## CHAPTER 1: GENERAL INTRODUCTION

Over the past 70 years, the metabolic adjustments occurring in birds that enhance their ability to deal with thermoregulatory challenges have been extensively studied (Dawson, 2003). These studies often involved assessing how metabolic parameters respond to either laboratory-induced or seasonal biotic and abiotic factors (McKechnie *et al*., 2006; Liknes *et al.,* 2002; Tieleman and Williams, 2000; Swanson and Weinacht, 1997; Swanson, 1991). More recently, the concept of phenotypic plasticity, i.e. the ability of a single genotype to express various phenotypes in response to different environmental stresses (Ernande and Dieckmann, 2004), has steadily been gaining exposure in studies exploring metabolic variation and adaptation. Phenotypic plasticity encompasses 'phenotypic flexibility', i.e. short-term, reversible, within individual variation; and/or 'developmental plasticity', which are the irreversible changes that occur as a result of developmental processes (Piersma and Drent, 2003).

To date, there have been several reports of phenotypic flexibility in birds, with parameters such as body mass ($M_b$), minimum wet thermal conductance ($C_{wet}$), body temperature ($T_b$), organ size, basal metabolic rate (BMR) and metabolic intensity changing either seasonally or in laboratory experiments (Vézina and Williams, 2003; Battley *et al*., 2000, 2001; Maddocks and Geiser, 1997; Piersma and Lindström, 1997).

Phenotypic plasticity in physiological traits is not unique to birds. As the response to reproductive demands in the nude house mice, *Mus musculus,* also mandate increases in metabolic rate and organ size (Speakman and McQueenie, 1996).

Basal metabolic rate (BMR) is the minimum metabolic rate of quiescent, non-reproductive, non-growing, post-absorptive endotherms in a thermoneutral environment.

It has become one of the standard baseline parameters used to assess the costs of thermoregulation, energy expenditure due to activities in the wild, the limits of metabolic scope and adjustments of metabolic rates to environmental stress (Williams and Tieleman, 2000; Ricklefs *et al*., 1996). In birds, BMR has been reported to be phenotypically plastic trait that can either increase (Liknes *et al.,* 2002) or decrease (Swanson, 1991) during winter. However, BMR can also remain static, while other associated metabolic parameters ($C_{wet}$, $T_b$ and $M_b$) exhibit plasticity to compensate for seasonal changes (Swanson and Weinacht, 1997). Similarly, laboratory experiments show that birds acclimated for three weeks to a low ambient temperature ($T_a$) have a higher BMR than individuals of the same species acclimated to a warmer $T_a$ (Klaassen *et al.*, 2004; William and Tieleman, 2000). Even though the most striking occurrences of metabolic flexibility are found in year-round residents at high latitudes and in long distance migrants (Battley *et al*., 2000 & 2001; Swanson and Olmstead, 1999), it is also present in non-migrant species from the tropics and sub-tropics (Tieleman *et al*., 2003a).

In essence, the question now is no longer whether avian metabolic rate is subject to change, but rather, how flexible is avian metabolic variation in response to short-term thermal acclimation? Investigations of metabolic responses to temperature are either undertaken in the field, examining the effects of seasonal acclimatization, or in the laboratory where the effects of induced acclimation can be monitored (Dawson, 2003). Acclimation experiments have an advantage over acclimatization experiments as advances in modern technology offer a precision that is not always possible to achieve in the field i.e. all variables except those being investigated can be kept constant during acclimation experiments (Costa and Sinervo, 2004).

Although adjustments in BMR and changes in organ sizes are often related, flexibility in BMR may also be linked to the metabolic intensity of certain organs. In the European Starling, *Sturnus vulgaris,* metabolic adjustments occur as a result of either increases or decreases in the oxidative activity of the enzyme citric synthase (Vézina and Williams, 2003). Secor and Diamond (1998, 1995) also reported increases in oxygen consumption, organ size and metabolic intensity of the Burmese python, *Python molurus*, during digestion of large infrequent meals.

The allometric relationship between BMR and body mass in birds was recently re-evaluated using 231 bird species. After the appropriate corrections for phylogeny, it was reported that smaller (<200g) captive-raised birds have a reduced overall BMR when compared to wild-caught birds (McKechnie *et al.,* 2006; McKechnie and Wolf, 2004a). We can therefore conclude that phenotypic plasticity in BMR occurs in most, if not all, birds. However, the costs of phenotypic plasticity are incurred at the level of the individual (Ernande and Dieckmann, 2004) and the effect of genotypic natural selection would also be responsible for the evolution of phenotypic flexibility of BMR (Schleucher and Withers, 2002; Tieleman and Williams, 2000).

## 1.2. RESEARCH OBJECTIVES

The general aim of this study was to explore phenotypic flexibility in BMR in the Laughing Dove, *Streptopelia senegalensis*, in response to short-term thermal acclimation. To this end, the following specific studies were undertaken in each of the following chapters:

**Chapter 2**: Basal metabolic rate adjustments in response to short-term thermal acclimation: magnitude, reversibility, repeatability and correlation with body composition.

- To investigate the effects of short-term thermal acclimation on BMR and associated metabolic parameters.
- To investigate whether the effects of short-term thermal acclimation are reversible and/or repeatable, and
- To identify a mechanism potentially responsible for adjustments in BMR.

**Chapter 3**: Temporal dynamics of basal metabolic rate adjustments to short-term thermal acclimation.

- To investigate the temporal dynamics of adjustments in BMR, during short-term thermal acclimation.

## 1.3. THE STUDY ANIMAL

The Laughing Dove, *Streptopelia senegalensis,* belongs to the cosmopolitan bird family Columbidae, which is comprised of more than 300 species of pigeons and doves (Schleucher, 2001) and is one of the most successful bird families in the world (Marder *et al*., 2003). This family displays considerable variability in their ecology and life history. They are found in virtually all habitat types, from deserts to rainforests, show contrasts in food specialization (granivorous vs.frugivorous) and flight techniques (strong flyers vs. ground-living species) (Schleucher, 2001, 2002). However, in spite of these different ecological adaptations, the columbidae are all similar enough that even the most exotic forms are recognised as being some sort of pigeon (Schleucher, 2001). The capacity to occupy such diverse habitats makes this bird family an interesting candidate for a physiological study.

Laughing Doves have a distribution that extends across sub-Saharan Africa, and are only absent from true desert habitats (Hockey *et al.*, 2005). It is a generalist granivorous species but do eat fruit when available, have an average life span of 3-5 years (although the longest-lived birds recorded in South Africa were between 7-18 years). In South Africa the species is currently classified as a non-threatened species with numbers greatly increasing, possibly as a response to habitat disturbance and changes in land use practices (Hockey *et al.*, 2005). The ability of this species to colonize varied habitats across South Africa undoubtedly arises in part from its physiology. Since scientific investigation into phenotypic plasticity in avian physiological traits is somewhat limited, with most studies concentrating on birds found in the Northern hemisphere, the Laughing

Dove is the ideal study animal to pursue an investigation into the physiological flexibility of BMR.

# CHAPTER 2: BASAL METABOLIC RATE ADJUSTMENTS IN RESPONSE TO SHORT-TERM THERMAL ACCLIMATION: MAGNITUDE, REVERSIBILITY, REPEATABILITY AND CORRELATION WITH BODY COMPOSITION

## 2.1. ABSTRACT

The question of whether basal metabolic rate (BMR) adjusts in response to short-term thermal acclimation was addressed in the Laughing Dove (*Streptopelia senegalensis*). Additionally, reversibility, repeatability and a possible mechanistic correlate of BMR adjustments were also assessed. By acclimating *S. senegalensis* to three air temperatures ($T_{acc}$) $10^{o}$, $22^{o}$ and $35^{o}$C for 21 days and subsequently reverse-acclimating them, also for 21 days, I was able to examine the response in BMR to short-term thermal acclimation.

After acclimation BMR was significantly influenced by $T_{acc}$ and was significantly higher in the $10^{o}$C group relative to the $35^{o}$C group. After reverse-acclimation BMR was again significantly influenced by $T_{acc}$, indicating reversibility in BMR as a response to short-term thermal acclimation. Repeatability of BMR was significant in only one temperature group (the $10^{o}$C group) with 25% of the observed variation being attributed to between-individual variability. The mechanistic correlate responsible for BMR adjustment is metabolic intensity, as organ size was relatively invariant. The only evidence for a change in body composition was that the intestines of the group acclimated to $10^{o}$C were significantly heavier than those of the $22^{o}$C group.

## 2.2. INTRODUCTION

Variation in avian physiological traits has received a great deal of attention over the last 70 years (Dawson, 2003). These studies usually focused on the allometric scaling of metabolic traits with body mass, the effects of phylogenetic inertia, and/or adaptation to biotic and abiotic determinates (McKechnie *et al*., 2006; McKechnie and Wolf, 2004a; Frappel *et al.,* 2001; Tieleman and Williams, 2000; Lasiewski and Dawson, 1967). Allometric equations are a valuable tool in comparative physiology as they allow us to assess whether a particular species deviates from the norm with respect to its body size, and how differences in design reflect different functions (Frappel *et al.*, 2001). However, comparisons across taxa need to be corrected to control for the effects of phylogenetic inertia (Felsenstein, 1985). These corrections give us the ability to clearly distinguish whether observed traits are a consequence of adaptive variation or an underlying effect of phylogeny.

Recently there has been an increasing interest in uncovering traits that exhibit phenotypic flexibility, as this flexibility may allow organisms to cope with environmental heterogeneity (Relyea, 2002). Phenotypic flexibility i.e. reversible within-individual variation (Piersma and Drent, 2003) is thought to be selected for in temporally unpredictable environments, since individuals with physiological flexibility may presumably cope better with fluctuating environmental conditions than individuals in which these parameters remain static. Phenotypic flexibility in metabolic traits has been documented in many endothermic animals (Broggi *et al*., 2004; Bozinovic *et al*., 2003, Nagy and Gruchacz, 1994), and several studies provide examples of how behavioural and ecological changes drive shifts in parameters such as body temperature ($T_b$), body mass

($M_b$), organ metabolic intensity, wet thermal conductance ($C_{wet}$), evaporative water loss and basal metabolic rate (BMR) (Tieleman *et al*., 2003a; Tieleman and Williams, 2000; Vézina and Williams, 2003; Liknes *et al.*, 2002; Williams and Tieleman; 2000; Swanson, 1991; Swanson in press).

Basal metabolic rate is the standard baseline parameter used to quantify the energy costs of thermoregulation, limits of metabolic scope, energy expenditure, and evolutionary adjustments in metabolic rates to biotic and abiotic constraints (Williams and Tieleman, 2000). Basal metabolic rate is always measured during the rest phase in postabsorptive, inactive, non-growing, non-reproductive individuals in a thermoneutral environment (McKechnie and Wolf 2004a; Lovegrove, 2000). Even though animals in their natural environment seldom function at basal levels, understanding BMR has increased our understanding of animal physiology and energetics, and given us insight into how these animals have evolved in response to different environmental and ecological stresses (Williams and Tieleman, 2000). There is increasing evidence that, like other physiological parameters, BMR is not static but is adjusted over short time scales (Klaassen *et al*., 2004; Piersma and Lindström, 1997).

The most extreme occurrences of flexibility in BMR are found in year-round residents at high latitudes and long distance migrants (Battley *et al*., 2001, 2000; Swanson and Olmstead, 1999), but it is also present in non-migrant tropical and sub-tropical species (Tieleman *et al*., 2003a). Seasonal adjustments in BMR vary in their direction and magnitude, and BMR has been reported to increase during winter in American Goldfinches, *Carduelis tristis,* (Liknes *et al.,* 2002) and Dark-eyed Juncos, *Junco hyemalis*, (Swanson, 1991), to decrease in the Australian Silvereye, *Zosterops*

*lateralis*, (Maddocks and Geiser, 1997) and to show no difference in the Northern Bobwhite, *Colinus virginianus* (Swanson and Weinacht, 1997). Laboratory experiments on Hoopoe Larks, *Alaemon alaudipes,* showed that birds acclimated for three weeks to a cool (15°C) air temperature ($T_a$) increase their BMR by ca. 42% relative to individuals acclimated to 35°C (Williams and Tieleman, 2000).

The realization that many species of birds exhibit phenotypic flexibility in physiological traits such as BMR recently led Tieleman *et al.,* (2003b) to hypothesize that birds inhabiting desert areas characterized by a high degree of environmental heterogeneity have a more flexible BMR than more mesic species. In five species of larks occupying habitats ranging from mesic to hyper-arid, there was no evidence to support this hypothesis (Tieleman *et al*., 2003b). However, acclimation to different air temperatures did induce changes in BMR, body mass and body composition (Tieleman *et al*., 2003b). Changes in BMR (by either acclimation or acclimatization) often reflect changes in an animal's morphology, most notably changes in body mass and the relative masses of organs associated with thermoregulatory heat production (Piersma and Lindström, 1997; Williams and Tieleman, 2000; Tieleman *et al*., 2002). Additionally, adjustments in BMR of migratory birds also reflect changes in body composition. For example, in a migratory flight from Australia to China, the Great Knot, *Calidris tenuirostris*, showed decreases in lean muscle mass, fat content and the masses of several organs (Battley *et al*., 2001, 2000). In this species the changes in body composition were so extreme that, aside from the lungs and brain, no organ remained homeostatic during its migratory flight (Battley *et al*., 2000).

There has also been a recent interest in assessing whether BMR is a repeatable trait. In female Kittiwakes, *Rissa tridactyla*, BMR has been revealed to be a significantly repeatable trait with between 35-52 % of the variation in BMR of breeding birds being attributed to between-individual variation (Bech *et al.*, 1999). Additionally, in Zebra Finches, *Taeniopygia guttata*, ca. 41% of the observed variation in BMR is repeatable over time scales as long as 2.5 years, making individual variation in BMR a possible target for natural selection (Rønning *et al.*, 2005). However, the repeatability of BMR before and following thermal acclimation has not yet been investigated. One possibility is that individuals that exhibit elevated BMR compared to other members of an experimental population before thermal acclimation exhibit similar relative BMR following phenotypic adjustments to a new thermal environment. In this case, relative BMR would be a heritable trait that is potentially subject to selection, despite the fact that BMR is temporally variable within individuals.

The 'energy demand' hypothesis states that an increase in energetic demands, due to either greater activity levels and/or low $T_a$s necessitates an increase in food consumption (Williams and Tieleman, 2000). This in turn stimulates the organs involved in the catabolic processes of digestion (stomach, intestines and liver), elimination of waste (kidneys) and transport of oxygen (lungs and heart) to the tissues into hypertrophy (Williams and Tieleman, 2000). Since all these organs have a high metabolic intensity, an increased demand on the performance of these organs may result in an increase in their size and/or metabolic intensity, thus elevating the BMR of the animal (Williams and Tieleman, 2000).

This study investigates the flexibility, reversibility and repeatability of BMR and associated metabolic responses of Laughing Doves, *Streptopelia senegalensis*, to short-term thermal acclimation. Specifically, I investigated whether metabolic adjustments in response to thermal acclimation are reversible over a time scale of several weeks. I predicted that the BMR of Laughing Doves would increase after acclimation to colder $T_a$s, and decrease after acclimation to warmer $T_a$s.

I also examined a mechanistic correlate that might explain variation in BMR, namely organ mass. I hypothesised that the increase in BMR would reflect an increased demand in oxygen consumption to vital organs necessary for the maintenance of cellular homeostasis, and would thus increase the weight of these organs (as they would be stimulated into hypertrophy) and increase the body mass of the bird itself.

