ALTITUDINAL AND SEASONAL VARIATION IN AMETHYST SUNBIRD PHYSIOLOGY

CLAIRE VICKY LINDSAY

Submitted in fulfilment of the academic requirements for the degree of

MASTER OF SCIENCE

in the Discipline of Zoology

School of Biological and Conservation Sciences

Faculty of Science and Agriculture

University of KwaZulu-Natal

Pietermaritzburg

2007
PREFACE

The experimental work described in this dissertation was carried out in the School of Biological and Conservation Sciences, University of KwaZulu-Natal, Pietermaritzburg from January 2006 to December 2007, under the supervision of Professor Colleen T. Downs and co-supervision of Mr Mark Brown.

This dissertation, submitted for the degree of Master of Science in the Faculty of Science and Agriculture, University of KwaZulu-Natal, Pietermaritzburg, 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.

..............................
Claire V. Lindsay
December 2007

I certify that the above statement is correct

..............................
Professor Colleen T. Downs
Supervisor

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Mark Brown
Co-supervisor
December 2007
Abstract

Southern Africa is characterised by an unpredictable environment with daily and seasonal temperature fluctuations. As a local or non-migratory endothermic species occurring over an altitudinal gradient from the Drakensberg to the coast of KwaZulu-Natal in southern Africa, Amethyst Sunbirds (*Chalcomitra amethystina*) experience challenging thermal conditions and increased energetic stress as a result of ambient temperature variation. Flexibility of metabolic rates within a species allows for the colonization of different habitats along an altitudinal and thus temperature gradient. It was predicted that over this altitudinal gradient Amethyst Sunbirds would exhibit variation in metabolic rates, particularly basal metabolic rates, pre- and post-acclimation, as well as variation in hematocrit levels in winter and summer trials. It was also predicted that Amethyst Sunbirds would exhibit seasonal variation in metabolic parameters.

Sunbirds were caught in a winter and summer season (2006-2007) using mist nets in three locations; Underberg (1553 m), Howick (1075 m) and Oribi Gorge (541 m). Upon capture, metabolic rate was measured indirectly by quantifying oxygen consumption (*VO₂*) using flow through respirometry, at 5 and 25°C. Birds were then acclimated at 25°C for 6 weeks on a 12L:12D cycle. *VO₂* was measured post-acclimation at 8 different temperatures (15, 5, 10, 20, 30, 28, 25 and 33°C). Hematocrit levels were taken pre-acclimation and pre-release. Winter and summer data were compared.

In the winter trials it was found that there was little variation in *VO₂* between individuals from the same locality, whereas significant variation was observed at the same temperatures between localities and thus between altitudes. The subpopulation from the highest altitudinal site had the highest basal metabolic rate (BMR). Summer
trials showed that metabolic rates did not differ significantly between altitudinal subpopulations of Amethyst Sunbirds, however, BMR was observed to decrease as altitude decreased. The comparison of seasonal data showed that Amethyst Sunbird subpopulations from Underberg and Howick showed higher post-acclimation VO$_2$ values per temperature in winter than in summer trials. Post-acclimation resting metabolic rate (RMR) values for Howick subpopulations were generally higher in winter than in summer, Underberg Amethyst Sunbirds showed a significant difference between summer and winter RMR at 5 and 10°C and Howick sunbirds showed a significant difference in RMR between seasons at 5°C. The Oribi Gorge subpopulation, however, showed no significant differences in metabolic rate between any temperatures when comparing a summer and a winter season. Thermal neutral zones of all of the subpopulations of Amethyst Sunbirds shifted between the winter and summer trial period.

This study thus emphasized the need to understand plasticity in metabolic rates and acknowledge altitudinal and seasonal differences within a species, in order to make accurate predictions about a species thermal physiology and responses to changes in ambient temperatures. In particular, the variation in BMR, which is usually used as a species specific value, should be acknowledged in comparative studies of avian metabolic rates or in climate change models.

Keywords: Altitude, Amethyst Sunbird, basal metabolic rate, metabolic rate, season, thermal neutral zones.
Acknowledgments

First and foremost, I would like to thank my supervisor Professor Colleen Downs for being patient with her time, and generous with her guidance and kindness, and for allowing me to write this thesis in my own way and time. You are a role model to women in science, due to your obvious passion for what you do. My co-supervisor Mark Brown, for so willingly helping me with field work, respirometer queries and statistics and for his guidance, moral support, friendship and patience over the last two years. Thirdly I would like to thank my family, Dad, Mom, Adrienne, Ant and Nate, for always supporting me in my studies, both in terms of moral and financial support. Children are a product of their families, and I would not be who I am without your love and faith in me. I would like to thank Dr Sue McConnachie for her invaluable assistance in understanding the respirometer, and for being a sounding board and source of advice for any ideas and problems I had. My office mates, Andrea, Eebee, Kirsten, Helen and Joy, with who so many stressful times, and times of fun procrastination have been spent. It has been a pleasure to work with all of you. Importantly, many thanks to the land owners who have so willingly “lent” me their sunbirds and welcomed me into their homes, Prof and Mrs Piper (Underberg), Pam and Bill Nicol (Howick) and Mike and Heidi Neethling (Oribi Gorge). I would like to thank the people who have kindly assisted me in my field work: Jaclyn Tennent, Tracy Odendaal, Kelly Brown, Ashton Musgrave, John Lindsay and finally Nicky Taylor, who also willingly helped me with proofing presentations, endless literature searches and pep talks. I would also like to thank Akeem Akilimali and Lungile Mvelase for assisting with the general sunbird upkeep. Lastly, but definitely not least, I would like to thank all of my friends for all their love, support, encouragement,
hugs, chocolate, dinners, gin and tonics, sense of humour boosters, and crazy sporting
endeavours, all of which assisted in the maintenance of my sanity.

“Nature unfolds her secrets only to the patient and persevering”
- Ian Player, In Men, Rivers and Canoes.
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Chapter 1

Introduction

In a climate like that of southern Africa, which is characterised by unpredictable daily and seasonal temperatures and precipitation (Schulz et al., 1997), fluctuations pose challenging thermal conditions for vertebrates, especially for mammals and birds, which are endothermic. As homeothermic endotherms, birds are able to maintain a constant body temperature over a broad range of ambient temperatures by adjusting their metabolism (Chaui-Berlinck et al., 2002). Many species of birds exist successfully in climates both cold and warm, as a result of variation in metabolic parameters between populations of the same species (Furness, 2003). Besides seasonal ambient temperature fluctuations, climate change, and thus more permanent temperature shifts, will influence many areas of species survival, as a result of differing abilities to adapt and move with the changing climate. Simmons et al. (2004) pose the question of whether some species will exhibit rapid adaptations to climate change.

Adaptation was defined by Mayr (1988) as “the morphological, physiological and behavioural equipment of a species or member of its species that permits it to compete successfully with individuals of its own species or members of another species that allows it to tolerate the extant physical environment.” Adaptation may refer to changes that occur within an individual in response to changes in the environment, which may help the animal respond to these changes (Garland and Adolph, 1991).

However, a more flexible form of adaptation has been proposed in the form of phenotypic plasticity, which implies the general capacity for change and production of
a range of relatively fit phenotypes within genotypes in response to changing environmental conditions (DeWitt et al., 1998; Kingsolver et al., 2002; Piersma and Drent, 2003). However, instead of using the term “phenotypic plasticity”, Piersma and Drent (2003) propose instead using the term “phenotypic flexibility” which refers to variation within a characteristic of a single individual which is reversible, and a function of both predictable and unpredictable fluctuations in environmental conditions. Birds are known to show flexibility in their thermoregulatory response, showing the ability to increase heat or cold resistance seasonally or in response to laboratory conditions, i.e. acclimation (Dawson, 2003). It is important to note that acclimation is taken to be a physiological change in an organism in response to a particular environmental factor in a laboratory, whereas acclimatization is a progressive change in an organism in response to changes in its natural environment (Hine and Martin, 2005).

Previous comparative studies of avian thermal biology have focussed on the origin of study birds in terms of captive bred or wild caught populations and the effect of this factor on basal metabolic rate (BMR) (e.g. Weathers et al., 1983; McKechnie et al., 2007; McKechnie, In Press), but do not take into consideration the geographic and altitudinal origin of the wild caught species as a possible source of variation. Alternatively, studies have looked at metabolic adaptations along an aridity gradient (e.g. Tieleman et al., 2002), the effects of seasonal and environmental changes on BMR of a species (Ambrose and Bradshaw, 1988), phenotypic flexibility in the BMR of one population as a representative of a species (McKechnie et al., 2007), or have assessed population responses to climate change as a mean response at the population level (Møller et al., 2004). Importantly, published data often represents a single RMR or BMR value per species, regardless of altitudinal origin or whether birds were
captive bred or wild caught (e.g. Bech, 1980; Cooper and Swanson, 1994; Boix-Hinzen and Lovegrove, 1998; Maddocks and Geiser, 2000; McKechnie and Lovegrove, 2001; López-Calleja and Bozinovic, 2003; Lovegrove and Smith, 2003; McKechnie et al., 2007; Smit et al., In Press), thus neglecting the need to acknowledge the role of phenotypic flexibility within a species.

Very few avian studies have looked at variation in the thermal physiology of a species over an altitudinal gradient (Soobramoney et al., 2003). According to Körner (2007), altitudinal gradients are very important ‘natural experiments’ for testing ecological and evolutionary responses to influences such as temperature. Variation would be expected in metabolic rates over an altitudinal gradient due to these changes in ambient temperature ranges, and thus the adaptations subpopulations show to these temperature ranges. More specifically, in this case, few studies have looked at phenotypic plasticity or flexibility within a subpopulation seasonally, between pre- and post-acclimation trials, and over an altitudinal gradient. This is an oversight in metabolic studies as McNab (2003) found that 99% of the observed variation in the BMR of birds of paradise was due to inter-specific variation in body mass, food habits and distribution over an altitudinal gradient. Although McNab (2003) focused on inter-species differences, one can conclude that if inter-species differences can be attributed to altitude, it can be assumed that subpopulations of the same species would also display variation in certain bioenergetic parameters as a result of existing in a non- or local migratory manner over an altitudinal gradient. As early as 1962, Hart noticed that a striking feature of small birds was their ability to withstand changes in ambient temperature with very little protection, but at greater metabolic cost. Similarly, Swanson and Weinacht (1997) noted that seasonal differences in metabolism are common in small passerine birds and that birds display phenotypic
flexibility in maintenance energy requirements, and are able to up or down regulate BMR over a period of time during thermal acclimation (McKechnie et al., 2007). The dynamic interaction between animals and the environment is reflected in the physiological plasticity of the animal, employed to avoid the problems of evolutionary history or lifestyle (Tieleman et al., 2002).

The ability to adapt and change is most pronounced in species living in fluctuating environments (Cossins et al., 2006). The ability to employ flexibility and regulation of maintenance energy is important within a species inhabiting locations over an altitudinal and thus temperature gradient. More so, small diurnal birds that are locally migratory or non-migratory, have to deal with seasonal changes in ambient temperature and thus would have to employ seasonal adjustments in their physiology in order to reduce the thermal stress placed on these small passerines (Maddocks and Geiser, 2000).

Individuals (and thus populations) that are able to adjust their thermal physiology in response not only to their thermal environmental range, but ecological factors leading to rapid environmental changes (shorter than their lifetime), may enjoy a selective advantage and thus higher fitness pay-offs than those who cannot (DeWitt et al., 1998; Piersma and Drent, 2003). Thus the role of phenotypic flexibility relative to changing environmental conditions needs to be evaluated, and the ecological factors able to predict inter-specific (as well as intra-specific) differences in response to climate change need to be identified (Møller et al., 2004).

Evaporative water loss (EWL) is a homeostatic mechanism used by animals when ambient temperatures increase and affect body temperature. In many small mammals, such as *Gerbillurus* species, short-term evaporative cooling is employed to deal with hyperthermia above the thermal neutral zone (Downs and Perrin, 1990).
Thus the rate at which water is, and can, be lost from an animal has important implications for thermoregulation and thus survival (McKechnie and Wolf, 2004). Birds living over an altitudinal gradient experience differing ranges of ambient temperatures and would have differing evaporative cooling requirements. Thus we would expect to see flexibility in the ability of subpopulations to employ EWL as a thermoregulatory mechanism.

