et al., 2004; Virkkala and Lehikoinen, 2014) and overwintering range (Austin and Rehfisch, 2005), surprisingly little data are available on the effects of climate change on avian metabolism. Hence the role of phenotypic flexibility relative to changing environmental conditions needs to be evaluated, and the ecological factors leading to inter- and

intra-specific differences in response to climate change need to be identified (McKechnie and Swanson, 2010; Møller et al., 2004).

Conclusions

While comparative studies are powerful tools for testing hypotheses about ecological and evolutionary processes, they may incorporate studies that have measured various physiological parameters (i) in species that differ in their behaviour, diet, habitat or phylogeny (Barceló et al., 2009), (ii) in different seasons or using different methods (Hails, 1983; Seavy and McNab, 2007), (iii) in animals that differ in terms of whether they were wild-caught or captive-raised (McKechnie et al., 2006; White et al., 2007a) or (iv) by conducting any of a variety of errors which may vary in magnitude (Withers, 2001), which may bias the data (e.g. Hayes et al., 1992), leading to interpretational problems (Tieleman et al., 2003a). Therefore, researchers should cite the respirometry equations that they used (Lighton and Halsey, 2011), and ideally their methods and conditions should be standardised for results to be reliable, accurate and comparable (Page et al., 2011). Even data from peer-reviewed journals may need to be scrutinised to ensure they are of high quality (McKechnie and Wolf, 2004a). Measurement duration should be sufficient to allow basal values to be reached within the measurement period, and yet the subjectivity of recording until 'a low, stable value is obtained' may introduce bias (Hayes et al., 1992). This time period differs significantly between various marsupial species (Cooper and Withers, 2009), and is likely to do so for different avian species as well. The calculation interval ('time over which metabolic rate is calculated') should be used as a covariate in comparative studies using respirometry data (Hayes et al., 1992), and the acclimation period should be stated (Bush et al., 2008a; Rezende et al., 2004).

Although whole-animal and mass-specific BMR may be positively correlated (Broggi et al., 2009), the two metrics may give divergent results (Hayes, 2001; Liknes et al., 2002), since mass-specific metabolism may not fully account for variation in body size (McNab, 1999; Packard and Boardman, 1988). Mass-specific rates may underestimate metabolic rate in fatter individuals (Liknes et al., 2002; Sharbaugh, 2001), since fat contributes to mass despite being relatively metabolically inert (Rezende et al., 2002; Weathers and Caccamise, 1978). Thus, analyses of covariance may be preferable to the use of ratios (that is, mass-specific metabolic rates) (Packard and Boardman, 1988).

When comparing BMR of different species, rather than classifying study species using a simple dichotomy (such as arid vs. mesic), species should be placed along a continuum that is dependent on various factors (such as altitude, continentality, rainfall and latitude), which may more accurately reflect reality (Tieleman et al., 2003a). In addition, the time of year during which BMR measurements are made could be standardised (McKechnie, 2008); for example, only winter values might be used, if these are the lowest seasonal measurements (McNab, 2009). Moreover, studies on the capacity for phenotypic change in metabolic rate may benefit from a wider approach that distinguishes between genetic and environmental influences on phenotypes of different species (Tieleman et al., 2003b). Finally, there is a need to recognise phenotypic flexibility in BMR within species (Williams and Tieleman, 2000), and that it may be inconsistent across species or populations (Lindsay et al., 2009b).

Study species

The model species chosen for this study is a small (10-12 g) passerine bird, the Cape White-eye (Fig. 1). Cape White-eyes are widely regarded as sexually monomorphic (Hockey et al., 2005; Oatley, 2011; Skead, 1967), thus both males and females are included in this study.

The Cape White-eye is a widespread southern African endemic species, listed as Least Concern on the IUCN Red List of Threatened Species (BirdLife International, 2012), due to its large range (Fig. 2). Cape White-eyes are arboreal and catholic in their choice of habitat, which includes evergreen and coastal forest, riverine bush, Drakensberg scrub vegetation, as well as parks, gardens, orchards, and even alien plantations, between sea-level and 2 800 m (Nuttall, 1997).

Fig. 1. Cape White-eyes *Zosterops virens* © L. Thompson

As of 10 November 2014, 17 215 Cape White-eyes had been ringed/banded (SAFRING, 2014), and although movements may range between 10-164 km, the majority of ringing recoveries suggest that Cape White-eyes do not migrate (Nuttall, 1997). Their occurrence in various habitats, covering a wide range of altitudes and hence temperatures, is suggestive of a high degree of flexibility in terms of their metabolic rate, making them an ideal study species for this

project. Furthermore, they are small, easy to catch and to handle, and have previously been used in various studies on captive birds (Brown and Downs 2003; Franke et al. 1997; Lobban et al. 2010; Wellman and Downs 2009a; Witteveen and Brown 2014), suggesting that they do well in captivity.
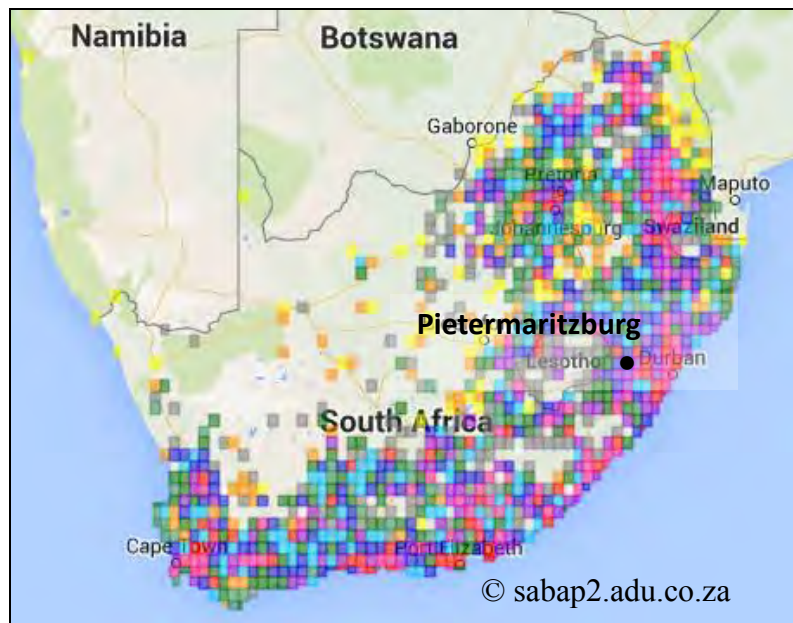
Figure 2. The range of the Cape White-eye (*Zosterops virens*), a southern African endemic species, and Pietermaritzburg, the city in KwaZulu-Natal, where the study took place (SABAP2, 2014). Coloured squares indicate reporting rates within pentads (where grey indicates incidental records; yellow <11%; orange 11-24%; olive green 24-38%; dark green 38-51%; light blue 51-62%; dark blue 62-71%; purple 71-79%; red 79-87%; pink 87 – 100%).

Numerous studies have been done on white-eye systematics (Oatley, 2011; Oatley et al., 2011; Oatley et al., 2012; Thompson and Taylor, 2014), behaviour (Craig, 1999; Lunt et al., 2004; Wellman and Downs, 2009), moult and breeding (Hulley et al., 2004; Symes et al., 2001), and food habits (Brown and Downs, 2003; Franke et al., 1997; Symes et al., 2011; Symes and

Yogananda, 2013; Witteveen and Brown, 2014), however, to date, there has been no investigation into the physiology of the Cape White-eye. Moreover, despite *Zosterops* being the most species-rich genus in the world (Oatley, 2011; Van Balen, 2008), there are only two metabolic studies on *Zosterops* species; those on the Australian Silvereye (*Z. lateralis*) (Maddocks and Geiser, 1997, 1999, 2000b) and the Chestnut-Flanked White-eye (*Z. erythropleura*) (Liu et al., 2005). This thesis therefore aims to address various knowledge gaps highlighted in this literature review, in terms of factors affecting flexibility in avian metabolic rate, using the Cape White-eye as the study species. In particular, we will investigate seasonal variation and circannual rhythm in various metabolic parameters, as well as the time necessary for thermal acclimation, the effects of long-term captivity on avian metabolism, and the potential effects of climate change-associated temperature increase on avian metabolism.

Study site

All Cape White-eyes used in this study were caught at or near the University of KwaZulu-Natal, Pietermaritzburg, South Africa (29°37'S, 30°24'E), which is within the range of this species (Fig. 2), and where there is an active community of bird ringers to assist with capturing birds. The University's Animal House provided a safe, clean place to house the birds, and the Zoology building contained constant-environment rooms, and a physiology lab with all of the gas analysers and other instruments necessary for respirometry trials. Cape White-eyes were caught, monitored, ringed, released and transported under permit number OP 5122/2012 from Ezemvelo KwaZulu-Natal Wildlife. Ethical approval for this study was granted by the Animal Ethics Sub-committee of the University of KwaZulu-Natal's Ethics Committee, reference: 071/13/Animal. After the study, Cape White-eyes were released at their capture sites.

Thesis structure

The remainder of this thesis comprises six chapters, five of which are experimental and formatted for publication in peer-reviewed journals. There is some inevitable repetition, since these chapters are intended to be published separately.

- Chapter 2 describes seasonal variation in metabolism and body mass of the Cape White-eye over two years.

- Chapter 3 is one of only a handful of studies globally on circannual rhythm in avian metabolic rate.

- Chapter 4 describes the effects of long-term captivity on the metabolic parameters of the Cape White-eye.

- Chapter 5 looks at thermal acclimation in freshly wild-caught Cape White-eyes, showing how weekly body mass, respiratory exchange ratio and resting metabolic rate vary over eight weeks.

- Chapter 6 describes how the resting metabolic rate of the Cape White-eye may be affected by a 4°C rise in mean ambient temperature, which is equal to that predicted for the range of this species by 2080.

The concluding chapter puts the main results from this study into a broader context.

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CHAPTER 2

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SEASONAL METABOLIC VARIATION OVER TWO YEARS IN AN AFROTROPICAL PASSERINE BIRD

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Abstract

Seasonal trends in metabolic parameters are well established in avian populations from highly seasonal environments, however, seasonal trends in metabolism of birds from lower latitudes (and of Afrotropical birds in particular) are not well understood. We aimed to investigate seasonal trends in metabolism for a small (10-12g) Afrotropical bird, the Cape White-eye (*Zosterops virens*), using flow-through respirometry in two summers and two winters. There was no seasonal difference in body mass between consecutive seasons, and the lower critical limit of thermoneutrality was lower in winter (23°C) than in summer (28°C), as expected for a small Afrotropical bird. In the two winters of the study, whole animal basal metabolic rates (BMR) of Cape White-eyes were significantly lower than in the first summer, however there was no significant difference in BMR between summer and winter in the second year of the study. This seasonal trend in BMR is similar to that shown by other small Afrotropical avian species, and

may represent energy conservation in an unpredictable climate with mild winters. Differences in mean temperature and mean rainfall between seasons could not explain the lack of a seasonal difference in BMR in the final year. We conclude that seasonal trends in avian BMR may vary between years, within a population.

1. Introduction

Small birds have higher mass-specific rates of energy expenditure, thermoregulatory costs and surface-area-to-volume ratios than larger birds (Arens and Cooper, 2005; McNab, 2009; Sharbaugh, 2001). To cope with changing environments, they need to make physiological adjustments in metabolism or insulation, at a rate equalling that at which they gain and lose heat from/to their environment (Chaui-Berlinck et al., 2002; Williams and Tieleman, 2001). Small birds lose heat to their environments more rapidly than larger birds, however, they can only carry a limited amount of insulation before their movement is affected and the risk of predation increases (Calder, 1984). Thus, in winter, many small species show higher summit metabolism (Cooper and Swanson, 1994; Dawson and Marsh, 1989), and higher fasting (Southwick, 1980; Weathers and Caccamise, 1978), basal (Liknes et al., 2002; McKechnie, 2008; Pohl and West, 1973; Swanson and Olmstead, 1999) and peak metabolic rates (Liknes and Swanson, 1996; O'Connor, 1995), possibly reflecting greater thermogenesis, and thus higher cold tolerance, in winter than in summer (Swanson, 1990).

Basal metabolic rate (BMR) is the minimum metabolic rate of postabsorptive homeotherms, measured at thermoneutrality during their inactive phase (Daan et al., 1990). BMR is one of the most commonly measured physiological variables of endotherms (Bech et al., 1999; Rønning et al., 2007), making it extremely useful for comparisons between avian taxa (Liknes and Swanson, 1996; McKechnie, 2008; McKechnie et al., 2006). Seasonal differences in avian metabolism have been well-studied in temperate climates in the Holarctic, where BMR is generally higher in winter than in summer (Liknes et al., 2002). For example, House Sparrows (*Passer domesticus*) from Wisconsin increased their whole animal BMR by 64% in winter (Arens and Cooper, 2005), and Downy Woodpeckers (*Picoides pubescens*) from South Dakota increased their mass-specific BMR by 40% (Liknes and Swanson, 1996). These winter increases in BMR seem to follow seasonal changes in energy expenditure, and most likely reflect an up-regulation in the metabolic machinery necessary for improved cold tolerance, which increases the chances of survival in extremely cold winter conditions (Cooper and Swanson, 1994; Dawson, 2003; Piersma et al., 1995).

In contrast, birds resident in the Afrotropics, Australasia and Indomalaya, are generally exposed to milder winters and less predictable climates than those in the Holarctic region (Lovegrove, 2000; Sinclair et al., 2003). Although fewer studies have investigated seasonal changes in metabolism in these tropical and subtropical bird species (Van de Ven et al., 2013), their peak metabolic rates are often significantly lower in winter than in summer (Wells and Schaeffer, 2012), and their BMR is often lower in winter than in summer (Table 1). For example, Australian Silvereyes (*Zosterops lateralis*) had significantly lower BMR (Maddocks and Geiser, 2000) and White-browed Scrubwrens (*Sericornis frontalis*) from arid Western Australia had

significantly lower standard metabolic rates in winter than in summer (Ambrose and Bradshaw, 1988). Similarly, Southern Red Bishops (*Euplectes orix)* from a milder, more predictable, coastal environment in South Africa's Eastern Cape province reduced their BMR in winter (Van de Ven, 2012). However, not all southern African birds follow this general trend of reducing their BMR in winter; Rock Kestrels (*Falco rupicolus*) showed no significant seasonal difference in BMR (Bush et al., 2008), while Kynsna Turacos (*Tauraco corythaix*) and Red-winged Starlings (*Onychognathus morio*) elevated their BMR in winter, suggestive of thermogenesis rather than energy conservation (Chamane and Downs, 2009; Wilson et al., 2011). It may be that timing of seasonal metabolic measurements influences the direction and magnitude of seasonal change (Chapter 3).

In light of the seeming disparity regarding seasonal trends in BMR of southern African bird species (Table 1), We investigated seasonal effects on BMR in a 12g Afrotropical bird, the Cape White-eye (*Zosterops virens*; Sundevall, 1850; Thompson and Taylor, 2014). This southern African endemic species has an extremely large range (Hulley et al., 2004; Smith and Bowie, 2005), and we anticipated that its ability to tolerate such a wide variety of habitats could reflect a high degree of flexibility in its BMR, making it an ideal candidate for showing seasonal variation in BMR.

We hypothesized that Cape White-eyes would show seasonal variation in BMR, evaporative water loss (EWL) and the lower critical limit (T_{lc}) of the thermoneutral zone (TNZ). We hypothesized that there would be no seasonal difference in body mass (M_b), since small birds generally do not rely on increased plumage insulation or subcutaneous fat for improved cold tolerance in winter (Zheng et al., 2014). We predicted that the BMR and T_{lc} of Cape White-eyes would be lower in winter than in summer, in accordance with the energy conservation generally

shown by small southern African bird species in winter (Smit and McKechnie, 2010; Table 1). Since ambient temperatures are lower in winter than in summer, evaporative cooling should not be as important for thermoregulation during winter, and so we predict that EWL will be lower in winter than in summer.

2. Materials and Methods

2.1 Experimental animals

Twelve Cape White-eyes were caught in February 2012 at the Darvill Bird Sanctuary, Pietermaritzburg, KwaZulu-Natal, South Africa (29°36'S, 30°26'E), using mist nets (Ecotone, Gdynia, Poland). Birds were transported 2.5 km to the Animal House of the University of KwaZulu-Natal, Pietermaritzburg, where they were housed in groups of 4 in outdoor aviaries (1 x 3 x 2 m). These aviaries were large enough to permit limited flight, and they ensured that the birds used in the study experienced the same environmental conditions encountered by wild birds in the same area. Fresh fruits (oranges, papayas, bananas and apples) and softbill pellet supplements (Avi-products, Durban, South Africa) were supplied daily, and water was given *ad libitum*. Breeding was discouraged with a lack of nesting materials. After the study, birds were released at their capture sites. A permit to capture, ring, transport, monitor and release Cape White-eyes was granted by Ezemvelo KwaZulu-Natal Wildlife, number OP 5122/2012. Ethical approval for this study was granted by the Animal Ethics Sub-committee of the University of KwaZulu-Natal, reference 071/13/Animal. Temperature data obtained from the South African Weather Service showed that in the three years prior to the study, mean maximum monthly

temperatures at the study site ranged from 30°C in February to 22°C in July, and mean monthly minima ranged from 19°C in February to 6°C in July (Table 4).

Immediately prior to respirometry trials, Cape White-eyes were scored for primary flight feather moult, based on feather length and age (De Beer et al., 2001). Scores ranged from 0 at pre-moult to 45 when primary moult was completed (Klaassen, 1995). This value was divided by 4.5 to give an index ranging from 0 to 10 (Thompson et al., 2015a). Sex determination involved drawing blood (<100 μl) from the brachial vein of each bird using a disposable Healthease® syringe and 29G x ½" (0.33 x 13 mm) needle (Neomedic Pty. Ltd., Riverhorse Valley East, South Africa). This blood was deposited onto FTA filter paper and stored in Eppendorf tubes®, before being sent to Molecular Diagnostics Services Pty. Ltd. (Westville, South Africa) for molecular sex determination.

2.2 Measurement of gas exchange by open-flow respirometry

Gas exchange measurements were conducted on Cape White-eyes in Aug – Sept 2012, Aug 2013, Feb – Mar 2013, and Jan – Mar 2014, during two austral winters and summers respectively, when birds housed in outdoor aviaries were assumed to have acclimatized to seasonal temperatures (July had the lowest mean T_a, and February the highest T_a). Data were collected using an open-flow respirometry system, and rates of oxygen consumption ($\dot{V}O_2$), carbon dioxide production ($\dot{V}CO_2$) and EWL were calculated using equations given in Withers (2001):

- $\dot{V}O_2 = \dot{V}_I (F_IO_2 - [F_EO_2 (1 - F_IO_2 - F_ICO_2 - F_IH_2O) / (1 - F_EO_2 - F_ECO_2 - F_EH_2O)])$

- $\dot{V}CO_2 = \dot{V}_I ([F_ECO_2 (1 - F_IO_2 - F_ICO2 - F_IH_2O) / (1 - F_EO_2 - F_ECO_2 - F_EH_2O)] - F_ICO_2)$

- $EWL = \dot{V}_I ([F_EH_2O (1 - F_IO_2 - F_ICO2 - F_IH_2O) / (1 - F_EO_2 - F_ECO_2 - F_EH_2O)] - F_IH_2O)$

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where $\dot{V}_I$ was the measured mass flow.

Birds were weighed using digital scales (model: AFB-3100L, Adam Equipment S.A. Pty. Ltd., Johannesburg), and individually placed into 2.8 L Perspex respirometry chambers, each of which contained a plastic mesh platform elevated 10 cm above the bottom of the chamber, where a 1 cm layer of liquid paraffin (AlphaPharm, Pietermaritzburg) would eliminate evaporation from excreta. These respirometers were placed within a temperature-controlled environmental chamber (CMP2244, Conviron, Winnipeg, Canada), set to 12L : 12D. Variation in day length is closely linked to change in season (Bradshaw and Holzapfel, 2010), however temperature, not photoperiod, appears to be the primary driver of flexibility in avian summit metabolism (Swanson et al., 2014), and thus we kept photoperiod consistent across seasons, assuming that temperature is the main driver of flexibility in BMR.

T_a of each chamber were recorded every 15 min. using i-Buttons® (model DS1922L-F5, Thermochron®, Maxim, CA, USA), which were calibrated to the nearest 0.1°C over 7 - 36°C in a circulating water bath, using a mercury-in-glass thermometer. Birds were fasted for 3 h before measurements began, to ensure they were post-absorptive (Wellman and Downs, 2009). The first 3 h of measurements (15:00 to 18:00) were discarded, to reduce the potential impacts of handling stress on metabolic measurements (Regel and Pütz, 1997; Weimerskirch et al., 2002), and so we evaluated raw data taken between 18:00 and 06:00. We did not measure body temperatures of the birds.

Environmental air was dried and scrubbed of CO_2 with silica gel, soda lime and then more silica gel, and then pumped (model PP2 pumps, Sable Systems, Las Vegas, Nevada, USA) through a flow measurement system (model FB8, Sable Systems), at ~ 800 mL.min^{-1} (i.e. flow rate per chamber). This flow rate maintained the O_2 depletion from the control chamber to each

respective bird's chamber between 0.1 and 0.5% (following Lighton, 2008). Air inlets were positioned at the bottom of the respirometry chambers and outlets at the top, to enable mixing of air. Air then passed through a flow multiplexer (model MUX, Sable Systems) whereafter excess air escaped through a manifold, and ~ 200 mL.min^{-1} passed through a subsampler (model SS4, Sable Systems). The effluent airstream flowed through a water vapour analyser (model RH300, Sable Systems), which was zeroed with N_2 and spanned by finding the water vapour pressure of a nearly-saturated airstream at a known temperature, generated by bubbling air from outside the building through water at room temperature, and then passing this air through water 5ºC cooler than room temperature (Cory Toussaint and McKechnie, 2012).

Air was dried with a minimal quantity of Drierite (Hammond Drierite Co. Ltd., Xenia, Ohio) that had previously been dried (following White et al., 2006), before passing through a CO_2 analyser (model CA-10, Sable Systems) which was zeroed using N_2 and spanned using a certified 964 ppm CO_2 in N_2 mix (AFROX, Pietermaritzburg, South Africa). Finally, air flowed through a fuel cell O_2 analyser (model FC-10, Sable Systems), which was spanned to 20.95% at the start of each night's trial using dry, CO2-free air.

A Universal Interface (model UI2, Sable Systems) transferred data from the gas analysers and flow meter to a desktop computer, equipped with ExpeData Version 1.4.8 data acquisition software (Sable Systems). The CO_2 and water vapour analysers were zeroed (and all three gas analysers were spanned) immediately prior to each season's work, and at regular intervals thereafter as required.

Due to the advantage of being able to measure metabolic rate of multiple birds each night, we used an interrupted sampling regime, beginning with a baseline measurement and then four birds in succession, each for six min. Using a switching system with multiple birds gives smaller

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errors than measuring one bird continuously for shorter periods (Cooper and Withers, 2010). This 30 min. cycle was repeated throughout the night (Page et al., 2011), with each bird being measured for 12 min. in total, every hour. The lowest hourly mean $\dot{V}O_2$ was taken as RMR for each night. Flow rate (mL.min^{-1}), O_2 and CO_2 concentrations (%), and water vapour density (μg.mL^{-1}) were recorded every 5 sec.

Each season, Cape White-eyes were measured over a range of T_a, from 5°C to 35°C, at 5°C intervals. Only one T_a was used per night, so that birds experienced the same T_a overnight from 15:00 until 06:00 the next morning. The order in which T_as were used was randomly determined. The T_as that we measured our birds at precluded determination of the upper critical limit. Nevertheless, Cape White-eyes seemed to be increasing their EWL at 35°C, and so at 35°C, birds were removed from the temperature-controlled environmental chamber (CMP2244, Conviron, Winnipeg, Canada) at ~ 21:15, to avoid the possible risk of dehydration. Standard EWL was recorded at the same time as RMR, whereas basal EWL was the minimal mean hourly EWL recorded each night; standard and basal EWL measurements did not necessarily fall at the same time of the night. There was an interval of at least one night between measurements.

Lag and drift correction were performed using a macro in ExpeData, and a 95% equilibration time of 11 min. was calculated using the equation given in Lasiewski et al. (1966). Hence, $\dot{V}O_2$, $\dot{V}CO_2$ and EWL were z-transformed before hourly means were calculated (Lighton, 2008; Lighton and Halsey, 2011).

2.3 Data analyses

Data analyses and figures were produced using the base package in R version 3.0.1 (R Core Team, 2014) and the packages '*ggplot2*' (Wickham, 2009) and '*gridExtra*' (Auguie, 2012) in R.

T_{lc} was objectively estimated using a piecewise linear model with the R package '*lme4*' (Bates et al., 2014), with 'sex' (male, female, or unknown, when we did not take a blood sample for that particular bird), 'M_b' (M_b at time of RMR) and 'T_a' as fixed factors, and 'BirdID' (individual) as a random factor. T_a at the study site were compared with Welch's two-sample unpaired t-tests in R. In addition, to determine the effects of various predictor variables on RMR, EWL and M_b of Cape White-eyes, we defined a set of candidate models *a priori* following Burnham and Anderson (2002), using the R package '*lme4*' (Bates et al., 2014) to perform linear mixed-effects analyses. Fixed effects included 'season' (summer or winter), 'year', 'M_b', 'moult' (primary wing moult score, ranging from 0 to 10), and 'sex'. To control for repeated measures 'BirdID' was included as a random effect. Visual inspection of residual plots showed no deviations from homoscedasticity or normality. Best approximating models were selected based on differences in corrected AIC values (AICc) between each candidate model and the best approximating model (ΔAICc), and on their corrected Akaike weights (AICcWt), produced using the R package '*AICcmodavg*' (Mazerolle, 2013). Comparisons of M_b, EWL, BMR, rainfall and temperature between seasons and years were made using *t*-tests in R, after a Bonferroni correction was applied for each set of *t*-tests: that is, for (i) M_b, (ii) EWL, (iii) BMR, (iv) rainfall, (v) minimum temperature, (vi) mean temperature, (vii) maximum temperature (Cabin and Mitchell, 2000).

3. Results

M_b of individual Cape White-eyes ranged from 7.9g to 13.8g (Table 2). There were no significant differences between mean M_b in consecutive seasons (Figure 3, for M_b in winter 2012 and summer 2013, $t_{155} = 1.64$, p = 0.103; for M_b in summer 2013 and winter 2013, $t_{164} = -0.376$,

p = 0.707; and for M_b in winter 2013 and summer 2014, $t_{168} = 0.833$, p = 0.406). The best approximating models for predicting M_b included 'BirdID' as a random effect, and 'year', 'season' and 'sex' as fixed effects, where 'sex' referred to male birds and 'season' to winter (Table 3).

There were no significant differences in mean T_a between the two winters ($t_{(3)} = -0.100$, p = 0.926) or the two summers of the study ($t_{(2)} = -0.3159$, p = 0.781) (that is, the mean T_a during the months in which the birds were measured in each season). However, there were significant differences in mean T_a between consecutive winters and summers (

Table S1), with mean T_a in summer 2013 significantly higher than that of winter 2012 ($t_{(2)} = -8.973$, p = 0.012) and of winter 2013 ($t_{(2)} = -14.454$, p = 0.004), and mean T_a in summer 2014 was significantly higher than mean T_a in winter 2013 ($t_{(4)} = -10.356$, p < 0.001).

After the Bonferroni correction had been applied, there was no significant difference in rainfall between the two summers ($t_{(1)} = 4.580$, p = 0.137) or the two winters ($t_{(2)} = -2.423$, p = 0.136). When comparing mean rainfall between different seasons, there was no significant difference between winter 2012 and summer 2013 ($t_{(1)} = -8.603$, p = 0.074), or between summer 2013 and winter 2013 ($t_{(1)} = 7.388$, p = 0.057), or between summer 2014 and winter 2013 ($t_{(2)} = -9.567$, p = 0.011).

In both years, T_{lc}, was lower in winter than in summer (Table 2 2, Fig. 1). In the final season of the study, our environmental cabinet sometimes could not reach the set T_a, deviating from it by up to 2.4°C, although our analysis accounted for this by including T_a as a fixed factor. A linear model of summer T_{lc} against the logs of the summer M_b (Table 1) produced the following results: $R^2 = 0.692$, $F_{(1, 8)} = 21.2$, p = 0.002, however a linear model of winter T_{lc} and winter M_b showed no relationship, with $R^2 = -0.076$, and $F_{(1, 8)} = 0.367$, p = 0.561.