## 2.3. MATERIALS AND METHODS

### *2.3.1. Capture and housing*

Eighty-two Laughing Doves were caught during July 2005 in Pietermaritzburg, South Africa (sixty-two were used in this experiment and the remaining 20 were used for a separate experiment running concurrently – see Chapter 3). Birds were trapped using two walk-in traps baited with a wild birdseed mix. They were initially housed in outdoor aviaries (1m wide x 3m high x 3m long) in the Animal House Facility of the School of Biological and Conservation Sciences at the University of KwaZulu-Natal in Pietermaritzburg with water, wild birdseed and grit available *ad libitum*. Following an initial outdoor period of four weeks, accustoming the birds to captivity and an *ad lib* feeding regime, they were marked with coloured split celluloid rings for individual

identification and moved to individual cages (ca. 40cm wide x 40cm high x 50cm long), in one of three indoor temperature-controlled ($10^{o}\pm2^{o}$, $22^{o}\pm2^{o}$ and $35^{o}\pm2^{o}$C) constant environment (CE) rooms. In the CE rooms they experienced a 12h L: 12h D photoperiod, approximately matching that prevailing outdoors, and were again given *ad lib* access to water, wild birdseed and grit. Birds were housed in the cages until the end of the thermal acclimation experiments in November 2005. The first 30 birds were weighed upon capture, and weighed weekly thereafter while in the outdoor aviaries. Mean body mass ($M_b$) of the doves upon capture was 93.05±6.25g, and after one week in captivity decreased by approximately 7.8% and then stabilized.

*2.3.2. Oxygen consumption and body temperature*

Metabolic rate (MR) was measured indirectly as oxygen consumption ($VO_2$) in an open flow-through respirometry system. Birds were weighed to two decimal places and placed individually into 3.96-L clear Perspex respirometry chambers (22cm high x 15cm long x 12cm wide). The respirometry chambers were placed in a $1m^3$ soundproof temperature controlled cabinet at either 16:30 or 23:30h, with measurements beginning at either 17:00 or 24:00h. Birds were removed from the respirometers at either 23:30h (the same day) or 06:30h the following morning. Removal and insertion of the birds into the respirometry chambers at 23:30h was done in the dark (with the aid of a red light) so that ambient light would not disrupt the natural circadian rhythm of the birds. Atmospheric air, acting as a control gas, was drawn from outside the building, dried by passing through a column of silica gel, and then pumped into the temperature cabinet. The dried air was then drawn in

from the base of each respirometer and pulled through the top using a variable speed air pump.

A flow rate of 750±190 ml.min$^{-1}$ was chosen to maintain <1% $O_2$ depletion rate between incurrent and excurrent airflow. The flow rate in each chamber was measured with a mass flow meter (Brooks thermal model 5810) calibrated with a soap bubble flow meter (Baker and Pouchot, 1983). Excurrent air from each respirometer was passed through a water condenser (consisting of copper tubing) where the air was partially dried by cooling it to approximately 3$^{o}$C. After passing through the condenser, air was sub-sampled using a system of solenoid valves, and further dried using a column of silica gel, before the fractional concentration of $O_2$ was measured with an $O_2$ analyzer (Ametek S-3A/I Applied Electrochemistry, Sunnyvale, California, USA). Solenoid valves coupled with individual pumps allowed the sampling of five respirometers and a control channel within a six minute cycle, one minute for each of the five experimental channels and one minute for the control channel. Analog signals from the temperature probes, mass flow meters and the $O_2$ analyzer were digitized using an A/D converter and recorded on a multi-channel Windows based recording program written by B. G. Lovegrove. The fractional concentration of $O_2$ in each experimental chamber was subtracted from that in the control chamber, effectively zeroed every six minutes, nullifying the effect of long-term drift in the $O_2$ analyzer. Fractional $O_2$ concentration, flow rate and air temperature in each respirometry chamber were measured in the last 5 seconds of each sub-sampling interval, allowing air from the previous channel to be purged from the system. The rate of oxygen consumption was calculated using the equation 3a from Withers (1977) and accounts for $CO_2$ that is still present in the air:

$$VO_2 = V_E(F_{IO2}-F_{EO2})/(1-F_{IO2}),$$

Where

$VO_2$ = $O_2$ consumption (ml $O_2$ $min^{-1}$),
$V_E$ = flow rate (ml $min^{-1}$),
$F_{IO2}$ = incurrent fractional $O_2$ concentration, and
$F_{EO2}$ = excurrent fractional $O_2$ concentration

A factor of 20.083 J.ml $O_2^{-1}$ was used to covert $VO_2$ to metabolic rate in Watts (W) (Schmidt-Nielsen, 1990). Body temperature ($T_b$) was recorded within 30 seconds of removing each bird from the respirometry chamber. A fine gauge thermocouple was inserted into each bird's cloaca, to a depth of ca. 1.5cm where a slight withdrawal did not result in a decrease in the reading.

### *2.3.3. Experimental protocol*

#### *2.3.3.1. Determination of the thermoneutral zone (TNZ)*

The TNZ is the air temperature range over which MR is minimal and does not change with $T_a$. The lower critical limit of thermoneutrality ($T_{lc}$) is the lower limit of the TNZ, below which MR increases for thermoregulation. To determine the $T_{lc}$ and TNZ, MR was measured in 15 birds (not used in the acclimation and reverse-acclimation trials described below) over a period of nine days and subjected to a series of air temperatures ($0^oC<T_a<32^oC$). Each bird spent a minimum of 2h at each $T_a$ ($0^o$, $5^o$, $10^o$, $15^o$, $20^o$, $24^o$, $28^o$ & $32^oC$). The mean of the three lowest $VO_2$ values from the last 30 minutes of each temperature were plotted against $T_a$ and a linear regression fitted. The $T_{lc}$ was calculated from the intercept of the linear regression and lowest observed $VO_2$ (assumed to be equivalent to the animal's BMR). Since the $T_{lc}$ is potentially subject to change induced

by acclimation, it was determined for each group following acclimation and again following reverse-acclimation.

All BMR measurements were made at or just above the $T_{lc}$ to ensure basal rates of metabolism were recorded.

*2.3.3.2. Experiment 1- BMR responses to Acclimation and reverse-acclimation.*

On completion of the determination of the $T_{lc,}$ initial BMR was measured in 30 birds from the outdoor aviaries. To ensure that the BMR of each bird was measured exactly at the $T_{lc}$, a temperature profile of $3^{o}$ or $5^{o}$C was used ($\pm 1^{o}$ or $2^{o}$C on either side of the previously determined $T_{lc}$). The birds experienced each $T_a$ for at least two hours and the mean of the three lowest consecutive values of $VO_2$ in the last 30 minutes at any one of the temperatures was considered to be the animals BMR. All data were examined, and non-steady state $VO_2$, i.e. where consecutive measures followed in a 10% range of the previous measure, was excluded from the analyses.

Following an initial BMR measurement each bird was randomly allocated to one of the three CE rooms ($10^{o}$, $22^{o}$ & $35^{o}$C), until each CE room had 10 birds. Each group was then allowed to acclimate to the thermal environment in the CE room for 21days. .

Toward the end of the 21day acclimation period the $T_{lc}$ was determined again. Determination of the new $T_{lc}$ was done with the 10 experimental birds over a period of four nights at $T_a$s of $5^{o}$, $10^{o}$, $15^{o}$, $20^{o}$, $23^{o}$, $26^{o}$, $29^{o}$ & $31^{o}$C (each $T_a$ was again experienced for 2 hours). Each bird spent a maximum of 36 hours outside of the CE rooms. Thereafter BMR was measured again. The 10 birds from each temperature group

were then randomly split into two groups of five and each group assigned to one of the two CE rooms not yet experienced (Fig. 1).

After a 21day reverse-acclimation period to the new acclimation air temperature ($T_{acc}$) the $T_{lc}$ was again determined for each group of birds and the subsequent (reverse-acclimated) BMR measures taken. On completion of this experiment the 30 birds were released.

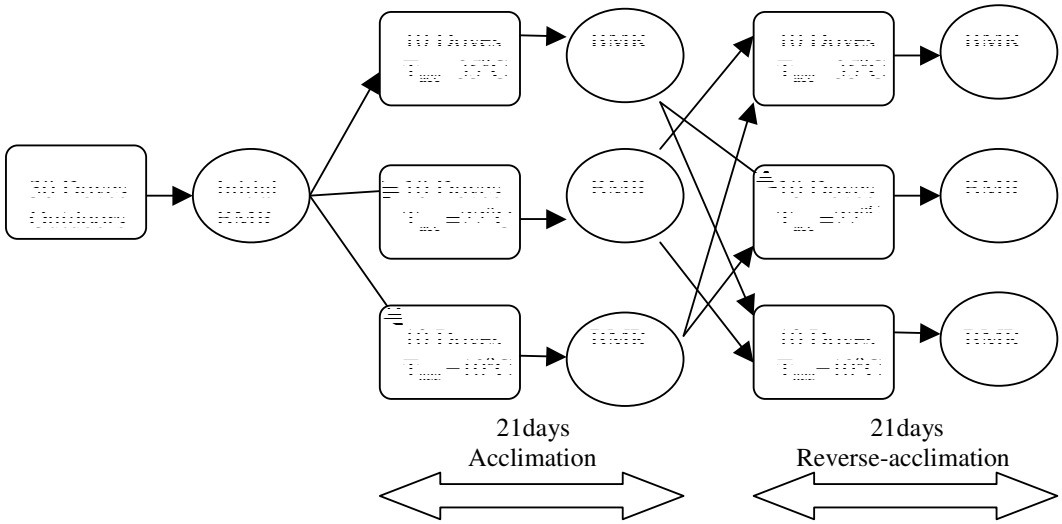


Fig 1. A flow diagram of the experimental protocol investigating flexibility and reversibility in BMR of Laughing Doves (*Streptopelia senegalensis*) in response to short-term thermal acclimation. Following an initial measurement of BMR the doves were acclimated for 21 days. After this period BMR was measured again and the 10 birds in each group were randomly divided into two groups of five, and moved into one of the other two constant-environment rooms for a second reverse-acclimation period of 21 days.

### *2.3.4. Minimum wet thermal conductance ($C_{wet}$)*

Wet thermal conductance (i.e. including evaporative heat loss) was calculated as:

$$C_{wet}\,(\text{ml } O_2\ g^{-1}\ h^{-1}\ {}^{o}C^{-1}) = MR/(T_b - T_a)$$

Where:

MR= metabolic rate in ml $O_2$ $g^{-1}$ $h^{-1}$ and

$T_a$ & $T_b$ = body & ambient temperature measured in $^{o}C$ (from Schleucher and Withers, 2001).

Wet thermal conductance was measured at either $1^{o}$ or $2^{o}C$ below the $T_{lc}$ and represent minimum $C_{wet}$. A conversion factor of 1 ml $O_2$ = 20.083J was used to convert $C_{wet}$ from ml $O_2$ $g^{-1}$ $h^{-1}$ $^{o}C^{-1}$ to J $g^{-1}$ $h^{-1}$ $^{o}C^{-1}$.

### *2.3.5. Repeatability of basal metabolic rate*

Repeatability of BMR was calculated from the variance components derived from a main effects ANOVA test, as described in Lessells and Boag (1987). Briefly, repeatability or '*r*' is given by the equation:

$$r = S^2_A / S^2 + S^2_A$$

Where:

$S^2_A$ = among individual variance, and

$S^2$ = within individual variance

Variance components were derived from mean squares in a one-way ANOVA where BMR was the dependant variable and treatment as the fixed factor:

$$S^2_A = (MS_A - MS_W) / N_0$$

Where:

$MS_W$ = error mean square

$MS_A$ = mean square among individuals, and

$N_0$ = the coefficient related to the sample size per individual (Lessells and Boag, 1987).

As BMR did not show a consistent dependence on $M_b$ (see below), the limitation of ordinary least square regressions underestimating the true allometric exponent scaling of BMR (Rønning *et al.*, 2005) was not a factor in this analysis.

### *2.3.6. Experiment 2 - Body composition and organ masses*

Thirty-two laughing doves were killed by carbon dioxide asphyxiation. Eight of the 15 that were used to determine the initial $T_{lc}$ (and had previously spent nine days in the 22°C CE room) were killed and are referred to in the analyses as the initial birds. The remaining 24, eight from each temperature group (10°C, 35° and 22°C) were killed after an acclimation period of 21 and 29 days respectively. To ensure doves were postabsorptive, and to eliminate gut contents contributing to the mass of the stomach and intestines, all doves prior to being killed were fasted for 20 hours - as gut retention time in both Black-faced Solitaires (*Myadestes melanops*) and European Blackbirds (*Turdus merula*) is no longer than two hours (Murray *et al*., 1994; Sorenson, 1984). Immediately after the birds were killed they were weighed to two decimal places and the heart, liver, stomach, intestines, kidneys and pectoral and supracoracoideus muscles (referred to collectively in the analyses as pectoral muscles) dissected out, weighed, and dried at 60°C to a constant mass. All tissue and muscle samples were weighed to two decimal places.

In the analyses organ tissue and muscle masses are expressed as percentage of the corresponding masses in the initial eight birds.

### *2.3.7. Data analysis*

To compare BMR, $T_b$, $M_b$ and $C_{wet}$ among groups and through time a repeated measures analysis of variance (RM-ANOVA) was used. Dependant variables were the parameters being investigated and the independent variables were time and $T_{acc}$. I did not use an analysis of co-variance (ANCOVA) to control for mass as a co-variate when analyzing BMR, as BMR was not consistently dependent on $M_b$ (see results). An ANOVA was used to identify significant differences in the relative changes of the organ masses among temperature groups. Unless otherwise stated results were considered significant if $P \leq 0.05$. An '*a posterior*' Tukey test for multiple comparisons was used to identify significant differences among groups (Zar, 1984). Results were analyzed using Statsoft Statistica Version 6.

## 2.4. RESULTS

### *2.4.1. Body mass*

Mean $M_b$ of the Laughing Doves upon capture was 93.05±1.39g (N = 30). After one week in captivity in the outdoor aviaries $M_b$ decreased significantly ($t = 9.3$; $P<0.001$) to 85.78±1.38g (N = 30), after which it showed no further significant change and stabilized at 88.68±1.43g (N = 30) after four weeks.

Following the initial measurements (before acclimation) of $M_b$ in all birds, $M_b$ increased slightly in all groups, with the 10°C reverse-acclimated group displaying the

largest mass gain of 7.5% (Table. 1). However, throughout the duration of the study $M_b$ did not change significantly ($F_{4,54}$=1.96; $P$>0.05) with either treatment or time after acclimation or reverse-acclimation.

Table 1. Mean ± SE body mass (g) of Laughing Doves (*Streptopelia senegalensis*) at three acclimation temperatures ($T_{acc}$) after the initial, acclimated and reverse-acclimated time periods. Body mass of the Laughing Doves did not differ significantly throughout the duration of the study.

| $T_{acc}$ | Initial (0 days) | Acclimated (21 days) | Rev-acclimated (42 days) | *N* |
|---|---|---|---|---|
| 10°C | 90.42±2.43 | 94.46±2.11 | 99.17±2.69 | 10 |
| 22°C | 95.77±1.81 | 97.52±2.45 | 93.62±2.55 | 10 |
| 35°C | 91.76±1.77 | 91.59±2.54 | 93.62±2.36 | 10 |

*2.4.2. Body temperature*

Initial $T_b$ of the Doves before allocation to their CE rooms did not differ significantly among the three groups ($F_{4,54}$= 3.27; $P$>0.05; Fig.2). After acclimation, $T_b$ of the 10°C and 22°C groups averaged 40.5±0.4 ($N$=10) and 39.8±0.4°C ($N$=10) respectively and was significantly higher ($F_{4,54}$= 3.27; $P$<0.05) from their initial $T_b$. The acclimated 35°C group averaged 38.4±0.4°C ($N$=10) which was not significantly different from their initial $T_b$ ($F_{4,54}$= 3.27; $P$>0.05; Fig.2) but was significantly lower than the $T_b$ of the acclimated 10°C group. No other significant differences were found between the acclimated or reverse-acclimated groups (Fig.2 & Table 2).