Møller et al. (2004) stated that the current knowledge of the effects of climate change on birds is mostly restricted to passerines from northern hemispheric temperate zones, and more work is needed on their southern hemisphere counterparts. The proposed study will aim to investigate differences in physiology between subpopulations of Amethyst Sunbirds, *Chalcomitra amethystina* (Shaw, 1811), over an altitudinal gradient within a latitudinal zone in KwaZulu-Natal (KZN). The Amethyst Sunbird is a relatively large African nectarivorous sunbird with a mean mass of approximately 15g (Cheke et al., 2001; Tree, 2005). Adult Amethyst Sunbirds exhibit sexual dimorphism. Adult males have blackish-brown plumage with purplish-copper on the throat and shoulders and silvery light green on their heads whereas females are grey-brown with pale grey brown underbellies (Cheke et al., 2001; Tree, 2005). The distribution of Amethyst Sunbirds, in KZN includes a latitudinal zone from the Drakensberg mountains to the coast (Tree, 2005). Their populations in KZN are described as being fairly sedentary, with some localised winter movement (Tree, 2005). This makes them a suitable species for a study on metabolic variation within a species over an altitudinal gradient. As the climate varies between the Drakensburg and the coastal regions of KZN, sites along this altitudinal gradient will allow for comparison between subpopulations of Amethyst Sunbirds living in different ambient temperature ranges. Thus the altitudinal and seasonal physiological adaptations and
inter-specific plasticity/flexibility of the subpopulations of Amethyst Sunbirds in these habitats can be determined.

Consequently, knowledge of the plasticity/flexibility in metabolic strategies employed to cope with ambient temperature fluctuations, both altitudinal and seasonal, is important in understanding terrestrial vertebrate survival in southern Africa, in terms of predicted climate change models. It was predicted that Amethyst Sunbirds will differ in thermal parameters, particularly BMR, resting metabolic rate and thermal neutral zone, within and between seasons.

This thesis is presented as chapters for submission to Journals:


Chapter 4. A comparison of summer and winter metabolic rates of Amethyst Sunbirds (*Chalcomitra amethystina*) over an altitudinal gradient.

with a final concluding chapter.

As chapters have been prepared as stand alone manuscripts, some overlap and repetition between chapters has been unavoidable.
References


**McKechnie, A.E.** Phenotypic flexibility in basal metabolic rate and the changing


**Sooobramoney, S., Downs, C.T. and Adams, N.J.** (2003). Physiological variability in


Physiological variation in Amethyst Sunbirds (*Chalcomitra amethystina*) over an altitudinal gradient. Part A: in winter.

Claire V. Lindsay\(^1\), Colleen T. Downs\(^1\) and Mark Brown\(^1\)

\(^1\) School of Biological and Conservation Sciences, University of KwaZulu-Natal, Private Bag X01, Pietermaritzburg, 3201, South Africa

Flexibility of metabolic rates within a species allows for the colonization of different habitats along an altitudinal and thus temperature gradient. The distribution range of Amethyst Sunbirds (*Chalcomitra amethystina*) within southern Africa includes an altitudinal gradient from the Drakensberg to the coast of KwaZulu-Natal. We expected that over this altitudinal gradient Amethyst Sunbirds would exhibit variation in metabolic rates, particularly basal metabolic rates (BMR), pre- and post-acclimation readings, as well as variation in hematocrit levels. Sunbirds from three locations; Underberg (1553 m), Howick (1075 m) and Oribi Gorge (541 m) were used for this study. Upon capture, metabolic rate was measured indirectly by quantifying oxygen consumption (VO\(_2\)) using flow through respirometry, at 5 and 25°C. Birds were then acclimated at 25°C for 6 weeks on a 12L:12D cycle. VO\(_2\) was measured post-acclimation at 8 different temperatures (15, 5, 10, 20, 30, 28, 25 and 33°C). Hematocrit levels were taken pre-acclimation and pre-release. We found little variation in VO\(_2\) between individuals from the same locality, whereas significant variation was observed in VO\(_2\) at the same temperatures between localities and thus
between altitudes. In particular, BMR decreased significantly with decreasing altitude post-acclimation. This study emphasizes the need to understand plasticity/flexibility in metabolic rates and acknowledge altitudinal differences within a species, in order to make accurate predictions about a species thermal physiology and responses to changes in ambient temperatures.

Corresponding author: downs@ukzn.ac.za

Keywords: Altitudinal variation, Amethyst Sunbird, metabolic rates, phenotypic plasticity, phenotypic flexibility.

Introduction

Phenotypic plasticity implies the general capacity for change and transformation within genotypes in response to changing environmental conditions (DeWitt et al., 1998; Kingsolver et al., 2002; Piersma and Drent, 2003). However, instead of using the term “phenotypic plasticity”, Piersma and Drent (2003) propose the use of the term “phenotypic flexibility” which refers to variation within a characteristic of a single individual which is reversible, and a function of both predictable and unpredictable fluctuations in environmental conditions.

The ability to employ flexibility and regulation of maintenance energy requirements is important within a species inhabiting locations over an altitudinal and thus temperature gradient. Avian species have shown the ability to enhance heat or cold resistance seasonally and in response to experimental conditions (Dawson, 2003). At higher altitudes, the effect of reduced oxygen partial pressure as well as decreasing ambient temperatures pose significant challenges to avian gas exchange and thus metabolic parameters (Clemens, 1988). Individuals (and thus populations) that are able to adjust their thermal physiology in response, not only to their thermal
environmental range, but to rapid environmental changes (shorter than their lifetime), may enjoy a selective advantage and thus higher fitness pay-offs than those who cannot (DeWitt et al., 1998; Piersma and Drent, 2003). Thus the role of phenotypic flexibility relative to changing environmental conditions needs to be evaluated, and the ecological factors leading to inter-specific (as well as intra-specific) differences in response to climate change need to be identified (Møller et al., 2004).

Birds are considered to be homeothermic endotherms, which implies the ability to maintain a constant body temperature over a broad range of ambient temperatures by adjusting their metabolism (Chaui-Berlinck et al., 2002). However small homeotherms have higher energetic demands at colder temperatures and require physiological adjustments in MR to counteract this (Downs and Brown, 2002; Soobramoney et al., 2003). As a result of this many birds display phenotypic flexibility in maintenance energy requirements, and are able to up or down regulate basal metabolic rate (BMR) over a period of time during thermal acclimation (McKechnie et al., 2007). Recent evidence suggests that winter BMR of species living in highly seasonal environments reflects the conditions in which the animal existed in immediately prior to metabolic measurements being taken (McKechnie, In Press) and thus it becomes important to differentiate metabolic measurements made pre-acclimation and those made post-acclimation (Smit et al., In Press). Klaasen (2004) suggested that it is important to recognize whether seasonal changes in BMR represented a separate acclimation or acclimatization response or was merely variation in working capacity.

In avian comparative studies, much focus is placed on the origin of study birds in terms of captive bred or wild caught populations and the effect of this factor on BMR (e.g. Weathers et al., 1983; McKechnie et al., 2007; McKechnie, In Press), but this does not take into consideration the geographic and consequent altitudinal origin
of the wild caught species as a possible source of variation. Previous studies in avian thermal biology have examined metabolic adaptations along an aridity gradient (e.g. Tieleman et al., 2002), the effects of seasonal and environmental changes on BMR of a species (e.g. Hart, 1962; Dawson and Carey, 1976; Weathers and Caccamise, 1978; Ambrose and Bradshaw, 1988; Maddocks and Geiser, 2000; Smit et al. In Press), phenotypic flexibility in BMRs of one population as a representative of a species (e.g. McKechnie et al., 2007), or have assessed population responses to climate change as a mean response at the population level (e.g. Møller et al., 2004). Published data often represents a single BMR or RMR value per species, regardless of altitudinal origin, or alternatively data from one population as a representative of an entire species (e.g. Bech, 1980; Cooper and Swanson, 1994; Boix-Hinzen and Lovegrove, 1998; Maddocks and Geiser, 2000; McKechnie and Lovegrove, 2001; Downs and Brown, 2002; López-Calleja and Bozinovic, 2003; Lovegrove and Smith, 2003; McKechnie et al., 2007), thus highlighting the need to acknowledge the role of phenotypic flexibility within a species.

Very few avian studies, however, have looked at variation in the thermal physiology of a species over an altitudinal gradient (Soobramoney et al., 2003). More specifically, in this case, few studies have looked at phenotypic plasticity or flexibility within a subpopulation pre- and post-acclimation, and over an altitudinal gradient, as well as examining altitudinal intra-specific variation in BMR. Thus the fact that plasticity may exist in phenotypic flexibility, with respect to physiological parameters, is not acknowledged.

This is an oversight in metabolic studies. For example, McNab (2003) found that 99% of the observed variation in the BMR of Birds of Paradise (Family Paradisaeidae) was due to inter-specific variation in body mass, food habits and distribution over an altitudinal gradient. Although McNab (2003) focused on inter-
species differences, one can assume that if inter-species differences can be attributed to altitude, that subpopulations of the same species would also display variation in certain bioenergetic parameters as a result of existing in a non- or local migratory manner over an altitudinal gradient.

Evaporative water loss (EWL) plays an important role in thermoregulation when ambient temperature exceeds body temperature. Thus the rate at which water is lost from an animal has important implications for thermoregulation and thus survival (McKechnie and Wolf, 2004).

The Amethyst Sunbird, *Chalcomitra amethystina* (Shaw, 1811), is a relatively large African nectarivorous sunbird with a mean mass of approximately 15g (Cheke et al., 2001; Tree, 2005). Adult Amethyst Sunbirds exhibit sexual dimorphism. Adult males have blackish-brown plumage with purplish-copper on the throat and shoulders and silvery light green on their heads whereas females are grey-brown with pale grey brown underbellies (Cheke et al., 2001; Tree, 2005). Amethyst Sunbirds occupy a broad geographical region within South Africa which includes an altitudinal gradient from the Drakensberg mountain range to the coast of KwaZulu-Natal (KZN) (Cheke et al., 2001). Their populations in KZN are described as being fairly sedentary, with some localised winter movement (Tree, 2005).

The current knowledge of the effects of climate change on birds is mostly restricted to passerines from northern hemispheric temperate zones, and more work is needed on their southern hemisphere counterparts (Møller et al., 2004). Thus this study aims to address this by looking at the way in which subpopulations of the same species survive over an altitudinal gradient and thus a range of temperatures, as well as how they adapt to changes in ambient temperature.

We predicted that the metabolic rates of subpopulations of Amethyst Sunbirds would vary over the altitudinal gradient due to acclimatization and adaptations to
different temperatures, as well as pre- and post-acclimation due to innate physiological differences in approach to acclimation to 25°C and the Pietermaritzburg altitude (660m). BMR is generally thought to be species specific, but we predicted plasticity/flexibility between subpopulations, within a species, due to temperature differences over altitude.

In addition, as hematocrit levels (% red blood cell concentration) generally increase with altitude in mammals (Willmer et al., 2005), it was predicted that in this avian species hematocrit values, would vary from high to low altitudes as a result of a change in oxygen concentration.

**Materials and Methods**

*Study site, Bird capture and maintenance*

Amethyst Sunbirds were captured in the winter of 2006 (May-June) at three different locations in KZN, South Africa, under permit from Ezemvelo KZN-Wildlife, using mist-nets. The three sites were: Underberg (29°47.614S, 29°30.319E, 1553 m above sea level, n = 9), Howick (29°28.203S, 30°13.316E, 1075 m above sea level, n = 6) and Oribi Gorge (30°40.067S, 30°15.316E, 541 m above sea level, n = 8). Study individuals were weighed and colour banded to allow for capture location and individual identification (Downs & Brown, 2002).

After capture birds were transferred to the University of KwaZulu-Natal (UKZN) Pietermaritzburg campus’s Animal house, at an altitude of 660 m, where they were housed individually in cages (1 x 0.35 x 0.5m) in a constant environment (CE) room. Room temperature was set at 25°C with a 12L:12D photoperiod for the study duration (May-September 2006). Artificial nectar (20% sucrose plus Ensure®), as well as water, were available *ad libitum* to birds from nectar feeders in cages.
Fruitflies were bred on rotting fruit in the room in which birds were housed, to supplement protein intake.

Birds were allowed to feed during the day prior to VO$_2$ measurements due to their rapid food transit times (Downs, 1997; Downs & Brown, 2002), but deprived of food during trials. Thus it was assumed that birds were post absorptive and that resting metabolic rate (RMR) was measured.

Metabolic Measurements and Protocol

Sunbirds were kept in the animal house for one night after capture before pre-acclimation respirometry trials to reduce the effect of transport stress. Acclimation is regarded as changes in the organism in response to changes in any component in the environment of the laboratory (Garland and Adolph 1991; Smit et al., In Press).

