

## Physiological variation in amethyst sunbirds (*Chalcomitra amethystina*) over an altitudinal gradient in winter

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### SUMMARY

**Flexibility of metabolic rates within a species allows for the colonization of different habitats along an altitudinal and thus temperature gradient. The distribution range of amethyst sunbirds (*Chalcomitra amethystina*) within southern Africa includes an altitudinal gradient from the Drakensberg to the coast of KwaZulu-Natal. We expected that over this altitudinal gradient amethyst sunbirds would exhibit variation in winter metabolic rates, particularly basal metabolic rates (BMR) and resting metabolic rate (RMR) pre- and post-acclimation. Sunbirds from three locations, Underberg (1553 m above sea level; asl), Howick (1075 m asl) and Oribi Gorge (541 m asl) were used for this study. Upon capture, metabolic rate was measured indirectly by quantifying oxygen consumption ( $\dot{V}_{O_2}$ ) using flow-through respirometry, at 5 and 25°C. Birds were then acclimated at 25°C for 6 weeks on a 12 h:12 h L:D cycle.  $\dot{V}_{O_2}$  was measured post-acclimation at eight different temperatures (15, 5, 10, 20, 30, 28, 25 and 33°C). We found little variation in winter  $\dot{V}_{O_2}$  between individuals from the same locality, whereas significant variation was observed in  $\dot{V}_{O_2}$  at the same temperatures between individuals from the different localities and thus between altitudes. In particular, winter BMR decreased significantly with decreasing altitude post-acclimation. This study emphasizes the need to understand plasticity/flexibility in metabolic rates and to acknowledge altitudinal differences within a species, to make accurate predictions about the thermal physiology of a species and its responses to changes in ambient temperatures.**

Key words: altitudinal variation, amethyst sunbird, metabolic rates, phenotypic plasticity, phenotypic flexibility.

### INTRODUCTION

Phenotypic plasticity implies the general capacity for change and transformation within genotypes in response to changing environmental conditions (DeWitt et al., 1998; Kingsolver et al., 2002; Piersma and Drent, 2003). However, instead of using the term ‘phenotypic plasticity’, Piersma and Drent (Piersma and Drent, 2003) proposed the use of the term ‘phenotypic flexibility’ which refers to variation within a characteristic of a single individual which is reversible, and a function of both predictable and unpredictable fluctuations in environmental conditions. Despite this the former is still used by some in the literature, however, we will use the latter term.

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

Birds are considered to be homeothermic endotherms, which implies the ability to maintain a constant body temperature over a broad range of ambient temperatures by adjusting their metabolism (Chaui-Berlinck et al., 2002). However small homeotherms have higher energetic demands at colder temperatures and require physiological adjustments in metabolic rate or insulation to counteract this (Downs and Brown, 2002; Soobramoney et al., 2003). As a result of this many birds display phenotypic flexibility in maintenance energy requirements, and are able to up- or downregulate basal metabolic rate (BMR) over a period of time during thermal acclimation (McKechnie et al., 2007; Bush et al., 2008a). Recent evidence suggests that winter BMR of species living in highly seasonal environments reflects the conditions in which the animal existed immediately prior to metabolic measurements being taken (McKechnie, 2008) and thus it becomes important to differentiate metabolic measurements made pre-acclimation and those made post-acclimation (Bush et al., 2008a; Smit et al., 2008). Klaasen et al. (Klaasen et al., 2004) suggested that it is important to recognize whether seasonal changes in BMR represent a separate acclimation or acclimatization response or merely variation in working capacity.