Table 2. Mean ± SE body temperature (°C) of Laughing Doves (*Streptopelia senegalensis*) at three acclimation temperatures ($T_{acc}$) after the initial, acclimated and reverse-acclimated time periods.

| $T_{acc}$ | Initial (0 days) | Acclimated (21 days) | Rev-acclimated (42 days) | *N* |
|---|---|---|---|---|
| 10°C | 37.9±0.4 | 40.5±0.4 | 39.2±0.6 | 10 |
| 22°C | 36.9±0.4 | 39.8±0.4 | 38.1±0.5 | 10 |
| 35°C | 38.3±0.3 | 38.5±0.4 | 38.6±0.4 | 10 |

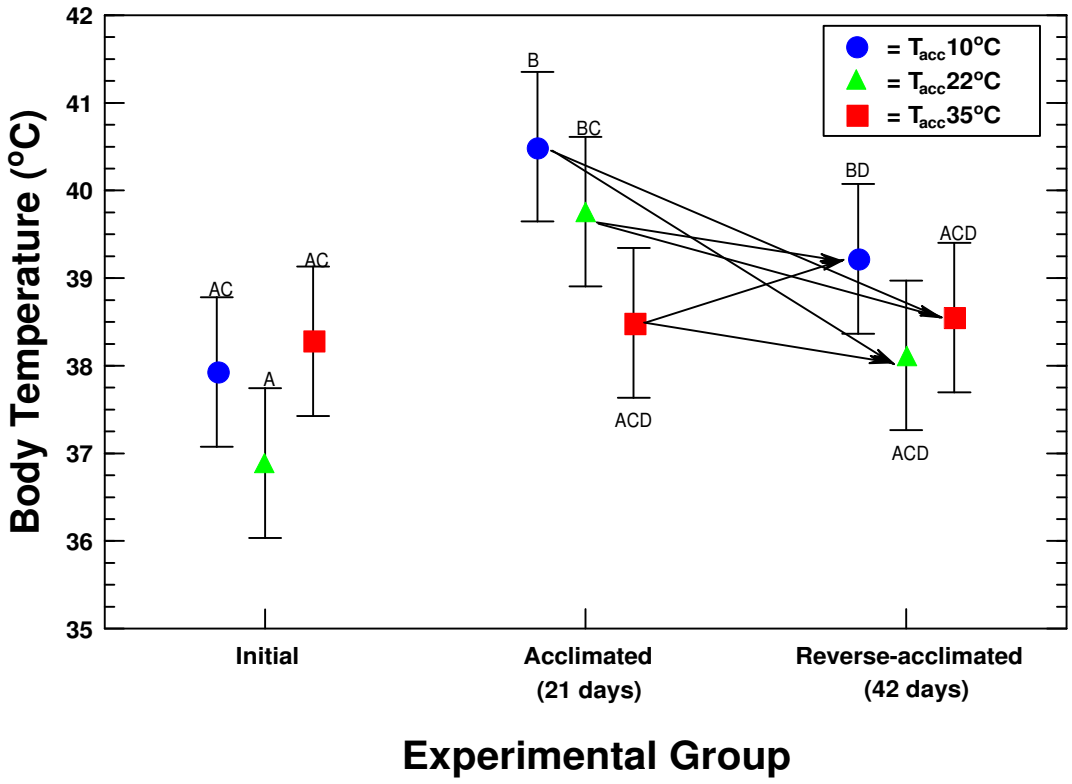


Fig. 2. The relationship between body temperature (°C), experimental group and acclimation temperature ($T_{acc}$) in Laughing Doves (*Streptopelia senegalensis*). Body temperature ($T_b$) of the three experimental groups were not significantly different from each other after their initial or reverse-acclimation measures. However, after acclimation $T_b$ of the 10°C group was significantly higher from their initial measure. Means followed by the same letter are not statistically different ($P$>0.05). Vertical bars denote 95% confidence intervals.

*2.4.3. Minimum wet thermal conductance*

The $C_{wet}$ of the Laughing Doves in the three $T_{acc}$ groups did not differ significantly either within or among groups throughout the duration of the experiment (Fig. 3). The 35°C group exhibited the most stable $C_{wet}$, which changed by <4.5 % throughout the duration of the study (Table 3).

Table 3. Mean ± SE minimum wet thermal conductance (J $g^{-1}$ $h^{-1}$ $^{o}C^{-1}$) of Laughing Doves (*Streptopelia senegalensis*) at three acclimation temperatures ($T_{acc}$) after the initial, acclimated and reverse-acclimated time periods. Doves at $T_{acc}$ = 35°C had the most stable $C_{wet}$ throughout the duration of the study.

| $T_{acc}$ | Initial (0 days) | Acclimated (21 days) | Rev-acclimated (42 days) | *N* |
|---|---|---|---|---|
| 10°C | 3.300±0.186 | 2.723±0.172 | 2.796±0.180 | 10 |
| 22°C | 3.459±0.163 | 3.160±0.206 | 3.366±0.263 | 10 |
| 35°C | 3.051±0.175 | 3.110±0.331 | 2.960±0.134 | 10 |

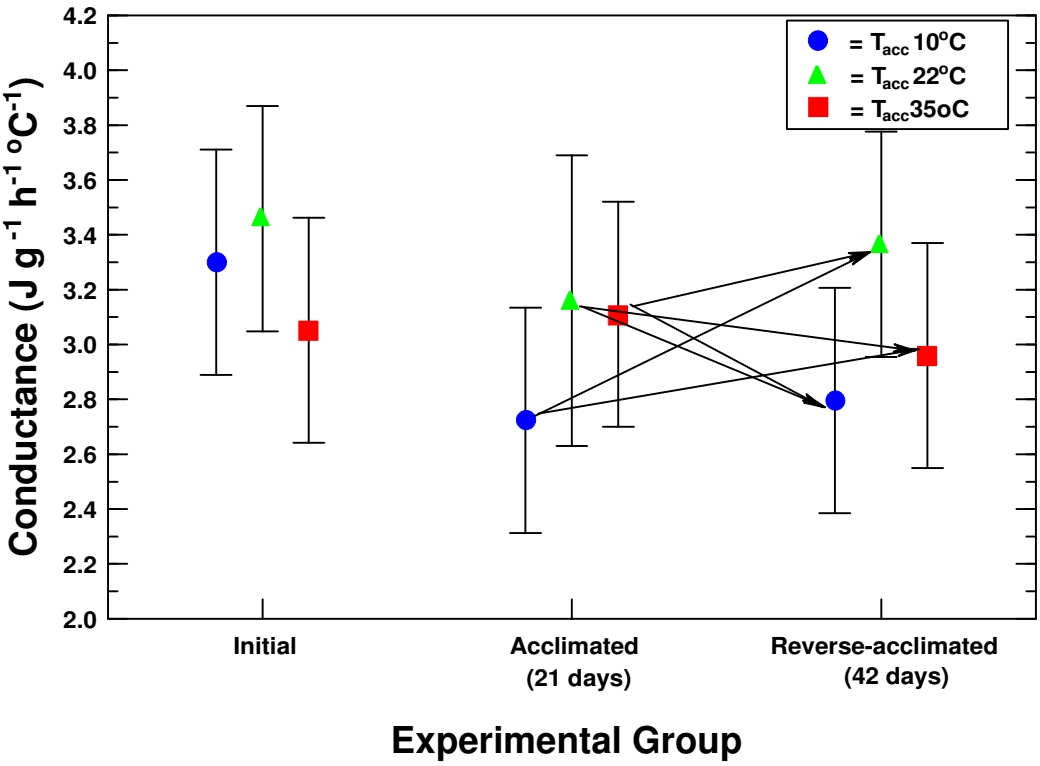


Fig. 3. Relationship between minimum wet thermal conductance (J $g^{-1}$ $h^{-1}$ $^oC^{-1}$), experimental group and acclimation temperature ($T_{acc}$) in Laughing Doves (*Streptopelia senegalensis*). Minimum wet thermal conductance ($C_{wet}$) did not differ significantly among the three temperature groups throughout the duration of the experiment. Vertical bars denote 95% confidence intervals.

### *2.4.4. Basal metabolic rate*

Basal metabolic rate was not consistently dependant on $M_b$, and in only one of nine measures was the effect of $M_b$ significant ($P$<0.05; Fig. 4). Hence, a RM-ANOVA was used to determine significant differences in BMR among temperature groups and through time.

Initial BMR measurements did not differ significantly ($F_{4,54}$= 1.20; $P$>0.05) between the three temperature groups. However, following the initial measurements BMR decreased significantly ($F_{4,54}$= 1.20; $P$<0.05) in the 22$^o$ and 35$^o$C groups after

acclimation and reverse-acclimation (Fig. 5). After acclimation and reverse-acclimation BMR was higher in the 10°C group when compared to the 35°C. This increase was however, only significant ($F_{4,54}$= 1.20; $P$>0.05; Fig. 5; Table 4) after acclimation. BMR of the 22°C group was similar to both the 10° and 35°C groups after acclimation and reverse-acclimation (Fig. 5).

To determine the effect of $T_{acc}$ on the BMR of the doves, linear regressions of $T_{acc}$ against BMR were fitted for the acclimated and reverse-acclimated measures (Fig. 6). Acclimation temperature directly influenced BMR, as both these regressions confirmed a significant relationship between $T_{acc}$ and BMR ($P$<0.05; Fig. 6). The BMR of the doves after acclimation and reverse-acclimation were lowest at 35°C, highest at 10°C, and intermediate at 22°C. By comparing the slope of the two regressions, using a modified *t*-test (Zar, 1984), I was able to determine that the effect of $T_{acc}$ on BMR was reversible. The two linear regressions did not differ significantly ($t_{0.05,2}$=0.058*; P*<0.05; Fig.6) and show that BMR of the doves after reverse-acclimation was readjusted in response to $T_{acc}$. This is the first evidence of reversibility of BMR in response to short-term thermal acclimation.

By ranking BMR (Fig. 7) of individual birds I was able to track which birds had the highest or lowest BMR before and after acclimation. Repeatability in BMR was only significant (see Fig.7 for statistics) for the 10°C group, with between-individual variation accounting for 25% of the observed variation in BMR.

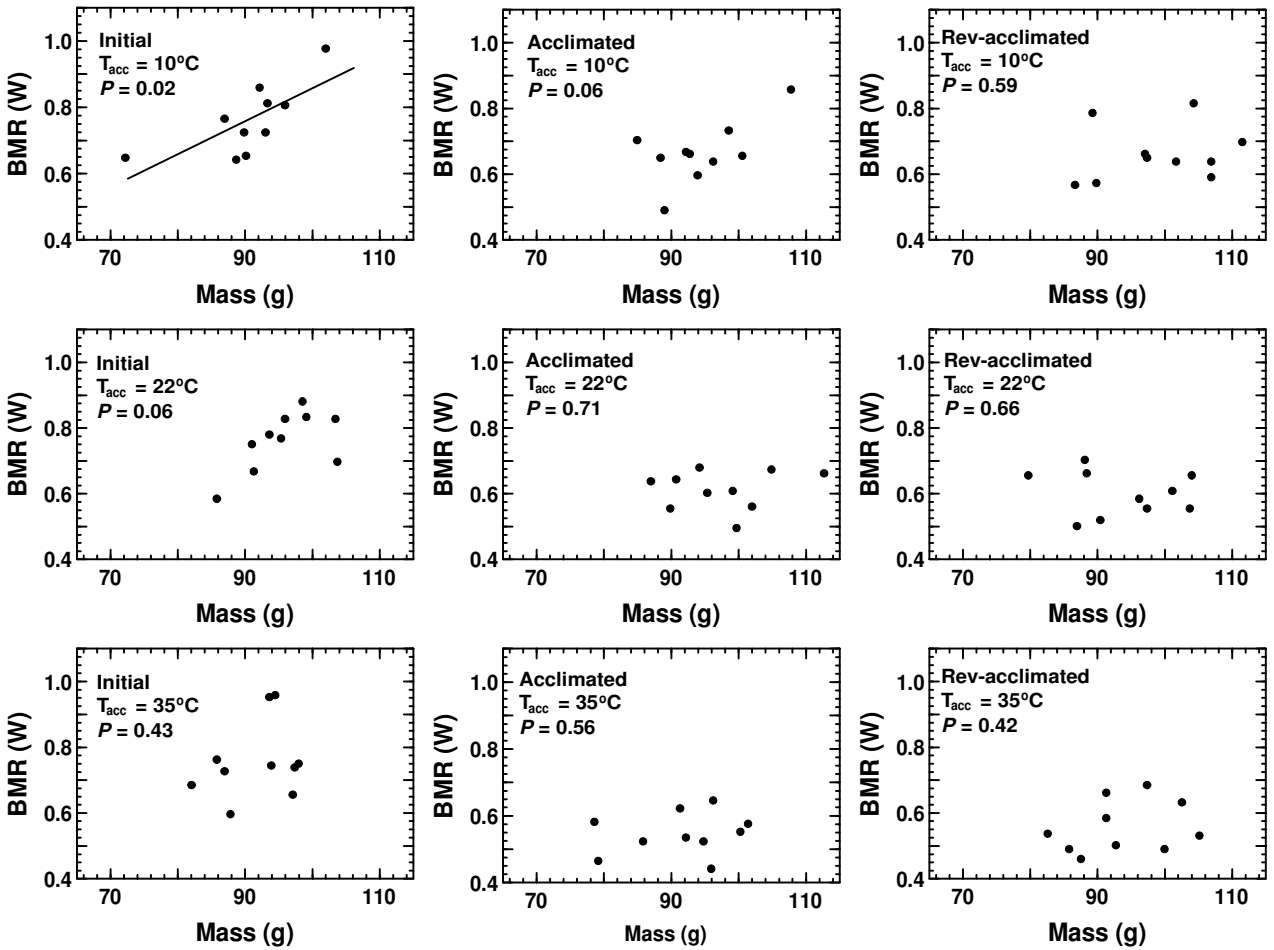


Fig. 4. The relationship between basal metabolic rate (BMR; W) and body mass ($M_b$; g) in Laughing Doves (*Streptopelia senegalensis*). A significant relationship ($P<0.05$) between BMR and $M_b$ was detected in only one of the nine measurements of BMR. The significant relationship ($P= 0.0195$) is indicated by the addition of a regression line ($BMR = -0.13+0.01M_b$; $r^2 =0.5148$).

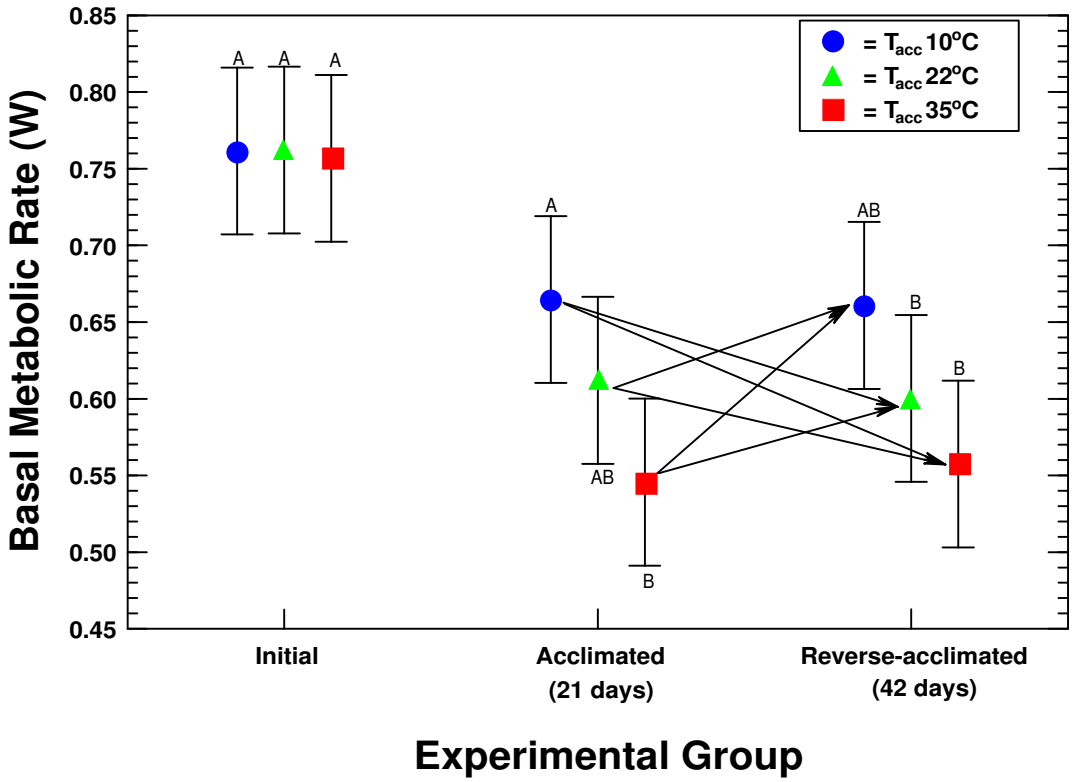


Fig. 5. The relationship between basal metabolic rate (BMR; W), time (days) and acclimation temperature ($T_{acc}$) in Laughing Doves (*Streptopelia senegalensis*). The three temperature groups display a similar pattern in BMR distribution after both acclimation and reverse-acclimation. Means followed by the same letter are not significantly different ($P$>0.05). Vertical bars denote 95% confidence intervals.