Metabolic rate was measured indirectly by quantifying oxygen consumption (VO$_2$) using a respirometer. The respirometer was switched on at least one hour prior to commencement of respirometry trials. Birds were weighed and then placed individually in respirometry chambers between 16h30 and 17h00. Respirometry chambers of clear perspex were used (volume = 3.96l), and contained a wooden perch as well as a wire grid at the base. Respirometry chambers were placed in a sound-proof Conviron® cabinet (1m$^3$). Light dark photoperiods were set in synchronization with that of the constant environment room where birds were housed (12L:12D). Conviron® cabinet temperature (T$_a$) was measured using thermistor probes calibrated with a standard mercury thermometer (0.05°C) in a water bath at temperatures 5-45°C.

Air flow was controlled using a computerized open flow-through system (Depocas and Hart, 1957; Hill, 1972). Atmospheric air was pumped in and partially dried using silica gel, before reaching the Conviron® cabinet. Flow rate was
maintained at a level that ensured <1% change in oxygen concentration, between 0.500 and 0.600 l.h\(^{-1}\) (Downs and Brown, 2002). The flow rate of each chamber was measured using a Brooks thermal mass flow meter (Model 580E) factory calibrated to STP. A steady flow of air through the chamber was ensured as air entered the bottom and was expelled through the top of the respirometry chambers. Simultaneous measurements of six chambers (five experimental and one control chamber) was achieved by using solenoid valves and a separate pump for each chamber.

Excurrent air was passed through a water condenser (a copper tube in which air was cooled to approximately 3°C, or below dew point) to remove water vapour, and soda lime, to remove Carbon Dioxide (CO\(_2\)). An oxygen analyzer (Model S-3A/1, Ametek) was used to determine the fractional concentration Oxygen (O\(_2\)) in dry air samples. The fractional concentration of O\(_2\) in the control chamber was measured at the start of every six minute cycle, and the O\(_2\) values from the experimental chambers were then subtracted from this value. The problem of long term drift in O\(_2\) analyzer outputs was thus limited to that which would occur in five minutes cycles. To further ensure the accuracy of measurements, a cell restore was run on the oxygen analyser every two weeks, and the oxygen analyser was calibrated regularly. Measurements of the various parameters for each chamber (T\(_a\), flow rate and fractional O\(_2\) concentrations) were recorded at the end of each 45s sampling interval, so as to allow sufficient time for the flushing of air from the previous channel from the ducting between relay valves and the sub sample tubing. VO\(_2\) was recorded digitally every six minutes, was corrected for standard temperature and pressure and expressed as a mass specific value. Thus ten readings per individual were recorded on an hourly basis.

Analog signals from the thermistor probes, mass flow meter and oxygen analyzer were recorded digitally using an A/D converter and software written by R. Van Zyl, UKZN.
The following equation was used to calculate mass specific oxygen consumption:

$$\text{VO}_2 = \frac{V_E(F_{IO2}-F_{EO2})/(1-F_{IO2})}{M_b} \text{ equation (1)}$$

Where $\text{VO}_2$ = metabolic rate (ml O$_2$/g/h), $V_E$ = flow rate (ml min$^{-1}$), $F_{IO2}$ = incurrent fractional O$_2$ concentration and $F_{EO2}$ = excurrent fractional O$_2$ concentration and $M_b$ = body mass (g) (Hill, 1972).

At 07h00 the following morning evaporative water loss (EWL) was recorded by measuring the amount of water collected from the excurrent air of each chamber by the water condenser. Birds were removed from the chambers, weighed and returned to their cages in the CE room in the Animal House. Food and water were available to them ad lib.

Pre-acclimation VO$_2$ values were measured at ambient temperatures of 25°C (assumed to be in the TNZ) and 5°C (assumed to be at an extreme) within four days of capture using an interspersed design within each subpopulation.

After the initial trials, birds were acclimated in the CE room for six weeks as described. This long acclimation period was necessary to ensure that sunbirds were all acclimated to the same conditions. After this time VO$_2$ measurements were repeated as in the earlier trials at randomly ordered ambient temperatures of 15, 5, 10, 20, 30, 28, 25 and 33°C, to ensure the absence of temperature acclimation. Birds were carefully monitored at 33°C and removed at approximately 21h00.
**Blood hematocrit measurements**

Hematocrit protocol was designed and carried out according to Soobramoney et al. (2005) and Yahav et al. (1996) with modifications. Blood samples for hematocrit measurements were taken upon capture from the brachial vein using 32 mm microcapillary tubes (5ul, Compur, Drummond). These were spun in a Compur M1101 mini-centrifuge for 8 minutes before % red blood cell readings were taken. Hematocrit measurements were repeated for each individual bird pre-release, approximately seven weeks after capture. Additional blood samples were taken for future genetic analyses.

**Release**

Birds were weighed and released back at the original capture site upon completion of respirometry trials.

**Statistical analyses**

Descriptive statistics were calculated in STATISTICA (Statsoft, Tulsa, USA) for each subpopulation of Amethyst Sunbirds. Hourly rates of VO\(_2\) for individuals from each subpopulation of sunbirds were determined and plotted against time for each T\(_a\). The minimum RMR at each of these temperatures for each individual was used in analysis to determine change with temperature using Generalized Linear Models (commonly called GLIM) Repeated Measures Analysis of Variance (RMANOVA). BMR was calculated by taking the lowest mean RMR per subpopulation. The TNZ was determined using Post-hoc Sheffé tests to determine over what range minimum RMR did not differ significantly. GLIM RMANOVA was further used for the comparison of VO\(_2\) measurements between populations at different altitudes and between pre- and post-acclimation data as well as to determine whether birds maintained constant blood
hematocrit levels. Post-hoc Sheffé tests were done to determine where significant interactions occurred (p < 0.05), between populations and within population pre- and post acclimation. Data were presented as mean ± SE of the individuals measured (n). Percentage change in MR (BMR or RMR) between populations was calculated by the following equation: (higher altitude MR- lower altitude MR)/ higher altitude MR)*100. Similarly, percentage change between pre- and post-acclimation MR values were calculated using the following equation: (Pre-acclimation MR- Post-acclimation MR)/ Pre-acclimation MR)*100).

Results

Pre- vs post-acclimation

Acclimation had a significant effect on VO\textsubscript{2} values for Amethyst Sunbird subpopulations at T\textsubscript{a} = 5°C when compared within and between sites (Fig. 1a, RMANOVA, F\textsubscript{(2, 8)} = 14.977, p = 0.002). Mean VO\textsubscript{2} values at 5°C changed significantly between pre- and post-acclimation for Underberg (n = 9) and Oribi Gorge (n = 8) subpopulations (Post-hoc Sheffé test, p < 0.05).

The Underberg subpopulation showed an 51.8% increase in VO\textsubscript{2} from pre- to post-acclimation at 5°C from 8.44 ± 0.170mlO\textsubscript{2}g\textsuperscript{-1}h\textsuperscript{-1} (0.047 W) to 12.81 ± 0.949mlO\textsubscript{2}g\textsuperscript{-1}h\textsuperscript{-1} (0.071 W). Oribi Gorge sunbirds showed a 52.5% decrease in VO\textsubscript{2} between pre-and post-acclimation trails at 5°C, from 12.59 ± 0.699 mlO\textsubscript{2}g\textsuperscript{-1}h\textsuperscript{-1} (0.070 W) to 5.98 ± 0.499 mlO\textsubscript{2}g\textsuperscript{-1}h\textsuperscript{-1} (0.033 W). Howick sunbirds did not show a significant difference between pre- and post-acclimation VO\textsubscript{2} at 5°C (Post-hoc Sheffé , p > 0.05), with a marginal 7.2% decrease in VO\textsubscript{2} between pre- and post-acclimation, from 15.94 ± 0.426 mlO\textsubscript{2}g\textsuperscript{-1}h\textsuperscript{-1} (0.089 W) to 14.79 ± 1.337 mlO\textsubscript{2}g\textsuperscript{-1}h\textsuperscript{-1} (0.083 W).

Further analysis of pre-acclimation VO\textsubscript{2} values at 5°C (Fig. 1a) showed a significant difference between Underberg and Howick (Post-hoc Sheffé, p < 0.05),
and Underberg and Oribi Gorge (Post-hoc Sheffé, p < 0.05), but not between Howick and Oribi Gorge subpopulations (Post-hoc Sheffé, p > 0.05). However, post-acclimation subpopulation comparisons indicated a significant difference between Underberg and Oribi Gorge (Post-hoc Sheffé, p < 0.05), Howick and Oribi Gorge (Post-hoc Sheffé, p < 0.05), but no significant difference in VO\(_2\) values between Underberg and Howick subpopulations (Fig. 1a, Post-hoc Sheffé, p > 0.05).

Acclimation had a significant effect on VO\(_2\) values for Amethyst Sunbird subpopulations at T\(_a\) = 25°C when compared within and between sites. (Fig. 1b, RMANOVA, F\(_{2, 10}\) = 10.345, p = 0.004). Significant variation existed between Underberg and Howick, and Underberg and Oribi Gorge subpopulations post-acclimation to 25°C, in 25°C trials (Post-hoc Sheffé, p < 0.05). The Underberg subpopulation showed very little change between pre- and post-acclimation trials at 25°C with a decrease of only 2.9% from 6.71 ± 0.146 mlO\(_2\)g\(^{-1}\)h\(^{-1}\) (0.037 W) to 6.52 ± 0.493 mlO\(_2\)g\(^{-1}\)h\(^{-1}\) (0.036 W) (Post-hoc Sheffé, p > 0.05). VO\(_2\) for Howick and Oribi Gorge subpopulations decreased significantly between pre- and post-acclimation trials (Post-hoc Sheffé, p < 0.05), with the VO\(_2\) of Howick subpopulations decreasing by 58.4% (from 7.20 ± 0.447 mlO\(_2\)g\(^{-1}\)h\(^{-1}\) (0.040 W) to 3.00 ± 0.386 mlO\(_2\)g\(^{-1}\)h\(^{-1}\) (0.017 W)) from pre- to post-acclimation, and Oribi Gorge sunbirds exhibiting a 48.7% decrease in VO\(_2\) (from 7.48 ± 0.742 mlO\(_2\)g\(^{-1}\)h\(^{-1}\) or 0.042 W to 3.84 ± 0.387 mlO\(_2\)g\(^{-1}\)h\(^{-1}\) or 0.021 W) from pre- to post-acclimation trials at 25°C.

The Underberg subpopulation of Amethyst Sunbirds (Fig. 2), showed a much greater within individual variation post-acclimation at 5°C than pre-acclimation at the same temperature, however very similar variation between pre- and post-acclimation at 25°C. At 5°C, Howick sunbirds showed greater between individual variation pre-acclimation, but a similar between individual variation pre- and post-acclimation at 25°C (Fig. 3). Oribi Gorge pre- and post-acclimation subpopulation data (Fig. 4)
showed more variation between individuals pre-acclimation at 5°C, but similar variation between pre- and post-acclimation at 25°C.

**Post-acclimation**

The VO$_2$ of different altitudinal subpopulations of Amethyst Sunbirds (Figs 5, 6 and 7) levelled off to RMR between 19h00 - 05h00. VO$_2$ increased pre-dawn (06h00) starting at approximately 05h00, and VO$_2$ started to decrease pre-sunset (18h00). Oribi Gorge displayed the lowest inter-individual variation (Fig. 7), and Underberg the highest inter-individual variation (Fig. 5) over the range of ambient temperatures. The Howick subpopulation of sunbirds displayed a high inter-individual variation in VO$_2$ at $T_a = 5-20, 33°C$ (Fig. 6), then a decrease in individual variation from $T_a = 25-30°C$, thus corresponding with the thermal neutral zone (TNZ). Inter-individual variation increased as altitude increased.

Mean resting metabolic rates for each of the different Amethyst Sunbirds subpopulations over the range of ambient temperatures are summarised in Figure 8. There was little variation in VO$_2$ between individuals from the same locality, whereas significant variation was observed at the same temperatures between localities and thus between altitudes. There was a significant effect of altitude and temperature when comparing VO$_2$ values for the subpopulations of sunbirds (RMANOVA, $F_{(14, 70)} = 21.039$, $p < 0.001$). There was a significant difference between the VO$_2$ values of Underberg and Oribi Gorge subpopulations at 5, 10, 15, 20, 28, 30°C (Post-hoc Sheffé, $p < 0.05$), and between Underberg and Howick subpopulations at 25 and 28°C (Post-hoc Sheffé, $p < 0.05$), and finally between Howick and Oribi Gorge subpopulations at 5, 10 and 15 °C (Post-hoc Sheffé, $p < 0.05$). BMR was determined for each subpopulation by using the mean of the lowest hourly individual RMRs: Underberg ($5.71 \pm 0.402 \text{ mlO}_2\text{g}^{-1}\text{h}^{-1}$ (0.032 W) at 33°C), Howick ($2.46 \pm 0.299$
mlO₂g⁻¹h⁻¹ (0.014 W) at 28°C) and Oribi Gorge (3.49 ± 0.312 mlO₂g⁻¹h⁻¹ (0.019 W) at 30°C).