Mean whole animal BMR of Cape White-eyes was significantly higher in summer 2013 than in winter 2012 ($t_{(54)}$ = -3.79, p < 0.001), while there was no significant difference in mean whole animal BMR between summer 2013 and winter 2013 ($t_{(56)}$ = 0.04, p = 0.969), and mean whole animal BMR was significantly higher in summer 2014 than in winter 2013 ($t_{(51)}$ = 4.68, p < 0.001, Fig. 3). The best approximating model for whole animal BMR of Cape White-eyes included 'season', 'year', 'moult', 'sex' and 'M_b' as fixed factors (Table S2).

Whole animal standard EWL of Cape White-eyes was generally higher at 35°C than at all other T_as (Fig. 2), so we could not use all EWL values within the TNZ in a linear mixed-effects model, because values taken at 35°C would distort the slope, resulting in a more positive intercept. Thus, we used only the values taken when the environmental chamber was set to 30°C, and the resulting best approximating model included only 'BirdID' as a random effect and 'season' as a fixed effect (Table 3). This model did not include EWL data for winter 2012, since in the first season of the study mineral oil was not used, and therefore the EWL data would not have been accurate, due to evaporation from excreta elevating EWL. There was no difference in whole animal standard EWL between summer and winter 2013 ($t_{(57)}$ = -0.11, p = 0.916), however, whole animal EWL was significantly higher in the final summer of the study than it was in the preceding summer ($t_{(36)}$ = -4.46, p < 0.001) and winter ($t_{(47)}$ = 3.98, p < 0.001; Fig. 3). For each of the different measures of EWL, the best approximating equations contained 'year', 'season', 'moult' and 'M_b' as fixed factors (Table S2). Standard whole animal EWL was the exception, since it did not include 'moult'.

4. Discussion

Our study was the first to analyse seasonal variation in metabolic parameters of an Afrotropical bird over two years. As predicted, Cape White-eye BMR was significantly lower in the first winter of the study than in the first summer; however, there was no difference in BMR between summer 2013 and winter 2013, and in the final year of the study there was no significant seasonal difference in BMR. Given that seasonal trends in whole animal BMR of these long-term captive birds reflect those of the local wild population (Thompson et al., 2015a), one might conclude any of three different scenarios from these results, depending when measurements began. These scenarios are that small Afrotropical birds (i) have significantly lower mean whole animal BMR in winter than in summer, (ii) show no seasonal difference in mean whole animal BMR, or (iii) have significantly lower mean BMR in summer than in winter. The first two results are in line with seasonal trends generally shown by small birds in areas where winters are not extremely cold; that is, they either have lower BMR in winter than in summer, or show no seasonal trend. The latter result is a trend more often shown by larger bird species (Wilson et al., 2011), although some other smaller species have shown similar seasonal trends in whole animal BMR (Nzama et al., 2010; Van de Ven et al., 2013).

The mean seasonal BMR values of Cape White-eyes from this study ranged from 40.07 ± 4.05 mL O_2 h^{-1} (mean $\pm$ s.d., n = 24, in summer 2014) to 45.12 ± 3.43 mL O_2 h^{-1} (n = 24, in summer 2013), and are similar to monthly BMR values reported for another group of Cape white-eyes from the same population but housed indoors at the same Ta as outdoors, when measured at 30ºC (41.24 ± 3.38 mL O_2 h^{-1}, n = 12, Thompson et al. 2015b). When converted to mass-specific values (using data in Table 2), the mean winter BMR values of Cape White-eyes from this study (3.63 ± 0.36 mL O_2 g^{-1} h^{-1} in winter 2012, and 3.84 ± 0.37 mL O_2 g^{-1} h^{-1} in

winter 2013) were higher than winter BMR values of another southern hemisphere congeneric species, the Australian Silvereye (2.30 ± 0.39 mL O_2 g^{-1} h^{-1}, n = 8) (Maddocks and Geiser, 1999).

Differences in mean seasonal T_a or rainfall could not be linked to seasonal differences in BMR of Cape White-eyes, however it may be that BMR is affected by T_a or rainfall on a time-scale shorter than an entire season, and so the time-scales used here may have been too coarse to explain the observed trends. Similarly, although the captive diet was largely consistent throughout the year, there may have been some variation in terms of variety and quality of fruits offered to birds, and ideally, consistent quantities and qualities of food should have been provided to each individual bird, throughout the year. Any potential difference in diet may have affected gross body composition and thus BMR (Pierce and McWilliams, 2014).

Van de Ven et al. (2013) suggested that seasonal variation in the BMR and summit metabolism of sub-tropical species may depend on the climatic conditions experienced by the birds. For example, various studies have found significant, negative correlations between mean minimum winter temperature and winter metabolic rate of Black-capped Chickadees (*Poecile atricapillus*) (Cooper and Swanson, 1994; Dutenhoffer and Swanson, 1996; Olson et al., 2010; Swanson and Olmstead, 1999). Other authors have suggested that seasonal patterns in BMR may be related to rainfall (Smit and McKechnie, 2010). However, we found no statistically significant differences in mean environmental T_a or in rainfall, between the two winters or the two summers at our study site, and therefore no reason to suggest that differences in T_a or rainfall may have caused the seasonal trends in Cape White-eye BMR that we observed. Thus our results do not support that idea that avian BMR is best correlated with medium-term (14-30d before testing) temperature variables (Swanson and Olmstead, 1999).

Our results for seasonal trends in M_b and T_{lc} of Cape White-eyes were as predicted, with no significant seasonal variation in M_b between consecutive seasons. This result is similar to that for another Zosteropid, the Australian Silvereye (Maddocks and Geiser, 2000), and the majority of southern African birds that have been studied (Table 1). The linear model showed a significant relationship between T_{lc} and log M_b of southern African bird species in summer, but not in winter. This increase in T_{lc} with log M_b in summer may be due to heavier birds having smaller surface area-to-volume ratios, and thus less need for heat production through thermoregulation, since they lose less heat than small birds. The lack of a relationship in winter may be due to different sized birds showing different seasonal trends in terms of their M_b and T_{lc}.

In both years, T_{lc} of Cape White-eyes was lower in winter than in summer, suggesting energy conservation in winter, since winters in the study site are relatively mild, but food is still scarce (Smit and McKechnie, 2010). This is in contrast to the seasonal trend in T_{lc} shown by the Australian Silvereye (Maddocks and Geiser, 2000). In summer, Cape White-eyes generally had higher T_{lc} than larger southern African birds, in accordance with the hypothesis that T_{lc} generally increases with smaller body size (Canterbury, 2002; McNab, 1970). Furthermore mean monthly $T_a < T_{lc}$ throughout the year at our study site, supporting the idea that most endotherms regularly experience $T_a < T_{lc}$ in most of the world for most of the year (Humphries and Careau, 2011).

Long-term captivity may influence physiological parameters in both mammals and birds (Geiser et al., 2000; Geiser et al., 1990), thus it is often preferable to measure freshly wild-caught individuals (Cooper and Withers, 2009). Indeed, long-term captive Cape White-eyes have been found to have higher BMR than freshly wild-caught conspecifics, although the seasonal trends in whole animal BMR shown by long-term captive Cape White-eyes were the same as those for

freshly wild-caught birds (Thompson et al., 2015a), lending support to the idea that seasonal trends shown here represent those of freshly wild-caught birds.

At T_a = 30°C, standard EWL of Cape White-eyes was lower in winter than in summer, as predicted, since there is a greater need for evaporative cooling in summer than in winter. However, standard EWL varied at different T_as within the TNZ, increasing exponentially as T_a increased. The TNZ is defined by the International Union of Physiological Sciences as the 'range of ambient temperature at which temperature regulation is achieved only by control of sensible heat loss, i.e., without regulatory changes in metabolic heat production (H) or evaporative heat loss' (I.U.P.S. Thermal Commission, 2001). Since we found that standard EWL values differed markedly at different T_as within the TNZ, as did Noakes et al. (2013), we propose the aforementioned definition of TNZ be re-examined, and perhaps modified. A revised definition of the TNZ could exclude the note about evaporative heat loss, such that TNZ is defined only in terms of metabolic rate.

We could not compare EWL between the two winters of the study, since in the first winter we did not use mineral oil in the chambers. However, standard and basal EWL values in the first summer were only 60% and 65% of those in the second summer. We were unsure as to why EWL would increase so much between years, particularly since there was no significant difference in either mean rainfall or mean T_a between the two summers. Nevertheless, other environmental variables such as humidity could be considered when conducting similar experiments in future. For example, House Sparrows acclimated for three weeks to dry conditions (6.5 g/m^3 absolute humidity) increased their cutaneous EWL by 36% compared with conspecifics acclimated to humid (31 g/m^3) conditions (Muñoz-Garcia et al., 2008).

In conclusion, the different seasonal trends in BMR observed in Cape White-eyes in this study highlight the fact that seasonal trends in BMR in Afrotropical birds may not be consistent across years, even within a population. Further studies on seasonal variation in avian BMR should focus on particular populations of wild birds, over multiple years, while recording various environmental parameters, to gain a deeper understanding of what drives or limits seasonal flexibility in BMR in Afrotropical birds.

Acknowledgements

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Tables

Table 1. Southern African avian species in which seasonal variation in metabolism has been investigated. Lower limit of the thermoneutral zone (T_{lc}), body mass (M_b), basal metabolic rate (BMR), and seasonal trends in winter (w) and summer (s) are presented. * indicates statistical significance.

Species	T_{lc} summer (°C)	T_{lc} winter (°C)	T_{lc}	Mean M_b (g) summer (n)	Mean M_b (g) winter (n)	M_b	BMR mass-specific	BMR whole-animal	Ref
Cape White-eye *Zosterops virens* (winter 2012, summer 2013)	28	23	w < s	11.7 (24)	11.7 (36)	w = s	w < s*	w < s*	1
Z. virens (winter 2013, summer 2013)	28	23	w < s	11.7 (24)	11.7 (36)	w = s	w = s	w < s*	1
Z. virens (winter 2013, summer 2014)	27	23	w < s	11.9 (24)	11.7 (36)	w = s	w = s	w = s	1
Southern Red Bishop *Euplectes orix* (inland site)	n/a	n/a	n/a	19.9 (14)	24.1 (18)	w > s*	w > s*	w > s*	2
E. orix (coastal site)	n/a	n/a	n/a	21.0 (17)	23.8 (18)	w > s*	w < s*	w = s	2
White-browed Sparrow-weaver *Plocepasser mahali*	26 - 29	26 - 29	n/a	40.6 (7)	40.6 (6)	w = s	w < s*	w < s*	3
Crimson-breasted Shrike *Laniarius atrococcinneus*	26 - 29	26 - 29	n/a	43.0 (6)	41.0 (5)	w = s	w < s*	w < s*	3
Fork-tailed Drongo *Dicrurus adsimilis*	26 - 29	26 - 29	n/a	44.8 (8)	42.6 (7)	w = s	w < s*	w < s*	3
African Scops-owl *Otus senegalensis*	26 - 29	26 - 29	n/a	62.2 (7)	55.4 (7)	w < s*	w < s*	w < s*	3
Pearl-spotted Owlet *Glaucidium perlatum*	26 - 29	26 - 29	n/a	66.4 (5)	70.3 (3)	w = s	w < s*	w < s*	3
Rose-ringed Parakeet *Psittacula krameri*	20	15	w < s	123.0 (10)	123.5 (10)	w = s	w < s*	n/a	4
House Sparrow *Passer domesticus*	25	25	w = s	n/a	n/a	w = s	w > s*	w > s*	5

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Red-winged Starling *Onychognathus morio*	15	30	w > s	n/a	n/a	w > s*	w > s*	n/a	6
Southern White-faced Scops-owl *Ptilopsis granti*	20	28	w > s	221.1	221.1	w = s	w = s	w = s	7
Knysna Turaco *Tauraco corythaix*	10	20	w > s	n/a	n/a	w = s	w > s*	w > s*	8
Rock Kestrel *Falco rupicolis*	20	15	w < s	214	214	w = s	n/a	n/a	9
F. rupicolis	21	21	w = s	214	214	w = s	w = s	n/a	9

This table only includes studies on species currently found in the wild in South Africa, and work done on birds that were either freshly wild-caught, or held in semi-natural conditions in outdoor aviaries prior to measurements being taken. 'n/a' indicates that a certain variable was not presented in a specific study, and 'w = s' shows that there was no significant seasonal variation in a certain variable. 1 = this study, 2 = Van de Ven et al. (2013), 3 = Smit and McKechnie (2010), 4 = Thabethe et al. (2013), 5 = Nzama et al. (2010), 6 = Chamane and Downs (2009), 7 = Smit et al. (2008), 8 = Wilson et al. (2011), 9 = Bush et al. (2008). We have included mass-specific BMR measurements because in some studies M_b was not provided. Note that strictly speaking, our summer measurements on Cape White-eyes should be classed as RMR not BMR, since birds showed some primary feather moult.

Table 2. T_{lc} (estimate, 95% CI, n) was calculated using piecewise regression with mixed-effects analyses using the '*lme4*' package in R (Mathot and Dall, 2013). All other variables (mean ± SD) were measured within the TNZ, at $T_a \geq T_{lc}$, and for these variables, sample sizes are shown in parentheses. EWL was not measured in winter 2012. 'Std' refers to standard EWL, taken at the same time as BMR, whereas basal EWL was the minimal mean hourly EWL recorded each night; the two measurements did not necessarily fall at the same time of the night.

Season	T_{lc} (°C)	BMR (mL.h⁻¹)	Std EWL (mg.h⁻¹)	Basal EWL (mg.h⁻¹)	M_b (g)
winter 2012	23.3 (19.5 - 26.9, 83)	42.42 ± 4.17 (36)	n/a	n/a	11.7 ± 1.3 (36)
summer 2013	28.0 (24.6, 29.8, 84)	45.12 ± 3.43 (24)	68.47 ± 24.17 (24)	58.87 ± 21.21 (24)	11.7 ± 1.0 (24)
winter 2013	23.4 (21.8, 24.8, 83)	44.97 ± 4.33 (36)	70.52 ± 40.87 (36)	52.81 ± 34.28 (36)	11.7 ± 0.9 (36)
summer 2014	27.4 (25.7, 28.8, 92)	40.07 ± 4.05 (24)	114.17 ± 43.94 (24)	90.32 ± 30.55 (24)	11.9 ± 0.9 (24)

Table 3. Ranking of models predicting metabolic parameters of Cape White-eyes. All candidate models included 'individual' (BirdID) as a random effect, and only those with an AICcWt > 0 are presented. The number of parameters (k) and corrected Akaike Information Criterion (AICc) scores for each respective model are given. Models were ranked on their Akaike weights (AICcWt) and on the difference between the best model and each candidate model (ΔAICc). Models with ΔAICc < 2, shown in bold, have considerable empirical support (Burnham and Anderson, 2002). For each response variable, only one candidate model had ΔAICc < 2, so there was no need for multimodel inference.

Response variable	Fixed effects	k	AICc	AICcWt	ΔAICc
Whole animal BMR	**'M_b' + 'sex' + 'moult' + 'season' + 'year'**	**8**	**536.52**	**1.00**	**0.00**
M_b	**'sex' + 'season' + 'year'**	**6**	**265.06**	**0.74**	**0.00**
	'sex' + 'moult'+ 'season' + 'year'	7	267.10	0.26	2.04
Whole animal standard EWL	**'M_b' + 'season' + 'sex'**	**6**	**341.47**	**0.79**	**0.00**
	'M_b' + 'moult' + 'season' + 'sex'	7	344.71	0.16	3.24
	'M_b' + 'moult' + 'season' + 'year'	7	346.67	0.06	5.20
Whole animal basal EWL	**'M_b' + 'moult' + 'season' + 'year'**	**7**	**278.84**	**1.00**	**0.00**
Mass-specific standard EWL	**'M_b' + 'moult' + 'season' + 'year'**	**7**	**170.38**	**0.99**	**0.00**
	'M_b' + 'moult' + 'sex'	6	**179.20**	**0.01**	**8.83**
Mass-specific basal EWL	**'M_b' + 'moult' + 'season' + 'year'**	**7**	**120.97**	**1.00**	**0.00**

Table 4. Variation in seasonal air temperature (mean ± SD) at the study site, in Pietermaritzburg, South Africa, where metabolic parameters and M_b were investigated in the Cape White-eye (*Zosterops virens*). Winter is June – August inclusive, and summer is December – February inclusive.

Year	summer temperature (°C)			winter temperature (°C)		
	minimum	mean	maximum	minimum	mean	maximum
2012	n/a	n/a	n/a	7.1 ± 2.0	15.0 ± 1.4	22.8 ± 0.9
2013	17.3 ± 0.2	22.4 ± 0.3	27.5 ± 0.6	7.0 ± 1.2	14.9 ± 0.7	22.8 ± 1.0
2014	17.6 ± 1.3	21.4 ± 0.0	27.7 ± 2.8	n/a	n/a	n/a
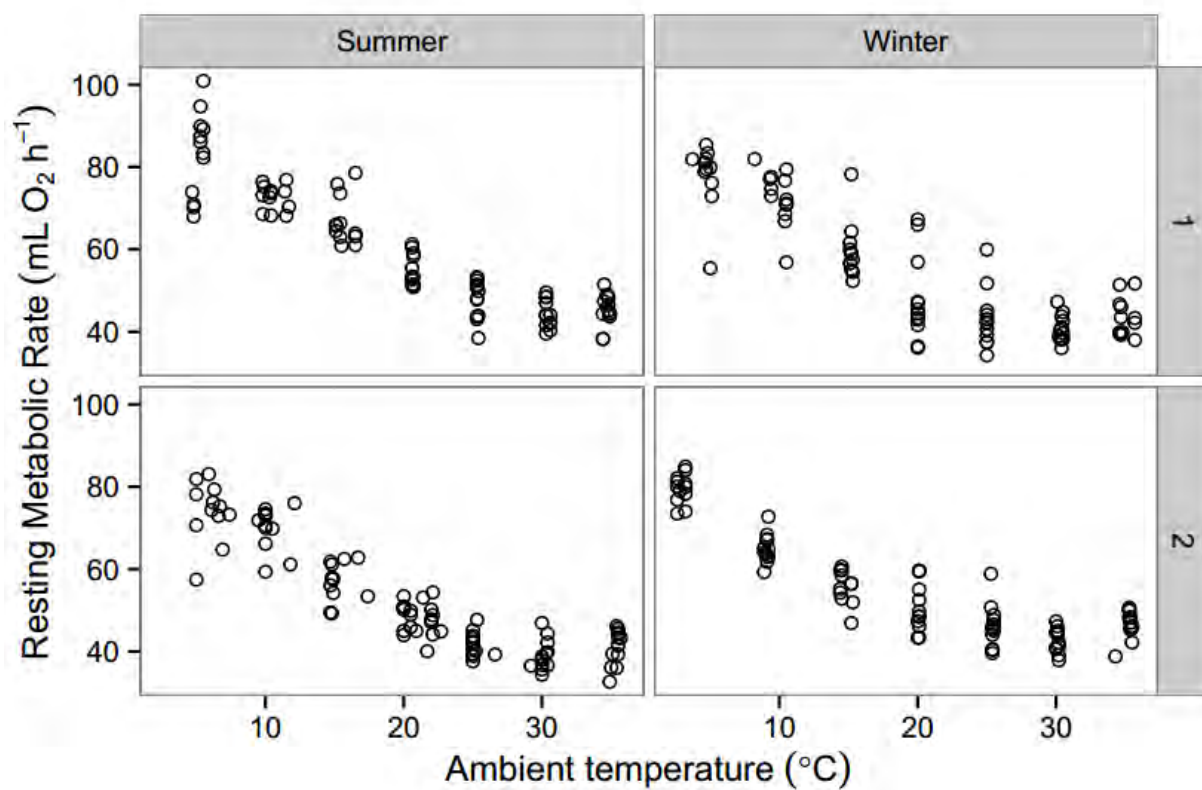
Figure 1. Whole animal resting metabolic rate (mL O_2 h^{-1}) of Cape White-eyes, at various ambient temperatures (ºC).
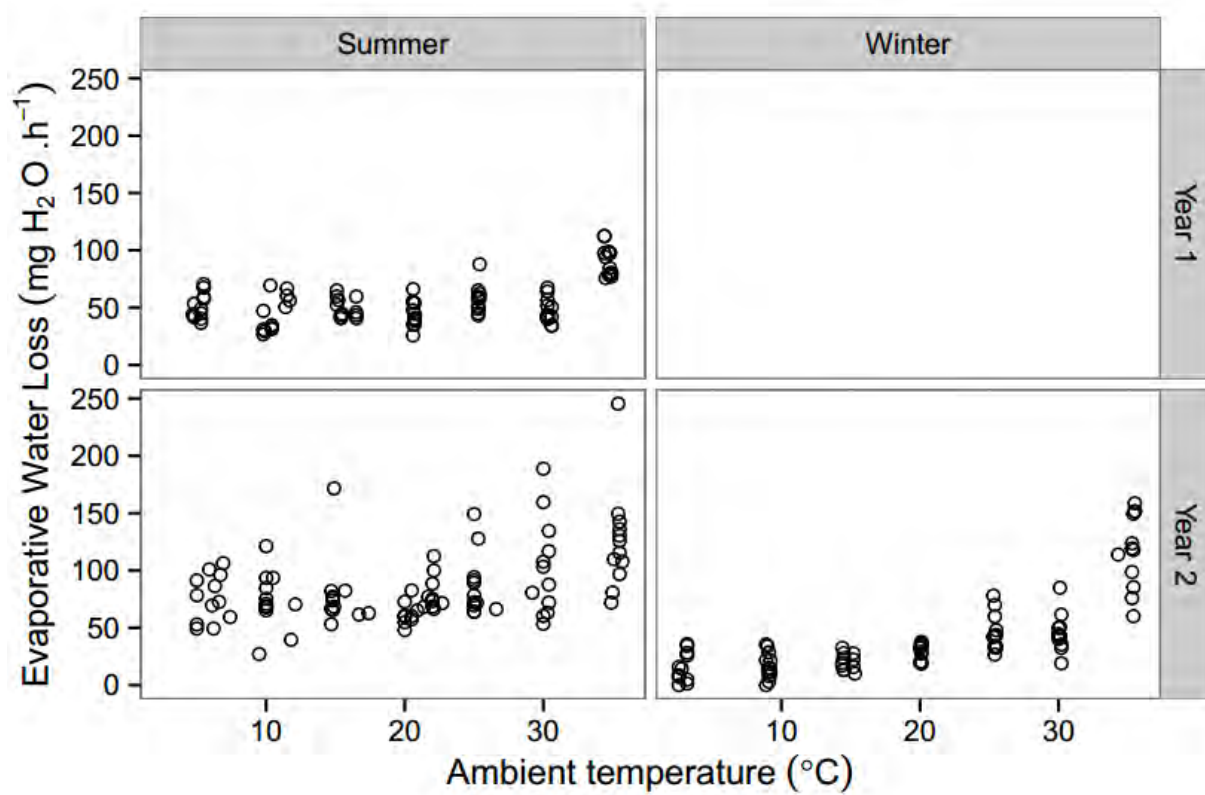
Figure 2. Whole animal evaporative water loss (mg H_2O h^{-1}) of Cape White-eyes, at various ambient temperatures (ºC). Note that mineral oil was not used in the respirometers in the first winter, and so we do not present the data for that season, since it will not be comparable with data from the other three seasons, when we did use mineral oil.
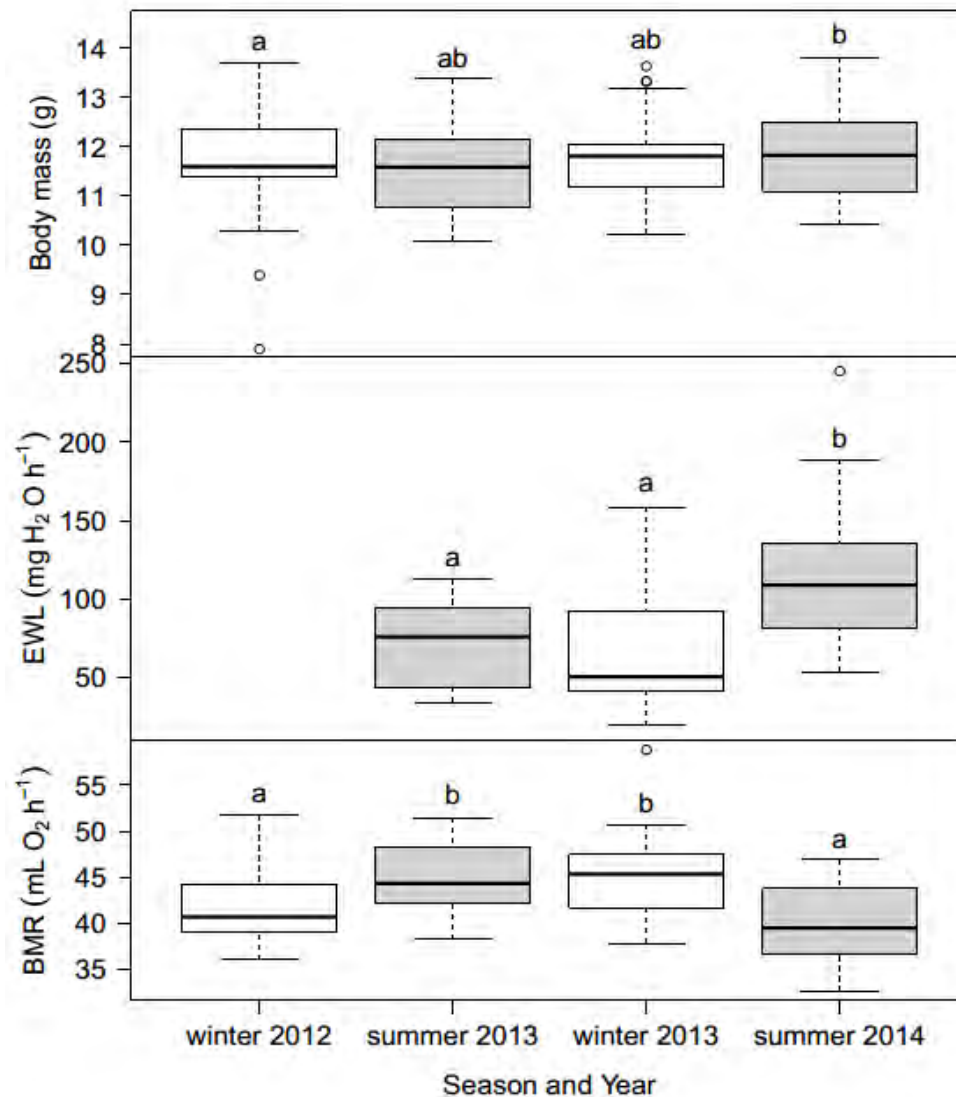
Figure 3. Seasonal variation in body mass (g), evaporative water loss (EWL, mg H_2O h^{-1}) and whole animal basal metabolic rate (BMR, mL O_2 h^{-1}, mean $\pm$ SD), in Cape White-eyes in Pietermaritzburg, KwaZulu-Natal, South Africa. The results for EWL in the first season of the study are not presented here, since mineral oil was not used in the chambers in that season. Thick lines represent medians, boxes contain 25th to 75th percentiles, whiskers show maximum and minimum values, and small circles represent outliers. Similar letters above boxplots indicate a lack of statistically significant differences, as determined using t-tests in R, with α levels adjusted using the Bonferroni correction.

Supplementary Materials

Table S1. Comparison of winter (June, July and August) and summer (Dec, January and February) ambient temperatures (T_a) in Pietermaritzburg, South Africa, over the study period. An asterisk indicates statistical significance after the Bonferroni correction was applied.

season	T_a	t	df	P
	minimum	-0.100	3.4	0.926
winter 2012 & winter 2013	mean	-0.055	2.8	0.960
	maximum	0.043	3.9	0.968
	minimum	-0.316	2.1	0.781
summer 2013 & summer 2014	mean	-0.197	2.1	0.862
	maximum	-0.141	2.2	0.900
	minimum	-8.973	2	0.012
winter 2012 & summer 2013	mean	-8.872	2.2	0.010
	maximum	-7.601	3.5	0.003*
	minimum	-14.454	2.1	0.004*
summer 2013 & winter 2013	mean	-18.216	2.7	0.001*
	maximum	-6.905	3.2	0.005*
	minimum	-10.356	4	< 0.001*
winter 2013 & summer 2014	mean	-6.278	2.4	0.015
	maximum	-2.869	2.5	0.079
	minimum	-7.729	3.4	0.002*
winter 2012 & summer 2014	mean	-5.363	3.6	0.008*
	maximum	-2.926	2.4	0.080

Table S2. Estimate sizes of fixed effects contained in the best approximating model for each respective response variable. In each case 'season' refers to winter, and 'sex' to male birds.