Table 4. Mean ± SE basal metabolic rate (BMR; W) of Laughing Doves (*Streptopelia senegalensis*) at three acclimation temperatures ($T_{acc}$) after the initial, acclimated and reverse-acclimated time periods.

| $T_{acc}$ | Initial (0 days) | Acclimated (21 days) | Rev-acclimated (42 days) |
|---|---|---|---|
| 10°C | 0.762±0.034 | 0.665±0.029 | 0.661±0.026 |
| 22°C | 0.762±0.028 | 0.612±0.019 | 0.600±0.021 |
| 35°C | 0.756±0.037 | 0.546±0.019 | 0.557±0.025 |

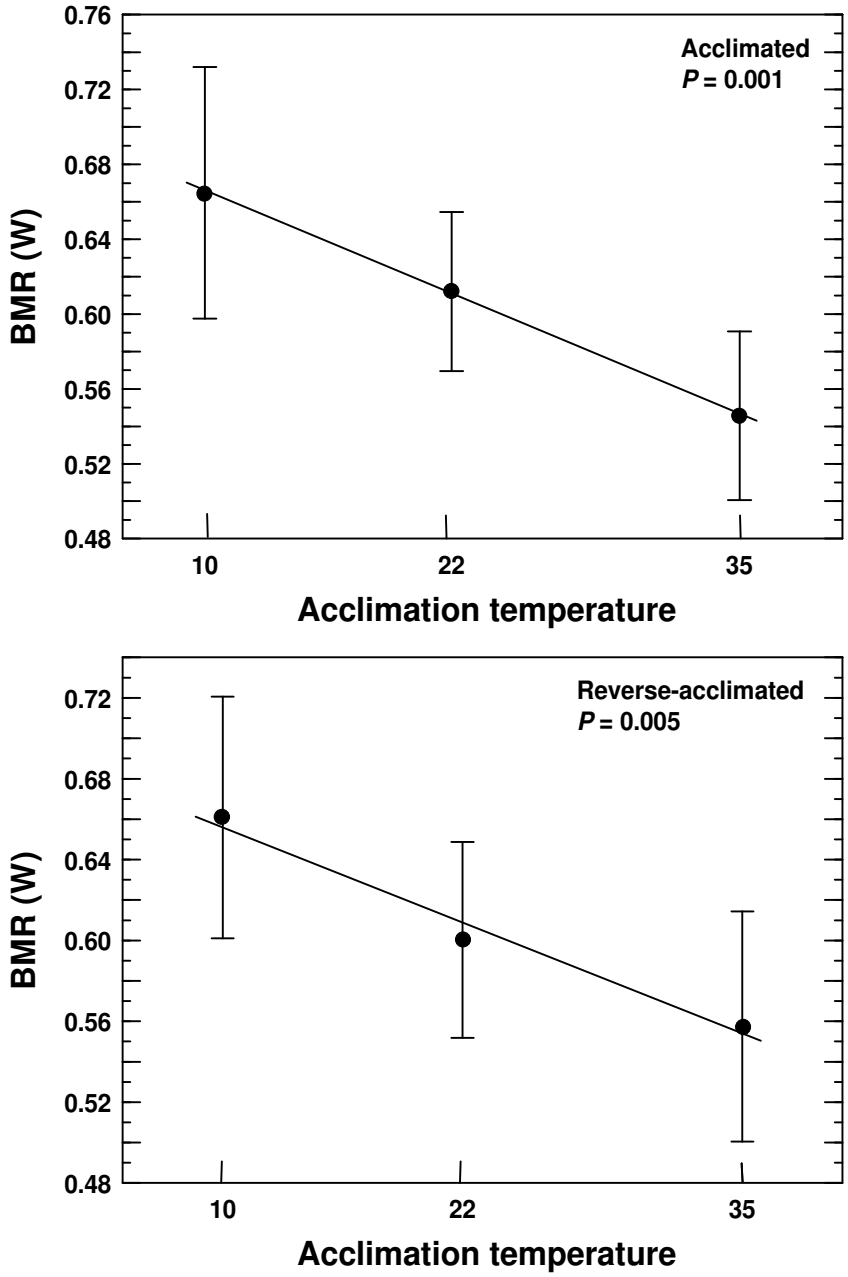


Fig. 6. The relationships between basal metabolic rate (BMR; W) and acclimation temperature ($T_{acc}$; $^{o}$C) after acclimation and reverse-acclimation in Laughing Doves (*Streptopelia senegalensis*). Both regression lines; Acclimated: BMR=0.714-0.0048$T_{acc}$ ($r^2$ = 0.33), and Reverse-acclimated: BMR= 0.698- 0.0041$T_{acc}$ ($r^2$ = 0.25) confirm a significant ($P$<0.05) relationship between $T_{acc}$ and BMR. A modified $t$-test (Zar, 1984) was used to compare the two regression lines, which were not significantly different ($t_{0.05,2}$ = 0.058; $P$<0.05) and indicates reversibility in BMR in response to $T_{acc}$.

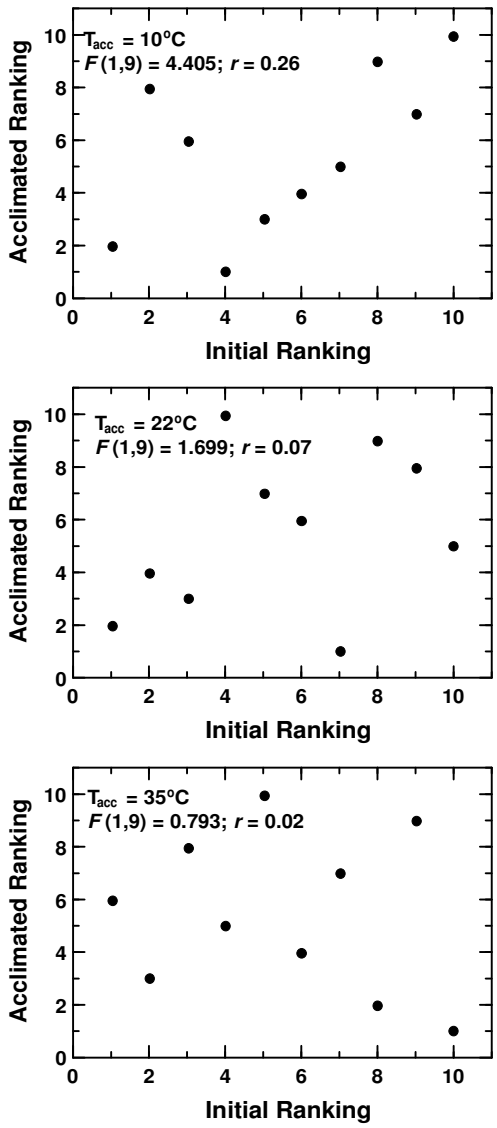


Fig. 7. Ranking of BMR in 30 individual Laughing Doves (*Streptopelia senegalensis*) before and after acclimation to three acclimation temperatures ($T_{acc}$). A value of 1 indicates lowest BMR. The '*r* '= repeatability values for the 10 individual birds at each temperature for initial and acclimated measures only.

### 2.4.5. *Experiment 2 - Body Composition and organ masses*

The $M_b$ of the four groups of birds did not differ significantly ($F_{3,28}$=1.88; $P$>0.05) from each other, and differences in BMR were similar to those of the acclimation experiment. BMR of the initial group was significantly higher ($F_{3,28}$=1.88; $P$<0.05; one-way ANOVA) than that of the three acclimated groups, and BMR of the 10°C group was also significantly higher ($F_{3,28}$=1.88; $P$<0.05) than the 35°C group (Fig. 8). No significant differences were found in the mean masses of the heart, stomach, liver, kidneys and pectoral muscles of the four experimental groups. The only organ to show a significant difference ($F_{3,28}$=2.49; $P$<0.05) was the intestines, where the 10°C group was heavier than the 22°C group (Fig.9).

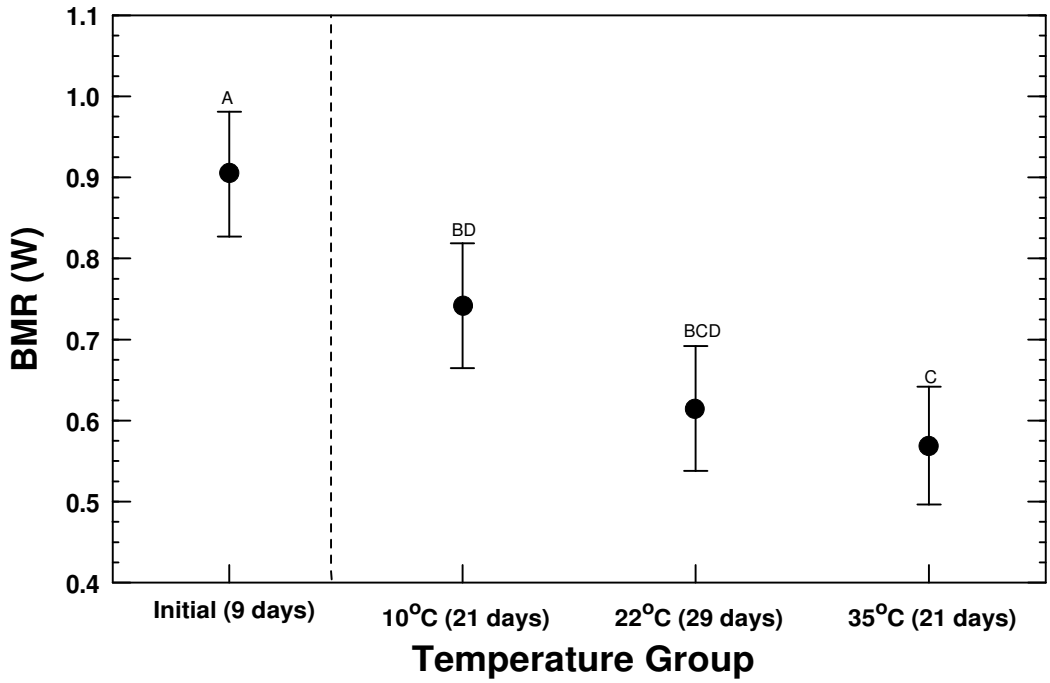


Fig. 8. The relationship between basal metabolic rate (BMR; W) in the initial group and the three acclimated temperature ($T_{acc}$) groups of Laughing Doves (*Streptopelia senegalensis*). The initial group spent 9 days in the 22°C constant environment (CE) room, while the other three $T_{acc}$ groups (10°, 35° & 22°C) spent 21 and 29 days in their respective CE rooms. BMR displays a similar distribution pattern as the acclimation experiment, where all the three $T_{acc}$ groups decrease BMR in response to acclimation. However, as observed in the acclimation experiment the BMR of the 10°C group is higher than the 35°C group. Means followed by the same letter are not significantly different ($P$>0.05). Vertical bars denote 95% confidence intervals.

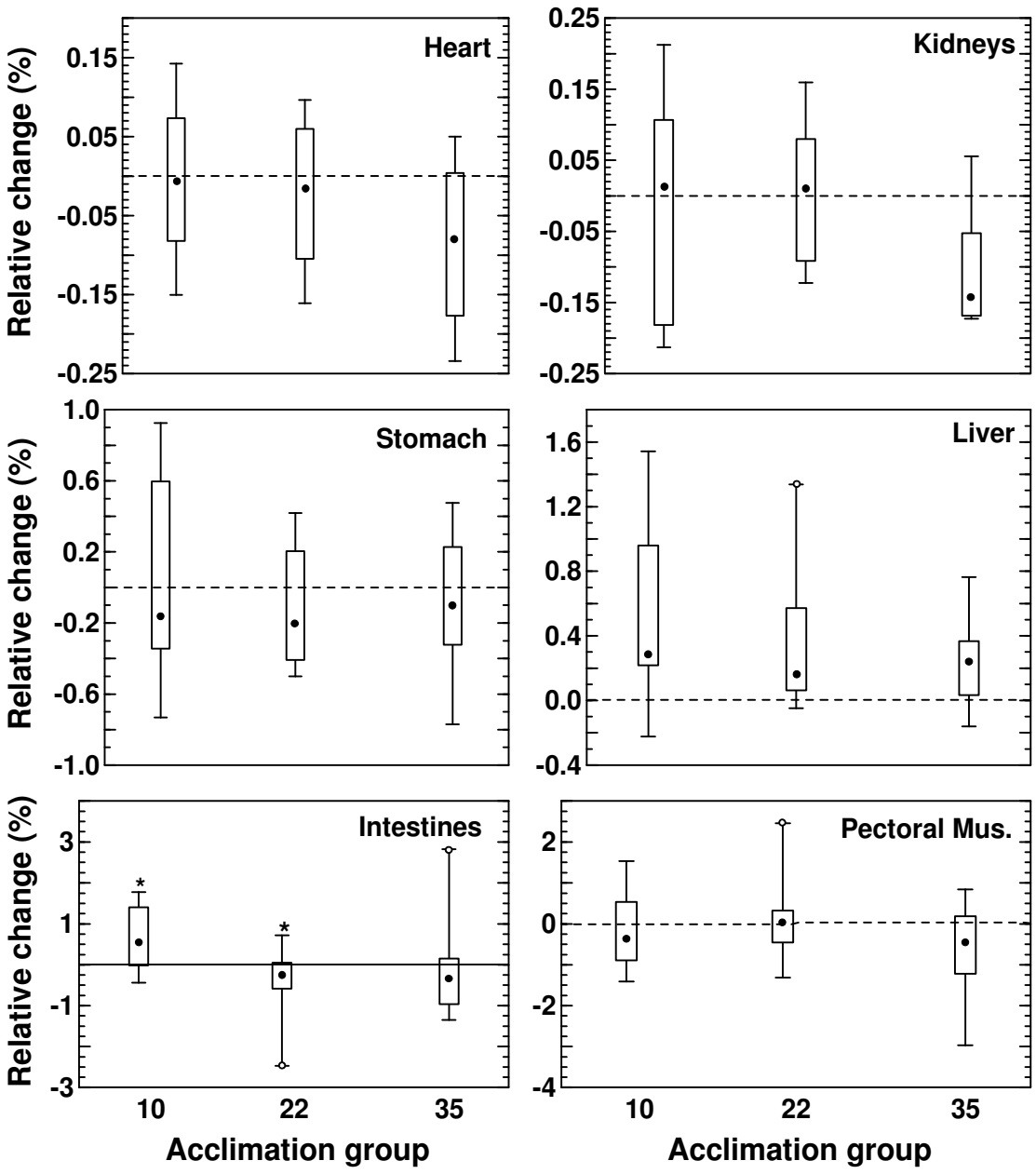


Fig.9. Percentage changes in dry organ mass in three groups of acclimated Laughing Doves (*Streptopelia senegalensis*) compared to initial body composition. Plots show the percentage changes in the organs of the acclimated 10°, 22° & 35°C temperature groups relative to the initial eight birds. Boxes show the 25th and 75th percentiles, whiskers show the 10th and 90th percentiles, closed circles show means and outliers are represented with open circles. The only organ that showed a significant change was the intestine (*) where the 10°C group was significantly heavier than the 22°C group ($P$>0.05).