Overall there was a significant effect of altitude on BMR (RMANOVA, F(2,10) = 15.183, p = 0.001). BMR decreased significantly from Underberg to Howick by 56.9% (Post-hoc Sheffé, p < 0.05), and by 38.9% from Underberg to Oribi Gorge (Post-hoc Sheffé, p < 0.05). However comparison of the BMR data from Howick to Oribi Gorge showed a non-significant increase of 41.7% (Post-hoc Sheffé, p > 0.05).

Post-hoc Sheffé tests (p < 0.05) were used to determine the thermal neutral zone (TNZ) and differences in the TNZ range between altitudinal subpopulations were evident, (Underberg = 15-33°C, Howick = 25-30°C and Oribi Gorge = 5-33°C), with the subpopulation at the lowest altitude having the broadest TNZ.

**Hematocrit**

There was a significant overall interaction of acclimation and altitude in hematocrit values of Amethyst Sunbirds (Fig. 9, RMANOVA , F(2, 10) = 5.010, p = 0.031). However only Oribi Gorge sunbirds showed a significant change in hematocrit levels post-acclimation (Post-hoc Sheffé test, p < 0.05). There was more variation about the mean pre-acclimation for all locations, than post-acclimation (Fig. 9).

**Evaporative water loss**

Evaporative water loss (EWL) of three altitudinal subpopulations of Amethyst Sunbirds over the range of experimental temperatures differed significantly (Fig. 10. RMANOVA, F(12, 60) = 4.8092, p < 0.001). However, post-hoc tests revealed that only the Oribi Gorge subpopulation’s EWL measured at 33°C differed significantly from other EWL measurements (Post-hoc Sheffé test, p < 0.05). Generally sunbirds had lower EWL below the TNZ.
Body Mass

There was no significant difference between mean pre- and post-acclimation mass (g) of Amethyst Sunbirds within sites nor was there a significant difference between pre- and post-acclimation masses between altitudinal subpopulations of Amethyst Sunbirds (Fig. 11, RMANOVA, \( F_{2, 22} = 0.345, p = 0.712 \)).

Ambient temperature

Ambient temperatures for the three altitudinal locations (January 2004- May 2007), Underberg (Shaleburn), Howick (Cedara), and Oribi Gorge (Paddock) are represented in Table 1. as per data obtained from the South African Weather Service. Underberg consistently had lower ambient temperatures over the winter months (May- August) than the other altitudinal locations. Howick had higher mean ambient temperatures than Oribi Gorge in the winter months.

Discussion

Many metabolic studies (particularly comparative studies), both avian and other, have used a mean BMR for a population regardless of capture location, or alternatively have used data from one population as a representative for an entire species (e.g. Bech, 1980; Cooper and Swanson, 1994; Boix-Hinzen and Lovegrove, 1998; Maddocks and Geiser, 2000; McKechnie and Lovegrove, 2001; López-Calleja and Bozinovic, 2003; Lovegrove and Smith, 2003; McKechnie et al., 2007; Smit et al., In Press). However, this study showed that populations respond differently to acclimation, possibly as a result of persistent underlying physiological differences, or persistent effects of altitudinal acclimatization. Recent evidence suggests that winter BMR of species living in highly seasonal environments reflects the conditions in which the animal existed in immediately prior to metabolic
measurements being taken (McKechnie, In Press). As a result of this, after the six week acclimation period to 25°C and an altitude of 660m, we would expect birds from all of the altitudinal subpopulations to react similarly if not uniformly to the range of ambient temperatures of the respirometry trials, and thus differently to pre-acclimation trials, thereby displaying phenotypic flexibility. However, differences in MR, and in particular BMR, between subpopulations in post-acclimation trials indicate that this is not necessarily the case. Altitudinal subpopulations still showed differences in MR post-acclimation, thus indicating that captive bred populations of birds would not represent the entire species as well as assumed, unless altitudinal origin of the original population is known, and laboratory populations represent subpopulations over the entire altitudinal gradient occupied by the species. It is also difficult to assess the end point of thermal acclimation (Rezende et al., 2004; Bush, 2007) and thus it is possible that this was merely a stage in the ability of Amethyst Sunbirds to change their thermal phenotype over a longer period of time.

The fact that there was no significant difference between the post-acclimation masses of the three subpopulations of Amethyst Sunbirds shows that this difference is not a result of the effects of body mass and thus could indicate a difference in acclimation strategies, most likely as a result of adapting to live in different altitudinal and thus thermal environments. This further emphasizes the need for knowledge of the origin of study populations, and not just in terms or captive vs. wild caught populations. In recent comparative avian reviews, much focus is placed on whether study birds were captive-bred or wild caught (e.g. Weathers et al., 1983; McKechnie et al, 2007; McKechnie, In Press) and on phenotypic flexibility. However, changing views in avian physiology as yet omit to recognize the altitudinal origin of the study population. A review by McKechnie (In Press) recognized that the data represented in the literature often uses a single BMR
value per species and is assumed to represent a fixed species specific value. Indeed, most studies of avian thermoregulatory abilities have used a mean BMR from one population as a representative for the entire species, which does not take into consideration the altitudinal origin of the study animals, nor does it acknowledge the fact that phenotypic flexibility, with respect to physiological parameters, may not be consistent throughout a species.

Amethyst Sunbirds exhibited significant differences in pre-acclimation metabolic rates at both 5 and 25°C, indicating that altitudinal acclimatization plays a big role in sunbird physiology at any point in time. The results also indicate that different subpopulations show different responses to acclimation, and that differences in TNZ were evident post-acclimation, which indicated that physiological differences were not just a result of acclimation to the temperature and altitude of the acclimation site. Post-acclimation results also showed significant differences in VO₂ between Underberg and Oribi Gorge subpopulations at 5, 10, 15, 20, 28, 30°C, and between Underberg and Howick Amethyst Sunbird subpopulations at 25, 28°C and between Howick and Oribi Gorge subpopulations at 5, 10 and 15°C. Similarly, Soobramoney et al. (2003) found that there was a difference in metabolic rates of the Common Fiscal (*Lanius collaris*) over an altitudinal temperature gradient as colder temperatures at high altitudes require an increase in metabolic heat production in homeotherms. However, Common Fiscals showed higher metabolic rates in subpopulations from the warmer altitudes, whereas Amethyst Sunbirds subpopulations from the warmer location (Oribi Gorge, lowest altitude) showed lower metabolic rates. As winter temperatures vary dramatically between the two habitats it would be expected that individuals that could survive in that range of ambient temperatures would be selected for over the generations and thus we would expect underlying physiological differences between altitudinal subpopulations.
Our data, and that of other altitudinal studies, emphasizes the need to acknowledge altitudinal differences between populations and not just use species means, as species means do not fully incorporate the effect of phenotypic plasticity/flexibility.

We predicted that as altitude increased, and atmospheric oxygen concentration decreased, hematocrit values would increase in order to facilitate a higher oxygen uptake to assist in regulating MR in a species that occupies habitats over an altitudinal gradient. A study by Soobramoney et al. (2005) on the Common Fiscal showed an altitudinal difference in hematocrit values (32.5 - 48.3%). In contrast, hematocrit levels between altitudinal subpopulations of Amethyst Sunbirds showed no significant differences pre- or post-acclimation. However, Oribi Gorge sunbirds showed a significant decrease in hematocrit levels between pre- and post-acclimation. Blood hematocrit levels for avian species typically range between 30- 45% (Willmer et al., 2005). The optimum for blood hematocrit levels is 50%, above which circulatory problems could result due to increased viscosity (Yahav et al., 1996). Overall, hematocrit levels for Amethyst Sunbirds were high when compared with other avian species and predicted values (e.g. Jones and Johansen, 1972; Soobramoney et al., 2005). However, Amethyst Sunbirds have achieved, if not exceeded a hematocrit level of 50%, pre-acclimation. Similar to this, Rosy finches (Leucosticte arctoa) and House Finches (Carpodacus mexicanus) (Clemens, 1988) showed mean hematocrit values of 53% and 64- 68% respectively at high altitudes (3800m). Broiler Chickens (Gallus domesticus) on the other hand, have a mean hematocrit level of approximately 30% (Yahav et al., 1996). Possibly, as a result of these discrepancies in expected and observed hematocrit ranges, the hematocrit range of avian species needs to be re-examined, taking into consideration the altitudinal origin of studied individuals.
As ambient temperature increases, evaporative cooling is required in order to assist endotherms in maintaining a constant body temperature, and thus we would expect higher EWL at higher ambient temperatures. A study on Spinifexbirds, *Eremiornis carteri* (body mass ± 13 g) (Ambrose and Bradshaw, 1988) showed an increase in EWL with increase in $T_a$ particularly in and above the TNZ. As is typical in most birds, the same trend was found in Amethyst Sunbird subpopulations over a range of ambient temperatures. However, the Oribi Gorge subpopulation showed the highest levels of EWL, which is to be expected for the altitudinal subpopulation adapted to the higher ambient temperature range and thus requiring higher levels of evaporative cooling. In many small mammals, such as *Gerbillurus* species, short-term evaporative cooling is employed to deal with hyperthermia above the thermal neutral zone (Downs and Perrin, 1990). However, Amethyst Sunbirds showed an increase in EWL, particularly Oribi Gorge sunbirds, within their thermal neutral zone at higher temperatures.

**Conclusion**

Variation exists in RMR, BMR and TNZ between populations of Amethyst Sunbirds over an altitudinal gradient. Variation persisted, if not increased, post-acclimation, indicating phenotypic flexibility within the species. Thus acclimation time should be taken into account. Hematocrit levels were generally high and did not change significantly. Physiological phenotypic flexibility within a species indicates differing abilities to adapt to climate change and thus may lead to different survival predictions for each population. Thus one subpopulation should not be used as a representative of a species, and location and altitude of experimental subpopulations should be taken into account when making species predictions or comparing species.
Acknowledgements

CL would like to thank the NRF and the Gay Langmuir bursary fund for financial assistance. We would also like to thank Mike and Heidi Neethling (Oribi Gorge), Bill and Pam Nicol (Howick) and Prof. and Mrs. Piper (Underberg) for allowing us to “borrow” their Amethyst Sunbirds for the study period. Nicolette Taylor, John Lindsay and Ashton Musgrave are thanked for their invaluable field trip assistance. Thanks also to students at UKZN for assisting with feeding and bird maintenance.

List of symbols and abbreviations

BMR Basal Metabolic Rate
RMR Resting Metabolic Rate
EWL Evaporative Water Loss
TNZ Thermal Neutral Zone
VO₂ Oxygen consumption

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**Figure 3.** Pre- vs. post-acclimation VO₂ (mLO₂g⁻¹h⁻¹) of the Howick subpopulation of Amethyst Sunbirds in winter, 17h00-07h00 at 5 and 25°C (mean ± SE, n = 6).

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Physiological variation in Amethyst Sunbirds (*Chalcomitra amethystina*) over an altitudinal gradient. Part B: in summer.

Claire Lindsay\(^1\), Colleen Downs\(^1\) & Mark Brown\(^1\)

\(^1\) School of Biological and Conservation Sciences, University of KwaZulu-Natal, Private Bag X01, Pietermaritzburg, 3201, South Africa

Amethyst Sunbirds (*Chalcomitra amethystina*) occur over an altitudinal gradient within KwaZulu-Natal, South Africa, from the Drakensberg mountain range to the coast. Sunbirds were caught in summer (November - December 2006) at three altitudinal locations within KwaZulu-Natal; Underberg (1553m), Howick (1075m) and Oribi Gorge (541m). Oxygen consumption (\(\text{VO}_2\)) was measured pre-acclimation at 5 and 25°C. After post-acclimation to 25°C and 660m for 6 weeks, \(\text{VO}_2\) measurements were taken at 8 different temperatures (15, 5, 10, 20, 30, 28, 25 and 33°C). Pre- and post-acclimation hematocrit levels were measured. Resting metabolic rates differed significantly between altitudinal subpopulations of Amethyst Sunbirds. Basal metabolic rate was observed to decrease as altitude decreased. This summer research complemented an earlier study on altitudinal variation in Amethyst sunbirds in winter. It again emphasized the need to acknowledge altitudinal differences between subpopulations and not just use species means, as species means do not fully incorporate the effect of phenotypic plasticity/flexibility.
Keywords: Altitudinal variation, Amethyst Sunbird, metabolic rates, phenotypic plasticity, phenotypic flexibility.