Response variable	Fixed effect(s)	Estimate size	s.e.m
Whole-animal RMR	'season'	- 7.90	1.90
	'year'	1.20	0.70
	'moult'	1.11	0.25
	'sex'	0.46	1.96
	'M_b'	0.20	0.39
M_b	'year'	0.25	0.16
	'season'	-0.22	0.16
	'sex'	0.10	0.38
Whole animal standard EWL	'season'	-28.22	13.85
	'sex'	11.96	12.96
	'M_b'	7.46	6.61
Mass-specific standard EWL	'year'	5.184	0.934
	'season'	-1.266	1.155
	'moult'	0.354	0.211
	'M_b'	0.113	0.372
Whole animal basal EWL	'year'	33.46	5.98
	'season'	-18.20	7.58
	'moult'	2.51	1.32
	'M_b'	0.71	2.49
Mass-specific basal EWL	'year'	3.316	0.547
	'season'	-1.754	0.693
	'moult'	0.243	0.121
	'M_b'	-0.127	0.228

CHAPTER 3

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CIRCANNUAL RHYTHM OF RESTING METABOLIC RATE OF A SMALL AFROTROPICAL BIRD

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Abstract

Seasonal variation in avian metabolic rate is well established in Holarctic and temperate species, while trends in Afrotropical species are poorly understood. Furthermore, given the relative paucity of data on circannual rhythm in avian metabolism, it is not known whether seasonal measurements made in summer and winter correspond with annual peaks and troughs in avian metabolic rate. Thus, we investigated how mean monthly body mass, resting metabolic rate (RMR) and evaporative water loss (EWL) of a small Afrotropical bird, the Cape white-eye (*Zosterops virens*), changed over the course of a year. Mean body mass was 12.2 ± 1.0 g throughout the study period. However, both EWL and RMR varied monthly, and peaks and troughs in RMR occurred in March and October respectively, which did not correspond to peaks and troughs in mean monthly outdoor ambient temperatures. These results suggest that measuring RMR at the height of summer and winter may underestimate the flexibility of which birds are capable in terms of their metabolic rate. We encourage further studies on this topic, to

establish whether the lag between environmental temperature and RMR is consistent in other species.

Keywords: Acclimatization; Circannual rhythm; Resting metabolic rate; Evaporative water loss; Afrotropical; Cape white-eye *Zosterops virens*

Abbreviations

BMR   Basal metabolic rate
EWL   Evaporative water loss
RMR   Resting metabolic rate
RER   Respiratory exchange ratio
$\dot{V}CO_2$ Rate of CO_2 production
$\dot{V}O_2$ Rate of O_2 consumption

1. Introduction

Basal metabolic rate (BMR) is the 'obligatory cost of living for endotherms' (Barceló et al., 2009), and it is measured in resting, post-absorptive, non-reproductive, adult endotherms at thermoneutrality during their inactive period (McKechnie et al., 2006; McNab, 1997). BMR is one of the most commonly measured physiological variables of endotherms (Bech et al., 1999; Rønning et al., 2007), and is a useful metric for comparing metabolic power output among avian taxa (Liknes and Swanson, 1996; McKechnie, 2008; McKechnie et al., 2006).

In birds, BMR is influenced by a multitude of factors (McKechnie and Swanson, 2010), however, it is not fixed, as was previously implied by studies that reported a single BMR value for a species (McKechnie, 2008; Speakman et al., 2004). On the contrary, avian BMR is now known to be a highly flexible trait (Piersma, 2002; Swanson et al., 2014; Van de Ven et al.,

2013a; Vézina et al., 2006; Zheng et al., 2013), and changes in avian BMR are temporary, reversible and repeatable (McKechnie et al., 2007; Piersma and Drent, 2003), which helps birds to adjust to spatially or temporally heterogeneous environments (DeWitt, 1998; DeWitt et al., 1998; Schlichting and Pigliucci, 1998; Tieleman et al., 2003; Via et al., 1995). For example, there are a plethora of studies showing seasonal flexibility in avian metabolic rate (Bush et al., 2008a; Cooper, 2002; Cooper and Swanson, 1994; Hart, 1962; McKechnie, 2008; Piersma et al., 1995; Pohl and West, 1973; Thabethe et al., 2013; Van de Ven et al., 2013b) and these changes in avian metabolism usually follow seasonal changes in energy expenditure (Dawson, 2003; Smit et al., 2008).

The higher winter metabolic rates of some avian species suggest increased thermogenic capacity, and are correlated with improved cold tolerance (Cooper and Swanson, 1994). For example, increased thermogenic capacity was found to be the main feature of winter acclimatisation in American goldfinches (*Carduelis tristis*) (Carey et al., 1978; Dawson and Carey, 1976), and winter-acclimatized house sparrows (*Passer domesticus*) are more tolerant of low temperatures than summer-acclimatized birds (Davis Jr., 1955; Kendeigh, 1949; Nzama et al., 2010). Avian BMR may show significant seasonal adjustments of up to 64% and 120% for whole animal and mass-specific BMR respectively (McKechnie, 2008; Nzama et al., 2010), and in environments with extremely cold winters, birds usually have higher BMR, cold tolerance, metabolic capacity, standard and peak metabolic rates, and resting metabolic rate (RMR), in winter than in summer (Dawson and Carey, 1976; Dawson and Marsh, 1989; Downs and Brown, 2002; Hart, 1962; McKechnie et al., 2007; Olson et al., 2010; Pohl and West, 1973; Weathers and Caccamise, 1978; Williams and Tieleman, 2000).

However, avian metabolic rate does not always increase with cold acclimation (Vézina et al., 2006; Williams and Tieleman, 2000); reduction of fasting metabolic rate in winter may allow improved maintenance of body temperature independent of low ambient temperature (Weathers and Caccamise, 1978). Furthermore, the direction of seasonal change may depend on body size, with some smaller birds and mammals reducing their metabolic rates in winter, to conserve energy (Heldmaier, 1989; Lovegrove, 2005; Smit and McKechnie, 2010). For example, in the Afrotropics, where winters are generally mild, some species have been shown to conserve energy during winter acclimatisation by lowering RMR (Bush et al., 2008b). However, in the southern subtropics, the direction of seasonal trends in metabolic rate are not yet well understood; Knysna turacos (*Tauraco corythaix*) and an inland population of southern red bishops (*Euplectes orix*) increased their BMR in winter (Van de Ven et al., 2013b; Wilson et al., 2011); no significant seasonal change in BMR was recorded in rufous-collared sparrows (*Zonotrichia capensis*), white-browed scrubwrens (*Sericornis frontalis*) and a coastal population of southern red bishops (Ambrose and Bradshaw, 1988; Maldonado et al., 2009; Smit and McKechnie, 2010; Van de Ven et al., 2013b); and white-browed sparrow-weavers (*Plocepasser mahali*), crimson-breasted shrikes (*Laniarius atrococcinneus*), fork-tailed drongos (*Dicrurus adsimilis*), African scops-owls (*Otus senegalensis*) and pearl-spotted owlets (*Glaucidium perlatum*) all reduced their BMR in winter (Smit and McKechnie, 2010).

There are numerous studies on seasonal variation in avian metabolism (McKechnie, 2008), often comparing metabolic rate between two seasons (Versteegh et al., 2012). However, relatively few studies have investigated circannual rhythms in avian metabolic rate (Klaassen, 1995; Piersma et al., 1995; Vézina et al., 2011; West, 1960; Zheng et al., 2008), belying the possible importance of the finer-scale understanding of circannual rhythm in metabolic rate to be

gleaned from more frequent, regular measurements. For example, Zheng et al. (2008) found distinct seasonal trends in BMR and body mass in freshly wild-caught Eurasian tree sparrows (*Passer montanus*), and yet their clustering of the data into seasonal values, rather than separation into monthly values, gives a coarser picture of circannual rhythm than it would had they presented monthly data, as did West (1960) and Vézina et al. (2011). Similarly, despite the importance of evaporative water loss (EWL) in avian thermoregulation, we could find no studies on circannual rhythms in avian EWL.

Given that seasonal metabolic adjustments in Afrotropical birds are relatively understudied and poorly understood compared with in temperate and Holarctic birds (Smit and McKechnie, 2010), and given the abovementioned scarcity of data on circannual rhythms in avian metabolic rate, we studied variation in RMR of a small Afrotropical bird throughout the year. The genus *Zosterops* contains ~ 75 species (Van Balen, 2008), making it the most species-rich bird genus in the world (Oatley, 2011), and yet barring work on the Australian silvereye (*Z. lateralis*) (Jurisevic et al., 1999; Maddocks and Geiser, 1997, 1999, 2000) and the chestnut-flanked white-eye (*Z. erythropleura*) (Liu et al., 2005), relatively little is known about the metabolism of this group. We hypothesized that Cape white-eyes (*Z. virens*) (Sundevall, 1850; Thompson and Taylor, 2014), a southern African endemic species, would show a distinct circannual rhythm of metabolism, leading to seasonal differences in RMR, and we predicted that they would reduce their RMR in winter, in accordance with many other small Afrotropical species, as an energy conservation mechanism (Smit and McKechnie, 2010).

2. Methods

2.1 Study animals and housing

Twelve Cape white-eyes were trapped as adults in February 2012 using mist-nets (Ecotone, Gdynia, Poland) in a garden in Pietermaritzburg, KwaZulu-Natal, South Africa (29°36'S, 30°26'E). Birds were housed in groups of 4, in outdoor aviaries (1 m wide x 3 m long, and 2 m tall), with cement floors, and walls and roofs of wire mesh. One third of each roof was covered in shade cloth to provide shelter from the sun, and each aviary was equipped with two wooden perches, but no nesting material, to discourage breeding. There were no refugia within cages. Each morning, birds were given a variety of fresh grated and whole fruits (papayas, bananas, oranges and apples) supplemented with softbill pellets (Avi-products, Durban, South Africa). Water was provided *ad libitum*.

2.2 Gas exchange measurements

Respirometry measurements were conducted in the first week of each month, from September 2012 to August 2013 inclusive. Four birds were measured each night, and T_a in the environmental chamber was kept constant throughout the night. Each bird was measured twice a month; once overnight at 20ºC, and once overnight at 25ºC. Measurements lasted from 15:00 until 06:30 the following morning, with 12 minutes per hour spent recording data from a reference chamber, and then from each bird in turn. Scotophase was set from 18:00 to 06:00, and all data recorded before 18:00 each evening were discarded, as live infrared video camera feeds (CCTV camera Jiange® model JG-811CM, China) revealed that birds were awake and active while the lights were on in the temperature-controlled environmental chamber (Conviron,

Winnipeg, Canada), but that within about 1 min. of the lights going off, they closed their eyes and generally remained inactive. These differences in levels of physical activity between scotophase and photophase were reflected in the $\dot{V}O_2$ traces. All 12 birds were measured once a month, every month, at both 20°C and 25°C, with a break of at least one night in between measurements. One bird died part-way through the study and was replaced. These temperatures (20°C and 25°C) were chosen on the assumption that they would fall within the thermoneutral zone of this population of Cape white-eyes. However, subsequent measurements on Cape white-eyes from the same population showed the lower critical limit of the thermoneutral zone to be 28ºC in summer, and 23ºC in winter (Chapter 2). Thus, $\dot{V}O_2$ values are referred to as RMR_{Ta} where ambient temperature inside the environmental chamber (T_a) was either 20 or 25°C. Similarly EWL_{20} and EWL_{25} indicate EWL values when birds were measured overnight at 20 and 25°C respectively.

Details of the respirometry setup are described in Thompson et al. (2015). Briefly, birds were placed into 2.8 L Perspex respirometers inside a temperature-controlled environmental chamber (CMP2244, Conviron, Winnipeg, Canada), set to 12L:12D. Open-flow, push mode respirometry was used to measure metabolic rate. Environmental air was drawn from outside the building, and water vapour and CO_2 were removed with silica gel and soda lime respectively (Withers, 2001). This air was then pumped (model PP2, Sable Systems, Las Vegas, Nevada, USA) through a flow measurement system (model FB8, Sable Systems), with flow rates set to approximately 800 mL min^{-1} for each channel, maintaining the depletion in O_2 concentration in each chamber between 0.1 and 0.5 % (following Lighton, 2008). Effluent air then flowed through a multiplexer (model MUX, Sable Systems) and a subsampler (model SS4, Sable Systems), whereafter 200 mL min^{-1} of the effluent airstream passed into a water vapour analyser

(model RH300, Sable Systems), before being dried with minimal quantities of Drierite (Hammond Drierite Co. Ltd., Xenia, Ohio). Air then flowed through a CO_2 analyser (model CA-10, Sable Systems) and a fuel cell O_2 analyser (model FC-10, Sable Systems). All gas analysers were connected to a Universal Interface (model UI2, Sable Systems), which transferred data to a computer using ExpeData (Sable Systems) Software.

From 15:30 until 07:00, flow rate (mL min^{-1}), O_2 and CO_2 concentrations (%), and water vapour density (µg.mL^{-1}) were recorded at a 5 s sampling interval. Each bird was measured for 12 min in total, each hour, throughout the night (following Page et al., 2011). The lowest hourly $\dot{V}O_2$ reading for each bird was taken as the RMR for that night, and $\dot{V}CO_2$ and $\dot{V}H_2O$ were taken at the same time as $\dot{V}O_2$ was recorded. Sampling regime may significantly affect estimates of certain physiological variables, however, the differences between sampling regimes are usually small, and due to time constraints we used multiple birds each night in a switching system, as this results in smaller errors than measuring one individual continuously for shorter periods (Cooper and Withers, 2010).

Lag and drift correction were conducted using a macro in ExpeData. A 95% equilibration time of 11 min (calculated using the equation of Lasiewski et al. 1966). Rates of O_2 consumption ($\dot{V}O_2$), CO_2 production ($\dot{V}CO_2$) and $\dot{V}H_2O$ were z-transformed prior to calculation of hourly. The lowest hourly $\dot{V}O_2$ reading for each bird was taken as the RMR for that night. $\dot{V}CO_2$ and $\dot{V}H_2O$ were taken at the same time as RMR.

2.3 Data analyses

$\dot{V}O_2$, $\dot{V}CO_2$ and EWL were calculated as follows, with equations on page 456 of Withers (2001):

- $\dot{V}O_2 = \dot{V}_I \, (F_IO_2 - [F_EO_2 \, (1 - F_IO_2 - F_ICO_2 - F_IH_2O) \, / \, (1 - F_EO_2 - F_ECO_2 - F_EH_2O)])$

- $\dot{V}CO_2 = \dot{V}_I \, ([F_ECO_2 \, (1 - F_IO_2 - F_ICO2 - F_IH_2O) \, / \, (1 - F_EO_2 - F_ECO_2 - F_EH_2O)] - F_ICO_2)$

- $EWL = \dot{V}_I \, ([F_EH_2O \, (1 - F_IO_2 - F_ICO2 - F_IH_2O) \, / \, (1 - F_EO_2 - F_ECO_2 - F_EH_2O)] - F_IH_2O)$

where $\dot{V}_I$ was measured mass flow. Respiratory exchange ratio (RER) was calculated as the ratio of CO_2 produced to O_2 consumed. RER during the study was 0.72 ± 0.04 (mean $\pm$ SD), indicating that Cape white-eyes were predominantly catabolising lipids (Walsberg and Wolf, 1995), as would be expected in a small bird, measured overnight.

Statistical analyses were executed in R version 3.0.1 (R Core Team, 2014). Visual inspection of residual plots showed no deviations from homoscedasticity or normality. Data were visualized using the base graphics in R, and the package *multcompView* (Graves et al., 2012). The relationship between the response variables (RMR, EWL and body mass) and fixed and random predictor variables were investigated using general linear mixed models with the R package *nlme* (Pinheiro et al., 2014). Fixed effects included 'T_a' (the temperature at which metabolic trials were done, either 20°C or 25°C), 'Month' and 'Body mass' (g, recorded at either the start or end of the night, or the mean of these two, depending on which was closer to the time of minimum hourly metabolic rate, or RMR, for each night). 'BirdID' (individual) was included as a random effect to control for repeated measures. Correlations were performed in R, and post hoc tests (Tukey's HSD) were performed using the R packages *multcomp* (Hothorn et al., 2008)

and *mvtnorm* (Genz et al., 2014). Values are presented as means ± SD. Repeatability of whole animal RMR was calculated using equations 2 to 5 in Lessells and Boag (1987).

3. Results

The mean monthly maximum outdoor ambient temperature peaked at 28.1°C in February and fell to 21.7°C in July, and mean monthly outdoor ambient minimum temperature peaked at 17.5°C in January, and dropped to 5.6°C in June, with peaks and troughs coinciding with the austral summer and winter respectively (Fig. 1). Mean monthly temperature was significantly correlated with monthly range in temperature (Pearson's product-moment correlation, $r = -0.725$, $P = 0.008$).

Mean monthly body mass of Cape white-eyes showed no significant variation between months ($F_{1, 11} = 1.515$, $P = 0.127$), with a mean body mass of 12.2 ± 1.0 g throughout the study period. Similarly, mean monthly body mass lost overnight showed little variation throughout the year, and was 1.4 ± 0.3 g whether birds were measured at 20°C or 25°C.

Whole animal RMR varied significantly between months ($F_{1, 11} = 13.711$, $P < 0.001$) and when birds were measured at 20°C vs. 25°C ($F_{1, 1} = 79.039$, $P < 0.001$). Monthly whole animal RMR was highest in March, and lowest in October (Fig. 2), whether birds were measured at 20°C or at 25°C, and at both temperatures, these differences were significant (Fig. 2). Whole animal RMR_{20} ranged from 40.078 ± 2.912 mL O_2 h^{-1} in October to 61.562 ± 6.760 mL O_2 h^{-1} in March, and RMR_{25} ranged from 41.651 ± 4.029 mL O_2 h^{-1} in October to 50.753 ± 4.518 mL O_2 h^{-1} in March. Thus, summer RMR_{20} and RMR_{25} values were 53.6 % and 21.9 % higher than winter values respectively. However, RMR measurements taken when mean outdoor ambient

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temperatures were highest (February) and lowest (June), resulted in a summer RMR_{20} and RMR_{25} values being only 7.5 % and 3.5 % higher than winter values respectively.

Whole animal EWL_{20} and EWL_{25} peaked at 63.480 ± 18.578 mg H_2O h^{-1} in April and 85.977 ± 38.702 in December respectively, and dipped to 28.173 ± 15.841 and 32.565 ± 10.659 in June, such that the maximum monthly EWL_{20} and EWL_{25} values were 125.3 % and 164.0% higher than the minima, and these differences between maxima and minima were significant (Fig. 3). Had EWL been measured in February and June, the hottest and coolest months of the year, then summer EWL_{20} and EWL_{25} values would have been 44.431 ± 11.859 and 84.003 ± 35.485 respectively, and winter EWL_{20} and EWL_{25} values would have been 28.173 ± 15.841 and 32.565 ± 10.659 respectively, resulting in summer EWL values being 57.7 % and 157 % higher than winter values at 20 and 25°C respectively, with only the latter seasonal difference being significant (Fig. 3).

There was a significant positive correlation between monthly mean temperature and mean monthly whole animal EWL_{20} (Pearson's product-moment correlation, $r = 0.636$, $P = 0.026$), but not between monthly mean temperature and mean monthly whole animal EWL_{25} ($r = 0.372$, $P = 0.233$).

When using the data from the nine Cape white-eyes for which we had a complete set of monthly whole animal RMR measurements at $T_a = 20°C$, repeatability (r) was 0.110 ($F_{8, 99} = 2.131$, $P = 0.041$). At $T_a = 25°C$, for the ten Cape white-eyes with complete sets of monthly whole animal RMR measurements, repeatability (r) was 0.349 ($F_{10, 121} = 107.139$, $P < 0.0001$).

4. Discussion

Whole animal RMR of Cape white-eyes was lower in winter than in summer, indicating energy conservation rather than thermogenesis (Smit and McKechnie, 2010) as expected. However, RMR dipped even lower after winter, reaching its minimum in October (the austral spring), and rose even higher after summer, reaching a peak in March (fall). Had we measured birds only in the height of summer (January and February) and in the middle of winter (June and July), we would have concluded that there was no seasonal difference in RMR. These results suggest that previous studies on seasonal variation in avian metabolic rate, which were conducted in the summer and winter months, may not have captured the extremes of resting and/or basal metabolic rate that birds may show throughout the year. This mismatch in timing may further complicate our understanding of seasonal variation in metabolic rate of avian species at lower latitudes, an area in which trends are not currently well defined, especially if the rate of delayed response to seasonal acclimatization varies between species.

The significant repeatability values suggest that at T_a = 20ºC and 25ºC, between-individual variation was responsible for 11% and 35% respectively of the variation in RMR of Cape white-eyes. Most studies on repeatability of avian metabolic parameters show significant repeatability values (Table 1). This individual repeatability of avian metabolic rate may be related to ranking in terms of social dominance (Bryant and Newton, 1994; Hogstad, 1987; Kerimov and Ivankina, 1999).

The consistency in body mass throughout the year was not surprising given that Cape white-eyes are a non-migratory species, and that their diet was kept constant throughout the year in captivity. Furthermore, although small endotherms have higher surface-area-to-volume ratios than larger endotherms, with concomitantly higher mass-specific energy expenditure rates and

thermoregulatory costs (Arens and Cooper, 2005; McNab, 2009; Sharbaugh, 2001), small birds can carry only limited amounts of insulation before their movement is affected, and so small birds might be expected to show behavioral and physiological adjustments to seasonal acclimatization, mainly by altering their metabolic rates (Chaplin, 1974; Dawson and Carey, 1976; Newton, 1969; Pohl and West, 1973; Weathers and Caccamise, 1978).

EWL was lowest in June, when the mean monthly outdoor temperature was also minimal, thus reducing the need for evaporative cooling. Conversely, monthly peaks in EWL occurred when mean monthly outdoor temperatures were highest, and when the need for evaporative cooling was greatest. At 25°C, EWL was significantly higher in December and February than in June, and at 20°C, EWL was significantly higher in December, January, February and March than in June or August. Measuring EWL at 20°C in February and July would have yielded a conclusion of 'no significant seasonal differences', as would measuring EWL at 25°C in January and July-August.

The only five studies (of which we are aware) on circannual rhythm in avian metabolism to date, generally suggest that circannual rhythm in metabolic rate may be modulated by phenotypic flexibility, although the main driver seems to be endogenous. Red knots (*Calidris canutus islandica*) kept at three thermal treatments showed coordinated and similar variation in body mass and metabolic rate throughout the year, as did captive and wild birds, suggesting regulation by an endogenous driver (Piersma et al., 1995; Vézina et al., 2011). Similarly, three subspecies of stonechat (*Saxicola torquata*) from areas differing in seasonality, maintained different annual cycles of body mass and BMR, even when kept under common environments, suggesting that the effects of phenotypic flexibility on circannual cycles were minor in comparison with the influence of genetic differences (Versteegh et al., 2012). Furthermore,

males of two subspecies of stonechats, raised and kept under similar laboratory conditions, showed differences in metabolic rate between the two subspecies which must have been genetically determined (Klaassen, 1995). In this study too, circannual rhythm of Cape white-eyes may be partly driven by genetics, since timing of peaks and lows in RMR do not coincide with those of outdoor ambient temperature. Alternatively, there may simply be a lag in time taken to acclimatize to environmental temperatures, although there is no evidence for an acclamatory period for metabolic rate of Cape white-eyes (Chapter 5). Perhaps surprisingly, the timing of peak RMR in Cape white-eyes did not coincide with their peak breeding season of October to December (Sinclair et al., 2011), a time when resting metabolic rate is usually elevated in birds (Nilsson and Råberg, 2001), which suggests little evidence for a large endocrine influence on metabolic rate in this species. However, future studies investigating the influence of reproduction on circannual rhythm in metabolic rate should use freshly wild-caught birds, rather than the long-term captive birds used here, where we discouraged breeding by not providing nesting materials.

In addition to being a phenotypically flexible trait (McKechnie, 2008), avian BMR is also heritable (Broggi et al., 2009; Bushuev et al., 2011; Rønning et al., 2007) and repeatable (Broggi et al., 2009; Hõrak et al., 2002; Nespolo and Franco, 2000; Rønning et al., 2005a; Versteegh et al., 2008), and thus birds may respond to environmental changes such as climate change, with both their phenotypic flexibility, and with genetic changes, that is microevolution, in that flexibility (Broggi et al., 2009; Gienapp et al., 2008).

5. Conclusions

Regardless of how much of the circannual variation in resting metabolism is due to genetics, and how much to phenotypic flexibility, it would seem that timing of measurements may be paramount in discovering the full range that birds are capable of in terms of their resting metabolism and EWL throughout the year. Indeed, the implicit assumption that extremes in RMR occur in summer and winter, may have been partly responsible for the confusion regarding seasonal trends in metabolic rate in Afrotropical and other subtropical bird species. Given that relatively few studies have focused on circannual cycles in avian metabolic rate to date, and the potential importance to understanding when peaks and troughs occur, we encourage further studies on this topic, particularly in non-migratory, subtropical species.

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Figure legends

Figure 1. Mean monthly maximum (max), minimum (min), mean, and range (maximum –
minimum) of outdoor ambient temperatures (T_a, °C) at the study site in Pietermaritzburg,
KwaZulu-Natal, South Africa, over the study period, September 2012 to August 2013. Data
supplied by the South African Weather Service.

Figure 2. Monthly resting metabolic rate (RMR, mL O_2 h^{-1}) of Cape white-eyes measured at
20°C (left) and 25°C (right), from September 2012 to August 2013. Dots represent outliers, thick
black line medians, and tops and bottoms of boxplot the 1st and 3rd quartiles. Similar letters
above boxplots indicate a values that are not significantly different, with the left and right graphs
being treated separately.

Figure 3. Monthly evaporative water loss (EWL, mg H_2O h^{-1}) of Cape white-eyes measured at
20°C (left) and 25°C (right), from September 2012 to August 2013. Dots represent outliers, thick
black line medians, and tops and bottoms of boxplot the 1st and 3rd quartiles. Similar letters
above boxplots indicate a values that are not significantly different, with the left and right graphs
being treated separately.