## 2.5. DISCUSSION

### *2.5.1. Basal metabolic rate*

There were two trends that emerged from the results of the acclimation and reverse-acclimation experiments, firstly a reduction in BMR in all $T_{acc}$ groups after acclimation, and secondly the relationship between BMR and $T_{acc}$ (Fig. 5). During acclimation BMR decreased by 12.7, 19.7 & 27.7% in the $10^{o}$, $22^{o}$ & $35^{o}$C temperature groups respectively. The first of two possible explanations for the initial decrease in BMR involves the doves' reduced activity levels in the cages compared to the aviaries, and the aerobic capacity model postulated by Bennett and Ruben (1979). The aerobic capacity model posits a direct mechanistic link between maximal and resting metabolic rate, and that selection acting to increase maximal metabolic rate results in an associated, albeit delayed, increase in resting metabolic rate (Hayes and Garland, 1995; Bennett and Ruben, 1979). Although the causal link is yet to be verified, and is probably related to cell membrane leakiness, the relationship between maximal metabolic rate (during flight) and BMR is typically a 5-10 fold increase (Hayes and Garland, 1995). Thus the reduction in exercise intensity would theoretically lead to a decrease in maximal metabolic rate and hence BMR. Additionally, the reduction in flight may have reduced the metabolic intensity of the pectoral muscles (the relative masses did not change significantly; Fig. 9). Metabolic activity in the pectoral muscles contributes a large fraction of BMR, as they are the largest avian muscle group and are involved in the most energy demanding task (Swanson in press), and a decrease in metabolic intensity at this site would involve a concomitant decrease in BMR. A reduction in metabolic intensity correlated to lack of exercise has been reported in another Columbid, *Columbia livia*, where sedentary pigeons

had a lower oxidative capacity in their pectoral muscles than more active pigeons (Saarela and Hohtola, 2003).

Another possible explanation could be that during the initial measurement of BMR birds were more stressed than during subsequent measures, and elevated stress levels contributed to a higher initial BMR. However, after comparing the BMR of the doves from this study (after acclimation) with Laughing Doves that had also been acclimated for 21 days but were more habituated to the respirometry chambers (see Chapter 3) no significant differences in BMR were found between either the 10°C group ($t = 1.4$, $P = 0.18$) or the 35°C group ($t = 0.1$, $P = 0.92$). Hence, it would appear that elevated stress levels do not significantly contribute to a higher BMR. The first two explanations are not mutually exclusive and a possible combinational effect may have been responsible for the observed decrease. This phenomenon is beyond the scope of this study and is an interesting area for further research.

Adjustments in BMR have been well documented in several seasonal acclimatization studies (Liknes *et al.*, 2002; Maddocks and Geiser, 1997; Swanson, 1991). However, evidence for a proximate effect of temperature on BMR is more limited, and to the best of my knowledge there is no published information on the reversibility of BMR as a direct response to temperature. In this study, significant linear regressions of $T_{acc}$ versus BMR after acclimation and reverse-acclimation verify a direct effect of temperature on BMR in Laughing Doves (Fig. 6). Additionally, a comparison of the slopes of these two relationships proved not to be significantly different, further indicating that the proximate effect of $T_{acc}$ on BMR is reversible over short time scales (Fig. 6). Quantitative analyses of the magnitude of the adjustments in BMR reveal that

doves acclimated to 35°C decrease BMR by 25.5 and 15.7% relative to the doves at 10°C after acclimation and reverse-acclimation respectively. Although adjustments of this magnitude have been reported for other birds as a proximate response to short-term thermal acclimation (Table. 5), this is the first study to show that the effects of short-term thermal acclimation on BMR are reversible. These results indicate that BMR is a phenotypically plastic trait that can be adjusted over time periods as short as weeks.

On comparison of observed BMR values with predicted allometric estimates for wild-caught birds of the same $M_b$ from McKechnie *et al.*, (2006):

$$\log BMR = 0.744 \log M_b - 1.670$$

I found that observed values displayed a large range of variation around predicted estimates, with the highest and lowest observed values of BMR being 5.2 and –12.8 % above and below the predicted estimates (Fig. 10; from McKechnie *et al.*, 2006). The above interspecific allometric equation was modeled on $M_b$ and BMR data from 137 bird species, assumes BMR is invariant and does not account for variation in BMR attributed to changes induced by acclimation. Accordingly, the variation of the observed BMR from predicted estimates may in part be because BMR is a phenotypically plastic trait that responds to short-term thermal acclimation. This aspect of variation of observed BMR values differing from predicted estimates as a consequence of thermal acclimation has been reported for other birds (Klaassen *et al*., 2004; Williams and Tieleman, 2000). In view of this, phenotypic flexibility in BMR has been suggested to be a generalized feature of the metabolic machinery found in all birds (Klaassen *et al*., 2004) and not necessarily a characteristic of evolutionary adaptation of bird populations restricted to

areas characterized by unpredictable resources and/or climatic conditions as suggested by Parsons (1987).

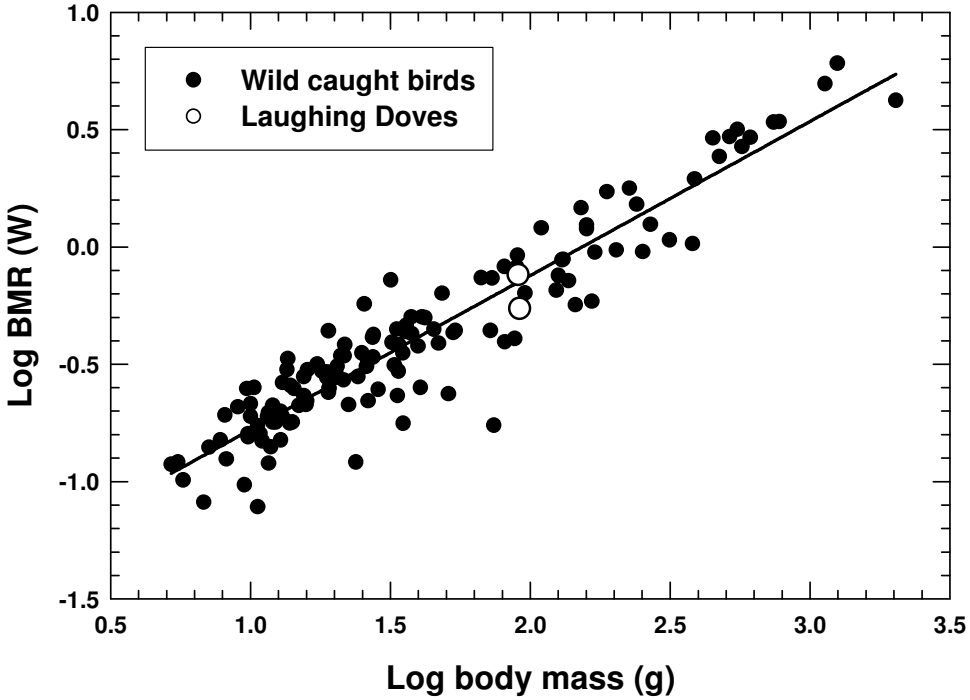


Fig. 10. The relationship between observed and predicted values of basal metabolic rate (BMR) in Laughing Doves (*Streptopelia senegalensis*). Deviations of the highest (initial 10°C group) and lowest (acclimated 35°C group) observed values (open circles) of BMR were 5.3% above and -12.8 % below the predicted estimates of wild caught birds (filled circles) of the same body mass ($M_b$).

After acclimation and reverse-acclimation, the BMR of the 10°C group was higher than that of the 35°C group, albeit only significantly higher after acclimation, a result that supports the 'energy demand' hypothesis. Doves at colder $T_a$ expended more energy on thermoregulation and upregulated their metabolic machinery in response to the elevated energy demand. This result is also consistent with the results from cold-acclimated Hoopoe Larks. The significantly higher BMR of cold-acclimated larks, relative to warm-

acclimated larks, supported the 'energy demand' hypothesis and was correlated to increased food consumption and enlargement of the organs associated with digestion, namely, the liver, kidney, intestines and stomach (Williams and Tieleman, 2000). However, the relationship between BMR, body composition and organ mass was not as direct in the Laughing Doves, and the higher BMR of the10$^{o}$C group is presumably more directly linked to metabolic intensity rather than organ size change (discussed below – Experiment 2).

An analysis of the repeatability of BMR following acclimation yielded a significant repeatability value for the 10$^{o}$C group. Indicating that 25% of the observed adjustments in BMR can be attributed to between-individual variation after acclimation (Fig. 7). In spite of the effect temperature has on BMR it is still a repeatable trait, in this temperature group, over short time scales. On comparison of these repeatability values with published literature I found that repeatability in the BMR of Laughing Doves is not similar to Zebra Finches, where the only independent variable was time (Rønning *et al*., 2005). After 48 days these Finches had a repeatability value of 56% (Rønning *et al*., 2005), almost twice as high as the Laughing Doves after acclimation.

### *2.5.2. Body temperature*

There was a trend for $T_b$ to be higher in the 10$^{o}$C group after acclimation and reverse-acclimation (Fig. 4). Since high $T_b$s are correlated with high internal heat production (Schleucher, 1999), this result is consistent with an animal encountering the effects of acclimation to cold $T_a$. Heat production increases to compensate for heat loss to colder $T_a$s, as reported by Swanson (1991) in the seasonal acclimatization changes in

the Dark-eyed Junco, *Junco hyemalis*. There is another stream of thought that posits high $T_b$s in birds pre-adapts them to off load a greater amount of heat to the environment non-evaporatively (Larcombe *et al.*, 2003). This pre-adaptation is however, more commonly found in desert birds where water scarcity is a determinate of rates of evaporative cooling (Larcombe *et al.*, 2003).

### *2.5.3. Conductance*

Comparison of observed $C_{wet}$ with predicted estimates from the re-evaluated equation of Schleucher and Withers (2001):

$$\log C_{wet\ (non\text{-}passerine\ inactive\ phase)} = \log 1.018 - 0.516 \log M_b$$

proved to be very inconsistent. The closest observed value of $C_{wet}$ was 25% greater than the predicted estimates, and deviations > 50 % were also found. However, previous studies of $C_{wet}$ in other Columbids have also proven to be troublesome, with observed values deviating by as much as –10 to 84% (Schleucher, 2002). As such, $C_{wet}$ of this family is possibly more variable and/or flexible than current literature suggests.

The general trend of increased $C_{wet}$ at higher $T_{acc}$s was not observed in these Laughing Doves, and a possible reason could be the small thermal gradient between the $T_b$ and $T_{acc}$ in the 35$^o$C group. The temperature difference might not have been large enough ($T_b$ was only 3.4$^o$±0.4$^o$C higher than the $T_{acc}$) to induce an increase in $C_{wet}$. As such, an increase in $C_{wet}$ would not facilitate increased rates of heat loss to the $T_a$, and it is more likely that there was an increase in evaporative water loss in the 35$^o$C group. Additionally, $C_{wet}$ in Laughing Doves takes longer than 21 days to respond to thermal

acclimation, as Laughing Doves acclimated to the same temperature did display significantly elevated $C_{wet}$, after a time period of 35 days (see Chapter 3)

Table 5. Percentage reductions in basal metabolic rate per $^{o}$C in birds after acclimation to different ambient air temperatures. An (*) indicates a significant difference in BMR.

| Species | Acclimation temperatures ($^{o}$C) | Acclimation period (days) | % change in BMR per $^{o}$C | Source |
|---|---|---|---|---|
| Skylark (*Alauda arvensis*) | 15, 35±2 | 21 | 1.3* | 1 |
| Woodlark (*Lullula arborea)* | 15, 35±2 | 21 | 0.2 | 1 |
| Hoopoe Lark (*Alaemon alaudipes*) | 15, 35±2 | 21 | 0.9* | 1 |
| Dunn's Lark (*Eremalauda dunni*) | 15, 35±2 | 21 | 1.5* | 1 |
| Spike-heeled lark (*Chersomanes albofasciata*) | 15, 35±2 | 21 | 0.6* | 1 |
| Garden Warblers (*Sylvia borin*) | 4, 24±5 | 150 | 0.9* | 2 |
| Laughing Doves (*Streptopelia senegalensis*) | 10, 22±2<br>22, 35±2<br>10, 35±2 | 21<br>21<br>21 | 0.3<br>0.5<br>0.9* | This study |

Sources:
1. Tieleman *et al.*, 2003b
2. Klaassen *et al.*, 2004

*2.5.4. Experiment 2 - Body composition and organ mass*

The results from the body composition analyses provide limited support for the 'energy demand' hypothesis, as the percentage mass of the intestines in the 10°C group was significantly heavier when compared to the 22°C group (Fig. 9). In Hoopoe Larks acclimated to a cold $T_a$ the size of the intestines increased by 66%, and it would appear that the intestine is the organ most intensely influenced during acclimation to cold $T_a$s. If this were indeed the case, in an animal where changes in BMR appear to be more directly linked with metabolic intensity, the organ most likely to show a size change would be the intestine – as observed in the Laughing Doves.

It has been reported that in pigeons' cold acclimation increases metabolic intensity by increasing mitochondrial density in the pectoralis muscle (Swanson in press). Since metabolic intensity has been reported to be a phenotypically plastic trait that can occur in the absence of organ mass changes (Vézina and Williams, 2003; Swanson in press) the metabolic intensity in the organs of the 10°C group after acclimation and reverse-acclimation must have been higher than the 22° and 35°C groups to meet the increased thermoregulatory demands of low $T_a$s. As changes in metabolic intensity occur at the cellular level and involve concentration of catabolic enzymes; increased capillary density; hematocrit levels; mitochondrial volume and/or mitochondrial cristae surface area (Swanson in press), adjustments of these factors would not be detected by evaluating size changes. Since it was probably metabolic intensity and not organ size responsible for the changes in BMR it is unsurprising that significant differences in the $M_b$s of the doves were not detected (Table 1). This is not uncommon, as no seasonal acclimation differences in $M_b$ were found in the Northern Bobwhite, *Colinus virginianus*

(Swanson and Weinacht, 1997). Body mass in Laughing Doves also takes longer to adjust than the 21 day acclimation period attempted in this experiment, as doves acclimated to the same temperatures for a longer time period did show significant adjustments in $M_b$ (see Chapter 3).

### *2.5.5. General*

The results of this study indicate that Laughing Doves display phenotypic flexibility in BMR and associated metabolic parameters in response to short-term thermal acclimation. This flexibility in BMR responds to short-term thermal acclimation, as BMR is higher at colder $T_a$s and lower at warmer $T_a$s, and the effect of short-term thermal acclimation in BMR is reversible and repeatable over time scales as short as weeks. The results also suggest that the mechanistic correlate of BMR in Laughing Doves is metabolic intensity, and not size of the organs. An unexpected outcome of the study was the decrease in BMR, possibly brought on by the reduction of exercise. I predicted that removal from the wild would induce a change in BMR and attempted to control for this by habituating the birds to captivity, however, the increased activity levels in the outdoor aviaries compared to the forced sedentary behaviour in the individual cages added an unforeseen variable that warrants further study.