Introduction

This is a continuation of an investigation into phenotypic plasticity/flexibility over an altitudinal gradient as described earlier (Chapter 2). Birds are considered to be homeothermic endotherms, which implies the ability to maintain a constant body temperature over a broad range of ambient temperatures by adjusting their metabolism (Chaui-Berlinck et al., 2002). According to McKechnie et al. (2007), many birds display phenotypic flexibility in maintenance energy requirements, and are able to up or down regulate basal metabolic rate (BMR) over a period of time during thermal acclimation.

Amethyst Sunbirds (*Chalcomitra amethystina*), relatively large African sunbirds (mean body mass 15g) (Cheke et al., 2001; Tree, 2005), occupy a relatively broad geographical region within South Africa which includes an altitudinal gradient within KwaZulu-Natal (KZN), from the Drakensberg mountain range to the coastal regions (Cheke et al., 2001). The distribution of Amethyst Sunbirds over an altitudinal gradient makes them an ideal species in which to study plasticity in thermal physiology within a species, as well as flexibility within a population.

As in the winter study, it was predicted that the metabolic rates of subpopulations of Amethyst Sunbirds would vary over the altitudinal gradient due to acclimatization and adaptations to different temperatures, as well as pre- and post-acclimation due to innate physiological differences in approach to acclimation to 25°C and the Pietermaritzburg altitude (660m). BMR is generally thought to be
species specific, however, it was predicted that plasticity/flexibility would occur between subpopulations, within a species, due to temperature differences over altitude. Similarly to the winter study, it was predicted that hematocrit values (% red blood cell concentration), would vary from high to low altitudes as a result of a change in oxygen concentration in atmospheric air and that evaporative water loss would increase with increasing ambient temperatures.

Materials and Methods

Study site, capture and maintenance

Amethyst Sunbirds were captured in the summer of 2006 (November-December) at three different locations in KZN, South Africa, under permit from Ezemvelo KZN-Wildlife, using mist-nets. The three sites were: Underberg (29°47.614S, 29°30.319E, 1553 m above sea level, n = 10), Howick (29°28.203S, 30°13.316E, 1075 m above sea level, n = 10) and Oribi Gorge (30°40.067S, 30°15.316E, 541 m above sea level, n = 10). Study individuals were weighed and colour banded to allow for capture location and individual identification (Downs & Brown, 2002).

After capture birds were transferred to the University of KwaZulu-Natal, Pietermaritzburg campuses’ Animal House, at an altitude of 660 m, where they were housed individually in cages (1 x 0.35 x 0.5m). Room temperature was set at 25°C with a 12L:12D photoperiod for the study duration (November 2006 - March 2007). Artificial nectar (20% sucrose plus Ensure®, Abbott Laboratories SA Ltd, Johannesburg, South Africa), as well as water, were available ad libitum to birds from nectar feeders in cages. Fruitflies were bred on rotting fruit in the room in which birds were housed, to supplement protein intake.
Birds were allowed to feed during the day prior to oxygen consumption (VO$_2$) measurements due to their rapid food transit times (Downs, 1997; Downs and Brown, 2002), but deprived of food during trials. Thus it was assumed that birds were post absorptive and that resting metabolic rate (RMR) was measured. None of the birds used in the experiments showed any indication of breeding activity, indicated by the absence of brood patches upon capture.

**Metabolic measurements and protocol**

Sunbirds were kept in the animal house for one night before pre-acclimation respirometry trials to reduce the effect of transport stress.

Metabolic rate was measured indirectly by quantifying oxygen consumption (VO$_2$) overnight, pre-acclimation at 5 and 25°C, and post-acclimation at 15, 5, 10, 20, 30, 28, 25 and 33°C, using a respirometry protocol as per Chapter 2. Flow rate was maintained as per winter trials, between 0.500 and 0.600 l.h$^{-1}$, ensuring <1% change in oxygen concentration.

**Blood hematocrit measurements**

Hematocrit protocol was performed according to Soobramoney et al. (2005) and Yahav et al. (1996) with modifications as per Chapter 2.

**Statistical analyses**

Descriptive statistics were calculated in STATISTICA (Statsoft, Tulsa, USA) for each subpopulation. The minimum RMR at each of these temperatures for each individual was used in analysis to determine change with temperature using Generalized Linear Models (commonly called GLIM) Repeated Measures Analysis of Variance.
BMR was calculated by taking the lowest mean RMR per subpopulation. The TNZ was determined using Post-hoc Sheffé tests to determine over what range minimum RMR did not differ significantly. GLIM RMANOVA was further used for the comparison of VO$_2$ measurements between populations at different altitudes and between pre- and post-acclimation data as well as to determine whether birds maintained constant blood hematocrit levels. Post-hoc Sheffé tests were done to determine where significant differences occurred (p < 0.05), between populations and within each population pre- and post-acclimation. Data were presented as mean ± SE of the individuals measured (n). As in winter trials percentage change in MR (BMR or RMR) between populations was calculated by the following equation: (higher altitude MR - lower altitude MR)/ higher altitude MR)*100. Similarly, percentage change between pre and post-acclimation MR values were calculated using the following equation: (Pre-acclimation MR - Post-acclimation MR)/ Pre-acclimation MR)*100).

Release

Birds were weighed and released back at the original capture site upon completion of respirometry trials.

Results

Pre- vs post-acclimation

There was no significant combined effect of altitude and acclimation on Amethyst Sunbird VO$_2$ at 5°C (Fig. 1a, RMANOVA, $F_{(2, 18)} = 1.634$, p = 0.223). However, all of the altitudinal subpopulations showed significant differences between pre- and post-acclimation VO$_2$ values at $T_a = 5°C$ (RMANOVA, $F_{(1, 9)} = 60.660$, p < 0.001). At 5°C,
the Underberg subpopulation of sunbirds showed a significant increase in VO\textsubscript{2} of 80.9\% between pre- and post-acclimation trials (Post-hoc Sheffé, p < 0.05), from 4.47 ± 0.263 mLO\textsubscript{2}g\textsuperscript{-1}h\textsuperscript{-1} (0.025 W) to 8.09 ± 0.511 mLO\textsubscript{2}g\textsuperscript{-1}h\textsuperscript{-1} (0.045 W). The Howick subpopulation showed a significant increase in VO\textsubscript{2} of 20.9\% (Post-hoc Sheffé, p < 0.05), from 6.08 ± 0.350 mLO\textsubscript{2}g\textsuperscript{-1}h\textsuperscript{-1} (0.034 W) to 7.35 ± 0.481 mLO\textsubscript{2}g\textsuperscript{-1}h\textsuperscript{-1} (0.041 W). The Oribi Gorge subpopulation, however, showed a significant decrease in VO\textsubscript{2} of 19.5\% (Post-hoc Sheffé, p < 0.05) between pre- and post-acclimation trials from 7.36 ± 0.450 mLO\textsubscript{2}g\textsuperscript{-1}h\textsuperscript{-1} (0.041 W) to 5.93 ± 0.398 mLO\textsubscript{2}g\textsuperscript{-1}h\textsuperscript{-1} (0.033 W). There was a significant difference in VO\textsubscript{2} values between sites (RMANOVA, F\textsubscript{(2, 18)} = 74.976, p < 0.001). Pre-acclimation VO\textsubscript{2} values at 5°C showed between site significance for all sites (Post-hoc Sheffé, p < 0.05). Post-acclimation, post-hoc tests showed a significant difference between Underberg and Oribi Gorge, and between Howick and Oribi Gorge subpopulations (Post-hoc Sheffé, p < 0.05), however differences in VO\textsubscript{2} values between Underberg and Howick subpopulations of sunbirds were not significant (Post-hoc Sheffé, p < 0.05).

At T\textsubscript{a} = 25°C there was a significant combined effect of altitude and acclimation on Amethyst Sunbird VO\textsubscript{2} values (Fig. 1b, RMANOVA, F\textsubscript{(2, 18)} = 5.266, p = 0.016). The Underberg subpopulation (Fig. 2) showed a significant increase of 85.2\% in VO\textsubscript{2} (Post-hoc Sheffé, p < 0.05) between pre-and post-acclimation values from 2.30 ± 0.143 mLO\textsubscript{2}g\textsuperscript{-1}h\textsuperscript{-1} (0.013 W) to 4.26 ± 0.255 mLO\textsubscript{2}g\textsuperscript{-1}h\textsuperscript{-1} (0.024 W). Sunbirds from Howick (Fig. 3) showed a significant 42.8\% increase in VO\textsubscript{2} (Post-hoc Sheffé, p < 0.05) from pre- to post-acclimation trials from 2.81 ± 0.352 mLO\textsubscript{2}g\textsuperscript{-1}h\textsuperscript{-1} (0.016 W) to 4.01 ± 0.230 mLO\textsubscript{2}g\textsuperscript{-1}h\textsuperscript{-1} (0.022 W). Oribi Gorge sunbirds, however, showed virtually no change in RMR between pre- and post-acclimation at T\textsubscript{a} = 25°C,
with a decrease of 0.3% from $3.86 \pm 0.278 \text{ mlO}_2\text{g}^{-1}\text{h}^{-1}$ (0.022 W) to $3.85 \pm 0.244 \text{ mlO}_2\text{g}^{-1}\text{h}^{-1}$ (0.021 W) (Post-hoc Sheffé, $p > 0.05$).

Comparison of pre-acclimation data indicated a significant difference between Underberg and Oribi Gorge (Post-hoc Sheffé, $p < 0.05$), and between Howick and Oribi Gorge subpopulations (Post-hoc Sheffé, $p < 0.05$), however, Underberg and Howick subpopulations were not significantly different pre-acclimation (Post-hoc Sheffé, $p > 0.05$). Post-acclimation VO$_2$ data did not show any significant differences between altitudinal subpopulations (Post-hoc Sheffé, $p > 0.05$).

Post-acclimation

Altitudinal Amethyst Sunbird subpopulations responses (Underberg, Howick and Oribi Gorge) to a range of temperatures indicated that that as $T_a$ increased, intra-subpopulation variation decreased (Figs 5-7). In general, RMR was reached between 19h00 and 20h00 over the range of ambient temperatures for all of the altitudinal subpopulations.

In summer there was a significant combined effect of altitude and temperature on resting metabolic rates of subpopulations of Amethyst Sunbirds in post-acclimation trials (Fig.8, RMANOVA $F_{(14, 126)} = 10.054$, $p < 0.001$). There was a significant difference in RMR between Underberg and Oribi Gorge at 5, 10, 15 and 20°C (Post-hoc Sheffé, $p < 0.05$), Howick and Oribi Gorge at 5, 10 and 15°C (Post-hoc Sheffé, $p < 0.05$), but no significant difference in RMR for Underberg and Howick (Post-hoc Sheffé, $p > 0.05$) when comparing between the same ambient temperatures. There was very little variation between individuals within a subpopulation at each of the ambient temperatures (Fig. 8).
When comparing Amethyst Sunbird BMR post-acclimation between altitudinal subpopulations in summer (Fig. 8) it was found that BMR values did not differ significantly (RMANOVA, $F_{(2, 18)} = 0.462, p = 0.637$), the Underberg subpopulation of sunbirds had the highest BMR ($3.50 \pm 0.245 \text{ mlO}_2\text{g}^{-1}\text{h}^{-1}$ or $0.020 \text{ W}$ achieved between 30-33°C), then Howick ($3.47 \pm 0.216 \text{ mlO}_2\text{g}^{-1}\text{h}^{-1}$ or $0.019 \text{ W}$ achieved between 28-33°C), with Oribi Gorge sunbirds displaying the lowest BMR ($3.29 \pm 0.232 \text{ mlO}_2\text{g}^{-1}\text{h}^{-1}$ or $0.018 \text{ W}$ achieved between 28-33°C). BMR decreased by 0.7% between Underberg and Howick subpopulations, by 5.9% between Underberg and Oribi Gorge, and by 5.3% between Howick and Oribi Gorge subpopulations, indicating a decrease in BMR as altitude decreased. Post-hoc Sheffé tests ($p < 0.05$) indicated relatively broad TNZ ranges for all of the subpopulations (Underberg ($T_a = 20-33°C$), Howick ($T_a = 20-33°C$) and Oribi Gorge ($T_a = 15-33°C$)) with the subpopulation at the lowest altitude having the broadest TNZ.