Figures
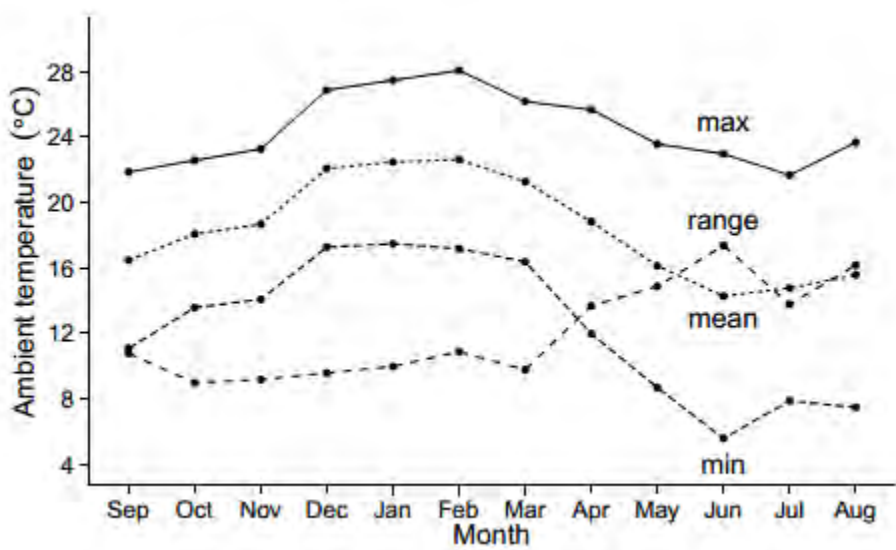
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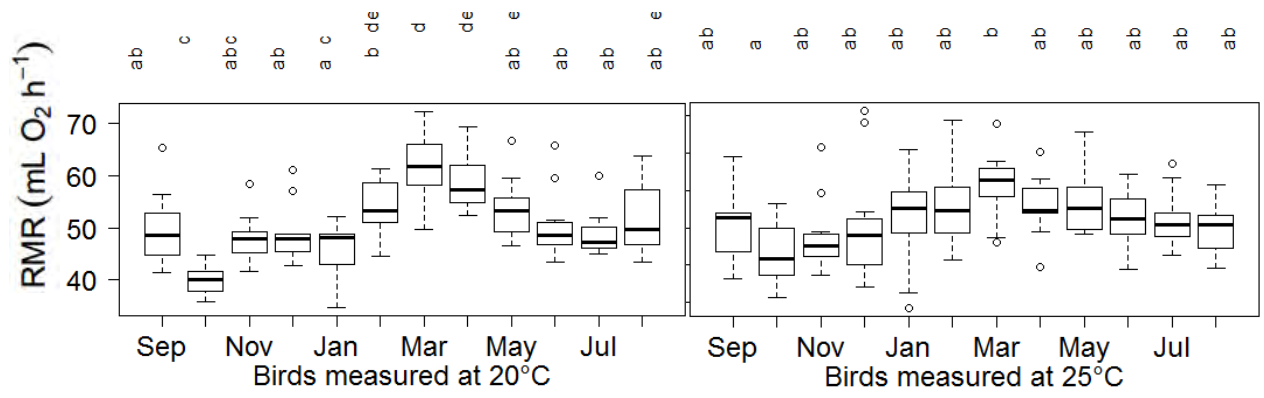
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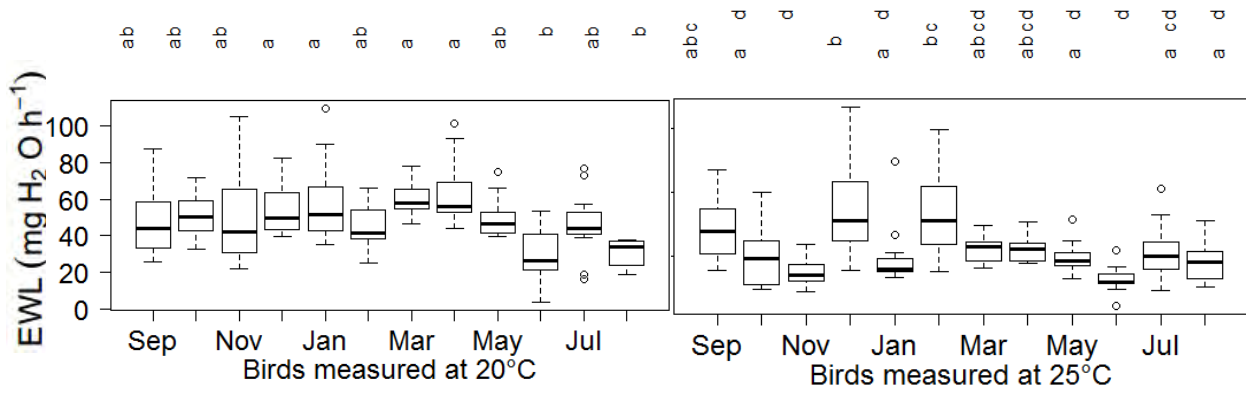
Figure 3.

Table legend

Table 1. Repeatability (r) values for various measures of avian metabolic rate.

* Note that Bech et al. (1999) measured BMR in breeding birds, and the sample size was stated as 8, suggesting that the degrees of freedom could possibly have been 7, not 8. A dash indicates that data was not provided. 1 = Bech et al. (1999); 2 = Fyhn et al. (2001); 3 = Chappell et al. (1996); 4 = Broggi et al. (2009); 5 = Hõrak et al. (2002); 6 = Versteegh et al. (2008); 7 = Tieleman et al. (2003); 8 = Rønning et al. (2005b).

Species	Measurement	n	r	F statistic	P-value	Reference
Kittiwake (*Rissa tridactyla*)	mass-specific BMR*	8	0.52	$F_{8,17}=4.25$	< 0.006	1
	mass-specific BMR	19	0.35	$F_{18,27}=2.28$	0.0028	1
Kittiwake (*Rissa tridactyla*)	field metabolic rate over 1 day	-	0.64	-	< 0.01	2
Red Junglefowl (*Gallus gallus*)	$\dot{V}O_{2max}$ over 2 h	11	0.90	$F=38.4$	<0.0002	3
	$\dot{V}O_{2max}$ over 28 days	31	0.90	$F=125$	<0.00001	3
	$\dot{V}O_{2max}$ over 56 days	30	0.93	$F=181$	<0.00001	3
	$\dot{V}O_{2max}$ over 180days	22	0.52	$F=7.29$	0.0137	3
Great Tit (*Parus major*)	total mass-specific BMR	93	0.36	$F_{1,169}=108.65$	< 0.0001	4
	within-year mass-specific BMR	65	0.40	$F_{1,108}=62.40$	< 0.0001	4
	between-year mass-specific BMR	48	0.32	$F_{1,77.4}=58.10$	< 0.0001	4
	total whole animal BMR	93	0.56	-	< 0.0001	4
	within-year whole animal BMR	65	0.56	-	< 0.0001	4
	between-year whole animal BMR	48	0.53	-	< 0.0001	4
Cape White-eye (*Zosterops virens*)	within-year whole animal RMR at T_a =20°C	9	0.11	$F_{8,99}=2.13$	0.0407	*this study*
	within-year whole animal RMR at T_a =25°C	10	0.35	$F_{10.121}=107.139$	< 0.0001	*this study*
Greenfinch (*Carduelis chloris*)	mass-specific BMR over 4 days	28	0.86	-	<0.001	5
	mass-specific BMR over 8 days	28	0.87	-	<0.001	5
	mass-specific BMR over 4 months	14	0.63	-	< 0.01	5
European Stonechat (*Saxicola torquata rubicola*)	winter BMR	18	0.56	-	0.002	6
	winter mass-specific BMR	18	0.60	-	<0.001	6
Skylark (*Alauda arvensis*)	whole animal BMR	14	0.17	$F_{13,17}=0.64$	0.788	7
Woodlark (*Lullula arborea*)	whole animal BMR	14	0.17	$F_{13,12}=1.40$	0.285	7
Spike-heeled Lark (*Chersomanes albofasciata*)	whole animal BMR	20	0.66	$F_{19,18}=4.88$	0.001	7

Dunn's Lark (*Eremalauda dunni*)	whole animal BMR	16	0.48	$F_{15,14} = 2.84$	0.029	7
Hoopoe Lark (*Alaemon alaudipes*)	whole animal BMR	14	0.57	$F_{13,12} = 3.63$	0.016	7
Zebra Finch (*Taeniopygia guttata*)	BMR	39	0.57	$F = 6.32$	<0.001	8
	BMR	36	0.57	$F = 6.24$	<0.001	8

Table 1.

CHAPTER 4

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THE EFFECTS OF LONG-TERM CAPTIVITY ON THE METABOLIC PARAMETERS OF A SMALL AFROTROPICAL BIRD

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Abstract

The few within-species studies on the effects of long-term captivity on avian physiological variables have small samples sizes and contradictory results. Nevertheless, many physiological studies make use of long-term captive birds, assuming the results will be applicable to wild populations. Here we investigated the effects of long-term captivity (> 1 year) and seasonal acclimatisation on a variety of physiological measurements in a relatively small (~12 g) southern African endemic bird, the Cape white-eye (*Zosterops virens*). Whole animal basal metabolic rate (BMR) and body mass (Mb) were influenced more by long-term captivity than by season, while mass-specific BMR, standard and basal whole animal and mass-specific evaporative water loss (EWL), and respiratory exchange ratio (RER), were all affected primarily by season, with long-term captivity having less of an effect. We therefore caution that whole animal BMR and Mb of long-term captive birds should not be used as representative of wild populations, and that the

origin of study birds should be considered when comparing EWL and RER of wild and long-term captive birds.

Key words   Avian basal metabolic rate -   Cape white-eye *Zosterops virens* - Evaporative water loss - Long-term captivity - Respiratory quotient - Seasonal effects

Abbreviations

BMR   Basal metabolic rate

EWL   Evaporative water loss

Mb   Body mass

RMR   Resting metabolic rate

RER   Respiratory exchange ratio

T_a Ambient temperature

$\dot{V}CO_2$ Rate of CO_2 production

$\dot{V}O_2$ Rate of O_2 consumption

Introduction

There is a multitude of studies that use respirometry to measure avian metabolic rate and associated parameters (see reviews by McKechnie 2008; McKechnie and Lovegrove 2002; Swanson et al. 2012; Bishop 1999; Jetz et al. 2008). Of these, many have used captive raised or long-term captive birds as study animals, assuming that the basal metabolic rate (BMR, the minimum maintenance energy requirement in normothermy (McNab 1997)) of these birds will be representative of wild individuals (e.g. McKechnie et al. 2006; Schleucher and Withers 2002). However, this is not always the case. Although studies have been conducted to determine the effects of captivity, particularly long-term captivity, on the metabolic rates of various avian

species (McKechnie et al. 2006; McKechnie et al. 2007; Weathers et al. 1983), the results are contradictory (Piersma et al. 1996). Generally, relatively small birds show either no change or increased BMR with long-term captivity (Table 1). Several variables may affect these metabolic parameters. For example, handling and capture stress may induce higher BMRs in freshly-caught birds (Hails 1983; Duriez et al. 2004; Speakman et al. 1993), while captive birds may experience a smaller temperature range and more moderate minimum temperatures than wild populations, resulting in reduced BMR (Tieleman et al. 2003; McKechnie et al. 2006). Furthermore, in species > 200 g, the confines of captivity may cause a reduction in pectoral muscle mass, leading to reduced BMR (McKechnie et al. 2006; McKechnie et al. 2007; McKechnie 2008; Davis Jr. 1955; Saarela and Hohtola 2003). However, food may be of higher quality and/or more freely available in captivity, which may lead to higher masses of metabolically active organs such as the intestines, liver and stomach, and therefore to elevated BMR (Mueller and Diamond 2001; Starck 1999; Dekinga et al. 2001; McKechnie et al. 2006; McNab 2009). Thus the effects of captivity, particularly long-term captivity, on avian metabolic rate may be complex and species-specific (McNab 2009; Weathers et al. 1983). In addition, there are almost no data on the effects of long-term captivity on avian metabolic parameters within species (McKechnie et al. 2006); a literature search revealed only a handful of such studies, some of which had very small sample sizes (Table 1). Furthermore, none of these studies commented on the effects of long-term captivity on other physiological and metabolic parameters including evaporative water loss (EWL), body mass (Mb) or respiratory exchange ratio (RER). Significant deviations from expected RER values may cause problems when estimating power consumption from CO_2 production (Walsberg and Wolf 1995). Thus, it would be useful to know whether or not long-term captivity has an effect on avian RER. At the same time, in the face of further climate

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change, there is an increasing need for predictive models to incorporate physiological information (Dillon et al. 2010), particularly EWL data, since water conservation will become ever more important for birds as ambient temperatures increase (Chown et al. 2010). Therefore, one needs to be certain that metabolic measurements, and particularly EWL data, obtained from captive birds are representative of those of wild individuals.

Therefore, given (i) the lack of consensus on whether or not long-term captivity affects avian BMR and associated physiological variables, (ii) the fact that it is sometimes impractical to measure wild-caught birds, particularly in instances where birds are endangered, elusive, or very rare (McNab 2009; McNab 2013), and (iii) the scarcity of within-species studies investigating the effects of long-term captivity on BMR, we aimed to investigate the effect of long-term captivity on avian BMR and other physiological parameters using a small southern African endemic bird, the Cape white-eye (*Zosterops virens*) (Thompson and Taylor 2014; Sundevall 1850). We hypothesised that long-term captivity would affect Mb, BMR and RER, but not EWL, in this species. We predicted that long-term captives would have higher Mb, BMR and RER values than freshly wild-caught birds, due to increased intake of higher quality and more readily available food. We did not expect that long-term captive birds would lose flight muscle mass or suffer resulting decreases in their BMR, since their cages were large enough to allow limited flight. Nor did we expect long-term captive birds to experience less extreme minimum environmental temperatures and consequent lower BMR (McKechnie et al. 2006), since the long-term captive birds were housed in outdoor aviaries, in order for them to experience the same rainfall, humidity, wind and ambient temperature (T_a) as wild birds. Avian BMR is negatively linked with T_a and annual temperature range (White et al. 2007), while whole animal avian metabolic rate may be significantly higher under artificial rainfall regimes than under no

rain (Wilson et al. 2004). Furthermore, increased wind speeds may elevate energy expenditure, particularly in small birds, as wind disturbs the layer of air within the feathers, penetrating below the coat surface and causing convective cooling at the coat surface (Williams and Tieleman 2001). This wind-related increase in metabolic rate results in elevated BMR (Wolf and Walsberg 1996), as would be expected, since BMR correlates with the energy expenditure of free-living animals (Daan et al. 1990; Nagy 1987). Thus, we wanted to ensure that the study birds experienced the same environmental conditions as wild birds, as far as possible, since the effect of captivity of metabolic rate could be linked to how closely captive conditions mimic the natural environment. Finally, we predicted that EWL measured in thermoneutrality would be similar in freshly wild-caught and long-term captive Cape white-eyes, since T_a and humidity levels experienced by long-term captives and freshly wild-caught birds would be similar. We used four different measures of EWL (basal whole animal, basal mass-specific, standard whole animal and standard mass-specific), to determine whether long-term captivity would have the same effect on all of them, and we predicted that it would. Since summer and winter values of BMR, EWL and Mb may differ significantly (McKechnie 2008; Thabethe et al. 2013; Zungu et al. 2013), and we thought it possible that the effect of captivity may differ between seasons, we recorded BMR, EWL and Mb of Cape white-eyes in long-term captive and freshly wild-caught Cape white-eyes in both summer and winter.

Materials and Methods

Animals

All Cape white-eyes used in this study were caught using mist-nets (Ecotone, Gdynia, Poland) at the Darvill Bird Sanctuary, Pietermaritzburg, KwaZulu-Natal, South Africa (29°36'S, 30°26'E), or in nearby gardens. A permit to capture, monitor, ring, release and transport Cape white-eyes was granted by Ezemvelo KwaZulu-Natal Wildlife, number OP 5122/2012, issued to CTD. Ethical approval for this study was granted to LJT by the Animal Ethics Sub-committee of the University of KwaZulu-Natal's Ethics Committee, reference: 071/13/Animal. After the study, birds were released at their capture sites.

The long-term captive group comprised 14 Cape white-eyes, captured as adults in February 2012. Captive birds were housed in groups of 4-5, in outdoor aviaries (1 x 3 m and 2 m tall), with a cement floor, and walls and roofs of wire mesh. One third of the roof was covered in shade cloth, and half of a small side was covered with chipboard to provide shelter. The aviaries were equipped with perches, but no nesting material was provided, to discourage breeding. Birds were given water *ad libitum*, and fed daily on a variety of grated and whole fruits (papayas, oranges, apples and bananas) supplemented with softbill pellets (Avi-products, Durban, South Africa). Respirometry measurements were made after these birds had been captive for at least one year: measurements were made during the austral winter in August 2013, and summer of February 2014. In addition, concurrent measurements were made on free-ranging birds which were measured within 48 h of being caught, and released the next morning. Resting metabolic rates (RMR) of both long-term captive and freshly wild-caught Cape white-eyes were measured

at 30°C, in both summer and winter, as this falls within the thermoneutral zone of this population in both seasons (Chapter 2) and therefore RMR_{30} was deemed to equate to BMR.

To account for the effects of moult on BMR, immediately prior to respirometry trials, birds were scored for primary flight feather moult, following guidelines given in the SAFRING Bird Ringing Manual (De Beer et al. 2001). Using this method, feathers were assigned a score, depending on their condition (old or new), and their length. The sum of the moult score of all nine primary feathers ranged from 0 (pre-moult) to 45 (primary moult completed), following Klaassen (1995), and this value was divided by 4.5 to obtain an index ranging from 0 to 10.

Gas exchange measurements

Mb (g) of each Cape white-eye was recorded to 2 d.p. using digital scales (model: AFB-3100L, Adam Equipment S.A. Pty. Ltd., Johannesburg) prior to each night's measurements. This Mb was used as Mb for the purposes of calculating mass-specific BMR, and for comparing Mb between seasons.

At 15:00 – 16:00, after being weighed, Cape white-eyes were placed into 2.8 L Perspex respirometry chambers within a temperature-controlled environmental chamber (CMP2244, Conviron, Winnipeg, Canada), set to 12L : 12D, and fasted for 3 h before measurements started, to ensure that they were post-absorptive, following Wellman and Downs (2009b) and to reduce the possible effects of handling stress. Each respirometry chamber contained a wooden perch, to allow normal sleeping posture (Wellman and Downs 2009a), and since a lack of a perch may result in higher CO_2 production (Wallgren 1954). Birds were placed onto a plastic mesh platform 10 cm above a 1 cm layer of liquid paraffin (AlphaPharm, Pietermaritzburg), to eliminate evaporation from excreta.

Metabolic rate was measured using an open-flow, push mode respirometry system. Water vapour and CO_2 were removed from environmental air with silica gel and soda lime respectively, following Withers (2001). Pumps (model PP2, Sable Systems, Las Vegas, Nevada, USA) pushed this air into five inlets of a flow measurement system (model FB8, Sable Systems), with flow rates set to approximately 800 mL.min^{-1}, such that the depletion in O_2 concentration within each respective chamber stayed within 0.1 and 0.5% (following Lighton, 2008).   In each respirometry chamber, the air inlet was located near the bottom, and the outlet near the top, to facilitate mixing of air within the chamber. Effluent air coursed through a flow multiplexer (model MUX, Sable Systems) and a subsampler (model SS4, Sable Systems), where a manifold allowed excess air to escape. Approximately 200 mL.min^{-1} of the effluent airstream then passed into a water vapour analyser (model RH300, Sable Systems), which was regularly zeroed using N_2, and spanned using a nearly-saturated airstream. Air was dried with minimal quantities of Drierite (Hammond Drierite Co. Ltd., Xenia, Ohio), which had previously been recharged, to reduce its affinity for CO_2 and thereby reduce CO_2 washout time (White et al. 2006). Air then passed through CO_2 analyser (model CA-10, Sable Systems) and a fuel cell O_2 analyser (model FC-10, Sable Systems). The CO_2 analyser was regularly zeroed using N_2, and spanned using a certified gas containing 964 ppm CO_2 in N_2 (AFROX, Pietermaritzburg, South Africa). The gas analysers and flow meter were connected to a Universal Interface (model UI2, Sable Systems), which transferred data to a desktop computer using ExpeData data acquisition software (Sable Systems). Temperatures within each respirometry chamber were recorded every 15 min. using i-Buttons® with a resolution of 0.0625°C, (model DS1922L-F5, Thermochron®, Maxim, CA, USA). All i-Buttons® were calibrated prior to use in a circulating water bath, at temperatures

from 7 - 36°C, with a mercury-in-glass thermometer (measurement precision = 0.1°C), with accuracy traceable to the US National Bureau of Standards.

From approximately 15:30 until 07:00 the next morning, the following variables were recorded at a sampling interval of 5 seconds: flow rate ($mL.min^{-1}$), O_2 and CO_2 concentrations (%), and water vapour density ($\mu g.mL^{-1}$). An interrupted sampling regime was used, starting with a baseline measurement for six min. and then four birds in succession for six min. each. This cycle was repeated such that each bird was measured twice (for 12 min. in total) every hour, throughout the night, following Page et al. (2011).

A macro was used to perform lag and drift correction in ExpeData, and a 95% equilibration time of 11 min. was calculated using the equation of Lasiewski et al. (1966). Thus, rate of O_2 consumption ($\dot{V}O_2$), CO_2 production ($\dot{V}CO_2$) and EWL were z-transformed before hourly means were calculated. The lowest hourly $\dot{V}O_2$ reading for each bird was taken as the RMR for that night. $\dot{V}CO_2$ and $\dot{V}H_2O$ (hereafter termed standard EWL) were taken at the same time as $\dot{V}O_2$ was recorded. The basal EWL measurement of each night was also recorded, since the time of minimum EWL and minimum $\dot{V}O_2$ usually did not coincide. $\dot{V}O_2$, $\dot{V}CO_2$, RER and EWL were calculated with the following equations from Withers (2001):

- $\dot{V}O_2 = \dot{V}_I (F_IO_2 - [F_EO_2 (1 - F_IO_2 - F_ICO_2 - F_IH_2O) / (1 - F_EO_2 - F_ECO_2 - F_EH_2O)])$

- $\dot{V}CO_2 = \dot{V}_I ([F_ECO_2 (1 - F_IO_2 - F_ICO2 - F_IH_2O) / (1 - F_EO_2 - F_ECO_2 - F_EH_2O)] - F_ICO_2)$

- $EWL = \dot{V}_I ([F_EH_2O (1 - F_IO_2 - F_ICO2 - F_IH_2O) / (1 - F_EO_2 - F_ECO_2 - F_EH_2O)] - F_IH_2O)$

- $RER = \dot{V}CO_2 / \dot{V}O_2$

where $\dot{V}_I$ was the measured mass flow.

Statistical analyses

All statistical analyses were performed using R version 3.0.1 . A set of candidate models was defined *a priori* following Burnham and Anderson (2002), and the R package '*lme4*' (Bates et al. 2014) was used to perform a linear mixed-effects analysis of the relationship between the response variables (BMR, EWL, RER and Mb) and fixed and random predictor variables. Fixed effects included 'Season' (summer or winter), 'Origin' (long-term captive or freshly wild-caught), 'Mass' (Mb at the start of the night'), 'Moult' (primary wing moult score, ranging from 0 to 10), and 'Origin*Moult' (the interaction between 'Origin' and 'Moult'). 'BirdID' (individual) was included as a random effect to control for repeated measures. Visual inspection of residual plots did not show any obvious deviations from normality or homoscedasticity. Best approximating models were objectively selected based on their second order (corrected for small sample size) Akaike weights (AICcWt) and differences in corrected AIC values (AICc) between each candidate model and the best approximating model (ΔAICc), which were produced using the R package '*AICcmodavg*' (Mazerolle 2013). Models with ΔAICc < 2 were averaged for the purpose of multimodel inference using the R package '*MuMIn*' (Bartoń 2013).

Results

In winter, all long-term captive and freshly-caught Cape white-eyes had the highest possible primary moult score of 10.0, indicating that all primary flight feathers were new and fully-grown. In summer, primary moult scores were much lower, 1.0 ± 1.5 (mean ± SD) for long-term captive birds and 6.6 ± 4.0 for freshly wild-caught birds, indicating that in summer, the primary flight feathers of freshly wild-caught birds were newer and in a more advanced stage of moult

than those of long-term captives. Nonetheless, in summer, both groups of birds were actively moulting their primaries.

T_a within the respirometry chambers was 30.2 ± 0.5°C (mean ± SD). At this temperature, mean whole animal BMR of long-term captive Cape white-eyes was significantly higher than that of freshly wild-caught birds, in both summer ($t_{(26)} = 6.47$, $p < 0.001$) and winter ($t_{(25)} = 6.21$, $p < 0.001$) (Fig. 1,Table 2).

Freshly wild-caught birds had a whole animal BMR that was 8.26 ml O_2 h^{-1} lower than that of long-term captives, and whole animal BMR was 2.83 ml O_2 h^{-1} higher in winter than in summer (Table 4). For every 1g increase in Mb, whole animal BMR increased by 2.49 ml O_2 h^{-1}. In summer and winter, for both long-term captive Cape white-eyes and freshly wild-caught birds, whole animal RMR declined sharply from 18:00 to 19:00, then more gradually, reaching a minimum around 21:00, which usually remained fairly constant until after 03:00, when RMR began to rise (Fig. 2).

Mb of Cape white-eyes was 0.09g lower in winter than in summer, and 0.67g lower in freshly wild-caught birds than in long-term captives (Fig. 1). In summer, the difference between mean Mb of freshly wild-caught and long-term captive birds was significant ($t_{(25)} = 2.85$, $p < 0.01$). In winter, this difference in mean Mb between freshly wild-caught and long-term captive birds was almost significant ($t_{(21)} = 2.06$, $p = 0.05$).

Only two candidate models for RER had ΔAICc < 2 (Table 3). Both of these contained 'Season' and 'Origin' as fixed factors, and the second best approximating model also contained 'Mass'. The averaged model was as follows: RER = 0.90 – 0.17*'Season' + 0.08*'Origin' – 0.01*'Mb', where 'Season' refers to winter, and 'Origin' refers to freshly wild-caught birds. The mean RER of captive birds was significantly lower than that of freshly-wild caught birds, in both

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winter ($t_{(20)}$ = -6.90, p < 0.001) and summer ($t_{(17)}$ = -4.90, p < 0.001, Table 2). In summer and in winter, for both freshly wild-caught birds and for long-term captive birds, RER was fairly consistent throughout the night (Fig. 2).

In winter, mean standard EWL of long-term captive Cape white-eyes was significantly lower than that of freshly wild-caught birds ($t_{(25)}$ = -2.72, p < 0.05), while there was no significant difference in standard EWL of long-term captive and freshly wild-caught birds in summer ($t_{(26)}$ = 0.52, p > 0.05 (Table 2). There was no significant difference in mean basal whole animal EWL of freshly wild-caught and long-term captive birds in either summer ($t_{(22)}$ = 0.84, p > 0.05) or winter ($t_{(24)}$ = -1.18, p > 0.05) (Fig. 1). Whole animal standard EWL decreased by 39.55 mg $H_2O.h^{-1}$ in winter, and increased by 7.95 mg $H_2O.h^{-1}$ in freshly wild-caught birds. The results for basal whole animal EWL were very similar whole animal basal EWL decreased by 32.44 mg $H_2O.h^{-1}$ in winter, and increased by 7.00 mg $H_2O.h^{-1}$ in freshly wild-caught birds. Thus 'Season' had a much greater effect on standard and basal whole animal EWL than 'Origin'. In both seasons, and in both long-term captive and freshly wild-caught Cape white-eyes, standard whole animal EWL dropped precipitously in the first few hours after dark, and then declined more slowly as the night went on, generally reaching a minimum in the hour before dawn (Fig. 2).

Discussion

Very few studies have investigated the effects of long-term captivity on physiological variables within avian species. This study demonstrated that some physiological data collected from Cape white-eyes held in long-term captivity were not directly comparable with those from freshly

wild-caught individuals. In particular, the effect of long-term captivity on whole animal BMR greatly outweighed the effect of season.

Mb was affected more by long-term captivity than by season. As predicted, Mb was lower in freshly wild-caught Cape white-eyes than in captive birds, presumably due to the constant, regular supply of perhaps higher quality food given to captive birds, compared with a possibly more erratic, lower quality diet in the wild. Ingestion of greater quantities of food by long-term captive birds would have resulted in an increase in the mass of small and large intestines and the liver (McWilliams and Karasov 2014), with concomitant elevations in BMR. Indeed, long-term captive birds had higher whole animal BMRs than freshly wild-caught birds, as predicted. This follows the general trend of relatively small birds showing either no change in BMR or having elevated BMR in captivity (Table 1).

It is also possible that the elevated whole animal BMR of long-term captive Cape white-eyes could be linked to chronic elevated stress levels in captivity, caused by the close proximity to individuals of other avian species, and by regular human disturbance. Increased stress results in increased heart rate and muscle tone which would be reflected in a higher metabolic rate (Careau et al. 2008). However, behavioural changes during the course of this study suggested that the long-term captive birds became more habituated over time, and so we do not suspect chronic stress to have caused elevations in BMR of captive birds. Conversely, unhabituated freshly wild-caught birds might be expected to experience more capture- and handling-induced stress (Hails 1983; Duriez et al. 2004; Speakman et al. 1993; Careau et al. 2008; McNab 2009) than long-term captives, which would be reflected in an elevation in their whole animal BMR, but this was not the case. Knowledge of captivity effects may be important for wildlife managers to better understand the implications of captive environments and husbandry techniques on

species' physiology. This could be critical for the success of captive breeding programmes for example.

Only adult Cape white-eyes were included in this study, however, long-term captive Cape white-eyes were six months older during the summer measurements than they were during the winter measurements. Thus, it is possible that age of Cape white-eyes may have had a confounding effect, since BMR is known to decrease as birds age (Broggi et al. 2010). This study would therefore have been improved by ensure that seasonal measurements were made on captive birds of the same age, however Cape white-eyes are difficult to age, so a different species that is easier to age could be used in future studies.