In avian comparative studies, much focus is placed on the origin of study birds in terms of captive bred or wild caught populations and the effect of this factor on BMR (e.g. Weathers et al., 1983; McKechnie et al., 2007; McKechnie, 2008), but this does not take into consideration the geographic and consequent altitudinal origin of the wild caught species as a possible source of variation. Previous studies of avian thermal biology have examined metabolic adaptations along an aridity gradient (e.g. Tieleman et al., 2002), the effects of seasonal and environmental changes on BMR of a

species (e.g. Hart, 1962; Dawson and Carey, 1976; Weathers and Caccamise, 1978; Ambrose and Bradshaw, 1988; Maddocks and Geiser, 2000; Bush et al., 2008b; Smit et al., 2008), phenotypic flexibility in BMRs of one population as a representative of a species (e.g. McKechnie et al., 2007), or have assessed population responses to climate change as a mean response at the population level (e.g. Møller et al., 2004). Published data often represents a single BMR or RMR value per species, regardless of altitudinal origin, or alternatively data from one population as a representative of an entire species (e.g. Bech, 1980; Cooper and Swanson, 1994; Boix-Hinzen and Lovegrove, 1998; Maddocks and Geiser, 2000; McKechnie and Lovegrove, 2001; Downs and Brown, 2002; López-Calleja and Bozinovic, 2003; Lovegrove and Smith, 2003; McKechnie et al., 2007), thus highlighting the need to acknowledge the role of phenotypic flexibility within a species.

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

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

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

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

We predicted that the winter metabolic rates of subpopulations of amethyst sunbirds would vary over the altitudinal gradient as a result of acclimatization and adaptations to different temperatures, as well as pre- and post-acclimation resulting from innate physiological differences to acclimation to 25°C and the Pietermaritzburg altitude (660 m). BMR is generally thought to be species specific, but we predicted phenotypic flexibility between subpopulations, within a species, because of the temperature differences occurring over altitude.

## MATERIALS AND METHODS

### Study site, bird capture and maintenance

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

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

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

### Metabolic measurements and protocol

Sunbirds were kept in the animal house for one night after capture before pre-acclimation respirometry trials to reduce the effect of transport stress. Acclimation is regarded as changes in the organism in response to changes in any component in the environment of the laboratory (Garland and Adolph, 1991; Smit et al., 2008).

Metabolic rate was measured indirectly by quantifying oxygen consumption ( $\dot{V}_{O_2}$ ) using a respirometer. Birds were weighed and then placed individually in respirometry chambers (volume=3.96 l) between 16:30–17:00 h. Respirometry chambers were placed in a sound-proof Conviron<sup>®</sup> (Winnipeg, Manitoba, Canada) cabinet (1 m<sup>3</sup>). Photoperiods were set in synchronization with that of the constant environment room where birds were housed (12 h:12 h L:D). Conviron<sup>®</sup> cabinet temperature ( $T_a$ ) was measured using thermistor probes calibrated with a standard mercury thermometer (0.05°C) in a water bath at temperatures between 5 and 45°C.

Air flow was controlled using a computerized open flow-through system (Depocas and Hart, 1957; Hill, 1972). Atmospheric air was pumped in and partially dried using silica gel, before reaching the Conviron<sup>®</sup> cabinet. Flow rate was maintained at a level that ensured <1% change in oxygen concentration, between 0.5 and 0.6 l h<sup>-1</sup> (Downs and Brown, 2002). The flow rate of each chamber was measured using a Brooks thermal mass-flow meter (Model 580E; Hatfield, PA, USA) factory calibrated to STP. A steady flow of air through the chamber was ensured as air entered the bottom and was expelled through the top of the respirometry chambers. Simultaneous measurements of six chambers (five experimental and one control chamber) was achieved by using solenoid valves and a separate pump for each chamber.