**Hematocrit**

Although there was no overall significant combined interaction between site and acclimation on hematocrit levels of Amethyst Sunbirds (Fig 9, RMANOVA, $F_{(2, 16)} = 2.617, p = 0.104$), hematocrit levels were significantly effected by acclimation (RMANOVA, $F_{(1, 8)} = 89.628, p < 0.001$). Post-hoc tests indicated that only Underberg sunbirds displayed a significant change post-acclimation (Post-hoc Sheffé, $p < 0.05$). Hematocrit data for the altitudinal subpopulations showed a significant difference between pre-acclimation hematocrit values for Underberg and Oribi Gorge subpopulations of sunbirds (Post-hoc Sheffé, $p < 0.05$). However, no significant difference was observed between subpopulations post -acclimation to 25°C and Pietermaritzburg altitude (Post-hoc Sheffé, $p > 0.05$).
Evaporative Water Loss

Evaporative water loss of three altitudinal subpopulations of Amethyst Sunbirds over the range of experimental temperatures increased marginally with increasing ambient temperature. There was a combined significant effect of temperature and altitude (Fig. 10, RMANOVA, $F_{(12, 108)} = 5.189, p < 0.001$). At 5°C EWL in summer trials was negligible.

Body Mass

There was no significant difference between pre- and post-acclimation body mass (g) for each subpopulation, nor between altitudinal subpopulations of Amethyst Sunbirds (Fig 11, RMANOVA, $F_{(2, 18)} = 0.935, p = 0.411$).

Ambient temperature

Summer ambient temperatures for January 2004- May 2007 (Table 1), showed that as altitude increased the mean monthly ambient temperature decreased.

Discussion

As was discussed in Chapter 2, avian metabolic data is often grouped together regardless of altitudinal origin of study birds, or alternatively one subpopulation is selected as a representative for the entire species in terms of metabolic measurements (e.g. Bech, 1980; Cooper and Swanson, 1994; Boix-Hinzen and Lovegrove, 1998; Maddocks and Geiser, 2000; McKechnie and Lovegrove, 2001; Downs and Brown, 2002; López-Calleja and Bozinovic, 2003; Lovegrove and Smith, 2003; McKechnie et al., 2007; Smit et al. In Press). However, avian BMR is emerging as a highly
flexible thermal trait, influenced by ambient temperature fluctuations, as well as other environmental changes (McKechnie, In Press).

As in the winter study, we predicted that the metabolic rates of subpopulations of Amethyst sunbird subpopulations would exhibit plasticity/flexibility over the altitudinal gradient due to acclimatization and adaptations to different ambient temperatures, as well as pre- and post-acclimation. Pre-acclimation data showed a significant difference between Underberg (highest altitude) and Oribi Gorge (lowest altitude), and between Howick (intermediate altitude) and Oribi Gorge (lowest altitude) subpopulations. As sunbird subpopulations would be acclimatized to different altitudes with differing variation in ambient temperatures respectively, it appears that they have adopted metabolic strategies to survive in the differing climatic conditions of their altitudinal sites and to minimize energy loss to metabolic heat production.

In summer Amethyst Sunbirds showed within species variation between altitudinal subpopulations in post-acclimation resting metabolic rates over the range of ambient temperatures, particularly at the lower ambient temperatures studied. Colder temperatures require that homeotherms increase metabolic heat production (Soobramoney et al. 2003). Underberg sunbirds were acclimatized to lower summer ambient temperatures than Oribi Gorge sunbirds, and similarly Howick sunbirds acclimatized to a lower range of summer ambient temperatures than Oribi Gorge birds. Consequently the differences in metabolic rate between these subpopulations shows a higher rate of metabolic heat production typical of higher altitude subpopulations compared with those at lower altitude in order to combat the effect of lower ambient temperatures and maintain homeothermy.
Although the effect of altitude on summer BMR of Amethyst Sunbird subpopulations was not significant, the higher altitude subpopulation (Underberg) exhibited the highest BMR, and the lowest altitude subpopulation (Oribi Gorge), the lowest BMR. This appears to be a consequence of the variation in ambient temperature at the differing localities along the altitudinal gradient where coldest mean monthly ambient temperature in Underberg was approximately 10°C lower that that of Oribi Gorge, and thus the colder temperatures at the higher altitudes would necessitate an increase in metabolic heat production by a homeothermic species (Soobramoney et al., 2003). Similarly, a summer study on Rosy Finches (Leucosticte arctoa) and House Finches (Carpodacus mexicanus) showed a decrease in BMR with decreased altitude from 3800m to 150m above sea level (Clemens, 1988). However, unlike those of Amethyst Sunbirds, these changes in BMR were significant, most likely as a result of a much greater difference in altitude between study sites. Thermal neutral zones for altitudinal subpopulations were similar, with only Oribi Gorge sunbirds (the warmest and lowest habitat) showing a slightly broader TNZ than the other two subpopulations, possibly due to natural acclimatization to the broader range of ambient temperatures experienced.

Evaporative water loss (EWL) plays an important role in thermoregulation when ambient temperature exceeds body temperature and consequently, the rate of water from an animal has important implications for thermoregulation and thus survival (Downs and Perrin, 1990; McKechnie and Wolf, 2004). Summer subpopulations of Amethyst Sunbirds all showed a marginal increase in EWL with increased ambient temperature, particularly within the TNZ.

It was predicted that summer hematocrit levels of Amethyst Sunbird subpopulations would increase with increasing altitude and decreasing atmospheric
oxygen concentration to aid in oxygen carrying capacity and thus metabolic rates. Pre-acclimation hematocrit values differed significantly between the highest (Underberg) and lowest altitude (Oribi Gorge) subpopulations of sunbirds. Soobramoney et al. (2005) found a similar trend in the hematocrit values of Common Fiscals *Lanius collaris* over the studied altitudinal gradient. Underberg sunbirds displayed a significant change post-acclimation. Hematocrit levels were high when compared with other avian species, for example, Broiler Chickens (*Gallus domesticus*) which had a mean hematocrit value of 30% (Yahav et al., 1996) which is within the typical range of hematocrit values for avian species suggested by Willmer et al. (2005) of between 30- 45%. High hematocrit values are possibly due to the small body size of Amethyst Sunbirds and the high metabolic cost of flight.

Along with the results of Chapter 2, these summer results emphasize the need to acknowledge the altitudinal origin of study populations and its effect on physiological parameters. Again it shows that in comparative avian studies the variation in physiological parameters of subpopulation is important, rather than just using a mean value for a species. This also has implications for models of the effects of climate change on the distribution of a species when physiological parameters, particularly thermal, are considered.

**Conclusion**

Similarly to chapter 2 summer studies of physiological parameters of Amethyst Sunbirds from different altitudinal subpopulations indicated variation in pre- and post-acclimation RMR, BMR and TNZ. This further emphasized the need to consider the altitudinal origin of study individuals, and to consider the effect of phenotypic flexibility on metabolic parameters, over an acclimation period.
Acknowledgements

CL would like to thank the NRF and the Gay Langmuir bursary fund for financial assistance. We would also like to thank Mike and Heidi Neethling (Oribi Gorge), Bill and Pam Nicol (Howick) and Prof. and Mrs. Piper (Underberg) for allowing us to “borrow” their Amethyst Sunbirds for the study period. Tracy Odendaal, Jaclyn Tennent, Kelly Brown & Akeem Akilimali are thanked for their invaluable field trip assistance. Thanks also to students at UKZN for assisting with feeding and bird maintenance.

List of symbols and abbreviations

BMR Basal Metabolic Rate
RMR Resting Metabolic Rate
EWL Evaporative Water Loss
TNZ Thermal Neutral Zone
VO$_2$ Oxygen consumption

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Figure 3. Pre- vs. post-acclimation VO$_2$ (mlO$_2$g$^{-1}$h$^{-1}$) of the Howick subpopulation of Amethyst Sunbirds in summer, 17h00-07h00 at 5 and 25°C (mean ± SE, n = 10).

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**Figure 11.** Pre- vs post-acclimation mean body mass (g) for Amethyst Sunbird subpopulations over an altitudinal gradient in summer.

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Chapter 4
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A comparison of summer and winter metabolic rates of Amethyst Sunbirds
(*Chalcomitra amethystina*) over an altitudinal gradient.

Claire Lindsay¹, Colleen Downs¹ & Mark Brown¹

¹ School of Biological and Conservation Sciences, University of KwaZulu-Natal,
Private Bag X01, Pietermaritzburg, 3201, South Africa

Southern Africa is characterised by an unpredictable environment with daily and
seasonal temperature fluctuations, thus posing challenging thermal conditions and
increased energetic stress for endothermic vertebrates. Amethyst Sunbirds
(*Chalcomitra amethystina*) are relatively large African sunbirds (15g). They are
considered non- or locally migratory and thus have to deal with the temperature
changes and physiological stresses a new season brings. This study compared
altitudinal subpopulations and the seasonal shifts between and within the
subpopulations in metabolic parameters. Amethyst Sunbirds were caught in summer
and winter at three different altitudinal subpopulations; Underberg, Howick and Oribi
Gorge. Upon capture, metabolic rate of the sunbirds were measured indirectly by
quantifying oxygen consumption (VO₂) using flow through respirometry, at 5 and
25°C. Birds then underwent a 6 week acclimation period at 25°C on a 12L: 12D
cycle. VO₂ was measured post-acclimation at 8 different temperatures (15, 5, 10, 20,
30, 28, 25 and 33°C). Hematocrit levels were taken pre-acclimation and pre-release. Experiments were repeated for a winter and summer season.

In general, Amethyst Sunbird subpopulations from Underberg and Howick showed higher post-acclimation resting metabolic rates per temperature in winter than in summer trials. Underberg and Howick subpopulations respectively showed a significant difference between summer and winter VO₂ at 5 and 10°C. Thermal neutral zones of all of the subpopulations of sunbirds shifted between winter and summer. Post-acclimation basal metabolic rate of sunbirds decreased significantly by 38.8% from winter to summer for the Underberg subpopulation, increased by 44.8% for the Howick subpopulation and did not change significantly for the Oribi Gorge subpopulation (5.8% decrease).

Introduction

Southern Africa is characterised by an unpredictable environment with daily and seasonal temperature variation (Schulz, 1997). These fluctuations pose challenging thermal conditions and increased energetic stress for vertebrates, especially for mammals and birds, which are endothermic. Climate can affect birds directly or physiologically, though its impact on energy maintenance and water balance, and/or indirectly (i.e. ecologically), through its influence on vegetation, food availability, photoperiod and thus available foraging time (Carey et al., 1980; Weather and Caccamise, 1978; Weathers and van Riper, 1982; Cooper, 2000; Cooper, 2002; Lovegrove and Smith, 2003; Crick, 2004). It would be a mistake to regard organisms that are exposed to the external environment as being passive to change, since they often show an ability to use physiological adjustment or plasticity to alleviate the effects of seasonal environment changes as well as experimental conditions (Dawson,
The ability to adapt and change is most pronounced in species living in fluctuating environments, which in this case could describe seasonal changes in ambient temperature, or alternatively changes in mean temperatures over an altitudinal gradient, and such species require seasonal acclimatization in order to facilitate thermoregulatory homeostasis (Cooper, 2000; Arens and Cooper, 2005; Cossins et al., 2006). As early as 1962, Hart noticed that a striking feature of small birds was their ability to withstand changes in ambient temperature with very little protection, but to a greater metabolic cost. Similarly, Swanson and Weinacht (1997) noted that seasonal differences in metabolism are common in small passerine birds. Weathers and van Riper (1982) showed that birds show a remarkable degree of physiological adjustment to differing climates. More so, small diurnal birds that are locally migratory or non-migratory, such as Amethyst Sunbirds (*Chalcomitra amethystina*) (Tree, 2005), have to deal with seasonal changes in ambient temperature and thus would have to employ seasonal adjustments in their physiology in order to reduce the thermal stress placed on these small passerines (Withers, 1992; Maddocks and Geiser, 2000), particularly as their small size restricts the ability of increased body insulation to assist in seasonal acclimatization (Clemens, 1988).