The realization that metabolic parameters are not invariant and have the ability to adjust over time periods as short as days or weeks in response environmental change, reproductive demands and behavioural goals is rapidly shaping studies of evolutionary eco-physiology. The idea proposed by Parsons (1987) that phenotypic and genotypic variation would be higher in organisms inhabiting areas of severe environmental stress is

not strongly supported by my results. Klaassen *et al*., (2004) suggested that phenotypic flexibility of metabolic machinery is more likely a trait common to all birds, an idea more consistent with my data, as these Laughing Doves were not restricted to areas of severe environmental stress and are able to adjust $T_b$, $M_b$, $C_{wet}$, BMR and possibly metabolic intensity in response to short-term thermal acclimation. Although the link between the phenotypic flexibility in BMR and ecological radiation is tenuous, the flexibility and reversibility of the metabolic machinery in these doves should have an effect on their distribution. McNab (2003) reported the effect ecology has on the energetics of the Paradisaeidae, and Schleucher (2001) has linked heterothermia to ecological radiation in three species of Columbidae. The ecological factors that appear to limit the distribution of Laughing Doves is water availability and primary productivity, as Laughing Doves are absent only from true desert areas (Hockey *et al.*, 2005). I believe that the rapidly adjustable and phenotypically plastic metabolic machinery of Laughing Doves must play a part in the distribution of this species throughout southern Africa.

# CHAPTER 3: TEMPORAL DYMAMICS OF BASAL METABOLIC RATE ADJUSTMENTS TO SHORT-TERM THERMAL ACCLIMATION

## 3.1. ABSTRACT

The temporal dynamics of adjustments in basal metabolic rate (BMR) in the Laughing Dove (*Streptopelia senegalensis*) during short-term thermal acclimation were addressed in this study. Following an initial BMR measurement 20 Laughing Doves were randomly divided into two groups of 10 and assigned to constant environments of 10$^{o}$ or 35$^{o}$C, where BMR was measured every 4-6 days for 34±3 days.

Both temperature groups decreased BMR in response to acclimation and sedentary behaviour in captivity. However, a definite effect of thermal acclimation was evident after 21 days as BMR of the 35$^{o}$C group was significantly lower than the 10$^{o}$C group. The response period of BMR lasted approximately 30 days, during which 99% of the observed change in BMR occurred. After 30 days BMR in both temperature groups converged on 0.68W, this convergence coincided with a change in the minimum wet thermal conductance ($C_{wet}$), where $C_{wet}$ began to increase significantly in the 35$^{o}$ group. During this response period BMR adjusted at a rate of 0.7 & 0.5 % day $^{-1}$ in the 10$^{o}$ and 35$^{o}$C temperature groups respectively.

## 3.2. INTRODUCTION

The metabolic adjustments that occur in birds as a response to seasonal acclimatization or to thermal acclimation under laboratory conditions have revealed considerable flexibility in the metabolic machinery that governs thermoregulation (Broggi *et al.,* 2004; Klaassen *et al*., 2004; Maddocks and Geiser, 1997, Swanson and Weinacht, 1997). Adjustments in body temperature ($T_b$), body mass ($M_b$), organ metabolic intensity; evaporative water loss, conductance ($C_{wet}$), and basal metabolic rate (BMR) have been reported for several bird taxa (Vézina and Williams, 2003; Liknes *et al*., 2002; Williams and Tieleman, 2000; Swanson, 1991; Swanson in press). Although metabolic flexibility is most pronounced in resident bird species at high latitudes and in long distance migrants (Battley *et al*., 2001; Piersma *et al*., 1995; Swanson in press), it is also present in resident species from the tropics and sub-tropics (Tieleman *et al*., 2003b). Plasticity in BMR is also evident in comparisons between captive and wild caught birds. Smaller (<200g) wild caught birds display a reduced BMR when compared to captive reared birds of the same mass (McKechnie *et al.*, 2006).

The realization that many species of birds exhibit plasticity in physiological traits, recently led Tieleman *et al.,* (2003b) to hypothesize that desert birds have a more flexible BMR than more mesic species. Although they found insufficient evidence to support this hypothesis, acclimation to different ambient air temperatures did induce changes in BMR, body mass and morphology (Tieleman *et al*., 2003b).

While emphasis is often placed on the magnitude of the induced changes, the temporal dynamics of BMR adjustments to thermal acclimation have, to the best of my knowledge, not been investigated. It is thus unclear how rapidly these adjustments occur,

or what shape defines the response curve of BMR to short-term thermal acclimation. The purpose of this study was to track the pattern of BMR adjustments during thermal acclimation over short time scales (days). Understanding the temporal dynamics of BMR adjustments to short-term thermal acclimation will provide a deeper understanding of short-term reversible flexibility in BMR.

## 3.3. MATERIALS AND METHODS

The Laughing Dove, *Streptopelia senegalensis,* was used in this study (see Chapter 1.3). For details of capture and housing, and the procedures involving metabolic measurements see Chapter 2.3.2.

### *3.3.1. Experimental protocol*

After four weeks in captivity BMR was measured in 20 Laughing Doves (following the procedures outlined in Chapter 2.3.2). Thereafter, they were randomly divided into two groups of 10 and moved into one of two ($10\pm2^{o}$ and $35\pm2^{o}$C) constant environment (CE) rooms. Birds in the $10^{o}$ and $35^{o}$C rooms will hereafter be referred to as the "cold-acclimated" and "heat-acclimated" groups respectively. I measured BMR in each bird every 4-6 days for a total of 34±3 days. A malfunctioning of the temperature cabinet during a BMR measurement in the heat-acclimated group on day 15 resulted in the death of four birds, reducing the sample size of the heat-acclimated group to six.

### *3.3.2. Data Analysis*

Since BMR was not significantly influenced by $M_b$, (see results) a *t* - test for dependent and independent samples was used to compare BMR, $T_b$, $M_b$ and $C_{wet}$ within and among the two temperature groups before and after acclimation. Unless otherwise stated all data are presented as means ± SE, and results considered significant if $P \leq 0.05$.

To determine the appropriate regression model for $C_{wet}$ and BMR against time I used the method described by Song *et al.,* (1997) where the coefficient of determination ($r^2$) of each fitted (exponential) model is compared with the $r^2$ of the linear regression of the predicted *y*-value of the fitted model versus the measured *y*-value. The fitted model with the highest $r^2$ in both the linear regression of the predicted *y*-value of the fitted model versus the measured *y*-value, and the exponential model was deemed the model of best fit. This approach was used since a direct comparison of the $r^2$ values for regression models cannot be made if the total sum of squares of both models differ (Song *et al.*, 1997).

## 3.4. RESULTS

### *3.4.1. Body mass*

The initial $M_b$ of the birds assigned to the cold and heat-acclimated groups averaged 92.04±1.63 (*N*=10) and 88.81±2.04g (*N*=10) respectively and did not differ significantly (*t*=1.2; *P*>0.23). After an acclimation period of 34±3 days, birds in the heat-acclimated group averaged 92.34±1.52g (*N*=6), which was not significantly heavier (*t*=1.21; *P*>0.05) from their initial $M_b$. However, after acclimation, $M_b$ of the cold group averaged 98.85±1.81g (*N*=10), significantly heavier (*t*=2.9; *P*<0.01) than their initial $M_b$, and significantly heavier (*t*=2.5; *P*<0.02) than the acclimated $M_b$ of the heat-acclimated group.

### *3.4.2. Body temperature*

After 35 days the $T_b$ of the heat-acclimated group changed from 39.2±0.3$^o$ (*N*=10) to 37.6±0.7$^o$C (N=6), a non-significant decrease (*t*=2.2; *P*>0.08). During acclimation, $T_b$ of the cold-acclimated group increased significantly (*t*=3.6; *P*<0.01) from 38.0±0.4$^o$ (*N*=10) to 40.4±0.4$^o$C (*N*=10). The acclimated $T_b$ of the cold group was also significantly higher (*t*=3.6; *P*<0.01) than that of the 35$^o$C group.

### *3.4.3. Minimum wet thermal conductance*

Initial $C_{wet}$ of the cold and heat-acclimated groups averaged 3.786±0.190 (N=10) and 3.141±0.107 J g$^{-1}$ h$^{-1}$ $^o$C$^{-1}$ (N=10) respectively. After acclimation, $C_{wet}$ of the cold group changed to 3.163±0.213 J g$^{-1}$ h$^{-1}$ $^o$C$^{-1}$ (N=10), which was not significantly lower (*t*=1.6; *P*>0,10) than their initial $C_{wet}$. After acclimation, birds in the heat-acclimated group

averaged 4.352±0.352 J $g^{-1}$ $h^{-1}$ $^oC^{-1}$ (N=6), which was also not significantly higher ($t$=1.9; $P$>0.1) than their initial $C_{wet}$, but was significantly higher ($t$=2.3; $P$<0.04) than the acclimated $C_{wet}$ of the cold group. In an analyses of the temporal dynamics of $C_{wet}$, it becomes apparent that after 30 days $C_{wet}$ begins to increase in the heat-acclimated group (Fig.1).

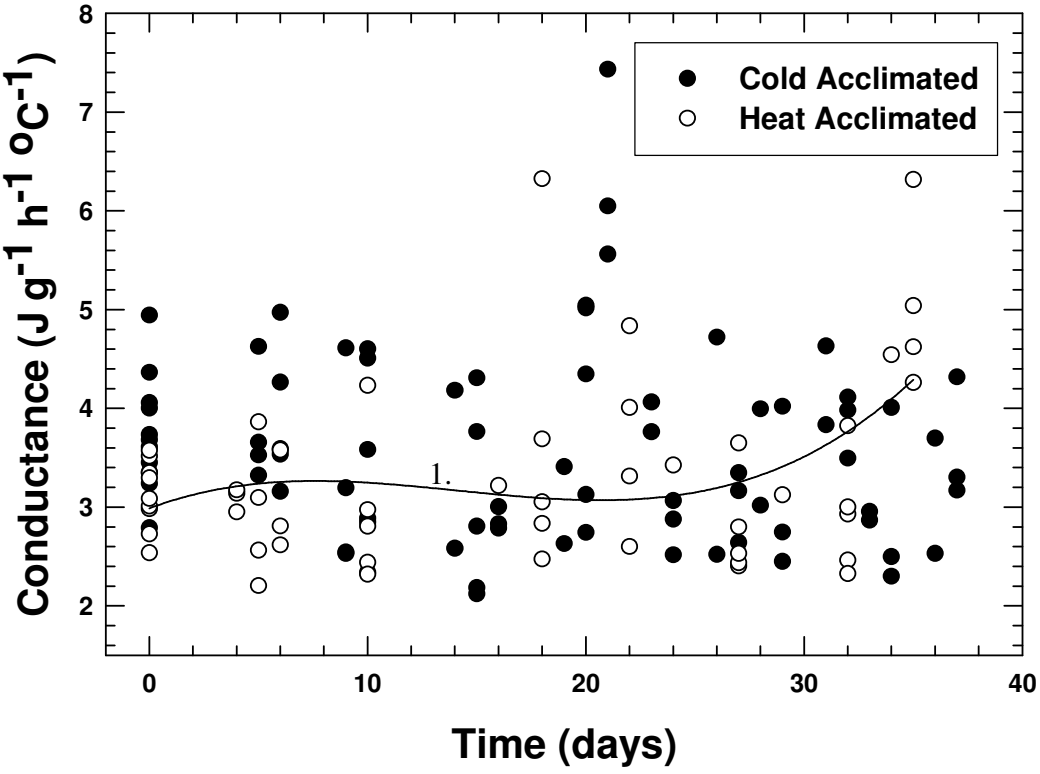


Fig. 1. The relationship between time (T; days) and minimum wet thermal conductance ($C_{wet}$; J $g^{-1}$ $h^{-1}$ $^oC$) in Laughing Doves (*Streptopelia senegalensis*) during acclimation to cold (10$^o$C) and hot (35$^o$C) air temperatures. The line indicates a best-fit regression model: (1) $C_{wet}$ vs. time in the heat-acclimated group (open circles) $C_{wet} = 0.149 + 0.004T - 3.705e^{-3}T^2 + 8.729e^{-6}T^3$ ($r^2$ = 0.17; $P$ = 0.038). After 30 days $C_{wet}$ begins to increases in the heat acclimated group.

*3.4.4. Temporal dynamics of basal metabolic rate*

Since BMR was not significantly related to $M_b$ (Fig. 2) there was no need to control for $M_b$ as a co-variate. Initial BMR between the cold and heat-acclimated groups did not differ significantly ($t$=1.2; $P$>0.23) and averaged 0.874±0.027 ($N$=10) and 0.822±0.032 W ($N$=10) respectively.

The shape of the response curves of BMR adjustment to short-term cold and heat acclimation (after 34±3 days) are defined by the equations; BMR = 0.533 + [(0.361x19.26)/(19.26xT)] ($r^2$= 0.37), and BMR = 0.625 + (0.204x$0.902^T$) ($r^2$= 0.27) respectively (Fig. 3; where T refers to time). Using these equations I determined that 99% of the adjustments in BMR (in both temperature groups) occur within the first 30 days of acclimation. Additionally, by calculating the rate of change in BMR per day as a percentage of the initial BMR (Fig. 4), I found that after 30 days BMR stabilizes at ±80% of the initial BMR (also in both temperature groups) and subsequently does not show any further significant change in response to acclimation, indicating steady state metabolism is achieved after 30 days to both captive conditions and thermal acclimation. As the data prior to 30 days more accurately depicts the true response period of BMR to captivity and acclimation, new sets of non-linear regressions were fitted to this time period, and are defined by the equations BMR = 0.564 + (0.331x$0.955^T$) ($r^2$= 0.38), and BMR = 0.504 + (0.323x$0.952^T$) ($r^2$= 0.34) for the cold and heat-acclimated groups respectively (Fig. 5; where T denotes time).

The largest significant ($t$=1.2; $P$=0.03) difference in BMR between the two temperature groups occurred during the response period after 21±2 days of acclimation. After this time BMR of the cold–acclimated group averaged 0.737±0.091 (N=10), which

was not significantly lower ($t$=1.67; $P$=0.12) than their initial BMR. The heat-acclimated group averaged 0.565±0.057W (N=6) and was significantly lower ($t$=5.93; $P$<0.04) than their initial BMR, and significantly lower ($t$=1.2; $P$=0.03) than the BMR of the cold-acclimated group after 21±2 days. The difference in BMR after this time is a direct response to thermal acclimation and not to captivity, as both temperature groups would have experienced the same captive conditions for the same time period and the only other independent variable would be acclimation temperature ($T_{acc}$).

In the first 30 days i.e. the response period, BMR decreased by 21.4 & 17.4 % in the cold and heat acclimated groups respectively. This translates into a rate of change in BMR of 0.7 & 0.5 % of BMR per day in the cold and heat acclimated groups respectively. At the end of the response period, i.e. after 30±2 days, the BMR of both temperature groups did not differ significantly ($t$=0.05; $P$>0.9), and averaged 0.687±0.02 (N=10) and 0.679±0.04 W (N=6) in the cold and heat acclimated groups respectively. At the end of the experiment (34±3 days) BMR averaged 0.688±0.02 (N=10) and 0.686±0.04W (N=6) in the cold and heat-acclimated groups, a difference of only 0.002W.

During the first 30 days BMR responded to both captivity and thermal acclimation, however, the response to temperature is evident in the divergence in BMR of the two temperature groups as they both have a BMR that is significantly different after 21 days. After 21 days there is still a response in BMR (as indicated by Fig. 4) until 30 days where the BMR of both temperature groups converge on 0.688 W.