The trends in timing of primary moult of the Cape white-eyes used in this study were in accordance with those from the same province (Symes et al. 2001) and elsewhere in South Africa (Hulley et al. 2004); birds were actively moulting in summer, and primaries were new and fully grown in winter. Just as with yellow-breasted buntings (*Emberiza aureola*) (Wallgren 1954), long-term captive and freshly wild-caught Cape white-eyes moulted their primary wing feathers in the same month. Thus the metabolic costs of maintaining tissues necessary for moult and of synthesising keratin (Klaassen 1995; Buchanan et al. 2001) should have been higher in summer if moult had a large effect on whole animal BMR. Yet, whole animal BMR of Cape white-eyes was higher in winter than in summer, contrary to the expected result of increased BMR during the moult period (Schieltz and Murphy (1997).

The direction and magnitude of seasonal adjustments in avian BMR are known to vary with Mb (Smit et al. 2008; Dawson 2003; Hayworth and Weathers 1984; McKechnie 2008; Swanson and Weinacht 1997). In areas where winters are severe, small birds (< 200 g) generally up-regulate their RMR and BMR in winter as a cold defence mechanism (Dawson 2003; Smit

and McKechnie 2010; Weathers and Caccamise 1978; Zheng et al. 2014). In contrast, small birds from the subtropics generally down-regulate basal, standard metabolic rates and RMR in winter, to conserve energy, or show no seasonal difference (Lill et al. 2006; Maddocks and Geiser 2000; McNab 2009; Smit and McKechnie 2010). While House sparrows (*Passer domesticus*) have been shown to elevate their whole animal BMR in winter (Nzama et al. 2010), this may be a phylogenetically conserved response, rather than an adaptation to local environmental conditions, since the species was originally from areas with more seasonal variation in T_a. Despite this general trend, Cape white-eyes elevated their whole animal BMR in winter, irrespective of whether they were wild or long-term captive. Although the number of studies on BMR of species from lower latitudes has increased in recent years, the seasonal trends in BMR in these birds are still unclear (Smit and McKechnie 2010), and may be partly related to the mean winter and summer temperatures each year. For example, there are significant, negative correlations between mean minimum winter temperature and winter metabolic rates of Black-capped Chickadees (*Poecile atricapillus*) (Swanson and Olmstead 1999; Dutenhoffer and Swanson 1996; Cooper and Swanson 1994; Olson et al. 2010). Alternatively, small birds resident in the subtropics that do not show seasonal variation in their metabolic rate may instead increase their plumage insulation in winter, thereby reducing conductance (Lill et al. 2006). Regardless of season and of origin, Cape white-eyes reduced their metabolic rates overnight, in accordance with the circadian cycles expected of small birds, reflecting energy conservation at night.

To our knowledge, this is the first within-species study on the effects of captivity on avian RER. RER, the ratio of CO_2 emitted to O_2 consumed, is an instantaneous indicator of the types of nutrients being metabolised by a living animal (Cortés et al. 2009). RER values close to 0.7 indicate that energy metabolism is occurring by the aerobic catabolism of fats, while RER

values close to 1.0 suggest the aerobic catabolism of carbohydrates, and intermediate RER values may reflect the catabolism of proteins, or of a mixture of proteins, carbohydrates and lipids (Lighton 2008). In this study, RER values (taken at the time of basal $\dot{V}O_2$) fell as low as 0.64 (in a captive bird during winter), similar to the value of 0.66 obtained for House sparrows by Walsberg and Wolf (1995). RER values < 0.7 may result from incomplete oxidation of lipids, and non-pulmonary loss of CO_2, and do not necessarily imply experimental error; indeed RER values of 0.6 to 0.7 may be normal in birds (King 1957). Conversely, the highest overnight RER value obtained for a Cape white-eye in this study was 1.02 (in a freshly wild-caught bird in summer), suggesting fat deposition (Powers 1991). Mean RER values in summer and winter, for both long-term captive and freshly wild-caught Cape white-eyes showed very little variation from 18:00 to 05:00, suggesting that substrates being catabolised did not vary during this period.

RER was lower in winter than in summer, suggesting that in winter, birds were metabolising substrates that would release more energy per mass of fuel catabolised. This was to be expected, since lower T_a would force Cape white-eyes to increase their metabolic rate, resulting in greater energy requirements. However, contrary to our predictions, RER values of long-term captive birds were lower than those of freshly wild-caught birds in both seasons. This suggests that long-term captive Cape white-eyes were catabolising more fats than their freshly wild-caught wild conspecifics, and it may reflect the increased metabolic costs associated with possibly larger digestive organs in the long-term captive birds. Alternatively, it may be because long-term captive birds were fatter than their freshly wild-caught counterparts and may have switched substrate due to the reliability and quality of food in captivity.

For all four measures of EWL, values were lower in winter than in summer. This seasonal difference was unexpected, since EWL was measured at thermoneutrality in both

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seasons. Long-term captivity had a much smaller effect on EWL than season, and as predicted, EWL was similar in freshly wild-caught and long-term captive Cape white-eyes, for all four measures of EWL. Standard EWL was higher than basal EWL. This probably occurred because EWL dropped precipitously in the first three hours after dark, and then gradually thereafter, and while basal $\dot{V}O_2$ (and thus standard EWL) could fall at any time throughout the night, basal EWL almost always fell in the hour before dawn.

Although in this study long-term captivity had a large effect on whole animal BMR and Mb, with less of an effect on EWL and RER measured within the thermoneutral zone, these are not the variables that will be most affected by accelerated climate change. Undoubtedly the daytime field metabolic rate of diurnal birds, and metabolic rates and EWL at temperatures above thermoneutrality, will be of more importance in studies on how climate change will affect birds physiologically (Chown et al. 2010). Therefore we propose that future studies on the effects of long-term captivity on avian metabolic parameters should focus on metabolic measurements at extremely high temperatures, which have been shown to have negative effects on avian populations (Altwegg et al. 2014). In addition, the factors affecting the direction and size of seasonal metabolic changes in small birds would be an interesting topic for further investigation. Finally, we suggest that future work on avian seasonal metabolic variation should include the post-measurement dissection of birds and weighing of their organs (Daan et al. 1990), to confirm (i) whether the masses of the digestive organs are more closely correlated with BMR than the masses of the locomotory organs, and (ii) whether the masses of the pectoralis muscle, heart and organs of the gastrointestinal tract are higher in long-term captives than in wild birds.

The use of captive birds in physiological studies remains important in many instances, particularly where wild individuals are rare or elusive (McNab 2013; McNab 2009), for

common-garden experimental setups (Wikelski et al. 2003; Klaassen 1995; Broggi et al. 2005), where repeatability of avian BMR is being investigated (Versteegh et al. 2008), and when isolating the effects of certain nutritional (Geluso and Hayes 1999) or environmental variables on avian metabolic rate (Goldstein 1983; Powers 1992). Nevertheless, we agree with Lovegrove et al. (2011), McKechnie et al. (2006), and Caro (2012), that some physiological parameters of captive-bred and long-term captive birds should not be compared to those of freshly wild-caught individuals, unless studies include a proper validation of their results using freshly wild-caught individuals. In particular, we refer to whole animal BMR and Mb, although long-term captivity also had a small effect on RER and on some measures of EWL in this study. We recommend that the origin and history of study animals should be stated where long-term captive birds are used for physiological studies.

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Table 1. Studies on the effects of captivity on avian BMR within species. We include an indication of whether results were significant or not, and period in captivity, where this information was provided. An x indicates that data was lacking. To our knowledge, this is an exhaustive list of within-species studies that investigated the effects of captivity on BMR.

Species	Latin name	Mass (g)	n (wild)	n (captive)	Time in captivity	Effect of captivity	Conclusion	References
Common amakihi	*Hemignathus virens*	11	6	6	> 2 yrs	None	Captivity does not affect mass-specific BMR	MacMillen 1974, 1981
Common amakihi	*Hemignathus virens*	13	x	x	x	Captive BMR ↑	Captives had higher BMR	McNab 2009
Cape white-eye	*Zosterops virens*	12	14-16	12-14	> 1 yr	Captive BMR ↑	Captivity increased whole-animal & mass-specific BMR	this study
Apapane	*Himatione sanguinea*	15	4	4	1 yr	None	Captivity does not affect BMR	Weathers et al. 1983
Yellow breasted bunting	*Emberiza aureola*	26	x	x	x	None	Captivity does not affect BMR	Wallgren 1954
House sparrow	*Passer domesticus*	29	x	x	x	None	Captivity does not affect BMR	Kendeigh 1949
Loria's satinbird	*Cnemophilus loriae*	77	1	2	x	None	Captivity does not affect BMR	McNab 2009, 2013
Red knot	*Calidris canutus canutus*	120	13	12	> 1 yr	Captive BMR ↓	Captives had significantly lower BMR	Piersma et al. 1996
Red knot	*C. c. islandica*	120	8	8	> 1 yr	Captive BMR ↓	Captives had lower BMR	Piersma et al. 1996
Papuan boobook	*Ninox theomacha*	151	1	2	x	Captive BMR ↓	Captives had slightly lower mass-specific BMR	McNab 2009, 2013
Merlin	*Falco columbarius*	200	9	4	7 mths - 3 yrs	Captive BMR ↑	Captives had higher BMR	Warkentin & West 1990

Table 2. Mean ± s.d. of body mass (Mb), basal metabolic rate (BMR), respiratory quotient (RER) and evaporative water loss (EWL) of long-term captive (> 1 year in outdoor aviaries) and freshly wild-caught Cape white-eyes *Zosterops virens*, in winter 2013 and summer 2014, at 30°C. 'n' is the number of birds in each group.

	Winter		Summer	
Origin (n)	captive (12)	wild (16)	captive (14)	wild (14)
Mb (g)	12.27 ± 0.78	11.69 ± 0.66	12.13 ± 0.840	11.33 ± 0.660
BMR (ml O_2.h^{-1})	42.803 ± 2.917	33.540 ± 4.920	39.905 ± 3.944	29.580 ± 4.483
BMR (ml O_2.g^{-1}.h^{-1})	3.491 ± 0.149	2.874 ± 0.425	3.290 ± 0.267	2.610 ± 0.363
RER	0.672 ± 0.015	0.754 ± 0.045	0.849 ± 0.025	0.930 ± 0.060
EWL (mg.h^{-1}) *standard*	45.577 ± 16.184	69.361 ± 29.500	102.233 ± 38.641	94.666 ± 39.597
EWL (mg.h^{-1}) *basal*	30.243 ± 6.866	33.344 ± 6.953	70.288 ± 19.716	65.061 ± 12.522
EWL (mg.g^{-1}.h^{-1}) *standard*	3.684 ± 1.189	5.931 ± 2.456	8.430 ± 3.174	8.401 ± 3.673
EWL (mg.g^{-1}.h^{-1}) *basal*	2.461 ± 0.552	2.863 ± 0.641	5.790 ± 1.598	5.748 ± 1.070

Table 3. Rank of models evaluating the effects of various factors on predicting physiological parameters of Cape white-eyes. All models with an AICcWt > 0 are shown, and the Akaike Information Criterion (AICc) scores and number of parameters in each model (k) are given. All candidate models included 'Individual' (1|BirdID) as a random effect. Models were ranked based on their Akaike weights (AICcWt), and on the difference between each candidate model and the best model (ΔAICc). Models for which ΔAICc < 2 are show in bold, as these are the models for which there is a substantial level of empirical support (Mazerolle 2006; Burnham and Anderson 2002), and which were averaged for the purpose of multimodel inference.

Response variable	Fixed effects	K	AICc	AICcWt	ΔAICc
Whole animal BMR	**'Season' + 'Origin' + 'Mass'**	**6**	**317.51**	**0.70**	**0.00**
	'Season' + 'Origin' + 'Mass' + 'Moult'	7	320.08	0.19	2.57
	'Season' + 'Origin*Moult' + 'Mass'	8	322.71	0.05	5.20
	'Origin' + 'Mass'	5	323.52	0.03	6.00
	'Season' + 'Origin'	5	325.09	0.02	7.57
Mass-specific BMR	**'Season' + 'Origin'**	**5**	**39.07**	**0.60**	**0.00**
	'Season' + 'Origin' + 'Mass'	6	41.17	0.21	2.10
	'Season'	7	43.68	0.06	4.60
	'Season' + 'Origin*Moult'	7	44.12	0.05	5.05
	'Origin*Moult'	6	44.88	0.03	5.81
	'Origin'	4	45.86	0.02	6.79
	'Season' + 'Origin*Moult' + 'Mass'	8	46.37	0.02	7.30
	'Origin' + 'Mass'	5	48.16	0.01	9.09
Whole animal standard EWL	**'Season'**	**4**	**558.41**	**0.44**	**0.00**
	'Season' + 'Origin'	**5**	**560.00**	**0.20**	**1.59**
	'Season' + 'Mass'	5	560.64	0.14	2.23
	'Season' +'Origin' + 'Mass'	6	561.66	0.09	3.25
	'Season' + 'Mass' + 'Moult'	6	563.09	0.04	4.68
	'Season' + 'Origin*Moult'	7	563.17	0.04	4.76
	'Season' + 'Origin' + 'Mass' + 'Moult'	7	564.21	0.02	5.80
	'Season' + 'Origin*Moult' + 'Mass'	8	565.19	0.01	6.78
	'Origin*Moult'	6	566.48	0.01	8.07
	'Moult'	4	567.30	0.01	8.88
Whole animal basal	**'Season' + 'Mass' + 'Moult'**	**6**	**449.51**	**0.21**	**0.00**

EWL	**'Season'**	**4**	**449.57**	**0.20**	**0.06**
	'Season' + 'Mass'	**5**	**449.63**	**0.20**	**0.12**
	'Season' + 'Origin' + 'Mass' + 'Moult'	**7**	**450.83**	**0.11**	**1.32**
	'Season' + 'Origin*Moult'	**7**	**450.97**	**0.10**	**1.46**
	'Season' + 'Origin'	5	451.88	0.06	2.36
	'Season' + 'Origin' + 'Mass'	6	452.01	0.06	2.49
	'Season' + 'Origin*Moult' + 'Mass'	8	452.21	0.05	2.70
Mass-specific standard EWL	**'Season'**	**4**	**284.03**	**0.34**	**0.00**
	'Season' + 'Origin'	**5**	**284.31**	**0.30**	**0.28**
	'Season' + 'Mass'	**5**	**285.82**	**0.14**	**1.80**
	'Season' + 'Origin' + 'Mass'	6	286.79	0.09	2.76
	'Season' + 'Origin*Moult'	7	288.09	0.05	4.06
	'Season' + 'Mass' + 'Moult'	6	288.16	0.04	4.14
	'Season' + 'Origin' + 'Mass' + 'Moult'	7	289.40	0.02	5.38
	'Season' + 'Origin*Moult' + 'Mass'	8	290.78	0.01	6.75
Mass-specific basal EWL	**'Season'**	**4**	**170.36**	**0.38**	**0.00**
	'Season' + 'Origin'	5	172.36	0.14	2.00
	'Season' + 'Mass'	5	172.45	0.13	2.09
	'Season' + 'Origin*Moult'	7	172.77	0.12	2.41
	'Season' + 'Mass' + 'Moult'	6	173.08	0.10	2.72
	'Season' + 'Origin' + 'Mass' + 'Moult'	7	174.50	0.05	4.14
	'Season' + 'Origin' + 'Mass'	6	174.77	0.04	4.41
	'Season' + 'Origin*Moult' + 'Mass'	8	175.10	0.04	4.73
RER	**'Season' + 'Origin'**	**5**	**-199.32**	**0.50**	**0.00**
	'Season' + 'Origin' + 'Mass'	**6**	**-198.55**	**0.34**	**0.77**
	'Season' + 'Origin' + 'Mass' + 'Moult'	7	-195.96	0.09	3.36
	'Season' + 'Origin*Moult'	7	-194.36	0.04	4.96
	'Season' + 'Origin*Moult' + 'Mass'	8	-193.35	0.03	5.97
Mb	**'Origin'**	**4**	**117.84**	**0.49**	**0.00**
	'Season' + 'Origin'	**5**	**119.58**	**0.21**	**1.74**
	'Season' + 'Origin' + 'Moult'	6	120.10	0.16	2.26
	'Season' + 'Origin*Moult'	7	122.67	0.04	4.83
	'Origin*Moult'	6	122.73	0.04	4.89
	'Season' + 'Moult'	5	122.97	0.04	5.13
	'Season'	4	125.66	0.01	7.82
	'Moult'	4	125.90	0.01	8.06

Table 4. Estimate sizes of fixed effects found in the best approximating model(s) for each response variable. Where there was > 1 model with ΔAICc < 2, the models were averaged using the '*MuMIn*' package in R (Bartoń 2013). In each case, 'Origin' referred to freshly wild-caught, and 'Season' referred to winter. Fixed effects are presented in order of decreasing magnitude of estimate size.

Response variable	Fixed effect(s)	Estimate size	s.e.m
Whole animal BMR	'Origin'	- 8.26	1.30
	'Season'	2.83	0.84
	'Mass'	2.49	0.78
Mass-specific BMR	'Origin'	-0.67	0.10
	'Season'	0.23	0.07
Whole animal standard EWL	'Season'	- 39.55	8.74
	'Origin'	7.95	8.73
Whole animal basal EWL	'Season'	-32.44	5.54
	'Origin'	7.00	6.28
	'Mass'	2.53	2.23
	'Moult'	-1.08	0.65
	'Origin*Moult'	-0.95	0.86
Mass-specific standard EWL	'Season'	-3.47	0.75
	'Origin'	1.10	0.75
	'Mass'	-0.38	0.48
Mass-specific basal EWL	'Season'	-3.08	0.27
RER	'Season'	-0.17	0.01
	'Origin'	0.08	0.01
	'Mass'	-0.01	0.00
Mb	'Origin'	-0.67	0.22
	'Season'	0.09	0.11
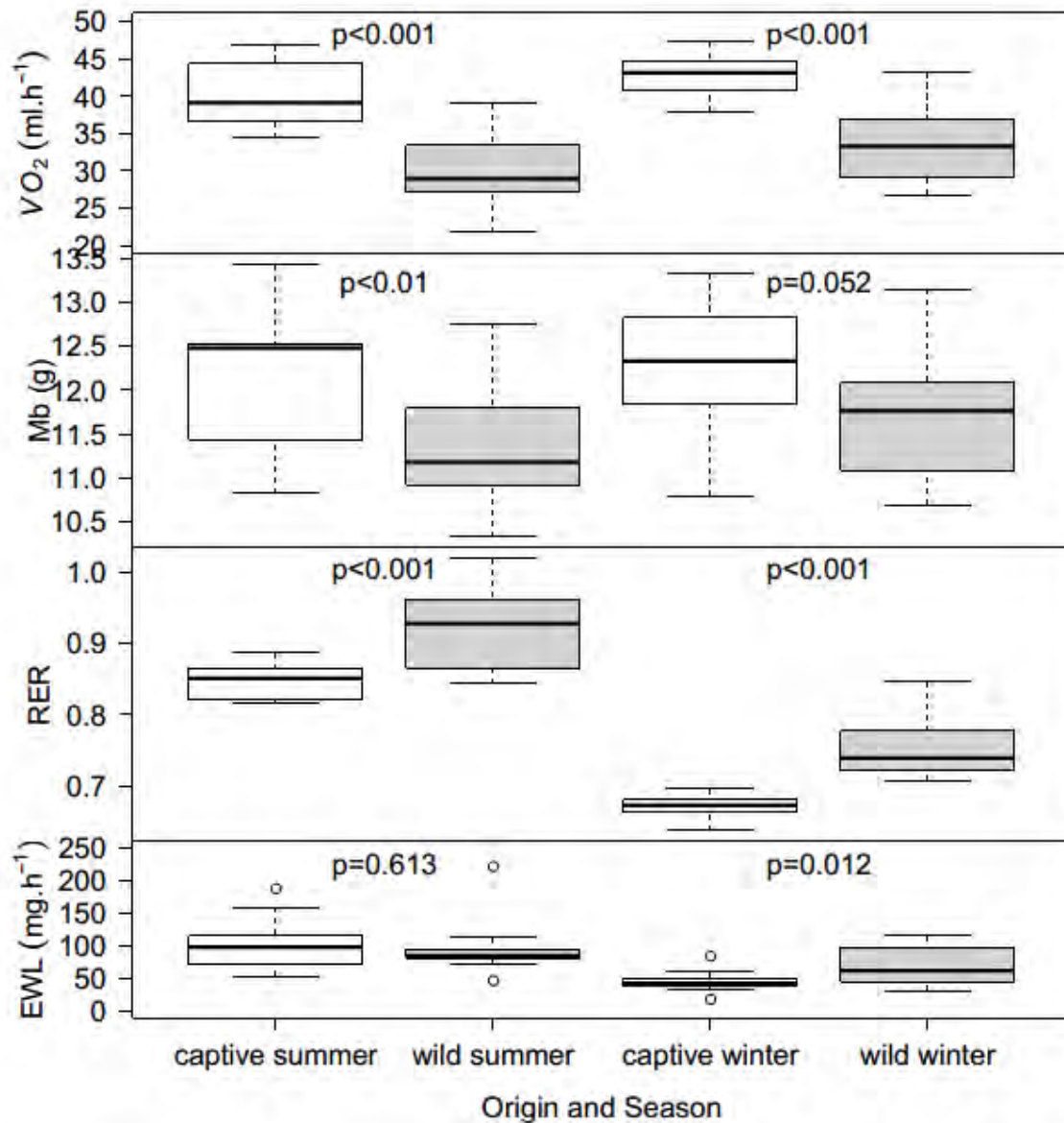
Fig. 1. Comparison of BMR (ml O_2 h^{-1}), body mass (Mb, g), RER and standard EWL (mg.h^{-1}) in long-term captive and freshly wild-caught adult Cape white-eyes in the austral summer and winter. Medians are indicated by the thick lines, and boxes contain the 25th to 75th percentiles. Whiskers show the minimum and maximum values, and outliers are shown with small circles. P-values were calculated using independent-sample t-tests in R.
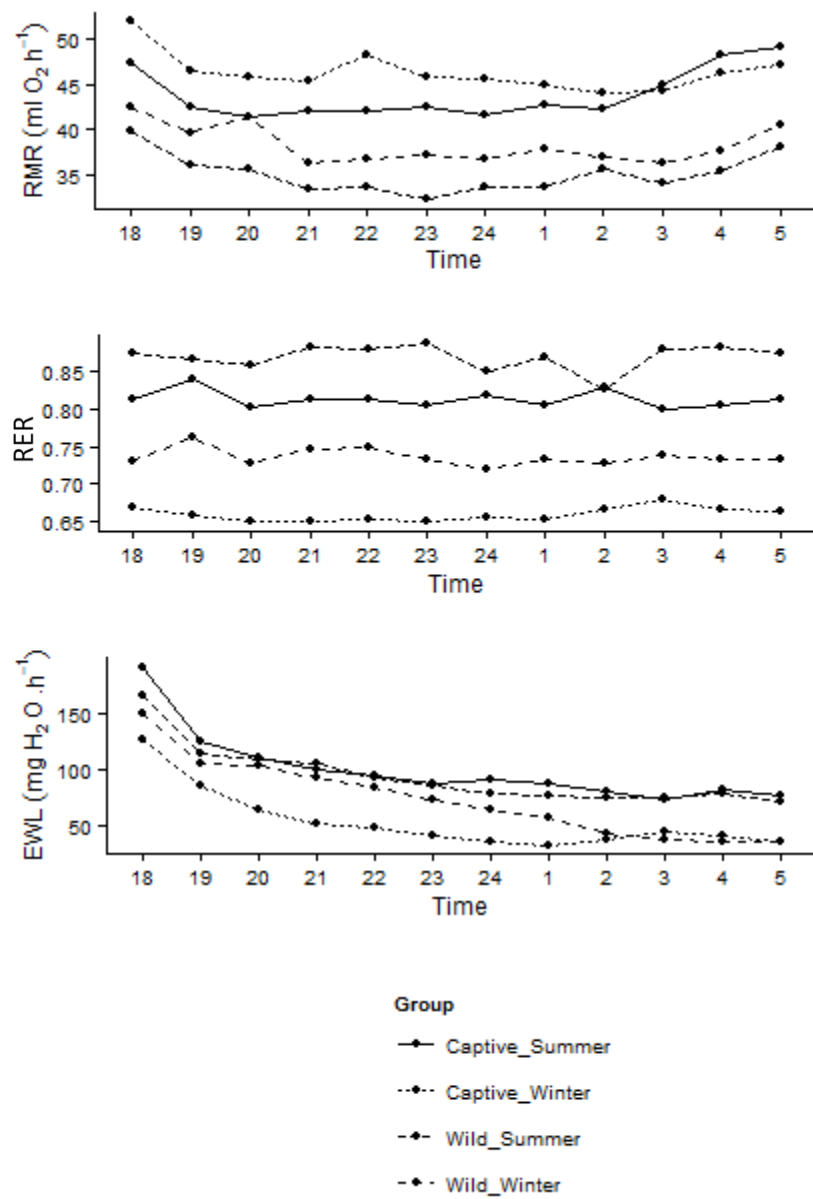
Fig. 2. Changes in mean whole animal resting metabolic rate (RMR, ml O$_2$ h^{-1}), mean respiratory exchange ratio (RER) and mean standard whole animal evaporative water loss (EWL, mg H$_2$O h^{-1}), of long-term captive and freshly wild-caught Cape white-eyes *Zosterops virens*, throughout the night, from 18:00 to 05:00.

CHAPTER 5

Formatted for: Comparative Biochemistry and Physiology, Part A

THERMAL ACCLIMATION IN A SMALL AFROTROPICAL BIRD

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Abstract

Wild-caught animals are regularly used in physiological studies, yet the length of time it takes for completion of their acclimation to laboratory conditions remains largely unknown. In particular, Afrotropical species are relatively understudied compared with temperate and Holarctic species. Thus we measured a number of metabolic variables in a 10-12g Afrotropical bird, the Cape white-eye (*Zosterops virens*), at weekly intervals, over an 8-week period, while acclimating to two different thermal environments (25°C and 29°C). Body mass increased significantly in the first three weeks, remaining approximately constant thereafter, with no significant difference between birds housed at 25°C and at 29°C. However, whole animal and mass-specific resting metabolic rates remained constant throughout the eight-week study period, with values for birds housed at 29°C lower than for birds housed at 25°C. Rather than pointing to a minimum time period necessary for thermal acclimation, these results suggest that in some

instances, freshly wild-caught small passerines may not need to be acclimated to laboratory conditions or respirometry equipment, prior to measurements of their resting metabolic rate.

Keywords: thermal acclimation; resting metabolic rate; Afrotropical; Cape white-eye; *Zosterops virens*

Abbreviations

BMR   Basal metabolic rate
EWL   Evaporative water loss
M_b Body mass
RMR   Resting metabolic rate
RER   Respiratory exchange ratio
$\dot{V}CO_2$ Rate of CO_2 production
$\dot{V}O_2$ Rate of O_2 consumption

1. Introduction

Basal metabolic rate (BMR) is a flexible trait, varying not only between populations, but also within individuals (Cavieres and Sabat, 2008; Klaassen et al., 2004; McKechnie et al., 2006). When wild-caught birds are brought into captivity for the purpose of laboratory experiments, their BMR may be affected by a number of factors that may differ between captive and wild environments. For example, handling and capture stress may elevate metabolism (Duriez et al., 2004; Hails, 1983; McKechnie et al., 2006; McNab, 2009; Saarela and Hohtola, 2003; Speakman et al., 1993; Warkentin and West, 1990) a captive diet may cause increases in body mass and possibly organ size, thereby affecting BMR (McNab, 1986, 1988, 2005), and a change in housing temperature may cause increases or decreases in BMR to different degrees, depending

on the direction and magnitude of the change in temperature, and whether it is constant or fluctuating (Hughes et al., 1982; Olson et al., 2010; Williams and Tieleman, 2000).

Acclimation involves adjustments to experimentally induced variation in certain climatic parameters (IUPS Thermal Commission 2001). Previous laboratory studies of short-term thermal acclimation in birds have compared BMR between two experimental groups, each acclimated to their own respective air temperatures, showing that various species alter the lower limit of metabolic heat production as their thermoregulatory demands alter (McKechnie et al., 2007; Tieleman et al., 2003). These physiological changes are reversible (McKechnie, 2008; McKechnie et al., 2007), and come about through phenotypic flexibility (Piersma and Drent, 2003).