Excurrent air was passed through a water condenser (a copper tube in which air was cooled to approximately 3°C, or below dew point) to remove water vapour, and soda lime, to remove CO<sub>2</sub>. An oxygen analyser (Model S-3A/1, Ametek, Pittsburgh, PA, USA) was used to determine the fractional concentration of O<sub>2</sub> in dry air samples. The fractional concentration of O<sub>2</sub> in the control chamber

was measured at the start of every 6 min. cycle, and the  $O_2$  values from the experimental chambers were then subtracted from this value. The problem of long-term drift in  $O_2$  analyser outputs was thus limited to that which would occur in 5 min cycles. Measurements of the various parameters for each chamber ( $T_a$ , flow rate and fractional  $O_2$  concentrations) were recorded at the end of each 45 s sampling interval, so as to allow sufficient time for the flushing of air from the previous channel from the ducting between relay valves and the sub sample tubing.  $\dot{V}_{O_2}$  was recorded digitally every 6 min, corrected for standard temperature and pressure, and expressed as a mass-specific value. Thus ten readings per individual were recorded on an hourly basis.

Analogue signals from the thermistor probes, mass flow meter and oxygen analyser were recorded digitally using an A/D converter and software written by R. Van Zyl, UKZN.

The following equation was used to calculate mass-specific oxygen consumption:

$$\dot{V}_{O_2} = [\dot{V}(FI_{O_2} - FE_{O_2}) / (1 - FI_{O_2})] / M_b, \quad (1)$$

where  $\dot{V}_{O_2}$  is the metabolic rate ( $\text{ml O}_2 \text{ g}^{-1} \text{ h}^{-1}$ ),  $\dot{V}$  is the flow rate ( $\text{ml min}^{-1}$ ),  $FI_{O_2}$  is the incurrent fractional  $O_2$  concentration and  $FE_{O_2}$  is the excurrent fractional  $O_2$  concentration, and  $M_b$  is body mass (g) (Hill, 1972).

At 07:00 h the following morning, birds were removed from the chambers, weighed and returned to their cages in the CE room in the animal house. Food and water were available to them *ad libitum*.

Pre-acclimation  $\dot{V}_{O_2}$  values were measured at ambient temperatures of 25°C [assumed to be in the thermal neutral zone (TNZ)] and 5°C (assumed to be at an extreme) within 4 days of capture using an interspersed design within each subpopulation.

After the initial trials, birds were acclimated in the CE room for 6 weeks as described. This long acclimation period was necessary to ensure that sunbirds were all acclimated to the same conditions. After this time  $\dot{V}_{O_2}$  measurements were repeated as in the earlier trials at randomly ordered ambient temperatures of 15, 5, 10, 20, 30, 28, 25 and 33°C, to ensure the absence of temperature acclimation. Birds were carefully monitored at 33°C and removed at approximately 21:00 h.

#### Release

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

#### Statistical analyses

Descriptive statistics were calculated in STATISTICA (Statsoft, Tulsa, USA) for each subpopulation of amethyst sunbirds. Hourly rates of winter  $\dot{V}_{O_2}$  for individuals from each subpopulation of sunbirds were determined and plotted against time for each  $T_a$ . The minimum RMR at each of these temperatures for each individual was used in analysis to determine change with temperature using generalized linear models (commonly called GLM) repeated measures analysis of variance (RM ANOVA). BMR was calculated by taking the lowest mean RMR per subpopulation. The TNZ was determined using *post-hoc* Sheffé tests to determine over what range minimum RMR did not differ significantly. GLM RM ANOVA was further used for the comparison of winter  $\dot{V}_{O_2}$  measurements between populations at different altitudes and between pre- and post-acclimation data. *Post-hoc* Sheffé tests were done to determine where significant interactions occurred ( $P < 0.05$ ) between populations and within populations pre- and post-acclimation. Data were presented as mean  $\pm$  s.e.m. of the individuals measured ( $N$ ). Percentage change in winter MR (BMR or RMR) between

populations was calculated using the following equation: (higher altitude MR – lower altitude MR) / (higher altitude MR)  $\times$  100. Similarly, percentage change between pre- and post-acclimation winter MR values were calculated using the following equation: (pre-acclimation MR – post-acclimation MR) / (pre-acclimation MR)  $\times$  100.