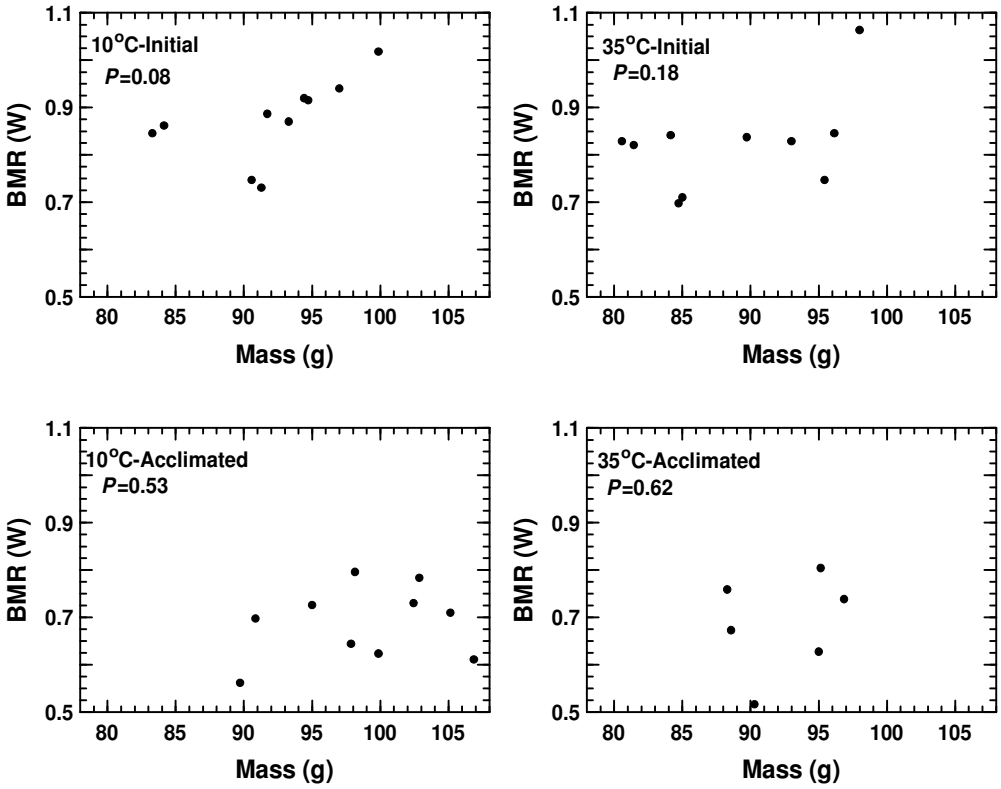


Fig. 2. The relationship between body mass ($M_b$; g) and basal metabolic rate (BMR; W) in Laughing Doves (*Streptopelia senegalensis*). In both temperature groups $M_b$ did not significantly influence BMR before or after acclimation.

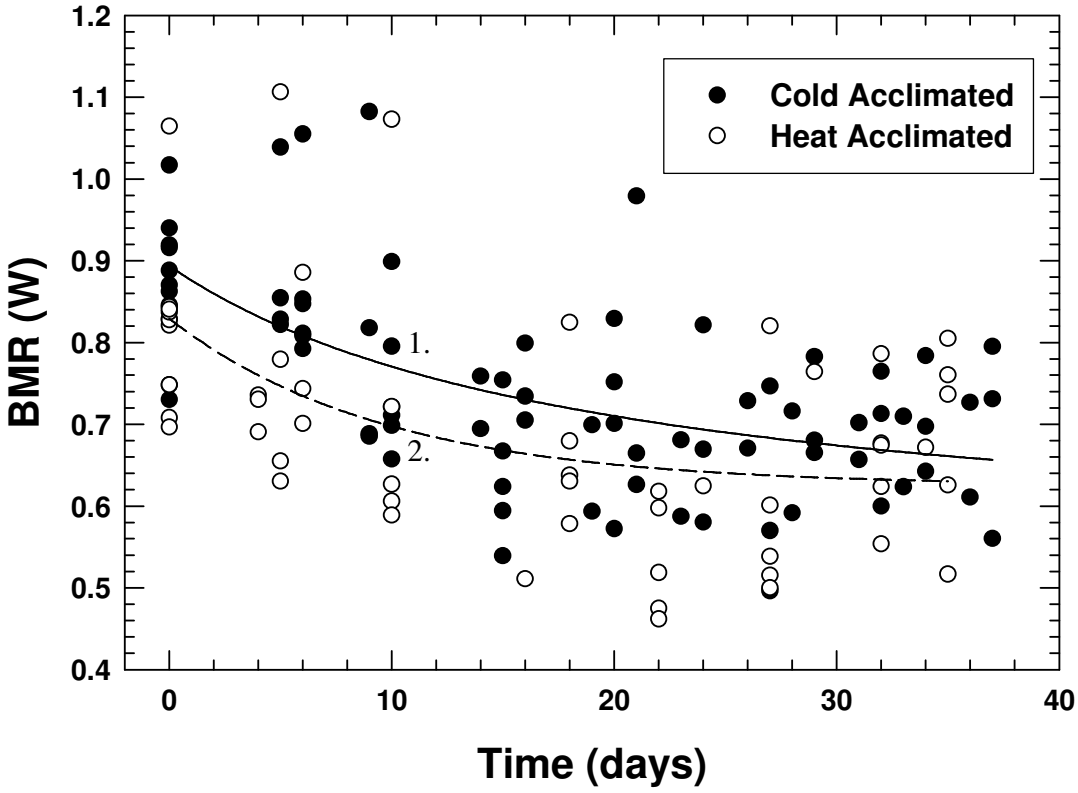


Fig. 3. Relationship between BMR (W) and time in days (T) in Laughing Doves (*Streptopelia senegalensis*). Lines indicate the best-fit regression models: (1) BMR vs. time in the cold-acclimated group (filled circles), BMR = 0.533 + [(0.361x19.26)/(19.26xT)] ($r^2$= 0.37; $P$<0.001); (2) BMR vs. time in the heat-acclimated group (open circles), BMR = 0.625 + (0.204x$0.902^T$) ($r^2$= 0.27; $P$<0.001). Approximately 99% of the observed change in BMR in both temperature groups occurs before 30 days.

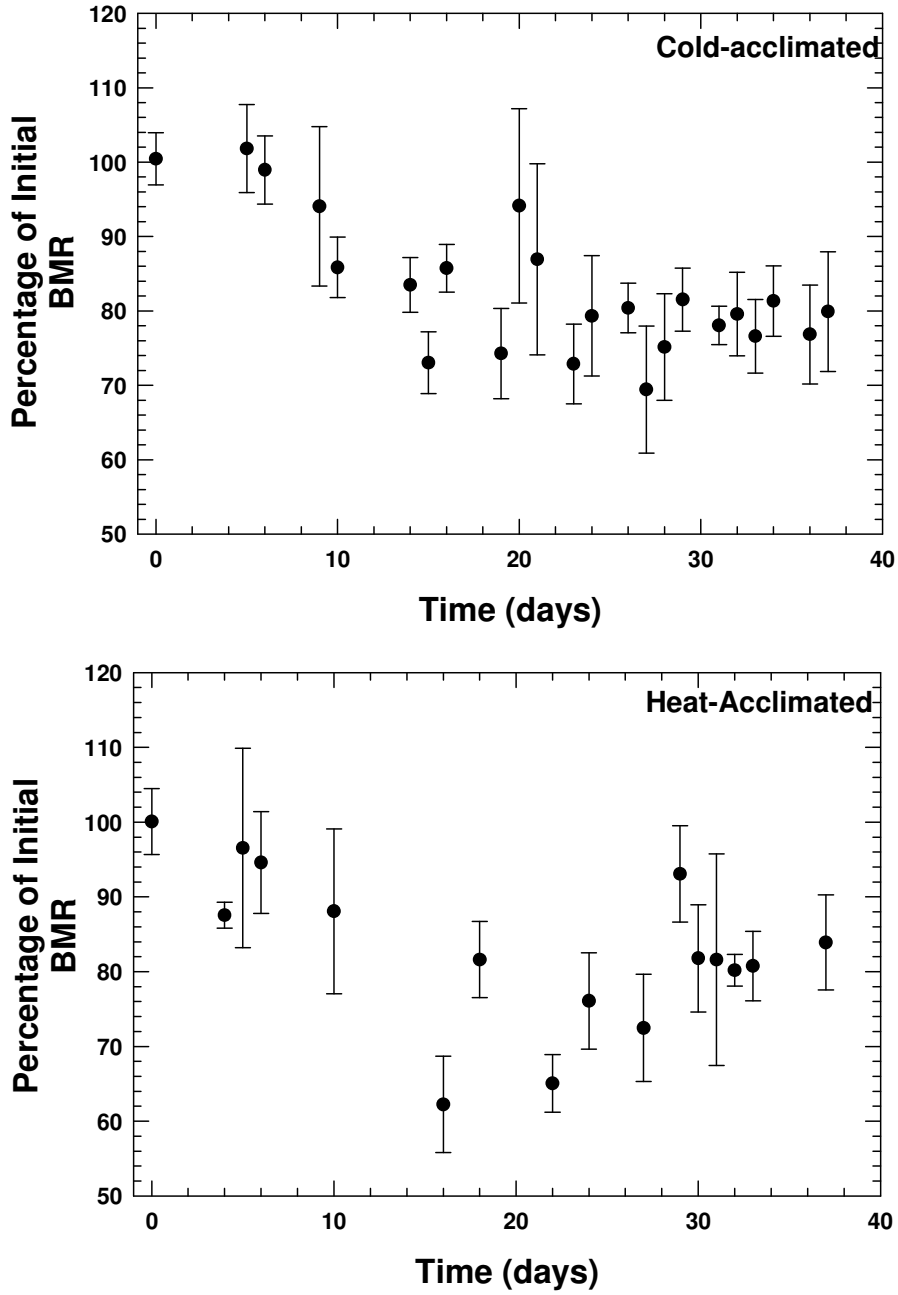


Fig. 4. Percentage change in BMR per day relative to initial BMR in cold and heat-acclimated Laughing Doves (*Streptopelia senegalensis*). Both temperature groups decrease BMR by ± 20% after 30 days and then stabilize indicating the onset of steady state metabolism at both acclimation temperatures.

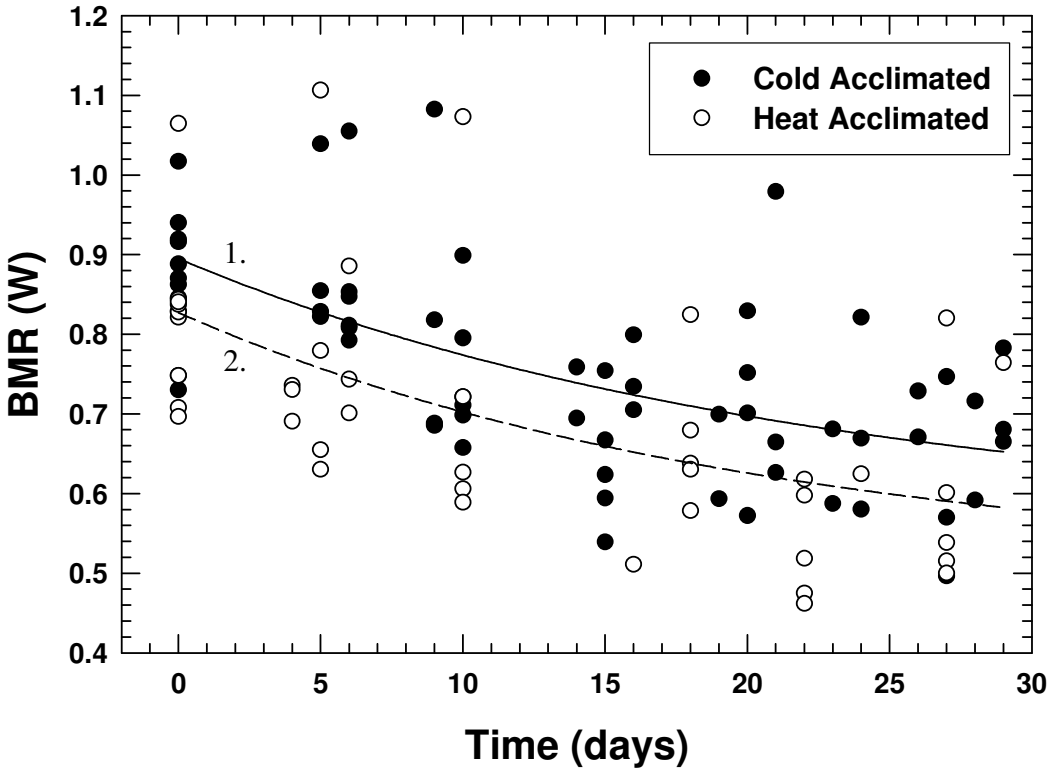


Fig. 5. Relationship BMR (W) and time in days (T) in Laughing Doves (*Streptopelia senegalensis*) before 30 days. Lines indicate the best-fit regression models: (1) BMR vs. time in the cold-acclimated group (filled circles), BMR = 0.564 + ($0.331 \times 0.955^{T}$) ($r^2$=0.38; $P$<0.001); (2) BMR vs. time in the heat-acclimated group (open circles), BMR= 0.504 + ($0.323 \times 0.952^{T}$) ($r^2$= 0.34; $P$<0.001). After 21±2 days BMR of the cold-acclimated group was significantly higher than the heat-acclimated group.

## 3.5. DISCUSSION

The $M_b$ gains of 6.1 & 3.8%, relative to initial $M_b$, of the cold and heat-acclimated groups are similar to those observed in Garden Warblers (*Sylvia borin*) acclimated to cold and warm temperatures (Klaassen *et al*., 2004). The larger mass gain in the cold-acclimated group is also consistent with acclimation in Hoopoe Larks, *Alaemon alaudipes*, (Williams and Tieleman, 2000) and is presumably due to increased energy demand (food consumption) for up-regulation of thermoregulatory heat production, as indicated by the higher BMR of the cold-acclimated group after 21 days.

The $T_b$ responses of both the cold and heat-acclimated groups reflect a response to $T_{acc}$. To compensate for heat loss to low $T_a$ the cold-acclimated group elevated their $T_b$ from 38.1±0.4 to 40.4±0.4$^oC$ and decreased $C_{wet}$ by 40%. The same response, in the opposite direction, was present in the heat-acclimated group; these birds decreased $T_b$ from 39.7±0.2 to 37.6±0.7$^oC$, just 2.7$^oC$ above the acclimation temperature, and increased $C_{wet}$ by 27.8%. Increased $C_{wet}$ at higher $T_a$s is a mechanism used to offload metabolic heat non-evaporatively (Larcombe *et al.,* 2003).

The basal metabolic rate of the doves from both temperature groups decreased after placement in the individual cages in the CE rooms. A similar reduction in BMR was observed in Chapter 2 and is thought to be linked to the reduction in exercise intensity associated with the individual cages (see Chapter 2). However, the change in BMR induced by acclimation is evident as the BMR of the two temperature groups show a significant difference after 21 days. The BMR in the cold-acclimated group was significantly higher than the heat-acclimated group. These results are consistent with other acclimation studies, where colder $T_a$s induce up-regulation of BMR to compensate

for increased thermoregulatory heat production (Klaassen *et al*., 2004; Williams and Tieleman, 2000). On comparison of the BMR of the cold-acclimated group (after 21 days) from this experiment with the Laughing Doves used in Chapter 2 no significant differences ($t$ = 1.4; $P$ = 0.18) were found between the BMR of these cold-acclimated doves relative to the doves acclimated to 10°C for 21days. A comparison of the heat-acclimated group with the acclimated 35°C group from Chapter 2 also did not yield significantly different ($t$ = 0.10; $P$ = 0.92) results. As the cold and heat-acclimated doves in this experiments and the 10° and 35°C doves from Chapter 2 were both acclimated to the same temperatures for essentially the same time periods I would not have expected the BMR in these birds to be different.

In the present study, after 30 days BMR of both temperature groups converge on 0.68W (Fig. 1), and the combination of changes in $T_b$, $M_b$ and particularly $C_{wet}$, which also begins to change after 30 days (Fig. 1), seem to drive BMR to an optimal rate in captivity. In view of this, it becomes apparent that during the acclimation process BMR displays plasticity to offset the costs of thermoregulation, while the other metabolic parameters ($M_b$, $T_b$, and $C_{wet}$) adjust to the new $T_a$. Collectively, these observations suggest that the temporal dynamics of metabolic adjustments to thermal conditions represent an interplay between $M_b$, $T_b$. $C_{wet}$ and metabolic heat production.