Avian basal metabolic rate (BMR) is beginning to be viewed as a highly flexible physiological trait influenced by environmental fluctuations, and in particular changes in ambient temperatures. Many non-migrants have been found to adjust their BMR seasonally, and winter-acclimatized and cold-acclimated birds have been known to exhibit higher basal metabolic rates, than those summer-acclimatised or warm acclimated birds (Klaasen et al., 2004; McKechnie, In Press). Recent evidence suggests that during winter, BMR of species resident in highly seasonal environments
reflects the prevailing conditions immediately before metabolic measurements (McKechnie, In Press). Summer responses may be less varied due to temperature fluctuations remaining within the thermal neutral zone (TNZ). It was predicted that BMR and TNZ would vary between bird subpopulations from different altitudinal localities, and between seasons. In addition, it is predicted that post-acclimation to a particular temperature over a period of six weeks, birds from different altitudes will respond similarly, in terms of physiology, to a range of ambient temperatures, and differ to pre-acclimation trials, therefore displaying phenotypic flexibility in physiological parameters, particularly thermal. Differences in altitudinal subpopulations post-acclimation were expected to indicate a difference in acclimation strategies, most likely as a result of adapting to live in different altitudinal and thus thermal environments.

Thus the ecological significance of seasonal acclimatization for animals living in a non- or locally migratory manner in a changing environment is obvious (Southwick, 1980) and consequently, knowledge of the plasticity in ecophysiological parameters and the strategies employed to cope with variability in food and water availability and extreme ambient fluctuations (in terms of seasonal and daily fluctuations) is important in understanding the survival of birds in southern Africa. Klaasen et al. (2004) questioned whether physiological flexibility in response to ambient temperature variation was a general feature of the metabolic properties of birds, irrespective of whether seasonal and daily temperature fluctuations were extreme. Thus, as most of the studies on small passerines have been on Holarctic species (McKechnie, In Press), substantially more research is required on the seasonal metabolic changes in small subtropical passerines. The necessity for further research into the phenotypic flexibility of metabolic rates (in particular BMR), as well as to
recognize altitudinal differences between subpopulations of the same species has been highlighted in Chapter 2 and 3. However seasonal differences and possible plasticity/flexibility of populations in physiological responses between seasons necessitates comparison between winter and summer studies. Here physiological data from Amethyst Sunbird subpopulations from KwaZulu-Natal (KZN), South Africa from winter and summer studies, and pre- and post-acclimation, in terms of resting metabolic rate (RMR), BMR, TNZ and evaporative water loss (EWL) were compared. In addition, changes in hematocrit values between seasons, and pre-and post-acclimation for subpopulations at each of the altitudinal study sites were compared.

Materials and Methods

Study site, Bird capture and maintenance

Amethyst Sunbirds were captured in summer (November 2006- December 2006) and winter (May 2006-June 2006) at three different locations in KZN, South Africa, under permit from Ezemvelo KZN Wildlife, using mist-nets. The number of sunbirds caught, capture co-ordinates and capture site altitudes and are shown in Table 1. Study individuals were weighed and colour banded to allow for capture location and individual identification (Downs & Brown, 2002). None of the birds used in the experiments showed any indication of breeding activity, as indicated by the absence of brood patches. Experimental protocol for metabolic measurements was kept standard between winter and summer trials, and these methods are described in chapters 2 and 3.

Weather data was obtained from the South African Weather Service (SAWS) for Shaleburn (representing Underberg), Cedara (representing Howick) and Paddock (representing Oribi Gorge) for January 2004- May 2007.
Statistical analyses

Descriptive statistics were calculated in STATISTICA (Statsoft, Tulsa, USA) for each subpopulation. The minimum RMR at each of these temperatures for each individual was used in analysis to determine change with temperature using Generalized Linear Models (commonly called GLIM) Repeated Measures Analysis of Variance (RMANOVA). BMR was calculated by taking the lowest mean RMR per subpopulation. GLIM RMANOVA was further used for the comparison of seasonal VO$_2$ measurements between populations at different altitudes, between pre- and post-acclimation data and for body mass comparison Post-hoc Sheffé tests were done to determine where significant differences occurred (p < 0.05). Pre-acclimation VO$_2$ data and weather data was analyzed using Factorial ANOVA. Post-hoc Sheffé tests were done to determine significance. Data are presented as mean ± SE of the individuals measured (n). Percentage change in Pre-acclimation MR and BMR between winter and summer was determined using the following equation: \((\text{Winter MR - Summer MR)} / \text{Winter MR}) \times 100\).

Release

Birds were weighed and released back at the original capture site upon completion of respirometry trials.

Results

Body Mass

Body mass (g) of Amethyst Sunbirds was compared between summer and winter, pre-acclimation and post-acclimation (Fig. 1). There were no significant differences
between pre- or post-acclimation masses between seasons or between altitudinal subpopulations within seasons (Fig. 1, RMANOVA, $F_{(2, 10)} = 1.106, p = 0.368$).

**Pre-acclimation Summer vs. Winter**

Pre-acclimation VO$_2$ of Amethyst Sunbirds was compared between altitudinal sites and between seasons for 5 and 25°C (Fig. 2a and b). At 5°C, all subpopulations showed a significant decrease in pre-acclimation VO$_2$ from winter to summer (Fig. 2a, RMANOVA, $F_{(1, 4)} = 102.19, p = 0.001$). Underberg showed a 47.0% decrease from $8.44 \pm 0.170 \text{mlO}_2\text{g}^{-1}\text{h}^{-1}$ or 0.047 W to $4.47 \pm 0.263 \text{mlO}_2\text{g}^{-1}\text{h}^{-1}$ or 0.025 W (Post-hoc Sheffé, $p < 0.05$), Howick a 61.9% decrease from VO$_2$ values of $15.94 \pm 0.426 \text{mlO}_2\text{g}^{-1}\text{h}^{-1}$ or 0.089 W to $6.08 \pm 0.350 \text{mlO}_2\text{g}^{-1}\text{h}^{-1}$ or 0.034 W (Post-hoc Sheffé, $p < 0.05$). Oribi Gorge subpopulations showed the lowest seasonal difference between the altitudes with a decrease in VO$_2$ from winter to summer trials of 41.6%, from $12.59 \pm 0.699 \text{mlO}_2\text{g}^{-1}\text{h}^{-1}$ or 0.070 W to $7.36 \pm 0.450 \text{mlO}_2\text{g}^{-1}\text{h}^{-1}$ or 0.041 W (Post-hoc Sheffé, $p < 0.05$).

A comparison of seasonal pre-acclimation Amethyst Sunbird VO$_2$ data at 25°C also showed a decrease in VO$_2$ values from winter to summer (Fig. 2b, RMANOVA, $F_{(1, 5)} = 83.600, p < 0.001$). From winter to summer, Underberg sunbirds showed a 65.7% decrease in VO$_2$ from $6.71 \pm 0.146 \text{mlO}_2\text{g}^{-1}\text{h}^{-1}$ or 0.037 to $2.30 \pm 0.143 \text{mlO}_2\text{g}^{-1}\text{h}^{-1}$ or 0.013 W (Post-hoc Sheffé, $p < 0.05$), Howick sunbirds showed a 61.0% decrease in VO$_2$ between seasons from $7.20 \pm 0.447 \text{mlO}_2\text{g}^{-1}\text{h}^{-1}$ or 0.040 W in winter to $2.81 \pm 0.352 \text{mlO}_2\text{g}^{-1}\text{h}^{-1}$ or 0.016 W in summer (Post-hoc Sheffé, $p < 0.05$). Oribi Gorge again showed the least change in VO$_2$ between seasons with a 48.4% decrease from $7.48 \pm 0.742 \text{mlO}_2\text{g}^{-1}\text{h}^{-1}$ or 0.042 W in winter to $3.86 \pm 0.278 \text{mlO}_2\text{g}^{-1}\text{h}^{-1}$ or 0.022 W in summer (Post-hoc Sheffé, $p < 0.05$).
**Post-acclimation Summer vs. Winter**

Post-acclimation summer and winter VO\(_2\) values of Amethyst Sunbirds were compared for each location (Figs 3a-c). In general, the Amethyst Sunbird subpopulation from the highest altitude, Underberg, showed higher post-acclimation VO\(_2\) values per temperature in winter than in summer trials (Fig. 3a, RMANOVA, \(F_{(7, 98)} = 46.372, p < 0.001\)). Post-acclimation VO\(_2\) values for Howick subpopulations were generally higher in winter than in summer (Fig. 3b, RMANOVA, \(F_{(7, 546)} = 195.710, p < 0.001\), however at 25 and 28°C summer VO\(_2\) values were higher than winter values, although not significantly so (Post-hoc Sheffé, \(p > 0.05\)). Underberg sunbirds showed a significant difference between summer and winter RMR values at 5 and 10°C (Figs 3a, Post-hoc Sheffé, \(p < 0.05\)), and Howick sunbirds showed a significant seasonal change in RMR at 5°C (Figs 3b, Post-hoc Sheffé, \(p < 0.05\)). The lowest altitude, Oribi Gorge, sunbirds showed no significant differences between a winter and a summer season between any of the ambient temperatures (Fig 3c, RMANOVA, \(F_{(7, 119)} = 1.369, p = 0.225\)).

Thermal neutral zones of Amethyst Sunbird subpopulations shifted between winter and summer seasons (Figs 3a-c). The TNZ for Underberg sunbirds in winter ranged from 10-33°C, but in summer it was narrower, between 20-33°C. The Howick sunbirds indicated a shift from a very narrow TNZ in winter \((T_a = 25-30°C)\) to a broader range of temperatures in summer \((T_a = 20-33°C)\). Oribi Gorge sunbirds displayed a smaller shift in TNZ between the winter \((T_a = 20-33°C)\) and summer season \((T_a = 15-33°C)\) with both TNZs relatively broad.

There was a significant effect of both season and altitudinal site on post-acclimation BMR (Fig. 4, RMANOVA, \(F_{(2, 10)} = 9.547, p = 0.005\)). Underberg
sunbirds significantly decreased BMR (Post-hoc Sheffé, p < 0.05) by 38.8% from winter to summer, from 5.71 ± 0.402 mlO₂g⁻¹h⁻¹ or 0.032 W to 3.50 ± 0.213 mlO₂g⁻¹h⁻¹ or 0.020 W (both at 30°C). Howick sunbirds showed a 44.8% increase in BMR from winter to summer, from 2.46 ± 0.299 mlO₂g⁻¹h⁻¹ or 0.014 W to 3.47 ± 0.216 mlO₂g⁻¹h⁻¹ or 0.019 W (at 28 and 30°C respectively), however this increase was not statistically significant (Post-hoc Sheffé, p > 0.05). There was very little effect of season on the BMR of the Oribi Gorge sunbirds (Post-hoc Sheffé, p > 0.05), with only a slight decrease of 5.8% in summer, from 3.49 ± 0.312 mlO₂g⁻¹h⁻¹ or 0.019 W to 3.29 ± 0.232 mlO₂g⁻¹h⁻¹ or 0.018 W (at 30°C).

Weather
Mean monthly temperatures per location were compared (Fig. 5, RMANOVA F(22, 61298) = 78.503, p < 0.001) and it was found that there was a significant difference between mean temperature (°C) per month for all of the locations (Post-hoc Sheffé, p < 0.05). The mean temperatures for February (hottest month) and June (coldest month) were compared for each of the locations as representing the seasonal extremes. Underberg showed the greatest seasonal extremes in temperature with a change in mean temperature between February and June of 12.2°C. Howick (10.4°C) and Oribi Gorge (6.9°C) which displayed less difference between seasons. Summer seasons showed less variability between altitudes than winter.

Discussion
It is thought that changes in body insulation in small birds are limited by their size and thus are not as marked as metabolic adjustments and only has a small role in seasonal acclimatization and adaptation to cold (Clemens, 1988; Cooper and Swanson, 1994;
Swanson and Weinacht, 1997). Studies on a small passerine the Australian Silvereye (Zosterops lateralis) by Maddocks and Geiser (2000), Monk Parakeets (Myiopsitta monachus) by Weathers and Caccamise (1978) and Black-capped Chickadees (Poecile atricapilla) by Cooper and Swanson (1994), indicated very little seasonal variation in body mass. Similarly, body mass in Amethyst Sunbirds did not vary seasonally or between altitudinal subpopulations within a season. This is contrary to most other species which displayed a 7% higher body mass in winter acclimatized birds (Weathers and Caccamise, 1978). As there was no significant change in body mass between seasons within altitudinal subpopulations, nor within seasons between altitudinal subpopulations, it can be concluded that changes in body fat stores were not an influencing factor in shifts in metabolic rates and thus seasonal acclimatization.