## RESULTS

### Pre- versus post-acclimation

Acclimation had a significant effect on winter  $\dot{V}_{O_2}$  values for amethyst sunbird subpopulations at  $T_a = 5^\circ\text{C}$  when compared within and between sites (Fig. 1A; RM ANOVA,  $F_{(2,8)} = 14.977$ ,  $P = 0.002$ ). Mean  $\dot{V}_{O_2}$  values at 5°C changed significantly between pre- and post-acclimation for the high altitude Underberg ( $N = 9$ ) and low altitude Oribi Gorge ( $N = 8$ ) subpopulations (*post-hoc* Sheffé test,  $P < 0.05$ ). The high altitude Underberg subpopulation showed a 51.8% increase in  $\dot{V}_{O_2}$  from pre- to post-acclimation at 5°C from  $8.44 \pm 0.170 \text{ ml O}_2 \text{ g}^{-1} \text{ h}^{-1}$  (0.047 W) to  $12.81 \pm 0.949 \text{ ml O}_2 \text{ g}^{-1} \text{ h}^{-1}$  (0.071 W). The low altitude Oribi Gorge sunbirds showed a 52.5% decrease in  $\dot{V}_{O_2}$  between pre- and post-acclimation trials at 5°C, from  $12.59 \pm 0.699 \text{ ml O}_2 \text{ g}^{-1} \text{ h}^{-1}$  (0.070 W) to  $5.98 \pm 0.499 \text{ ml O}_2 \text{ g}^{-1} \text{ h}^{-1}$  (0.033 W). The intermediate altitude Howick sunbirds did not show a significant difference between pre- and post-acclimation  $\dot{V}_{O_2}$  at 5°C (*post-hoc* Sheffé,  $P > 0.05$ ), with a marginal 7.2% decrease in  $\dot{V}_{O_2}$  between pre- and post-acclimation, from  $15.94 \pm 0.426 \text{ ml O}_2 \text{ g}^{-1} \text{ h}^{-1}$  (0.089 W) to  $14.79 \pm 1.337 \text{ ml O}_2 \text{ g}^{-1} \text{ h}^{-1}$  (0.083 W).

Further analysis of pre-acclimation winter  $\dot{V}_{O_2}$  values at 5°C (Fig. 1A) showed a significant difference between Underberg and Howick (*post-hoc* Sheffé,  $P < 0.05$ ), and Underberg and Oribi Gorge (*post-hoc* Sheffé,  $P < 0.05$ ), but not between Howick and Oribi Gorge

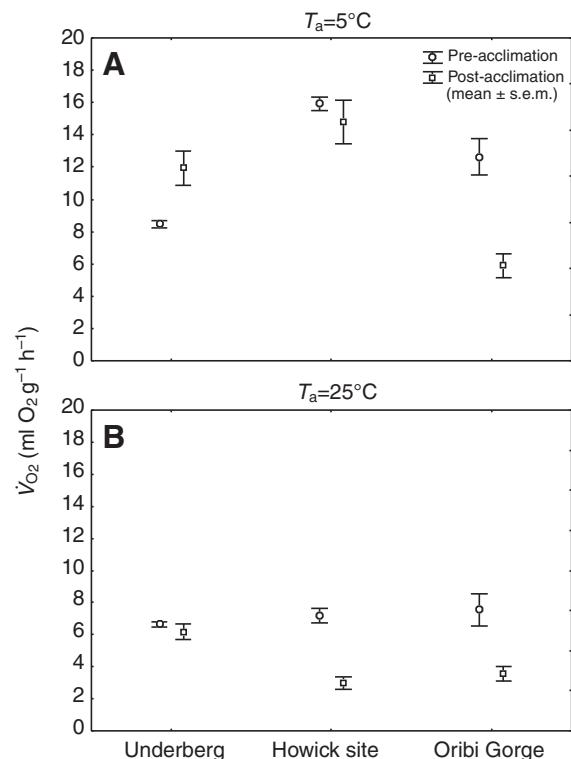


Fig. 1. Pre- and post-acclimation  $\dot{V}_{O_2}$  values for three subpopulations of amethyst sunbirds in winter (Underberg 1555 m asl, Howick 1075 m asl, and Oribi Gorge 541 m asl) at 5°C (A) and 25°C (B).