There have been several studies reporting the effects of seasonal acclimatization (Dawson and Olson, 2003; Liknes *et al.*, 2002; Maddocks and Geiser, 1997; Swanson and Weinacht, 1997; Swanson, 1990), and short-term thermal acclimation (McKechnie and Wolf, 2004b; Williams and Tieleman, 2000) on the metabolic machinery of birds. However, to the best of my knowledge this is the first study where the response in BMR

to thermal acclimation and/or to captivity has been monitored through time and the rate of change in BMR during the response period been described. As such, comparisons with published results were not possible. However, the percentage rate of change in BMR, 0.7 & 0.5 % per day in cold and heat-acclimated groups respectively, indicates BMR can rapidly shift over periods as short as days. After 30 days the effect of both temperature and captivity did not elicit further significant responses in BMR. A possible reason for this is that within 30 days $T_b$, $M_b$, and $C_{wet}$ had already adjusted to both captivity and thermal acclimation and further adjustments in BMR after this time period were not required for the maintenance of homeothermy in these animals.

The rapid response of the metabolic parameters in Laughing Doves is possibly an adaptation that in part explains the wide distribution of this species. It is however, premature to make broad scale assumptions of this nature without further evidence and thus investigating the response rate of metabolic parameters in other animals with similar distributions would be an interesting area for further research.

## 4.1. CONCLUDING DISCUSSION

The results of this study reveal that BMR in Laughing Doves is a phenotypically flexible trait and can be rapidly adjusted in response to short-term thermal acclimation. Furthermore, the effects of short-term thermal acclimation are reversible. BMR is also a repeatable trait in spite of changes induced by acclimation. Adjustments in the BMR of Laughing Doves also appear to be more directly associated with metabolic intensity rather than organ size. The response to short-term thermal acclimation in Laughing Doves is most evident after 21 days, however, the response period of BMR to both thermal acclimation and reduced levels of exercise extends to approximately 30 days. During this response period BMR is adjusted to offset the cost of acclimation and habituation to captivity while other associated metabolic parameters such as $C_{wet}$, $M_b$, and $T_b$, adjust to the thermal environment.

The results from this study support the notion that phenotypic flexibility in BMR is a trait common to all birds, as speculated by Klaassen *et al*., (2004) and not just to birds restricted to areas of harsh conditions or unpredictability as posited by Parsons (1987). The Laughing Doves used in this study were not from an area with harsh or unpredictable conditions and yet displayed phenotypic flexibility in their metabolic machinery. The flexible and rapidly adjustable metabolic machinery of Laughing Doves might play a part in the ecological radiation of these birds.

A further outcome of this study is the relationship between BMR reductions brought on by a possible lack of exercise. All birds that were in the outdoor aviaries and subsequently moved indoors into individual cages, where they could not fly, reduced BMR in response to decreased activity levels. The decrease in BMR that was most likely

induced by the sedentary lifestyle in the individual cages added a confounding variable, and I suggest that in future studies, wild caught birds should be habituated to the captive conditions they would experience during experimentation. Future studies should also attempt to minimize the degree of temperature fluctuations between measurements, as we have no idea how this variable would have influences the BMR of the birds. I also think that a comparison of BMR from bird species with a wide distribution and birds with a restricted distribution would be able to reveal whether phenotypic flexibility in metabolic machinery is a feature of all birds, or of birds restricted to areas of certain environmental stresses. It would also would be interesting to determine the effect that sedentary habituation has on BMR. McKechnie *et al.*, (2006) has already reported that BMR in captive raised birds scales differently to BMR in wild caught birds, but several unanswered questions still remain. How long does a bird have to be in captivity before BMR is affected? In the Laughing doves this time period was approximately 30 days, but the response to thermal acclimation was a confounding variable and needs to be removed to test this idea more thoroughly. Additionally, McKechnie *et al.*, (2006) reported that smaller (<200g) captive raised birds have a BMR that is lower than wild caught birds of the same mass. Can BMR in captive raised birds be upregulated as a response increased activity levels? These are interesting biological questions that need to be addressed in order to increase our understanding of avian evolutionary eco-physiology.

**Literature Cited:**

**Baker, W. C. and Pouchot, J. F.** (1983). The measurement of gas flow. Part I. *Journal of the Air Pollution Control Association* **33**, 66-72.

**Battley, P. F., Dekinga, A., Dietz, M. W., Piersma, T., Tang, S. and Hulsman, K.** (2001). Basal metabolic rate declines during long-distance migratory flight in great knots. *Condor* **103**, 838-845.

**Battley, P. F., Piersma, T., Dietz, M. W., Tang, S., Dekinga, A. and Hulsman, K.** (2000). Empirical evidence for differential organ reductions during trans-oceanic bird flight. *Proceedings of the Royal Society of London B* **267**, 191-195.

**Bech, C., Langseth, I. and Gabrielsen, G. W.** (1999) Repeatability of basal metabolism in breeding female kittiwakes *Rissa tridactyla*. *Proceedings of the Royal Society of London* **266,** 2161-2167.

**Bech, C., Rønning, B. and Moe, B.** (2004). Individual variation in the basal metabolism of Zebra finches *Taeniopygia guttata*: no effect of food quality during early development. *International Congress Series* **1275**, 306-312.

**Bennett, A. F. and Ruben, J. A.** (1979). Endothermy and activity in vertebrates. *Science* **206**, 649-654.

**Bozinovic, F., Gallardo, P.A., Visser, G.H., Cortes, A.** (2003). Seasonal acclimatization in water flux rate, urine osmolality and kidney water channels in free-living degus: molecular mechanisms, physiological processes and ecological implications. *The Journal of Experimental Biology* **206**, 2959-2966

**Broggi, J., Orell, M., Hohtola, E. and Nilsson, Å.** (2004). Metabolic response to temperature variation in the great tit: an interpopulation comparison. *Journal of Animal Ecology* **73**, 967-972.

**Costa, D. P. and Sinervo, B.** (2004). Field physiology: new insights from animals in nature. *Annual Review of Physiology* **66**, 209-38.

**Dawson, W. R.** (2003). Plasticity in avian responses to thermal challenges - an essay in honor of Jacob Marder. *Israeli Journal of Zoology* **49**, 95-109.

**Dawson, W. R. and Olson, J. M.** (2003). Thermogenic capacity and enzymatic activities in the winter-acclimatized dark-eyed junco (*Junco hyemalis*). *Journal of Thermal Biology* **28**, 497-508.

**Ernande, B. and Dieckmann, U.** (2004). The evolution of phenotypic plasticity in spatially structured environments: implications of intraspecific competition, plasticity costs and environmental characteristics. *Journal of Evolutionary Biology* **17**, 613-628.

**Felsenstein, J.** (1985). Phylogenies and the comparative method. *American Naturalist* **125**, 1-15.

**Frappell, P. B., Hinds, D. S. and Boggs, D. F.** (2001). Scaling of respiratory variables and the breathing pattern in birds: an allometric and phylogenetic approach. *Physiological and Biochemical Zoology* **74**, 75-89.

**Hayes, J. P. and Garland, T.** (1995). The evolution of endothermy: testing the aerobic capacity model. *Evolution* **49**, 836-847.

**Hockey, P.A.R., Dean, W.R.J and Ryan, P.G.** (eds) (2005). Birds of Southern Africa VII10th edition. *The Trustees of the John Voelcker Bird Book Fund*, Cape Town, 281-284

**Klaassen, M., Oltrogge, M. and Trost, L.** (2004). Basal metabolic rate, food intake, and body mass in cold- and warm-acclimated Garden Warblers. *Comparative Biochemistry and Physiology A* **137**, 639-647.

**Larcombe, A. N., Withers, P. C. and Maloney, S. K.** (2003). Thermoregulatory physiology of the Crested Pigeon *Ocyphaps lophotes* and the Brush Bronzewing *Phaps elegans. Journal of Comparative Physiology B* **173**, 215-222.

**Lasiewski, R. C. and Dawson, W. R.** (1967). A re-examination of the relation between standard metabolic rate and body weight in birds. *Condor* **69**, 13-23.

**Lessells, C. M. and Boag, P. T.** (1987). Unrepeatable repeatabilities: a common mistake. *Auk* **104**, 116-121.

**Liknes, E. T., Scott, S. M. and Swanson, D. L.** (2002). Seasonal acclimatization in the American Goldfinch revisited: to what extent do metabolic rates vary seasonally? *Condor* **104**, 548-557.

**Lovegrove, B. G.** (2000). The zoogeography of mammalian basal metabolic rate. *American Naturalist* **156**, 201-219.

**Maddocks, T. A. and Geiser, F.** (1997). Energetics, thermoregulation and nocturnal hypothermia in Australian silvereyes. *Condor* **99**, 104-112.

**Marder, J., Withers, P. C. and Philpot, R. G.** (2003). Patterns of cutaneous water evaporation by Australian pigeons. *Israeli Journal of Zoology* **49**, 111-129.

**McKechnie, A. E. and Wolf, B. O.** (2004a). The allometry of avian basal metabolic rate: good predictions need good data. *Physiological and Biochemical Zoology* **77**, 502-521.

**McKechnie, A. E. and Wolf, B. O.** (2004b). Partitioning of evaporative water loss in white-winged doves: plasticity in response to short-term thermal acclimation. *Journal of Experimental Biology* **207**, 203-210.

**McKechnie, A. E., Freckleton, R. P., and Jetz, W.** (2006). Phenotypic plasticity in the scaling of avian basal metabolic rate. *Proceedings of the Royal Society of Biology. Published online* (doi: 10.1098/rspb.2005.3415)

**McNab, B. K.** (2003). Ecology shapes bird bioenergetics. *Nature* **426**, 620-621.

**Murray, K. G., Russell, S., Picone, C. M., Winnett-Murray, K., Sherwood, W., and Kuhlmann, M, L.** (1994). Fruit laxatives and seed passage rates in frugivores: consequences for plant reproductive success. *Ecology* 75 (**4**), 989-994.

**Nagy, K. A., Gruchacz, M. J.** (1994). Seasonal water and energy metabolism of the desert-dwelling kangaroo rat (*Dipodomys merriami*). *Physiological and Biochemical Zoology* **67**, 1461-1478.

**Parsons, P.A.** (1987). Evolutionary rates under environmental stress. *Evolutionary Biology* **21**, 311-347.

**Piersma, T., Cadée, N. and Daan, S.** (1995). Seasonality in basal metabolic rate and thermal conductance in a long distance migrant shorebird, the knot (*Calidris canutus*). *Journal of Comparative Physiology* **165**, 37-45.

**Piersma, T. and Drent, J.** (2003). Phenotypic flexibility and the evolution of organismal design. *Trends in Ecology and Evolution* **18**, 228-233.

**Piersma, T. and Lindström, A.** (1997). Rapid reversible changes in organ size as a component of adaptive behaviour. *Trends in Ecology and Evolution* **12**, 134-138.

**Relyea, R.A.** (2002). Costs of Phenotypic Plasticity. *The American Naturalist* **159,** 272-282.

**Ricklefs, R. E., Konarzewski, M. and Daan, S.** (1996). The relationship between basal metabolic rate and daily energy expenditure in birds and mammals. *American Naturalist* **147**, 1047-1071.

**Rønning, B., Moe, B. and Bech, C.** (2005). Long-term repeatability makes basal metabolic rate a likely heritable trait in the zebra finch *Taeniopygia guttata. The Journal of Experimental Biology* **208,**4663-4669.

**Saarela, S. and Hohtola, E.** (2003). Seasonal thermal acclimatization in sedentary and active pigeons. *Israeli Journal of Zoology* **49**, 185-193.

**Schleucher, E.** (1999). Energetics and body temperature in two convergent dove species from extreme habitats. *Ornis Fennica* **76**, 199-210.

**Schleucher, E.** (2001). Heterothermia in pigeons and doves reduces energetic costs. *Journal of Thermal Biology* **26**, 287-293.

**Schleucher, E.** (2002). Metabolism, body temperature and thermal conductance of fruit-doves (Aves: Columbidae, Treronidae). *Comparative Biochemistry and Physiology A* **131**, 417-428.

**Schleucher, E. and Withers, P. C.** (2001). Re-evaluation of the allometry of wet thermal conductance for birds. *Comparative Biochemistry and Physiology A* **129**, 821-827.

**Schleucher, E. and Withers, P. C.** (2002). Metabolic and thermal physiology of doves and pigeons. *Physiological and Biochemical Zoology* **75**, 439-450.

**Schmidt-Nielsen, K.** (1990). Animal physiology: adaptation and environment. Cambridge: Cambridge University Press.

**Secor, S. M. and Diamond, J.** (1995). Adaptive responses to feeding in Burmese pythons: pay before pumping. *Journal of Experimental Biology* **198**, 1313-1325.

**Secor, S. M. and Diamond, J.** (1998). A vertebrate model of extreme physiological regulation. *Nature* **395**, 659-662.

**Song, X., Körtner, G. and Geiser, F.** (1997). Thermal relations of metabolic rate reduction in a hibernating marsupial. *American Journal of Physiology* **273**, R2097 - R2104.

**Sorenson, A. E.** (1984). Nutrition, energy and passage time: experiments with fruit preference in European blackbirds *(Turdus merula)*. Journal of Animal Ecology **53,** 545-557

**Speakman, J. R. and McQueenie, J.** (1996). Limits to sustained metabolic rate:the link between food intake, basal metabolic rate, and morphology in reproducing mice, *Mus musculus*. *Physiological Zoology* **69,**746-769.

**Swanson, D. L.** (1990). Seasonal variation in cold hardiness and peak rates of cold-induced thermogenesis in the dark-eyed junco (*Junco hyemalis*). *Auk* **107**, 561-566.

**Swanson, D. L.** (1991). Seasonal adjustments in metabolism and insulation in the Dark-eyed Junco. *Condor* **93**, 538-545.

**Swanson, D. L. and Olmstead, K. L.** (1999). Evidence for a proximate influence of winter temperatures on metabolism in passerine birds. *Physiological and Biochemical Zoology* **72**, 566-575.

**Swanson, D. L. and Weinacht, D. P.** (1997). Seasonal effects on metabolism and thermoregulation in northern bobwhite. *Condor* **99**, 478-489.

**Swanson, D. L. In Press.** Season metabolic variation in birds: functional and mechanistic correlates. *Current Ornithology* **17.**

**Tieleman, B. I. and Williams, J. B.** (2000). The adjustment of avian metabolic rates and water fluxes to desert environments. *Physiological and Biochemical Zoology* **73**, 461-479.

**Tieleman, B. I., Williams, J. B. and Bloomer, P.** (2003a). Adaptation of metabolic rate and evaporative water loss along an aridity gradient. *Proceedings of the Royal Society of London* **270**, 207-214.

**Tieleman, B. I., Williams, J. B., Buschur, M. E. and Brown, C. R.** (2003b). Phenotypic variation of larks along an aridity gradient: are desert birds more flexible? *Ecology* **84**, 1800-1815.

**Vézina, F. and Williams, T. D.** (2003). Plasticity in body composition in breeding birds: what drives the metabolic costs of egg production? *Physiological and Biochemical Zoology* **76**, 716-730.

**Williams, J. B. and Tieleman, B. I.** (2000). Flexibility in basal metabolic rate and evaporative water loss among hoopoe larks exposed to different environmental temperatures. *Journal of Experimental Biology* **203**, 3153-3159.

**Withers, P. C.** (1977). Measurement of VO2, VCO2, and evaporative water loss with a flow- through mask. *Journal of Applied Physiology* **42**, 120-123.

**Zar, J.** (1984). Biostatistical Analysis. New Jersey: Prentice Hall.