Maddocks and Geiser (2000) found that variation within populations of Silveryeyes, in terms of MR, was higher in summer than in winter. However, subpopulations of Amethyst Sunbirds consistently showed greater variation around the mean in winter than in summer. Maddocks and Geiser (2000) observed a shift in TNZ from 25.4 -33.5°C in summer, to a slightly narrower TNZ range between 27.0-33.6°C in winter. Dawson and Carey (1976), found the zone of thermal neutrality for the American Goldfinch (Carduelis tristis) ranged from approximately 23- 35°C in both summer and winter. Monk Parakeets (Weathers and Caccamise, 1978), showed a shift in thermal neutral zones between winter and summer from 24.5- 38.5°C to 28.0-40.0°C. Amethyst Sunbird subpopulations, however, showed mixed responses to seasonal TNZ changes. The higher altitude Underberg sunbirds showed a decrease in TNZ range between winter and summer, possibly as a result of reduction in temperature extremes in summer. Howick and Oribi Gorge sunbirds, however, showed an increase in TNZ range from winter to summer. Thus Howick and Oribi
Gorge sunbirds showed a similar response to that found by Maddocks and Geiser (2000). This response was to be expected due to exposure to a broader range of ambient temperatures in summer, and thus we expected birds to be able to cope with a broader range of temperatures. The absence of significant differences in winter and summer BMR values in Goldfinches (13g), tested over a range of temperatures in the laboratory (34 to -33°C), showed a striking ability to maintain and regulate MR and thus reduce energy expenditure (Dawson and Carey 1976), thus displaying flexibility in metabolic response enabling homeothermy. Similarly, seasonal studies on the Northern Bobwhite, *Colinus virginianus*, (Swanson and Weinacht, 1997), a relatively large bird (210-225g), and the Southern White-faced Scops-owl, *Ptilopsis granti* (±220g) (Smit et al., In Press), showed no significant difference in BMR between a winter and a summer season.

Hart (1962), found a significant difference between winter and summer in MR for Pigeons (*Columba livia*) and House Sparrows (*Passer domesticus*), but no significant seasonal difference in VO₂ in Starlings (*Sturnus vulgaris*) and evening Grosbeaks (*Hesperiphona vespertina*). House Sparrows displayed higher oxygen consumption in winter when compared with summer, however the pigeons studied showed a decrease in VO₂ for winter as compared to summer trials (Hart, 1962). However, at the lower temperatures tested, summer birds (except starlings) showed lower oxygen consumption than winter birds. This however was not a measure of BMR as it was done for only one hour during the day. Mountain Chickadees (*Poecile gambeli*) and Juniper Titmice (*Baeolophus griseus*), largely non-migratory passerines, showed a significantly greater winter BMR than summer BMR (Cooper, 2002), and Black-capped Chickadees indicated a significantly higher RMR referred to as standard metabolic rate in winter than summer (Cooper and Swanson, 1994).
However, Sharbaugh (2001), studied a population of Black-capped Chickadees in the much colder climate of Alaska and found no seasonal difference in RMR. Maddocks and Geiser (2000) found an increase of 25.2% in the BMR of Silvereyes between winter and summer acclimated birds and concluded that RMR reduction in winter is a common occurrence in small passerines. Weathers and Caccamise (1978) measured fasting metabolic rate in Monk Parakeets at night and found it significantly lower in winter than in summer. White-crowned Sparrows (Zonotrichia leucophrys) showed increased oxygen consumption in winter (Southwick, 1980). Similarly, BMRs of cold acclimated Garden Warblers (Sylvia borin) were found to be 18.5% higher than warm-acclimated birds (Klaasen, 2004). Weathers and Caccamise (1978) suggest that in a small species, winter fasting MR show a strong tendency to exceed summer fasting MR, while the opposite generally applies to larger birds. One suggested explanation was that due to their size, small birds are unable to use significant increases in body insulation as a compensatory technique in response to winter conditions and thus may resort to increased metabolic heat production at lower ambient temperatures (Dawson and Carey, 1976; Weathers and Caccamise, 1978). However, even Mute swans (Cygnus olor) (Bech, 1980), a much larger species (± 5-12kg), showed a higher RMR in winter than summer. This is contrary to predictions by Weathers and Caccamise (1978) that birds > 200g would show the opposite trend to smaller birds and decrease MR in winter.

Amethyst Sunbirds, as relatively small birds, do not show significant differences in mass between seasons (implying a negligible change in body insulation and organ mass). However, in our study, different altitudinal subpopulations responded differently, in terms or metabolic parameters, to seasonal changes. In general Underberg and Howick subpopulations decreased RMR between winter and
summer, whereas the lowest altitude subpopulation, Oribi Gorge, showed very little seasonal change in RMR. This was expected as Oribi Gorge did not show a great change in seasonal ambient temperatures, whereas Underberg and Howick weather data showed a more marked seasonal shift in ambient temperature. The Underberg subpopulation showed a decrease in BMR of 38.8% from winter to summer. Howick sunbirds increased BMR by 44.8% in summer, whereas the Oribi Gorge subpopulation showed very little difference between a winter and summer season (5.8%). As a relatively small avian species, Amethyst Sunbirds do not respond according to predictions for the effect of season on the MR of small birds, thus serving to further emphasize the need to acknowledge the importance of altitudinal origin of a studied species. Our results may also explain the conflicting results from other studies, where some small birds have been found to increase BMR in winter (Hart, 1962; Southwick, 1980), some to decrease BMR in winter (Hart, 1962; Maddocks and Geiser, 2000; Weathers and Caccamise, 1978) and some to show no change in BMR seasonally (Hart 1962). Different subpopulations of Amethyst Sunbirds showed each of these seasonal responses, indicating that altitude can vastly affect studies of seasonal changes in BMR. In comparison, although such variations are more likely to be prominent in sedentary birds, even long-distance migratory birds (in particular the Knot, *Calidris canutus*) have been found to have pronounced seasonal variation in BMR (Piersma et al., 1995).

Climate change, or long terms shifts in average weather, affects the MR of birds by requiring changes in energy expenditure (Crick, 2004). Consequently, one of the factors that could inhibit the ability to adapt to climate change, is a lack of phenotypic flexibility and thus the inability to adapt to climate change (Crick, 2004). According to Bernardo et al. (2007) there is a urgent need in conservation biology and
climate change research to find criteria for assessing the susceptibility of a species to climate change induced extinction. Many species of birds are found to exist in cold and warm climates, with metabolic parameters varying between populations of the same species (Furness, 2003). Surely, a promising indicator of a species ability to survive in a changing climate, is the ability to adapt and survive in response to seasonal as well as altitudinal shifts in temperature thus displaying flexibility/plasticity in metabolic parameters and consequently the ability to survive changes in ambient temperatures. This study on Amethyst Sunbirds showed that within a species, different altitudinal subpopulations and thus populations exposed to different ranges of ambient temperatures are able to adapt and survive in changing environments. Seasonal variation in BMR and RMR in Amethyst Sunbirds and other avian species thus questions the relevance and accuracy of predictions made in avian comparative studies which have used a mean BMR or RMR per population, and not considered the variation around the mean, particularly as a consequence of altitude or season, as an effect on these physiological parameters. Similarly, non- or local migratory species, such as Amethyst Sunbirds show the ability not only to endure temperature shifts between seasons, but also reduced foraging time and changes in food availability, and thus ecological constraints.

**Conclusion**

When considering a population to use as a representative for the species in physiological studies, not only does one have to take altitudinal origin into account, but also the season to which the study population is acclimatized to. It may be possible to acclimate birds to the same temperature and altitude over a period of time, but our results have shown that underlying physiological differences can persist post-
acclimation. Besides this, data from birds acclimated to pseudo environmental conditions may not represent the wild populations accurately, as significant differences were observed in metabolic parameters between pre- and post-acclimation trials.

Acknowledgements

CL would like to thank the NRF and the Gay Langmuir bursary fund for financial assistance. Temperature data was obtained from the South African Weather Service. We would also like to thank Mike and Heidi Neethling (Oribi Gorge), Bill and Pam Nicol (Howick) and Prof. and Mrs. Piper (Underberg) for allowing us to “borrow” their Amethyst Sunbirds for the study period. Tracy Odendaal, Jaclyn Tennent, John Lindsay, Kelly Brown, Nicolette Taylor, Ashton Musgrave, Akim Akilimali are thanked for their invaluable field trip assistance. Thanks also to students at UKZN for assisting with feeding and bird maintenance.

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**Figure 4.** Comparison of basal metabolic rates (VO$_2$) of altitudinal subpopulations of Amethyst Sunbirds for a winter and summer season respectively.

**Figure 5.** Weather data from each of the three capture locations January 2004- May 2007 (Solid bar indicates winter months, hollow bar indicates summer months).

**Table 1.** Location and number of Amethyst Sunbirds captured in a winter and summer seasons.
Figure 1. Comparison of pre (a) and post-acclimation (b) body masses of Amethyst Sunbird subpopulations between sites and seasons.
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Table 1. Location and number of Amethyst Sunbirds captured in a winter and summer seasons.

<table>
<thead>
<tr>
<th>Location</th>
<th>Altitude (m)</th>
<th>GPS Co-ordinates</th>
<th>No. of Sunbirds caught</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Latitude</td>
<td>Longitude</td>
<td>Winter</td>
</tr>
<tr>
<td>Underberg</td>
<td>1553</td>
<td>29°47.614S 29°30.319E</td>
<td>9</td>
</tr>
<tr>
<td>Howick</td>
<td>1075</td>
<td>29°28.203S 30°13.316E</td>
<td>6</td>
</tr>
<tr>
<td>Oribi Gorge</td>
<td>541</td>
<td>30°40.067S 30°15.316E</td>
<td>8</td>
</tr>
</tbody>
</table>
Bernardo et al. (2007) acknowledges an urgent need in conservation biology and climate change research to find criteria for assessing the susceptibility of a species to climate change induced extinction. Despite the knowledge that species distributions are shifting according to changes in climate (Pörtner, 2002), we have little understanding of the relationship between a species physiology and its vulnerability to climate changes and more permanent shifts in ambient temperature (Calosi et al., In Press). Simmons et al. (2004) posed the question of whether some species will exhibit rapid adaptations to climate change. However, avian species show remarkable plasticity in their thermoregulatory responses and many species of birds are able to exist in cold and warm climates, and an increasing number of studies are showing variation and flexibility in metabolic parameters, both seasonally and in response to experimental conditions, between populations of the same species (Dawson, 2003; Furness, 2003; McKechnie, In Press).

Differences between altitudinal subpopulations of Amethyst Sunbirds in pre- and post-acclimation results (Chapters 2 and 3) indicate plasticity in physiological responses within a species, as well as in the ability of the species to cope with and adapt to changes and differences in ambient temperatures. In combination with this, the ability of a species to exist in a variable environment in a non-or locally migratory manner, indicates an ability to shift metabolic responses between seasons, and the data showed this shift (Chapters 2, 3 and 4). As a result of this plasticity/ flexibility in metabolic parameters, Amethyst Sunbirds indicate that they will be able to shift their metabolism in response to predicted changes in climate (Chapters 2, 3 and 4).
Consequently, variation in basal metabolic rate (BMR) and resting metabolic rate (RMR) in Amethyst Sunbirds and other avian species calls into question the relevance and accuracy of predictions made in comparative avian studies which have used a mean BMR or RMR per species, or alternatively a single population as a representative of a species (e.g. Cooper and Swanson, 1994; Boix-Hinzen and Lovegrove, 1998; Maddocks and Geiser, 2000; McKechnie and Lovegrove, 2001; Downs and Brown, 2002; López-Calleja and Bozinovic, 2003; McKechnie et al., 2007). None of these studies considered altitude or season as a source of variation in these physiological parameters. In particular, the effect of altitude on seasonal variation in BMR of Amethyst Sunbirds indicates that the conflicting results of seasonal changes in BMR of other small bird species may be a reflection of population acclimatization to a particular geographical location, as apposed to species differences.

Thus in conclusion, a promising indicator of a species ability to survive in a changing climate, is the ability to adapt in response to seasonal and altitudinal shifts in temperature by displaying flexibility/plasticity in metabolic parameters. As a result of this, when considering subpopulations to use as a representative for a species in physiological data collection, and ensuing climate change survival predictions, not only does one have to take altitudinal origin into account, but also the season to which the study population is acclimatized to. Furthermore, the variation around the mean for a particular thermal parameter needs to be considered.

Further studies should include genetic analysis and for this purpose blood samples were taken from all birds sampled. As a consequence of time constraints, metabolic measurements in the current study were only undertaken during one winter and one summer season. Ideally seasonal data needs to be repeated and field
metabolic rates obtained for birds over the altitudinal gradient. In addition, more sites should be sampled over a broader altitudinal gradient and the full range of temperatures should be tested pre- as well as post-acclimation. However, there are numerous constraints to achieving data sets that accomplish all of these criteria.

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