Unfortunately, due to constraints on the number of birds that can be accommodated by respirometry equipment within a certain period, it is not always possible to take BMR measurements on the same day that birds are caught, not is it perhaps preferable, if the aim is to investigate BMR (Jacobs and McKechnie, 2014). Thus, in some instances, birds must be kept in indoor or outdoor aviaries before they can be measured experimentally. However, as discussed above, birds may acclimate to a new thermal environment by altering their BMR, and if this happens during the experimental period, it will presumably influence the results, possibly even changing the outcome of an experiment.

It would therefore be beneficial to know precisely how long each species takes to acclimate to a new thermal environment in captivity. However, while there is a vast number of studies on avian metabolic rate, relatively few of these have focussed on determining the minimum period necessary for acclimation to laboratory conditions, or to a new thermal environment in particular (McKechnie et al., 2007; Rezende et al., 2004). Furthermore, physiological studies of subtropical

bird species are relatively few, compared with those on birds from temperate and Holarctic regions (Smit and McKechnie, 2010).

Therefore, we aimed to measure the time it took for certain physiological variables to reach steady state conditions in a small Afrotropical passerine bird, the Cape white-eye (*Zosterops virens*) (Sundevall, 1850; Thompson and Taylor, 2014). We hypothesised that body mass, resting metabolic rate (RMR), evaporative water loss (EWL), and respiratory exchange ratio (RER, the ratio of CO_2 produced to O_2 consumed) of freshly wild-caught Cape white-eyes housed at two constant temperatures would diverge from a common value and reach a steady state after 3-4 weeks, since this is the period often deemed necessary for thermal acclimation or acclimation to laboratory conditions in various previous studies (e.g. Barceló et al., 2009; Buehler et al., 2012; Bush et al., 2008; Tieleman et al., 2003; Williams and Tieleman, 2000). We predicted that body mass and RMR of both groups of birds would initially increase, since Cape white-eyes have higher BMRs in outdoor aviaries in captivity than in the wild (Thompson et al., 2015). After an initial increase, we predicted that BMR would plateau, and maintain a constant difference, with birds housed at a slightly warmer temperature having lower RMR than birds housed at a slightly cooler temperature, since birds housed at a cooler temperature may be expected to increase their metabolic rate (Klaassen et al., 2004). We also predicted that birds housed at a slightly warmer temperature would have lower EWL and body mass than birds housed at a slightly cooler temperature, and we did not expect RER to fluctuate over the course of the study.

2. Materials and Methods

2.1 Animals

Twenty-two adult Cape white-eyes were caught from 14 November to 4 December 2012, in and near the Botanical Gardens of the University of KwaZulu-Natal in Pietermaritzburg, South Africa (29°37' S, 30°24' E), using mist nets (Ecotone, Gdynia, Poland). Birds with brood patches were immediately released, and not kept captive for the experiment. Individuals were randomly assigned to one of two thermal treatments, both with a constant photoperiod of 12L:12D; one group (n = 11) was maintained at a constant ambient temperature of 25°C, and the other (n = 11) at 29°C. This temperature difference was chosen to prepare the birds for a study on the effects of increased temperature, associated with climate change, on avian metabolic rate, where the two groups would be maintained at a constant temperature difference of 4°C. Within these two rooms, birds were housed in cages (90 x 40 x 100 cm) in groups of 3-4. Water was provided *ad libitum*, and each morning, birds were supplied with a variety of freshly grated and whole fruits (bananas, papayas, oranges and apples) supplemented with softbill pellets (Avi-products, Durban, South Africa).

2.2 Respirometry System

Overnight respirometry trials were conducted over a period of ten weeks, from 14 November 2012 to 23 January 2013, with each bird being measured overnight at 25°C once a week. At 15:00, after being weighed to 1 d.p. using digital scales (model: AFB-3100L, Adam Equipment S.A. Pty. Ltd., Johannesburg), Cape white-eyes were individually placed into 2.8 L Perspex respirometers inside a temperature-controlled environmental chamber (CMP2244,

Conviron, Winnipeg, Canada), set to 12L:12D. Birds were fasted for 3 h before measurements began, to ensure they were post-absorptive (Wellman and Downs, 2009b). Each respirometer contained a wooden perch to allow normal sleeping posture (Wellman and Downs, 2009a), and since a lack of a perch may result in higher CO_2 production (Maddocks and Geiser, 1999). In addition, each respirometer contained a plastic mesh platform, elevated 10 cm above a 1 cm layer of liquid paraffin (AlphaPharm, Pietermaritzburg), to eliminate evaporation from excreta.

Metabolic rate of Cape white-eyes was measured using an open-flow respirometry system. Air drawn from outside the building was passed through a column containing layers of silica gel, soda lime and more silica gel, for removing water vapour, CO_2, and finally for adsorbing the water vapour released by the soda lime. This air was then pumped (model PP2, Sable Systems, Las Vegas, Nevada, USA) through a flow measurement system (model FB8, Sable Systems), with flow rates set to approximately 800 mL.min^{-1}, to maintain the O_2 depletion in the chambers within 0.1 and 0.5% (Lighton, 2008). In each respirometer, the air inlet was located near the bottom, and the outlet near the top, to facilitate mixing of air. Effluent air passed through a flow multiplexer (model MUX, Sable Systems), and approximately 200 mL.min^{-1} of the effluent airstream flowed through a subsampler (model SS4, Sable Systems), with excess air escaping through a manifold. Air then passed into a water vapour analyser (model RH300, Sable Systems), which was regularly zeroed using N_2, and spanned using a nearly-saturated airstream. Air was dried with minimal quantities of previously recharged Drierite (Hammond Drierite Co. Ltd., Xenia, Ohio), which reduced its CO_2 affinity and hence the CO_2 washout time (White et al., 2006). Air then coursed through a CO_2 analyser (model CA-10, Sable Systems) and a fuel cell O_2 analyser (model FC-10, Sable Systems). The CO_2 analyser was frequently zeroed with N_2, and spanned with a certified gas containing 964 ppm CO_2 in N_2 (AFROX, Pietermaritzburg, South

Africa). The O_2 analyser was spanned to 20.95% at the start of each overnight trial. Voltage outputs from the gas analysers and flow meter were acquired using an analog-digital converter (model UI-2, Sable Systems) and ExpeData data acquisition software (Sable Systems).

From 15:00 until 07:00, the following variables were recorded every 5 s: flow rate (mL.min^{-1}), O_2 and CO_2 concentrations (%), and water vapour density (µg.mL^{-1}). An interrupted sampling regime was used, beginning with a baseline measurement for six min, and then four birds in succession for six min each. This cycle was repeated continually through the night, so that each bird was measured for 12 min per hour. Sampling regime can significantly affect measurements of physiological variables, however, the differences between sampling regimes are generally small, and due to time constraints we chose to use multiple birds each night in a switching system, as this results in smaller errors than measuring one bird continuously for shorter periods (Cooper and Withers, 2010). Lag and drift correction were performed in ExpeData using a macro, and a 95% equilibration time of 11 min was calculated using the equation of Lasiewski et al. (1966). Rates of O_2 consumption ($\dot{V}O_2$), CO_2 production ($\dot{V}CO_2$) and $\dot{V}H_2O$ (hereafter termed standard EWL) were z-transformed before hourly means were calculated. The lowest hourly $\dot{V}O_2$ reading for each bird was taken as the RMR for that night, and $\dot{V}CO_2$ and $\dot{V}H_2O$ were recorded at the same time as RMR.

2.3 Scoring of Moult and Sex Determination

Moult may be costly (Lindström et al., 1993; Schieltz and Murphy, 1997), and so may affect BMR, thus Cape white-eyes were individually scored for primary flight feather moult prior to each respirometry trial, according to feather length and age (De Beer et al., 2001). Moult

scores ranged from 0 at pre-moult to 45 when primary moult was completed (Klaassen, 1995). This value was then divided by 4.5, to produce an index ranging from 0 to 10.

Cape white-eyes are sexually monomorphic (Fry et al., 2000; Hockey et al., 2005), and so blood tests were necessary for sex determination. Thus, after the completion of all metabolic trials, a small amount (<100 μl) of blood was drawn from the jugular vein of each bird using a disposable Healthease® syringe and 29G x ½" (0.33 x 13 mm) needle (Neomedic Pty. Ltd., Riverhorse Valley East, South Africa). This blood was deposited onto FTA filter paper and stored in Eppendorf tubes®, before being sent to Molecular Diagnostics Services (Westville, South Africa) for molecular sex determination.

2.4 Data Analysis

$\dot{V}O_2$, $\dot{V}CO_2$, RER and standard EWL were calculated as follows, with equations on page 456 and 446 of Withers (2001):

- $\dot{V}O_2 = \dot{V}_I (F_IO_2 - [F_EO_2 (1 - F_IO_2 - F_ICO_2 - F_IH_2O) / (1 - F_EO_2 - F_ECO_2 - F_EH_2O)])$

- $\dot{V}CO_2 = \dot{V}_I ([F_ECO_2 (1 - F_IO_2 - F_ICO2 - F_IH_2O) / (1 - F_EO_2 - F_ECO_2 - F_EH_2O)] - F_ICO_2)$

- $EWL = \dot{V}_I ([F_EH_2O (1 - F_IO_2 - F_ICO2 - F_IH_2O) / (1 - F_EO_2 - F_ECO_2 - F_EH_2O)] - F_IH_2O)$

- $RER = \dot{V}CO_2 / \dot{V}O_2$

where $\dot{V}_I$ was the measured mass flow.

A set of candidate models was defined *a priori* (Burnham and Anderson, 2002), and the effect of number of weeks in captivity on BMR and other variables was investigated using general linear mixed models (GLMMs) with the *lme4* package (Bates et al., 2014) in R 3.1.0 (R Core Team, 2014). Response variables included whole animal and mass-specific RMR, standard

EWL and RER. Predictor variables included week (in captivity, where the first week was classed as week 1), body mass (g), sex, moult score, bird identity, and the Julian date of each individual's capture (the latter two as random effects). Visual inspection of residual plots did not show any deviations from homoscedasticity or normality. Best models were objectively selected based on their second order (corrected for small sample size) Akaike information criterion weights (AICcWt) and differences in corrected Akaike information criterion (AICc) values between each candidate model and the best approximating model (ΔAICc), produced using the R package *AICcmodavg* (Mazerolle, 2013). Models with ΔAICc < 2 were averaged for multimodel inference using the R package *MuMIn* (Bartoń, 2013). Post hoc tests (Tukey's HSD) were performed using the *mvtnorm* (Genz et al., 2014) and *multcomp* (Hothorn et al., 2008) packages in R. Values given are means ± SD.

3. Results

Individual body mass of Cape white-eyes ranged from 7.8 g (in week 1) to 12.0 g (in week 3), with a body mass of 10.4 ± 0.8 g for all birds throughout the study period. 'Week' was contained in the best model for predicting body mass (Table 1), and mean body mass was significantly lower in the first three weeks than it was thereafter, with no significant difference between body mass in weeks 1 and 2, or in weeks 2 and 3. The best model for predicting body mass did not contain 'Room' (Table 1), suggesting no difference in body mass between birds housed at a constant temperature of 25 and 29°C. Body mass increased during the first few weeks in captivity, in birds housed at constant temperatures of both 25 and 29°C (Fig. 1). Birds housed at 25°C increased their mean body mass from 10.1 ± 0.8 g in week 1, to 11.0 ± 0.5 g in

week 4, an increase of 8.9 %, while birds housed at 29°C increased their mean body mass from 9.3 ± 0.8 g in week 1 to 10.4 ± 0.5 g in week 3, an increase of 11.8 %.

RER of Cape white-eyes showed very little variation throughout the night (Thompson et al., 2015), and RER of all birds during the study was 0.710 ± 0.041, indicating that Cape white-eyes were catabolising only lipids (Walsberg and Wolf, 1995), as would be expected in a small bird measured overnight. There was no significant difference between mean weekly RER values ($F_{1, 138} = 0.115$, $P = 0.735$).

There was a highly significant positive correlation between body mass and RMR of Cape white-eyes (Pearson's product-moment correlation $r = 0.426$, $P < 0.001$). However, 'Week' was not contained within the best models for predicting whole animal or mass-specific RMR (Table 1), suggesting that Cape white-eyes showed no difference in mean weekly whole animal or mass-specific RMR over the course of the study (Fig. 1). Whole animal RMR increased as body mass increased, was higher in male birds than in females, and was lower in birds housed at 29°C than in those housed at 25°C (Table 2). Mass-specific RMR was also higher in males than in females, and lower in birds housed at 29°C than in those housed at 25°C (Table 2), however mass-specific RMR declined with increasing body mass, as expected (McNab, 1999). Whole animal standard EWL was significantly higher in birds housed at 29°C than in those housed at 25°C ($t = -3.553$, $P < 0.001$), and while EWL seemed to decline with time in captivity (Fig. 1, Table 2), there was no significant difference in EWL between weeks ($F_{7, 136} = 0.704$, P = 0.669), nor was the interaction of 'Week' and 'Room' significant ($F_{7, 128} = 0.644$, P = 0.719).

Neither the standard deviations of whole animal RMR nor of mass-specific RMR of Cape white-eyes decreased significantly with successive measurements ($r^2 = 0.059$, $P = 0.561$, and r^2

= 0.265, $P = 0.192$). Similarly, there was no significant decrease in the time taken for RMR to be reached with successive measurements ($r^2 < 0.001$, $P = 0.833$).

4. Discussion

We aimed to determine the minimum period necessary for thermal acclimation to be completed in a small Afrotropical bird (that is, for a stable end point to be reached), to aid future physiological studies in similar species. Given that Cape white-eyes took at least three full weeks in captivity to become acclimated in terms of body mass, we suggest that at least three weeks should be allowed for birds to become acclimated, prior to the start of measurements.

Meanwhile, time in captivity had no significant effect on the RMR of Cape white-eyes. This was in contrast to the results of Jacobs and McKechnie (2014), who found a significant decrease in the BMR of acclimated budgerigars (*Melopsittacus undulatus*) over a five week period, and of McKechnie et al. (2007) who found a significant decrease in the BMR of wild-caught laughing doves (*Streptopelia senegalensis*) after a 21 day acclimation period, with the magnitude of this decrease related to acclimation temperature. Conversely, Bush et al. (2008) found that after 21 days of acclimation to 25°C, mass-specific BMR of long-term captive rock kestrels (*Falco rupicolis*) was significantly higher than it was pre-acclimation. It is possible that in this study, habitation of Cape white-eyes to experimental procedure and a subsequent decrease in RMR over time (Jacobs and McKechnie, 2014) may be masking an initial increase in metabolic rate due to captivity-induced stress (Swanson and King, 2013), resulting in a constant RMR between weeks. An alternative explanation for the stability of RMR of Cape white-eyes between weeks might be that in areas such as the Afrotropics where climates are more seasonally

stable, birds have a smaller response to thermal acclimation in terms of their BMR than in areas with extreme climatic seasonality (Cavieres and Sabat, 2008).

Although the temperature difference between the two rooms was only 4°C, much smaller than in most thermal acclimation studies, it was enough to elicit a difference in RMR, with birds housed at 29°C showing RMR that was 11.7% lower than that of birds housed at 25°C. This result is similar to that shown by hand-raised garden warblers (*Sylvia borin*), where, after five months in rooms set to either 4 or 24°C, BMR was 18% higher in birds housed at 4°C than in those housed at 24°C (Klaassen et al., 2004), however body mass in these birds also increased, and the authors concluded that birds were storing fuel in response to the lower temperatures. Similarly, when acclimated to constant environments of either 15 or 35°C, BMR of woodlarks (*Lullula arborea*) and skylarks (*Alauda arvensis*) were 4.7% and 25.3% higher respectively, in birds acclimated to 15°C than in conspecifics acclimated to 35°C (Tieleman et al., 2003). Indeed, cold-acclimation usually seems to result in a higher BMR than warm-acclimation (e.g. Cavieres and Sabat, 2008). In addition, although in this study photoperiod was set to 12L:12D, whereas the natural outdoor photoperiod was slightly different, temperature seems to have a greater role than photoperiod on avian metabolic rate (Swanson et al., 2014). Total EWL was lower in cold-acclimated birds for two lark species, similar to the trend shown by Cape white-eyes, even though the temperature difference was much lower in this study (Tieleman et al., 2003).

Mean weekly body mass of Cape white-eyes increased significantly over the initial weeks of the study, similar to trends shown by freshly wild-caught American goldfinches (*Spinus tristis*), which significantly increased their body masses during their first two weeks in captivity, after which it remained stable (Swanson and King, 2013). In contrast, over a 21 day acclimation period, there was no significant change in the body mass of laughing doves in captivity

(McKechnie et al., 2007), nor was there a significant change in body mass of amethyst sunbirds (*Chalcomitra amethystina*) after six weeks of acclimation to 25°C in summer or in winter (Lindsay et al., 2009a; Lindsay et al., 2009b), and rock kestrels showed no difference in body mass pre- and post-acclimation to 25°C for 3 weeks (Bush et al., 2008). Nor was there a significant difference in the body mass of budgerigars during habituation to experimental procedure (Jacobs and McKechnie, 2014). In some of these studies (Bush et al., 2008; Jacobs and McKechnie, 2014), this consistency in body mass was most likely because the birds were either captive-bred or long-term captive, and therefore already accustomed to a captive diet, for at least a few weeks prior to metabolic measurements, whereas the Cape white-eyes used in this study were initially freshly wild-caught, and thus their masses increased in captivity, where the diet was presumably more regular and of higher quality than in the wild. Whether or not birds gain mass in captivity may also be related to acclimation temperature or season (Southwick, 1980); hoopoe larks (*Alaemon alaudipes*) kept at 15°C for 3 weeks gained mass, whereas those kept at 36°C did not (Williams and Tieleman, 2000). Finally, the lack of an effect of acclimation temperature on body mass in this study might not simply be because of the small difference in temperature between the two rooms; McKechnie and Wolf (2004) found no difference in body mass between birds acclimated to 21.7 or 42.6°C.

In conclusion, the time spent in captivity prior to an experiment should be stated, since captivity and thermal history may affect some physiological parameters. Furthermore, we reiterate the point made by Jacobs and McKechnie (2014), that authors should report the number of occasions on which their study animals experienced the experimental conditions, before metabolic rate was measured, to make studies more comparable.

Acknowledgements

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Figure legend

Figure 1. Weekly mean body mass (g), resting metabolic rate (RMR, ml O_2 h^{-1}), and evaporative water loss (EWL, mg H_2O h^{-1}), of Cape white-eyes *Zosterops virens* housed at constant temperatures of either 25°C (left) or 29°C (right), from November 2012 to February 2013. Dots represent outliers, thick black line indicates medians, and bottom and top of the boxplot show the 1st and 3rd quartiles.
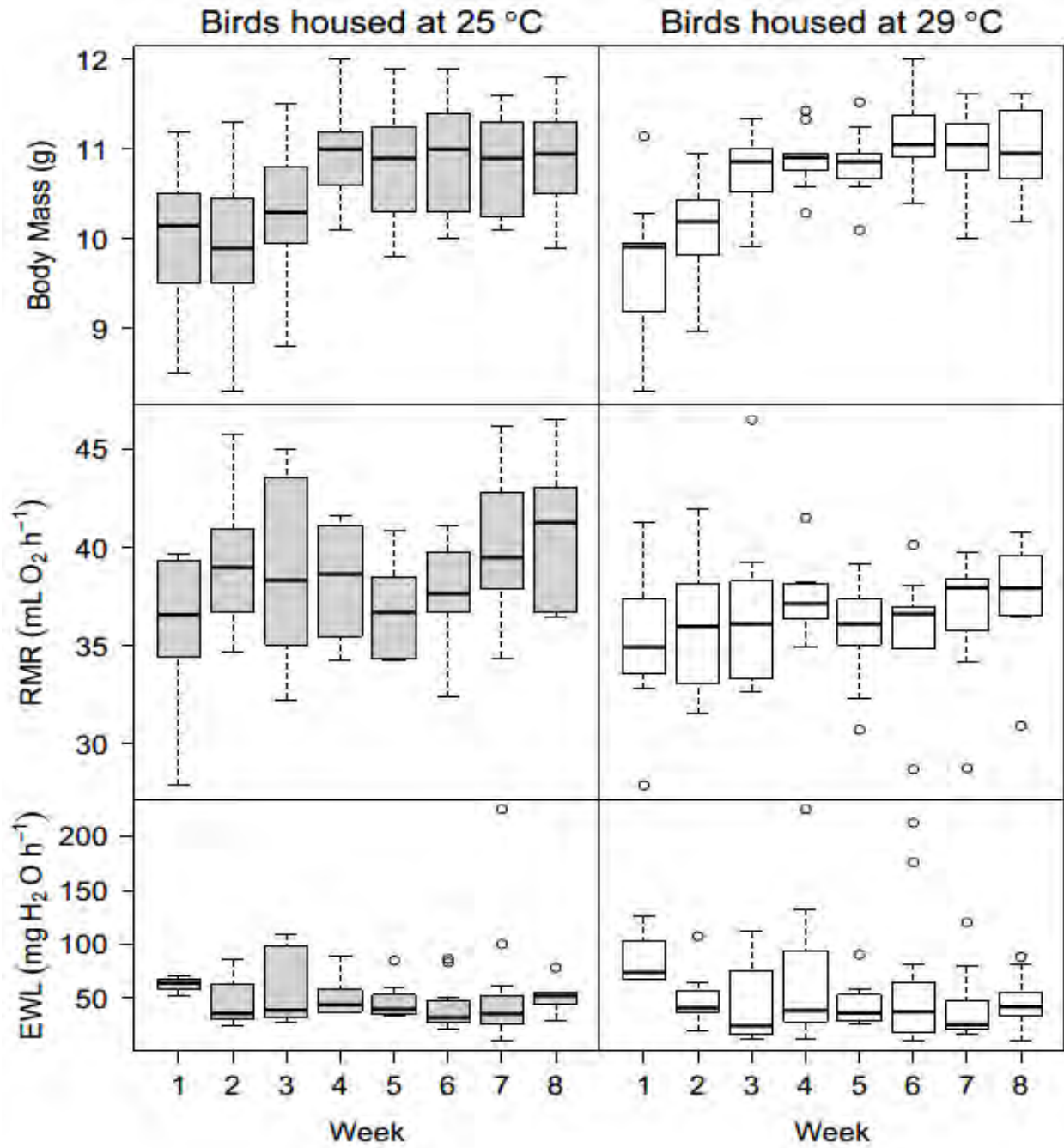
Figure 1.

Table 1. Rank of models evaluating the effects of various factors on physiological parameters of Cape white-eyes. Models were ranked based on their corrected Akaike information criterion weights (AICcWt), and on the difference between each candidate model and the best model (ΔAICc). AICc scores and the number of parameters in each model (k) are presented for all models with an AICcWt > 0. All models included 'Individual' and 'Capture date' as random effects. Models with ΔAICc < 2 (shown in bold) have substantial empirical support (Burnham and Anderson, 2002; Mazerolle, 2006), and were averaged for multimodel inference. 'Room' refers either to the 25°C or 29°C room in which birds were housed.

Response variable	Fixed effects	K	AICc	AICcWt	ΔAICc
Whole animal RMR	**Room + Sex + Body mass + Moult**	**8**	**335.82**	**0.70**	**0.00**
	Week + Room + Sex + Body mass + Moult	9	338.65	0.17	2.83
	Room + Sex + Moult	7	339.54	0.11	3.71
	Week + Sex + Moult	7	342.30	0.03	6.48
Mass-specific RMR*	**Body mass**	**5**	**73.64**	**0.27**	**0.00**
	Room + Body mass	**6**	**74.70**	**0.16**	**1.06**
	Sex + Body mass	**6**	**75.15**	**0.13**	**1.51**
	Body mass + Moult	6	75.74	0.10	2.10
	Week + Body mass	6	75.89	0.09	2.24
	Room + Body mass + Moult	7	76.63	0.06	2.99
	Room + Sex + Body mass + Moult	8	77.55	0.04	3.91
	Week + Body mass + Moult	7	78.18	0.03	4.54
	Week + Room + Sex + Body mass	8	78.40	0.03	4.75
Whole animal standard EWL	**Week + Room + Sex + Body mass**	**8**	**881.05**	**0.41**	**0.00**
	Room + Sex + Body mass + Moult	**8**	**881.41**	**0.34**	**0.35**
	Week + Room + Moult	6	883.29	0.13	2.24
	Week + Moult	9	883.50	0.12	2.45
Body mass	**Week + Sex + Moult**	**7**	**185.04**	**0.99**	**0.00**
	Week + Sex	6	195.04	0.01	10.00

* Only models with AICcWt > 0.02 are shown for mass-specific RMR

Table 2. Estimate sizes of fixed effects found in the best approximating model for each response variable. When more than one model had ΔAICc < 2, these models were averaged using the *MuMIn* package in R (Bartoń, 2013). 'Room' refers to the 29°C room, and 'Sex' refers to male.

Response variable	Fixed effect(s)	Estimate size	s.e.m
Whole animal RMR	'Room'	-3.121	2.447
	'Sex'	2.728	2.322
	'Body mass'	0.952	0.786
	'Moult'	0.101	0.120
Mass-specific RMR	'Body mass'	-0.245	0.077
	'Room'	-0.079	0.173
	'Sex'	0.051	0.140
Whole animal standard EWL	'Week'	-0.811	1.912
	'Room'	31.669	10.469
	'Sex'	-0.976	9.106
	'Body mass'	10.046	7.109
	'Moult'	-0.082	0.679
Body mass	'Week'	0.16	0.03
	'Sex'	-0.05	0.32
	'Moult'	0.03	0.02

THE POTENTIAL EFFECTS OF CLIMATE CHANGE-ASSOCIATED TEMPERATURE INCREASES ON THE METABOLIC RATE OF A SMALL AFROTROPICAL BIRD.

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Running head: Effects of climate change on a small bird

Abstract

Studies have only recently begun to underline the importance of including data on species' physiological flexibility when modelling their vulnerability to extinction from climate change. We investigated the effects of a 4°C increase in ambient temperature (T_a), similar to that predicted for southern Africa by the year 2080, on certain physiological variables of a 10-12g passerine bird endemic to southern Africa, the Cape white-eye *Zosterops virens*. There was no significant difference in resting metabolism, body mass and intraperitoneal body temperature between birds housed indoors at 4°C above outside ambient temperature and those housed

indoors at outside ambient temperature. We conclude that Cape white-eyes' physiological flexibility will aid them in coping with the 4°C increase predicted for their range by 2080.

KEY WORDS: Climate change, Avian resting metabolic rate, Evaporative water loss, Thermal PIT tags, Cape white-eye, *Zosterops virens*.

List of abbreviations

BMR — basal metabolic rate

Control — birds housed indoors, at the outdoor ambient temperature (T_a)

Control+4°C — birds housed indoors, at 4°C above the outdoor ambient temperature (T_a +4°C)

EWL — evaporative water loss (mg H_2O h^{-1})

Group — birds were either housed in the 'control' group or in the 'control+4°C' group

M_b — body mass (g)

RMR — resting metabolic rate (ml O_2 h^{-1})

T_a — ambient temperature (°C)

T_b — intraperitoneal body temperature (°C)

TNZ — thermoneutral zone

$\dot{V}CO_2$ — volumetric rate of carbon dioxide produced by the bird (mL $CO_2.h^{-1}$)

$\dot{V}O_2$ — volumetric rate of oxygen consumed by the bird (mL $O_2.h^{-1}$)

Introduction

Birds on every continent have been directly affected by anthropogenic climate change (Møller, 2013; Parmesan, 2006; Şekercioğlu et al., 2012; Wormworth and Mallon, 2006). One of the

direct impacts of this rapid change in climate is an increased frequency of extreme heat events (Easterling et al., 2000; I.P.C.C., 2012; Meehl and Tebaldi, 2004) to which birds are particularly vulnerable, because of their diurnal habits and small body sizes (Coumou and Rahmstorf, 2012; McKechnie et al., 2012; McKechnie and Wolf, 2010). Extreme weather events may drive local avian population dynamics (Parmesan et al., 2000), and can exert strong natural selection pressures (Bumpus, 1899), favouring the evolution of avian morphometric traits (Boag and Grant, 1984). Extreme heat events pose the most risk to species with narrower thermal ranges and consequently lower thermal tolerances (Jiguet et al., 2006; Khaliq et al., 2014).

In addition to surviving extreme heat events, birds will also need to cope with higher mean surface air temperatures (I.P.C.C., 2013a), which in turn will increase the frequency of exteme heat events (NASA, 2012). An A2 emissions scenario, where emissions related to land use are expected to continue increasing rapidly (I.P.C.C., 2000) for southern Africa (from the equator to 45°S and from 5° to 55°E), predicted a 3.7 to 4°C increase by the 2080s (Mlingwa, 2000), similar to the mean increase in surface temperature of 3.7°C predicted by a Representative Concentration Pathway 8.5 scenario by 2081-2100, relative to 1986-2005 (I.P.C.C., 2013b). Mean seasonal temperatures and dry periods are predicted to increase throughout sub-Saharan Africa, with El Niño Southern Oscillation effects, fires and severe weather anomalies likely to become more common in southern Africa (I.P.C.C., 2013a; Müller et al., 2014). Indeed, Africa, is projected to have 'above-average' climate change in the 21st century (I.P.C.C., 2007), and therefore is the continent where global warming will have the greatest effects on biodiversity (Simmons et al., 2004). Distributions of southern African bird species are predicted to contract towards the Cape (Huntley et al., 2006), yet little else is known of how African birds may move or adapt in response to climate change (Parmesan, 2006; Simmons et al., 2004).

Species may cope with environmental changes through micro-evolutionary adaptation (Karell et al., 2011) and/or phenotypic plasticity; the latter in particular is potentially crucial for projections of extinction risk due to climate change (Chase, 2013; Hoffmann and Sgrò, 2011). Small birds generally cope with elevated temperatures through behavioural adaptation, and physiologically, by reversibly altering their metabolic rate (McKechnie, 2008; McKechnie et al., 2007), body temperature (T_b) (Tieleman and Williams, 1999) and evaporative water loss (EWL) (Williams and Tieleman, 2000). Attention to how species' attributes change with temperature will help to improve the forecasting on the impacts of climate change (Berg et al., 2010), since species' physiological capabilities may buffer the predicted effects of global warming (Khaliq et al., 2014). Yet, the role of phenotypic plasticity and flexibility in species' physiological responses has been relatively underappreciated in climate change modelling (Chown et al., 2010; Vedder et al., 2013). We investigated how a relatively small passerine bird would cope with the sustained temperature increase predicted for its range by the year 2080. We chose the 10-12g southern African endemic Cape white-eye *Zosterops virens* (Sundevall, 1850; Thompson and Taylor, 2014) as our model species. This species has a large range across southern Africa (Hockey et al., 2005), and so should exhibit a fair degree of phenotypic flexibility in terms of its metabolic rate. We aimed to investigate how Cape white-eyes would perform metabolically with the sustained 4°C increase predicted for their range by the year 2080 (I.P.C.C., 2013b; Hudson and Jones, 2002). We hypothesized that Cape white-eyes would alter their resting metabolic rate (RMR), T_b and EWL when faced with a 4°C increase over a protracted period of time. We predicted that RMR would be lower, and T_b and EWL higher, in birds acclimated to higher temperatures, than in control birds also housed indoors, but at outdoor ambient temperatures.

Results

Mean, minimum and maximum monthly temperatures peaked in January and February, and dipped to their lowest points in June and July (Fig. 1). Mean monthly M_b of Cape white-eyes varied throughout the year, with lows in July and Nov-Jan, and a peak in March-April (Fig. 2). Male Cape white-eyes were significantly heavier than female birds ($\chi^2 = 205.4$, p < 0.001), however, there was no significant difference in M_b between control birds and those housed at control+4°C ($\chi^2 = 2.7$, p = 0.101, Table 1, 2).

During overnight metabolic measurements, intraperitoneal T_b, RMR and EWL of Cape white-eyes generally dropped precipitously in the first 3 h of darkness (from 18:00 – 21:00, Fig. 3 and 4). Intraperitoneal T_b and RMR rose again in the three hours before photophase, while EWL dropped continuously throughout the night. There was a weakly positive but highly significant correlation between T_b and whole animal RMR (r = 0.185, p = 0.004).

During scotophase, mean hourly T_b measurements of individual Cape white-eyes ranged from 36.6°C (at 23:00, in April 2014, in a control bird, measured at 20°C) to 43.4°C (at 18:00, in March 2014, in a control bird measured at 25°C). T_b was 0.9 ± 0.4°C lower in male birds than in females ($\chi^2 = 132.7$, p < 0.001), but there was no significant difference in T_b between birds housed at T_a+4°C and those housed at T_a ($\chi^2 = 1.1$, p = 0.303, Table 2, Fig. 5). Mean monthly T_b peaked in September-October, and dropped to a minimum in November-December.

Mean whole animal RMR of Cape white-eyes was lower in the austral winter and spring (August-December) than in summer and autumn (February-May, Fig. 4, 5). Whole animal and mass-specific RMR showed very similar circannual rhythms, and predictive models for each of these variables included the same fixed effects (Table 1). Whole animal RMR of male white-eyes was significantly lower than that of females ($\chi^2 = 495.8$, p < 0.001), and the temperature at

which birds were measured had a significant effect on whole animal RMR (χ^2 = 177.2, p < 0.001), however the effect of treatment on whole animal RMR was not significant (χ^2 = 1.3, p > 0.05).

Mean monthly whole animal EWL of Cape white-eyes showed distinct seasonal trends, being higher in September/October and March/April, than in May-July and November-February (Fig. 5). In September, when temperatures were cooler (Fig. 1), white-eyes lost water at a faster rate overnight than they did in February, when temperatures were slightly higher. Whole animal standard EWL was 2.313 mg H_2O h^{-1} lower in males than in females (Table 2) and this difference was significant (χ^2 = 774.8, p < 0.001), however there was no significant difference in whole animal standard EWL between birds housed at control+4°C and control birds (χ^2 = 0.2, p = 0.654). Trends were similar for mass-specific standard EWL of Cape white-eyes (Table 2). Similarly, there was no significant difference in basal EWL of control birds and those housed at control+4°C (χ^2 = 2,8, p = 0.096), however basal EWL was 0.015 mg H_2O h^{-1} lower in males than in females (Table 2, (χ^2 = 643.0, p < 0.001). Trends were similar for mass-specific basal EWL (Table 2).

Discussion

Experimental studies on the potential effects of climate change-associated temperature increases on the metabolic parameters of endotherms are valuable but rare. This year-long study showed that a 4°C increase in housing temperature had very little effect on intraperitoneal T_b and EWL, and no significant effect on the whole animal RMR of Cape white-eyes, suggesting that the phenotypic flexibility of the Cape white-eye will be more than sufficient for physiologically coping with the mean temperature increase predicted across its range by 2080.

Mean M_b of Cape white-eyes showed no clear trend over the duration of the study, which was very similar to what was found in a group of 12 Cape white-eyes caught at the same study site and housed in outdoor aviaries for one year (Chapter 3). Although white-eyes in this study increased their M_b prior to winter, there was nevertheless very little variation in M_b throughout the year, in stark contrast to circannual trends in M_b shown by migratory bird species (Zimmerman, 1965). The small fluctuations in M_b of Cape white-eyes between months may have been due to variation in their diet in captivity, in that while certain fruits were available year round, others were not. Birds housed at T_a were 0.5 g heavier than those housed at T_a +4°C, which may have been due to increased food intake and increased fat deposits, although this was not investigated here. Although *Z. virens* is widely accepted as a sexually monomorphic species (Hockey et al., 2005; Oatley, 2011; Skead, 1967), male Cape white-eyes were slightly heavier, and had significantly lower whole animal RMR, than females.

Thermal PIT tags have been used in physiological studies on reptiles (Bittner et al., 2002; Roark and Dorcas, 2000) and small mammals (Cory Toussaint and McKechnie, 2012), but to our knowledge this is the first study in which these PIT tags have been successfully used for a long-term study in small birds. Mean hourly intraperitoneal T_b of Cape white-eyes showed a marked circadian rhythm typical of small diurnal avian insectivores and nectarivores (Clarke and Rothery, 2008; McKechnie and Lovegrove, 2002).

Circannual rhythm in T_b did not seem to follow circannual rhythm in T_a, and there was little similarity between circannual trends in T_b and RMR in this study, even though T_b influences (and is influenced by) metabolic rate (Clarke and Rothery, 2008). The higher T_b levels shown in this study may be from nights when RMR fell earlier in the evening, since T_b usually took at least 3 hours after dark to fall to minimal levels, whereas RMR sometimes occurred within the

first 3 hours of darkness. We cannot explain the abrupt October-November crash in mean T_b of Cape white-eyes.

Sex may have a significant effect on T_b in passerines, presumably due to widespread sexual size dimorphism (Clarke and Rothery, 2008). Indeed, male Cape white-eyes had significantly lower T_b than females, possibly due to their marginally larger body size, although since the difference in M_b between the sexes was so little, we would not have expected a difference in T_b. Nevertheless the estimated effect of sex on T_b was double that of increasing the housing temperature by 4°C, suggesting that the 4°C increase in T_a, predicted for southern Africa by the year 2080, will have a negligible effect on core T_b in this population of Cape white-eyes. Similarly, the T_b of Cape white-eyes measured at 25°C was only 0.2°C different from that of birds measured at 20°C. This result is in line with the findings of Bucher (1981), who reported that a medium-sized parrot *Amazona viridigenalis* showed no significant difference in T_b between T_as of 10 and 27°C. Since our birds were measured at T_as close to or below the lower critical limit of their TNZ (Chapter 2), water conservation should not have been of concern, and thus there was no need for them to become hyperthermic (Tieleman et al., 1999).

Cape white-eyes in this study had lower whole animal RMR in spring than in autumn. However, the RMR trend does not precisely fit that of outdoor T_a at the study site. Cape white-eyes housed at T_a+4°C had a whole animal and mass-specific RMR that was marginally lower than for birds housed at Ta. However, this difference was not significant effect. Thus, it would seem that coping with the mean air temperature increase of 4°C predicted for southern Africa by 2080 (Hudson and Jones, 2002) should be well within the physiological capabilities for this

species. However, although a 4°C increase induced very little change in RMR of Cape white-eyes, the long-term fitness consequences of this change are unknown (Burton et al., 2011).

M_b, T_b and EWL all dipped around December, coinciding with an unseasonal drop in environmental temperature at the study site, when the range of temperatures was also the lowest. Since housing birds at 4°C above T_a had a smaller effect on whole animal and mass-specific standard EWL than either sex or of measuring at 25°C versus 20°C, we can conclude that the 4°C increase in temperature predicted for 2080 will have little direct effect on EWL in this population of Cape white-eyes.

EWL showed a general increase over the study period. This result goes against the notion of EWL having a circannual pattern, since start and end values were so different. We cannot explain what may have caused the overall increase in EWL over the study period, nor do we have any evidence to link the increase in EWL to any potential physiological effects of long-term captivity.

Mean BMR of captive Cape white-eyes housed indoors in the room set to T_a (41.235 ± 3.380 ml O_2 h^{-1}, mean ± s.d., n = 12) was very similar to that of conspecifics housed in outdoor aviaries (42.405 ± 3.139 ml O_2 h^{-1}, n = 13), when both groups were measured at 30°C (which is within the TNZ for this population of Cape white-eyes) in the same month. However, long-term captivity in outdoor aviaries is linked to a significant increase in BMR in Cape white-eyes (Thompson et al., 2015), and so we can assume that wild birds would have lower RMR than presented in this study. Indeed, the ecological relevance of laboratory studies has been questioned (Chown et al., 2010), so ideally this experiment should be repeated with birds housed in large outdoor temperature-controlled aviaries. In addition, field metabolic rate may be of more use than BMR or RMR in determining the effects of increased temperature associated with

climate change on avian physiology. Although extreme heat events will undoubtedly pose a greater risk to survival that the smaller temperature increase experienced globally over time, it is also important to quantify the effects of the increased temperature associated with climate change on physiological variables of animals most at risk on temperature increases (that is, small animals), in regions most at risk from climate change.

Moreover, although we acclimated the birds to indoor conditions, and to their different temperature regimes, in reality, birds are unlikely to experience such an immediate and continual 4°C increase in T_a, thus we accept that wild birds' responses to climate change may be somewhat different to the trends we observed here. While smaller bird species are more at risk from climate change due to their higher metabolic rates and reduced ability for fat storage (Simmons et al., 2004), we nevertheless feel that since all of our study birds coped with an abrupt increase of 4°C, they should be more than capable of coping with a far more gradual temperature increase associated with climate change in the coming decades. Indeed, when the constant-environment rooms overheated one night, maintaining a T_a of 43°C and 39°C for ~16 hours in the Control+4°C and Control groups respectively, only 2 of the 22 birds expired. That most of the birds could survive something akin to an 'extreme heat event' is testament to their great physiological flexibility, and this, along with their generalist feeding habits (Fry et al., 2000), and their use of a wide range of habitats (Hulley et al., 2004; Smith and Bowie, 2005), should be in their favour in the face of increased climate change (Jiguet et al., 2007; Julliard et al., 2003; Knowlton and Graham, 2010; Schwartz et al., 2006; Thomas et al., 2004). However, the effects of climate change on birds are numerous (Şekercioğlu et al., 2012), and may for example include mismatches in the timing of breeding and the timing of peak prey abundance. Thus the potential physiological effects of a 4°C increase on Cape white-eyes should be viewed within a natural,

environmental context, where diet, breeding, predation and movements may all affect individual survival and fitness.

During the study period, mean monthly temperatures were lowest in July and highest in February, corresponding with austral winter and summer respectively. Had we elected to measure our birds only in these two months, for a study on a seasonal variation in metabolic parameters, we would have concluded that our birds reduced their RMR and T_b in winter, which would suggest that birds were conserving energy in winter (Smit and McKechnie, 2010). Yet the observed peaks and troughs in RMR and T_b did not correspond to the peaks and troughs in housing temperature. Instead, T_b was lowest around Nov-Dec, and highest in Sept-Oct, while RMR was lowest in Dec and highest in April. This suggests that studies that investigate RMR only in summer and winter may not be recording the full range of values exhibited by the study animals.

Conclusions

Species may adjust to global warming through phenotypic plasticity in their thermal responses or though alterations in the genetic composition of populations (Pulido and Berthold, 2004). Currently, there is no documented case of a genetic shift towards increased thermal tolerance in any bird population (Bradshaw and Holzapfel, 2006; Bradshaw and Holzapfel, 2010; Gienapp et al., 2008). The adaptive capacity (sensu Dawson et al., 2011) of Cape white-eyes seems to be high. In conclusion, our results support the ideas of McClelland (2004) and the results of Khaliq et al. (2014) and of Vedder et al. (2013); that the temperature increase forecast for our study area by 2080 is within the range of temperature tolerance of our population of Cape white-eyes, and

that the direct effect of increased air temperature alone may not pose a severe threat to this southern African endemic species.

Materials and methods

Capture and maintenance

All research was conducted at the University of KwaZulu-Natal (UKZN), Pietermaritzburg, South Africa (29°37'S 30°24'E). Cape white-eyes were caught in UKZN's botanical garden using mist nets (Ecotone, Gdynia, Poland) and baited walk-in traps, from November 2012 to January 2013, and moved to UKZN's Zoology building, where they were randomly assigned to one of two groups. These two groups were initially acclimated to captivity at 25ºC and 29ºC respectively for eight weeks (Chapter 5) and then kept in the same two groups, and in the same two rooms, with (i) the birds acclimated at 25ºC becoming a control group (n=10, including five males and five females), housed in a room with T_a set to match the outdoor temperature of the previous day, and (ii) the birds acclimated at 29ºC becoming an experimental group (n=10, including two females, six males, and two of unknown sex), housed in a room with T_a set to 4°C higher than the outdoor temperature from the previous day, hereafter called 'control+4°C'. Temperatures in the two rooms were controlled by manually programming an Alerton® global controller (Redmond, WA). Each morning, a maximum and minimum temperature reading from the previous day was read from a max/min thermometer placed inside a Stevenson screen in UKZN grounds, Pietermaritzburg. Four times a day, every day, for a year, T_a in the control room was manually changed, to match the (i) minimum, (ii) mean (of the minimum and maximum), (iii) maximum and (iv) mean (of the minimum and maximum, again)

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temperatures of the previous day (Fig. 1). Where possible, maximum T_a was set just after midday, minimum in the early hours of the morning, and the two means approximately half-way in between. At the same time, T_a in the experimental room was set at 4°C higher than T_a in the control room. In this way we hoped that the birds would experience the same range of T_a as they would in the wild, whilst maintaining one group of birds 4°C higher than the control group.

No-one entered the rooms besides the same individual who fed the birds each morning, cleaned cages once a week, and handled the birds for metabolic measurements. The Cape white-eyes used in this study were initially acclimated for eight weeks, which is more than enough time for birds from this population to acclimate to captivity, in terms of their body mass, resting metabolic rate, evaporative water loss and respiratory quotient (Chapter 5). During this acclimation period, the resting metabolic rates of all birds were measured weekly (Chapter 5), thus avoiding stress-related elevations in RMR in this study (Jacobs and McKechnie, 2014). We then kept the birds for a year, to avoid biasing our results to one particular season, since recent, rapid climate change is having a more rapid effect on winter temperatures than on summer temperatures (Bradshaw and Holzapfel, 2008; Bradshaw and Holzapfel, 2010).

Only ten birds from each room were used in experiments; but two additional birds were caught with the original group, and one housed in each of the two rooms as a 'spare', so that there were 22 birds indoors in total, but only 20 were used in the experiments. Both rooms were artificially lit with 12 L:12 D, which remained constant throughout the year, since we were primarily interested in the effect of a 4°C difference in housing temperature. Although change in day length is closely tied to change in season (Bradshaw and Holzapfel, 2010), temperature, rather than photoperiod, seems to be the main driver of flexibility in avian metabolic rate (Swanson et al., 2014). Primary moult in the birds used in this study occurred from February to

June, which corresponded with the timing of moult in birds from the same population housed at the study site in outdoor aviaries, and with moult in other populations of Cape white-eyes in the same province (Earlé, 1981; Symes et al., 2001). Thus the timing of primary moult did not change when birds were brought into captivity, even though the photoperiod of the rooms they were housed in was slightly different from the natural photoperiod when the birds were caught.

Within the two rooms, birds were housed in cages (90 x 40 x 100 cm) in groups of 3 or 4. Since Cape white-eyes show no sexual dimorphism (Hockey et al., 2005; Oatley, 2011; Skead, 1967), we could not determine sex by looking at the birds, and so in some instances males and females were housed together. A variety of fruits (papayas, bananas, oranges and apples) and softbill pellet supplements (Avi-products, Durban, South Africa) were supplied daily, and water was given *ad libitum*. Breeding was discouraged with a lack of nesting materials. After all metabolic trails were completed, blood (<100 μl) was drawn from the jugular vein of each Cape white-eye using a disposable Healthease® syringe and 29G x ½" (0.33 x 13 mm) needle (Neomedic Pty. Ltd., Riverhorse Valley East, South Africa), and sent to Molecular Diagnostics Services Pty. Ltd. (Westville, South Africa) for molecular DNA sexing. Birds were moved into outdoor aviaries for three weeks, and then soft-released at their capture sites. Birds were captured, transported, ringed, monitored and released under permit (number OP 5122/2012) from Ezemvelo KwaZulu-Natal Wildlife, and ethical approval for this study was granted by UKZN's Animal Ethics Sub-committee (reference: 071/13/Animal).

Body temperature measurements and moult score

Intraperitoneal T_b of Cape white-eyes were measured to the nearest 0.1°C using 12 mm x 2.1 mm, 0.06 g, temperature-sensitive passive integrated transponder devices (PIT tags, Biomark, Boise, Idaho, USA), injected into the intraperitoneal cavity of each bird using a 12-gauge needle. Signals from the tags were detected using two racket antennae (Model FS2001F-ISO, Biomark) positioned next to metabolic chambers. These antennae in turn were connected to PIT tag readers (Destron Fearing, St. Paul MN, USA), programmed to record data every 15 min. These data were then used to obtain mean hourly T_b measurements. Only five birds from each of the two rooms were injected with PIT tags. Thus, of the four birds placed into the temperature-controlled cabinet each night, only two contained PIT tags; more than this caused the tags' signals to interfere with each other.

A sample of ten PIT tags was calibrated in a circulating water bath following Cory Toussaint and McKechnie (2012), from 5 to 45°C, using a mercury-in-glass thermometer with a measurement precision of 0.1°C, and accuracy traceable to the US National Bureau of Standards. A linear regression was applied to the data, giving the following equation: $y = 1.015\ x - 0.983$ ($r^2 = 0.9993$), where y = actual temperature in °C, and x = measured temperature in °C. The variation between tags was low, with standard deviation ranging from 0.148 at 44.9 °C to 0.196 at 39.9 °C.

Moult is energetically expensive and thus likely to increase metabolic rate (Cyr et al., 2008; Lindström et al., 1993; Portugal et al., 2007), and so each Cape white-eye was scored for primary feather moult immediately prior to metabolic trials, using methods described by De Beer et al. (2001). The score for each feather ranged from 0 (for an old feather) to 5 (for a new

feather). The sum of the scores for all nine primaries was then divided by 4.5 to give an index ranging from 0 to 10.

Gas exchange measurements

We used open-flow respirometry to indirectly measure the metabolic rate of Cape white-eyes. Birds fasted for 3 h before measurements started, to ensure that they were post-absorptive (Wellman and Downs, 2009), and to reduce the possible effects of handling stress. From 15:00 until 07:00 the next morning, flow rate ($mL.min^{-1}$), O_2 and CO_2 concentrations (%), and water vapour density ($\mu g.mL^{-1}$) were recorded every 5 sec. An interrupted sampling regime was used, beginning with a baseline measurement for six min, and then four birds for six min each. This sequence was repeated so that each bird was measured twice (for 12 min in total) per hour. Due to time constraints, we measured multiple birds each night in a switching system, which results in smaller errors than measuring one bird continuously for shorter periods (Cooper and Withers, 2010).

   All birds were measured once a month, at both 20 and 25°C, for one year, with at least one night between measurements. These temperatures were chosen on the assumption that they would fall below and within the thermoneutral zone (TNZ, the range of ambient temperatures over which temperature regulation is achieved without changes in metabolic heat production or evaporative heat loss (I.U.P.S. Thermal Commission, 2001) respectively for this population. However, the lower critical limit of the TNZ of this population of Cape white-eyes was subsequently found to be 27°C to 28°C in summer (Chapter 2), and so the measurements we made at 20°C and 25°C are likely to be below or close to the lower critical limit of the TNZ,

hence we refer to them as RMR. We therefore included metabolic measurements in summer and winter at 30°C, which is within the TNZ of this population of Cape white-eyes (Chapter 2), for a seasonal comparison of basal metabolic rate (BMR), that is, the minimum maintenance energy requirements of non-reproductive, post-absorptive, resting, adult normothermic endotherms (McKechnie et al., 2006; McNab, 1997), and to provide BMR values which may then be compared with other studies.

Metabolic rate was indirectly measured every month, from May 2013 to April 2014 inclusive, using open-flow, push-mode respirometry. At 15:00, after being weighed to the nearest 0.01g using digital scales (model: AFB-3100L, Adam Equipment S.A. Pty. Ltd., Johannesburg), Cape white-eyes were placed onto wooden perches inside 2.8 L Perspex respirometers, within a temperature-controlled environmental chamber (CMP2244, Conviron, Winnipeg, Canada), set to 12L : 12D. Inside each respirometer, a plastic mesh platform was positioned 10 cm above a 1 cm layer of liquid paraffin/mineral oil (AlphaPharm, Pietermaritzburg), to eliminate evaporation from excreta.

Water vapour and CO_2 were removed from environmental air with silica gel, soda lime and more silica gel. This air was then pumped (model PP2, Sable Systems, Las Vegas, Nevada, USA) into five inlets of a flow measurement system (model FB8, Sable Systems). Flow rates were set to ~ 800 mL.min^{-1}, to maintain O_2 depletion in each respective chamber between 0.1 and 0.5% (Lighton, 2008). In each respirometer, the air inlet was located near the bottom, and the outlet near the top, to aid mixing of air inside the chamber. Effluent air flowed through a flow multiplexer (model MUX, Sable Systems) and then excess air escaped through a manifold, while the remainder was pumped through a subsampler (model SS4, Sable Systems) at 200 mL.min^{-1}. This air then flowed through a water vapour analyser (model RH300, Sable Systems),

which was regularly spanned using a nearly-saturated airstream and zeroed using N_2. Air was then dried with minimal quantities of Drierite (Hammond Drierite Co. Ltd., Xenia, Ohio), that had previously been recharged to reduce its affinity for CO_2 and therefore to reduce CO_2 washout time (White et al., 2006). Air then flowed through a CO_2 analyser (model CA-10, Sable Systems) and an O_2 analyser (model FC-10, Sable Systems). The CO_2 analyser was regularly zeroed with N_2, and spanned with a certified gas of 964 ppm CO_2 in N_2 (AFROX, Pietermaritzburg, South Africa). The flow meter and gas analysers were connected to a Universal Interface (model UI2, Sable Systems), which transferred data to a computer using ExpeData data acquisition software (Sable Systems). Temperatures within each respirometry chamber were recorded every 15 min. with i-Buttons® at a resolution of 0.0625°C, (model DS1922L-F5, Thermochron®, Maxim, CA, USA). All i-Buttons® were calibrated before use in a circulating water bath, from 7 - 36°C, with a mercury-in-glass thermometer (measurement precision = 0.1°C), with accuracy traceable to the US National Bureau of Standards.

Lag and drift correction were performed in ExpeData using a macro, and a 95% equilibration time of 11 min. was calculated using the equation of Lasiewski et al. (1966). Therefore, rates of O_2 consumption ($\dot{V}O_2$), CO_2 production ($\dot{V}CO_2$) and EWL were *Z*-transformed (Lighton, 2008; Lighton and Halsey, 2011) before hourly means were calculated. We also checked the washout characteristics of our system for water vapour without a bird in the respirometer, since water may adhere to Perspex, and found the measurement duration (6 min. of each 30 min. continuously spent on each respective bird) to be sufficient for accurate EWL readings. For each night, the lowest hourly $\dot{V}O_2$ reading was taken as the RMR. $\dot{V}CO_2$ and $\dot{V}H_2O$ (hereafter termed standard EWL) were taken at the same time as RMR was recorded. The

basal EWL measurement of each night was also recorded, since the time of minimum EWL and minimum $\dot{V}O_2$ usually did not coincide.

$\dot{V}O_2$, $\dot{V}CO_2$, RER and EWL were calculated as follows, with equations from page 456 and 446 of Withers (2001):

- $\dot{V}O_2 = \dot{V}_I \, (F_IO_2 - [F_EO_2 \, (1 - F_IO_2 - F_ICO_2 - F_IH_2O) / (1 - F_EO_2 - F_ECO_2 - F_EH_2O)])$

- $\dot{V}CO_2 = \dot{V}_I \, ([F_ECO_2 \, (1 - F_IO_2 - F_ICO2 - F_IH_2O) / (1 - F_EO_2 - F_ECO_2 - F_EH_2O)] - F_ICO_2)$

- $EWL = \dot{V}_I \, ([F_EH_2O \, (1 - F_IO_2 - F_ICO2 - F_IH_2O) / (1 - F_EO_2 - F_ECO_2 - F_EH_2O)] - F_IH_2O)$

- $RER = \dot{V}CO_2 / \dot{V}O_2$

where $\dot{V}_I$ was the measured mass flow. Body mass (M_b, g) and standard EWL were taken at the same time as RMR each night.

Data analyses

Statistical analyses were conducted using the base program in R version 3.1.0 (R Core Team, 2014), and figures were produced using the R package '*ggplot2*' (Wickham, 2009). We defined a set of candidate models *a priori*, following (Burnham and Anderson, 2002) and linear mixed-effects models were performed with the '*lme4*' package in R (Bates et al., 2014) to determine the effects of various predictor variables on RMR, standard EWL, T_b and M_b. Fixed effects included 'T_a' (temperature at which birds were measured overnight, either 20°C or 25°C), 'group' (whether birds were housed at T_a or at T_a+4°C), 'M_b', 'moult' (ranging from 0 to 10), and 'sex' (male, female, or unknown). 'Date' (on which metabolic measurement was done) and 'BirdID' (individual) were included as a random effect to control for repeated measures. Predictors with possible biological importance were included in the global model regardless of whether they

were statistically significant or not (Cheng et al., 2010). Visual inspection of residual plots showed no deviations from homoscedasticity or normality. Akaike's information criterion weights (AICWt), and delta AIC values (ΔAIC, the differences between each respective candidate model and the best approximating model), produced using the R package '*AICcmodavg*' (Mazerolle, 2013), were used to select the best approximating models, following Burnham and Anderson (2002) and Wagenmakers and Farrell (2004). Models with ΔAICc < 2 were averaged for multimodel inference using the R package '*MuMIn*' (Bartoń, 2013). The significance of fixed effects was determined using analyses of variance in R (Knoblauch and Maloney, 2012).

Competing interests

The authors declare no competing financial interests.

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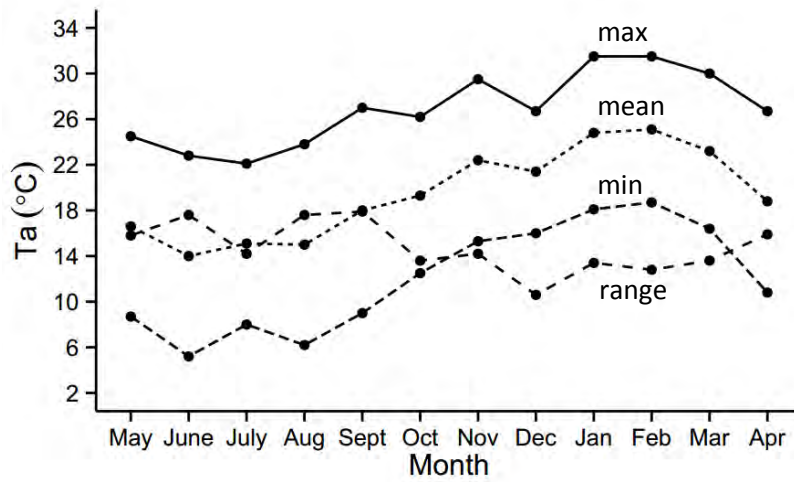
Fig. 1. Mean monthly maximum (max), minimum (min), mean (of maximum and minimum) and range (maximum – minimum) of outdoor ambient temperatures (T_a, °C), recorded at the study site in Pietermaritzburg, South Africa, during the study period, May 2013 to April 2014.
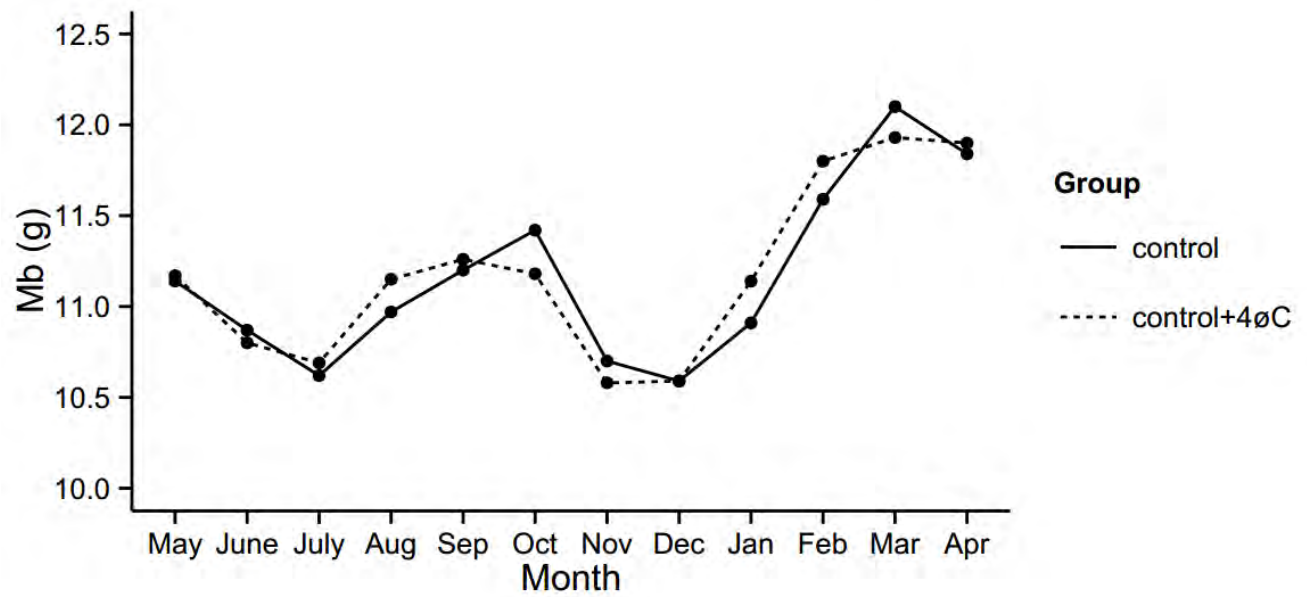
Fig. 2. Mean monthly body mass (M_b, g) of Cape white-eyes (n = 20) from May 2013 to April 2014. Birds were housed in the control group (solid line) or in the control+4°C group (dotted line), and were measured overnight.
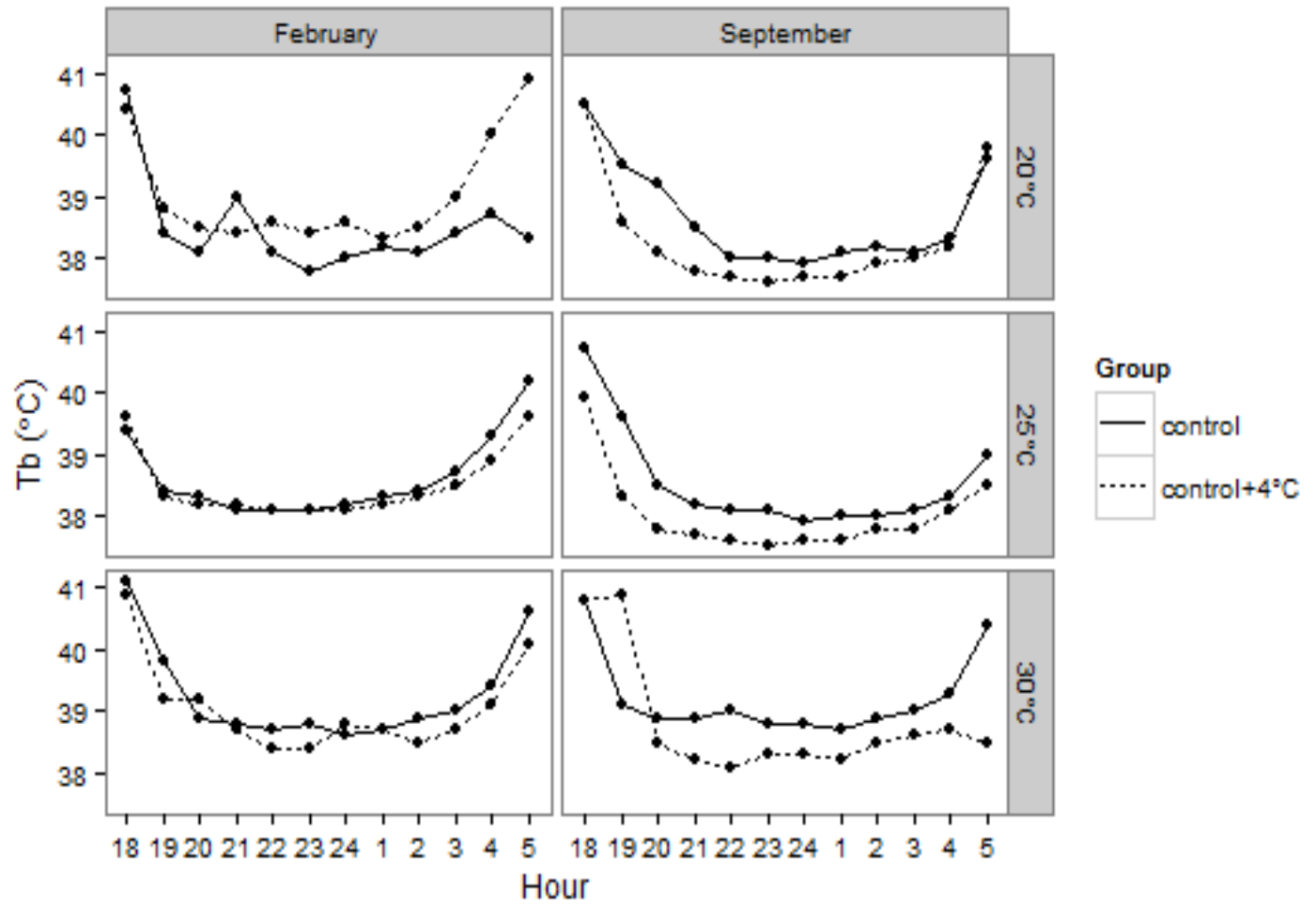
Fig. 3. Mean hourly intraperitoneal body temperature (T_b, °C) of Cape white-eyes housed at T_a (n = 5) and at T_a+4°C (n = 5), measured at T_a = 20°C (top row), 25°C (centre row) and 30°C (bottom row), in February 2014 (left) and September 2013 (right). Scotophase lasted from 18:00 to 06:00.

Fig. 4. Mean hourly resting metabolic rate (RMR, ml O$_2$ h^{-1}) of Cape white-eyes housed at T_a **(n = 10) and at** T_a**+4°C (n = 10), measured at** T_a **= 20°C (top row), 25°C (centre row) and 30°C (bottom row), in February 2014 (left) and September 2013 (right).** Scotophase lasted from 18:00 to 06:00.
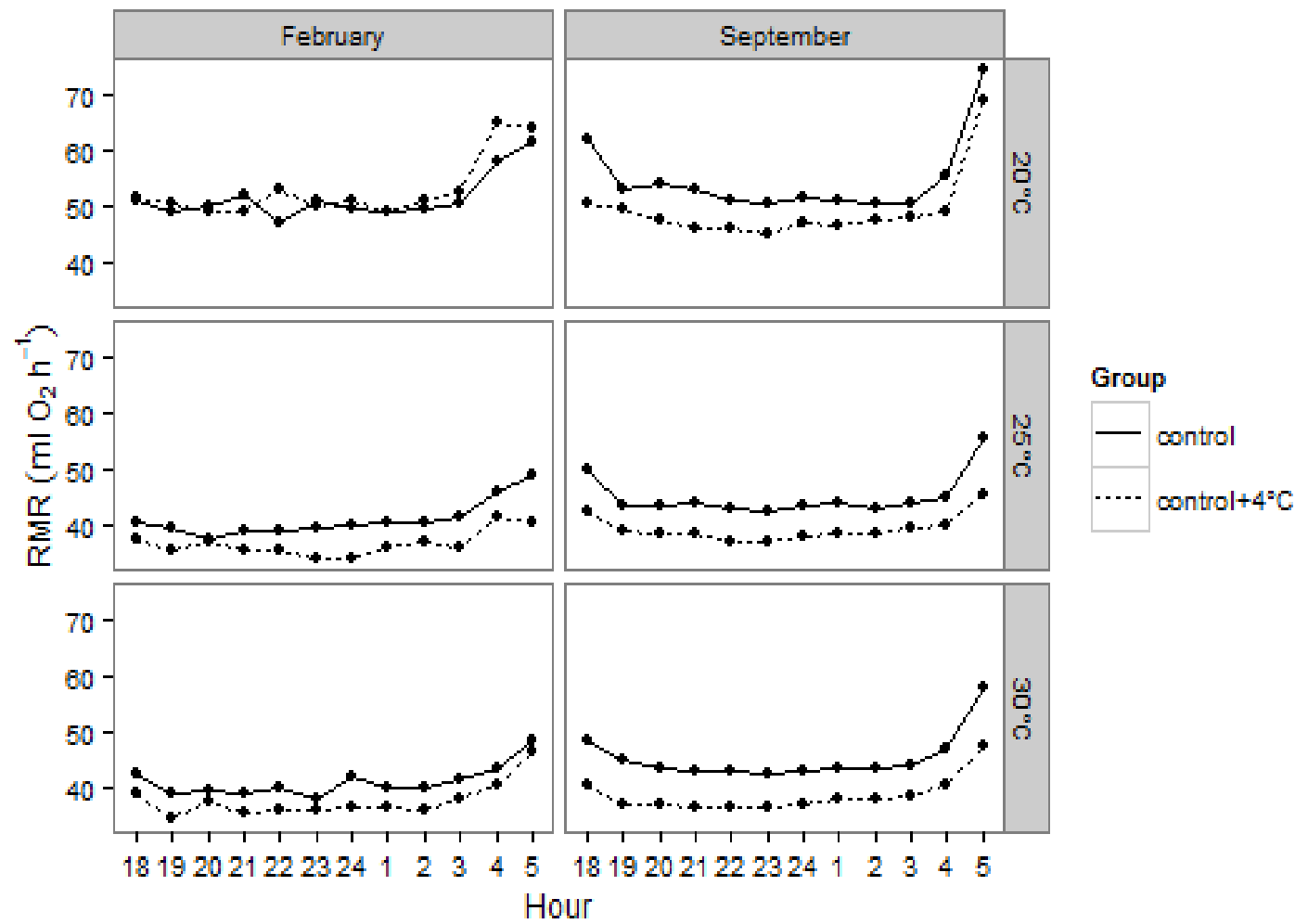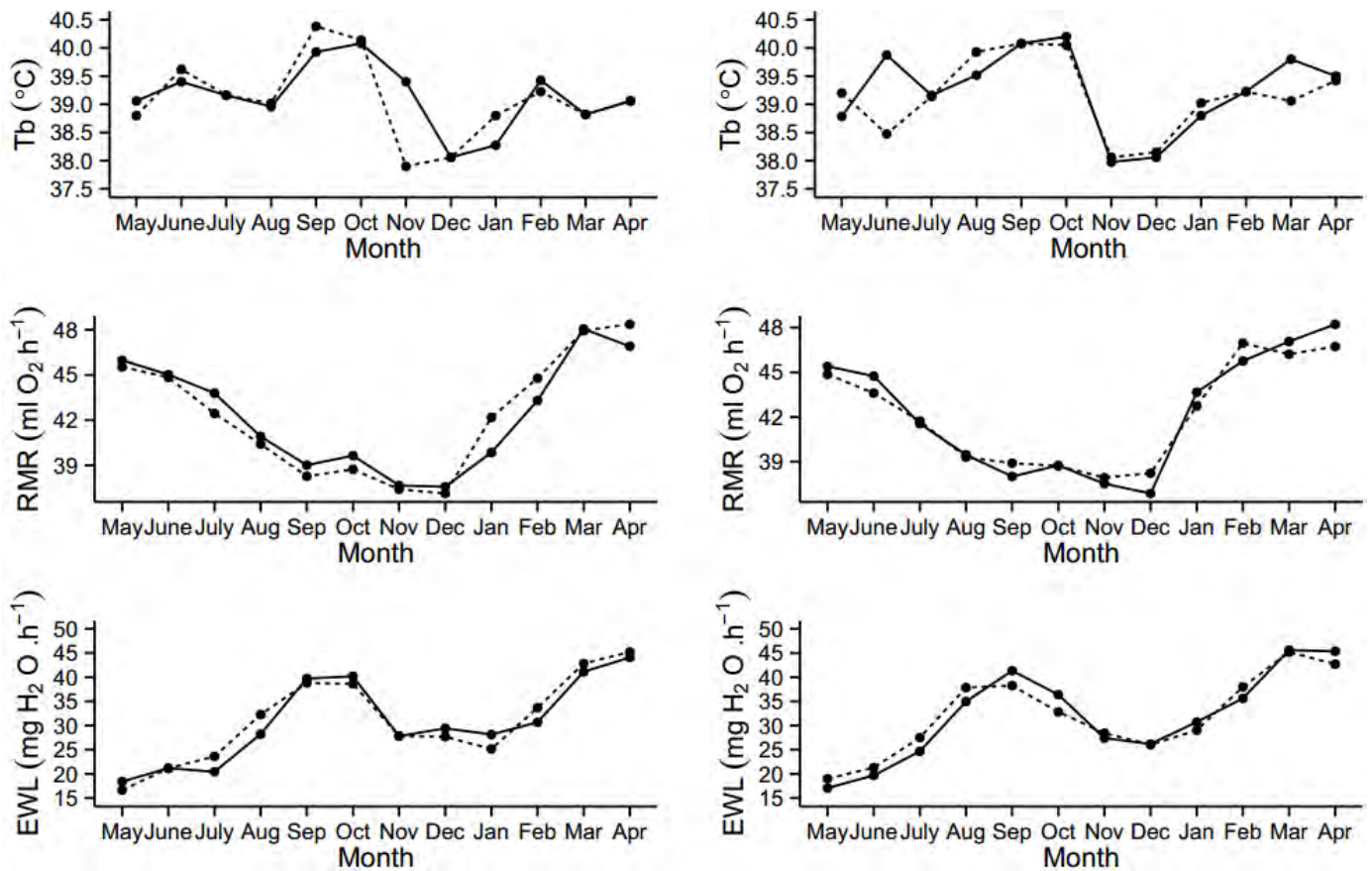
Fig. 5. Mean monthly intraperitoneal body temperature (T_b, °C), resting metabolic rate (RMR) and standard evaporative water loss (EWL, mg H_2O h^{-1}) for Cape white-eyes housed in the control group (solid lines) and in the control+4°C group (dotted lines). Birds were measured at $T_a = 20°C$ (left) and $T_a = 25°C$ (right) each month.

Tables

Table 1 Ranking of models predicting metabolic parameters of Cape white-eyes. All candidate models included 'individual' and 'date' as random effects, however, only those with an AICcWt > 0 are presented. The number of parameters (k) and Akaike Information Criterion (AIC) scores for each respective model are given. Models were ranked on their Akaike weights (AICWt) and on the difference between the best model and each candidate model (ΔAIC). Models with ΔAIC < 2 have considerable empirical support (Burnham and Anderson, 2002) and are shown in bold. See list of abbreviations for meaning of fixed effects.

Response variable	Fixed effects	k	AIC	AICWt	ΔAIC
T_b	**'M_b' + 'sex'+ 'T_a' + 'group'**	**8**	**635.39**	**0.69**	**0.00**
	'M_b' + 'moult' + 'sex'+ 'T_a' + 'group'	**9**	**637.01**	**0.31**	**1.63**
whole-animal RMR	**'M_b' + 'moult' + 'sex'+ 'T_a' + 'group'**	**9**	**2075.32**	**0.94**	**0.00**
	'M_b' + 'sex'+ 'T_a' + 'group'	8	2080.85	0.06	5.53
M_b	**'moult' + 'sex' + 'group'**	**7**	**790.94**	**1.00**	**0.00**
whole-animal standard EWL	**'M_b' + 'moult' + 'sex'+ 'T_a' + 'group'**	**9**	**3625.1**	**1.00**	**0.00**
mass-specific standard EWL	**'M_b' + 'moult' + 'sex'+ 'T_a' + 'group'**	**9**	**1705.3**	**0.99**	**0.00**
	'M_b' + 'sex'+ 'T_a' + 'group'	8	1715.3	0.01	9.95
whole-animal basal EWL	**'M_b' + 'moult' + 'sex'+ 'T_a' + 'group'**	**9**	**2961.11**	**0.00**	**1.00**
mass-specific basal EWL	**'M_b' + 'moult' + 'sex'+ 'T_a' + 'group'**	**9**	**1273.32**	**0.00**	**1.00**

Table 2 Estimate sizes of fixed effects contained in the best approximating model(s) fitted by restricted maximum likelihood estimation (REML) for each respective response variable. In each case, 'T_a' refers to metabolic measurement of birds at 25°C (as opposed to 20°C), 'sex' refers to male, and 'group' to the group of birds kept at T_a+4°C (as opposed to those kept at T_a). The estimate sizes given below for T_b are the result of model averaging. See list of abbreviations for meaning of fixed effects.

Response variable	Fixed effect	Estimate size	s.e.m
T_b	'sex'	-0.85	0.40
	'group'	0.44	0.42
	'T_a'	-0.20	0.15
	'M_b'	0.15	0.11
whole-animal RMR	'T_a'	- 7.33	0.38
	'M_b'	1.87	0.25
	'sex'	-1.79	1.24
	'group'	-1.27	1.26
	'moult'	0.12	0.05
M_b	'group'	-0.50	0.32
	'sex'	0.33	0.32
	'moult'	0.03	0.01
whole animal standard EWL	'T_a'	5.62	3.39
	'M_b'	3.39	1.64
	'sex'	-2.31	3.73
	'group'	1.83	4.33
	'moult'	-0.88	0.33
mass-specific standard EWL	'T_a'	0.50	0.30
	'sex'	-0.23	0.33

	'group'	0.22	0.38
	'M_b'	-0.15	0.15
	'moult	-0.08	0.03
whole animal basal EWL	'group'	-5.21	3.22
	'T_a'	-0.98	2.57
	'moult	-0.80	0.19
	'M_b'	-0.69	1.07
	'sex'	-0.02	2.71
mass-specific basal EWL	'group'	-0.41	0.31
	'M_b'	-0.33	0.11
	'T_a'	-0.10	0.25
	'moult'	-0.08	0.02
	'sex'	-0.02	0.26

GENERAL CONCLUSION

Summary of findings and implications

Avian basal metabolic rate (BMR) is known to be a highly flexible trait (McKechnie 2008), affected by a wide range of environmental factors (McKechnie and Swanson 2010). However, certain aspects of this flexibility are not yet well understood. For example, there is no clear trend in the direction and magnitude of seasonal change in the BMRs of subtropical bird species. Similarly, long-term captivity may cause both increases and decreases in avian metabolic rate, while studies may nevertheless implicitly assume that metabolic rates of long-term captive birds are representative of those of wild conspecifics. This study aimed to investigate flexibility in avian metabolic rate, using a small Afrotropical bird, since subtropical species are relatively understudied compared to temperate and Holarctic species (Van de Ven et al. 2013).

Seasonal variation was studied over multiple years, as recommended by Van de Ven (2012), and the results showed that small Afrotropical birds may vary in the magnitude of seasonal change in BMR between years. Environmental temperature and rainfall did not vary significantly between years at the study site, and therefore cannot have caused the reversal of seasonal trends in metabolic rate that was observed (Chapter 2). Thus seasonal trends in BMR in Afrotropical birds may not be consistent across years, even within a population.

There are only a handful of studies on circannual rhythm in avian metabolic rate, even though such studies give a much greater understanding of variation in metabolic rate within species and individuals than seasonal studies do. Petit et al. (2013) found that BMR of black-capped chickadees (*Poecile atricapillus*) peaked at the coldest time of year, however in this

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study peaks and troughs in monthly RMR of Cape white-eyes did not correspond to peaks and troughs in mean monthly outdoor ambient temperature, suggesting that measuring RMR at the height of summer and winter may in some instances underestimate the physiological flexibility of which birds are capable (Chapter 3). That some species of birds may have a lag between environmental temperature and RMR is important, and may be crucial for understanding seasonal variation of metabolic rate in subtropical bird species.

Wild-caught birds have been used in many physiological studies, yet the length of time it takes for completion of their acclimation to laboratory conditions in terms of their metabolic rate remains largely unknown. Weekly measurements of various metabolic parameters of Cape white-eyes over an 8-week period, showed that body mass increased significantly in the first three weeks of acclimation, remaining approximately constant thereafter (Chapter 5). However, whole animal and mass-specific RMR remained constant throughout the eight-week acclimation period. These results suggest that in some instances, freshly wild-caught small passerines may not need to be acclimated to laboratory conditions or respirometry equipment, prior to RMR measurements.

Since all of the results in this study were obtained from long-term captive birds, a comparison was made of BMR in wild birds and long-term captives, revealing that whole animal BMR and body mass of Cape white-eyes were influenced more by long-term captivity than by season, while mass-specific BMR and EWL were affected primarily by season, with long-term captivity having less of an effect (Chapter 4). These results support the conclusions from the few within-species studies on the effects of long-term captivity on avian physiological variables, which generally suggest that whole animal BMR and body mass of long-term captive birds should not be used as representative of wild populations (Caro 2012; Lovegrove et al. 2011;

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McKechnie et al. 2006). This knowledge may be important for wildlife managers, particularly those managing captive breeding programmes of endangered avian species, since the captive environment may significant affect various avian physiological variables.

Besides helping birds to survive with spatially and temporally variable environments, flexibility in BMR may help birds to cope, physiologically, with increased mean ambient temperatures associated with anthropogenic climate change (Khaliq et al. 2014). Yet many predictive models do not take species' physiological flexibility into account (Chown et al. 2010; Vedder et al. 2013). By the year 2080, a 4°C increase in mean surface air temperature is predicted for southern Africa (Hudson and Jones 2002). A sustained 4°C increase in housing temperature of Cape white-eyes was associated with 4.5% reduction in body mass, a 2.8% reduction in whole animal RMR, and an increase of 0.4 ± 0.4°C in intraperitoneal body temperature (Chapter 6). This suggests that Cape white-eyes' physiological flexibility may aid them in coping with the 4°C increase predicted for their range by 2080, however, the physiological (sensu Jimenez and Williams 2014) and long-term fitness costs of these small physiological changes need to be investigated, and although this study was novel and important, it is likely that the effects of extreme weather events such as heat waves and droughts, will be a far greater test of the physiological limits of this species.

Limitations

This study investigated flexibility in avian resting and basal metabolism in only one species. Therefore, extrapolation of conclusions from this study to other species, or even to other populations of the same species in other areas, and for other measures of metabolic rate, should be avoided; such claims are not within the scope of this study, but better left to a

phylogenetically-informed comparative analysis of avian metabolic rate. Similarly, this study did not include measurements on stress hormones or organ masses, and so the data presented here are mainly observational, preventing any comment on the mechanisms behind the observed trends.

Future work

Future work should focus on elucidating seasonal trends (and indeed whether there is a set trend) in metabolic rate of Afrotropical bird species, paying attention to the effects of body mass, phylogeny and various environmental drivers. In addition, further acclimation studies could be performed using two conspecific groups of birds, one freshly-wild caught and the other long-term captive, and therefore already accustomed to a captive diet and captive housing condition, sensu Jacobs and McKechnie (2014), to determine whether it is possible that in this study, increased metabolic rates due to captivity stress were perhaps offsetting a decrease in metabolism due to habituation to experimental procedure. Moreover, although climate change-related increases in mean ambient temperature may not pose an immediate physiological threat to this species, it is likely that extreme heat events would be a threat, particularly to small birds which have low thermal inertia (Wolf and Walsberg 1996b), and therefore may be more vulnerable to increased frequency of extreme heat events than larger bodied birds. Thus, it may be important to determine the upper critical limit of the thermoneutral zone for many Afrotropical species, and the effect of heat waves on small birds, with a similar approach as was used for other taxa (Fischer et al. 2014).

Future studies should also include a more comprehensive approach, looking not only at effects of climate change-associated temperature increases on avian physiology, but also on other

aspects of the avian ecology, including the availability of food and water, potential habitat loss, and whether their predators will be similarly affected, particularly when the results may be used in predictions for species of conservation concern. Avian metabolic studies are also encouraged to measure intraperitoneal body temperature of birds using PIT tags, and to use only freshly wild-caught birds, where possible (McKechnie et al. 2006).

A brief search of the literature revealed no published account on the merits of using certain materials for respirometers when studying evaporative water loss. A manual for measuring metabolic rates by Lighton (2008) briefly discusses the advantages of various materials for tubing, but not for respirometers, although the principles undoubtedly remain the same. Recent studies have used respirometers made predominantly of plastic (Minnaar et al. 2014), PVC (Ganey et al. 1993; Tomlinson et al. 2012), glass (McKechnie and Wolf 2004; Withers and Cooper 2011), Perspex (Cooper and Withers 2009; Versteegh et al. 2008), steel (Williams 1999; Williams and Tieleman 2000) and aluminium (Wolf and Walsberg 1996a), while some studies do not report the material of which their respirometers were made (Cavieres and Sabat 2008; Cortés et al. 2009). A future study could compare the water retention properties of respirometers made of different materials, to confirm whether there is a significant difference between materials in terms of washout times for water vapour.

Conclusion

In accordance with current widely held views that physiological flexibility aids birds in coping with temporal and spatial variation in a range of environmental factors (DeWitt 1998; DeWitt et al. 1998; Schlichting and Pigliucci 1998; Tieleman et al. 2003; Via et al. 1995), flexible changes in their metabolic rate allowed Cape white-eyes to cope physiologically with seasonal and

circannual changes, as well as changes associated with captivity and with acclimation to different thermal environments, and even with the same temperature increase this species may encounter in the coming decades with anthropogenic climate change. However, it seems that not all avian species respond to environmental changes in the same way, and these responses may even differ within populations. This has implications for researchers selecting studies on avian metabolic rate for comparative analyses, and indeed the factors affecting variation in avian resting metabolism, as well the potential influence of phenotypic flexibility, should be carefully considered, before drawing conclusions and making inferences.

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