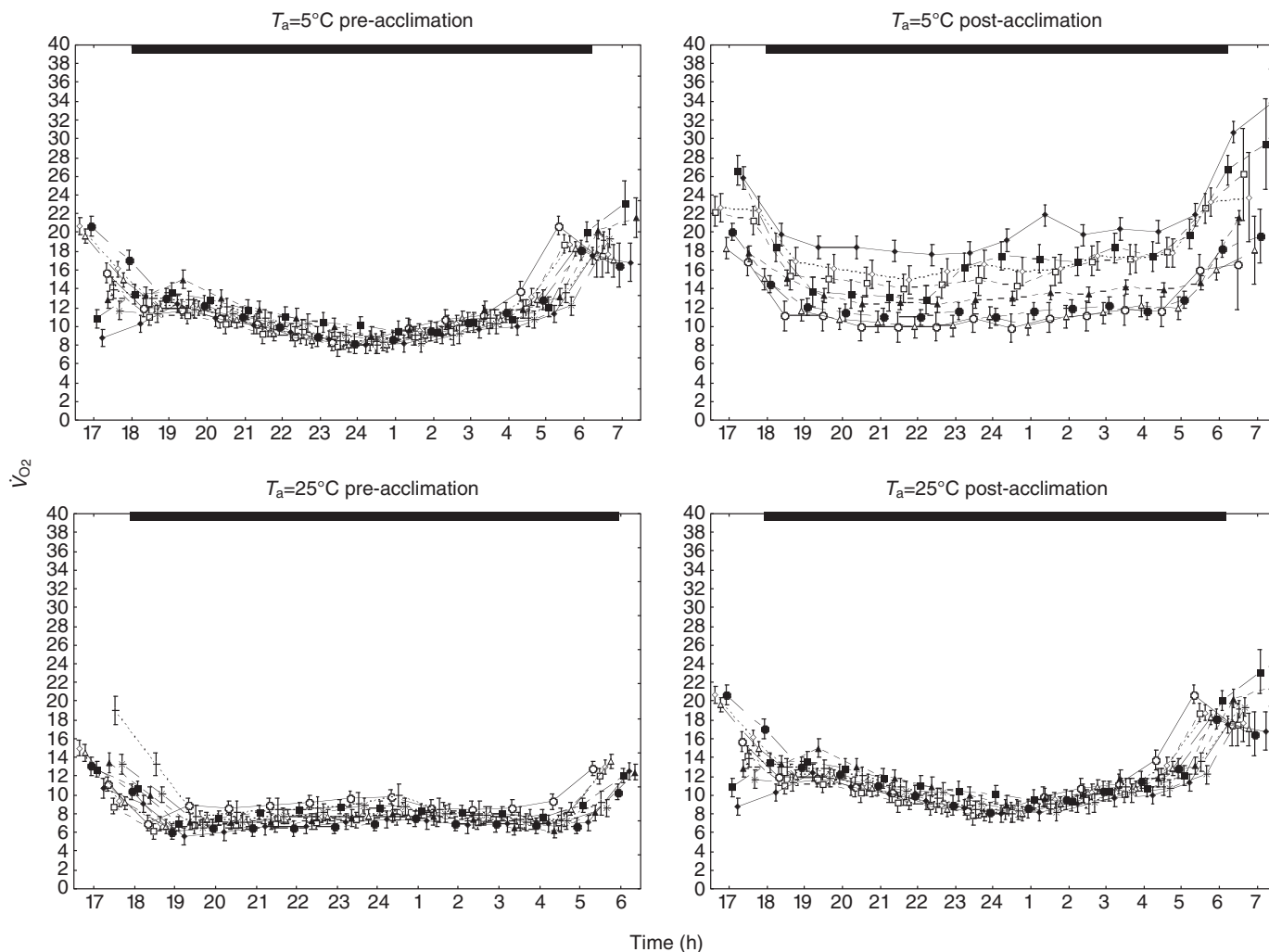


Fig. 2. Pre- versus post-acclimation  $\dot{V}_{O_2}$  (ml O<sub>2</sub> g<sup>-1</sup> h<sup>-1</sup>) of the Underberg subpopulation of amethyst sunbirds in winter, 17:00–07:00 h at 5 and 25°C (mean  $\pm$  s.e.m.,  $N=9$ ).

subpopulations (*post-hoc* Sheffé,  $P>0.05$ ). However, post-acclimation subpopulation comparisons indicated a significant difference between Underberg and Oribi Gorge (*post-hoc* Sheffé,  $P<0.05$ ), Howick and Oribi Gorge (*post-hoc* Sheffé,  $P<0.05$ ), but no significant difference in  $\dot{V}_{O_2}$  values between Underberg and Howick subpopulations (Fig. 1A; *post-hoc* Sheffé,  $P>0.05$ ).

Acclimation had a significant effect on winter  $\dot{V}_{O_2}$  values for amethyst sunbird subpopulations at  $T_a=25^\circ\text{C}$  when compared within and between sites (Fig. 1B; RM ANOVA,  $F_{(2,10)}=10.345$ ,  $P=0.004$ ). Significant variation existed between Underberg and Howick, and Underberg and Oribi Gorge subpopulations post-acclimation to 25°C, in 25°C trials (*post-hoc* Sheffé,  $P<0.05$ ). The high altitude Underberg subpopulation showed very little change between pre- and post-acclimation trials at 25°C with a decrease of only 2.9% from  $6.71\pm 0.146$  ml O<sub>2</sub> g<sup>-1</sup> h<sup>-1</sup> (0.037 W) to  $6.52\pm 0.493$  ml O<sub>2</sub> g<sup>-1</sup> h<sup>-1</sup> (0.036 W; *post-hoc* Sheffé,  $P>0.05$ ).  $\dot{V}_{O_2}$  for Howick and Oribi Gorge subpopulations decreased significantly between pre- and post-acclimation trials (*post-hoc* Sheffé,  $P<0.05$ ), with the  $\dot{V}_{O_2}$  of Howick subpopulations decreasing by 58.4% [from  $7.20\pm 0.447$  ml O<sub>2</sub> g<sup>-1</sup> h<sup>-1</sup> (0.040 W) to  $3.00\pm 0.386$  ml O<sub>2</sub> g<sup>-1</sup> h<sup>-1</sup> (0.017 W)] from pre- to post-acclimation, and the low altitude Oribi Gorge sunbirds exhibiting a 48.7% decrease in  $\dot{V}_{O_2}$  (from  $7.48\pm 0.742$  ml O<sub>2</sub> g<sup>-1</sup> h<sup>-1</sup> or 0.042 W

to  $3.84\pm 0.387$  ml O<sub>2</sub> g<sup>-1</sup> h<sup>-1</sup> or 0.021 W) from pre- to post-acclimation trials at 25°C.

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

#### Post-acclimation

The winter  $\dot{V}_{O_2}$  of different altitudinal subpopulations of amethyst sunbirds (Figs 5, 6 and 7) levelled off to RMR between 19:00–05:00 h.  $\dot{V}_{O_2}$  increased pre-dawn (06:00 h) starting at approximately 05:00 h, and  $\dot{V}_{O_2}$  started to decrease pre-sunset (18:00 h). The low altitude Oribi Gorge sunbirds displayed the lowest inter-individual variation (Fig. 7), and the high altitude Underberg sunbirds the highest inter-individual variation (Fig. 5) over the range

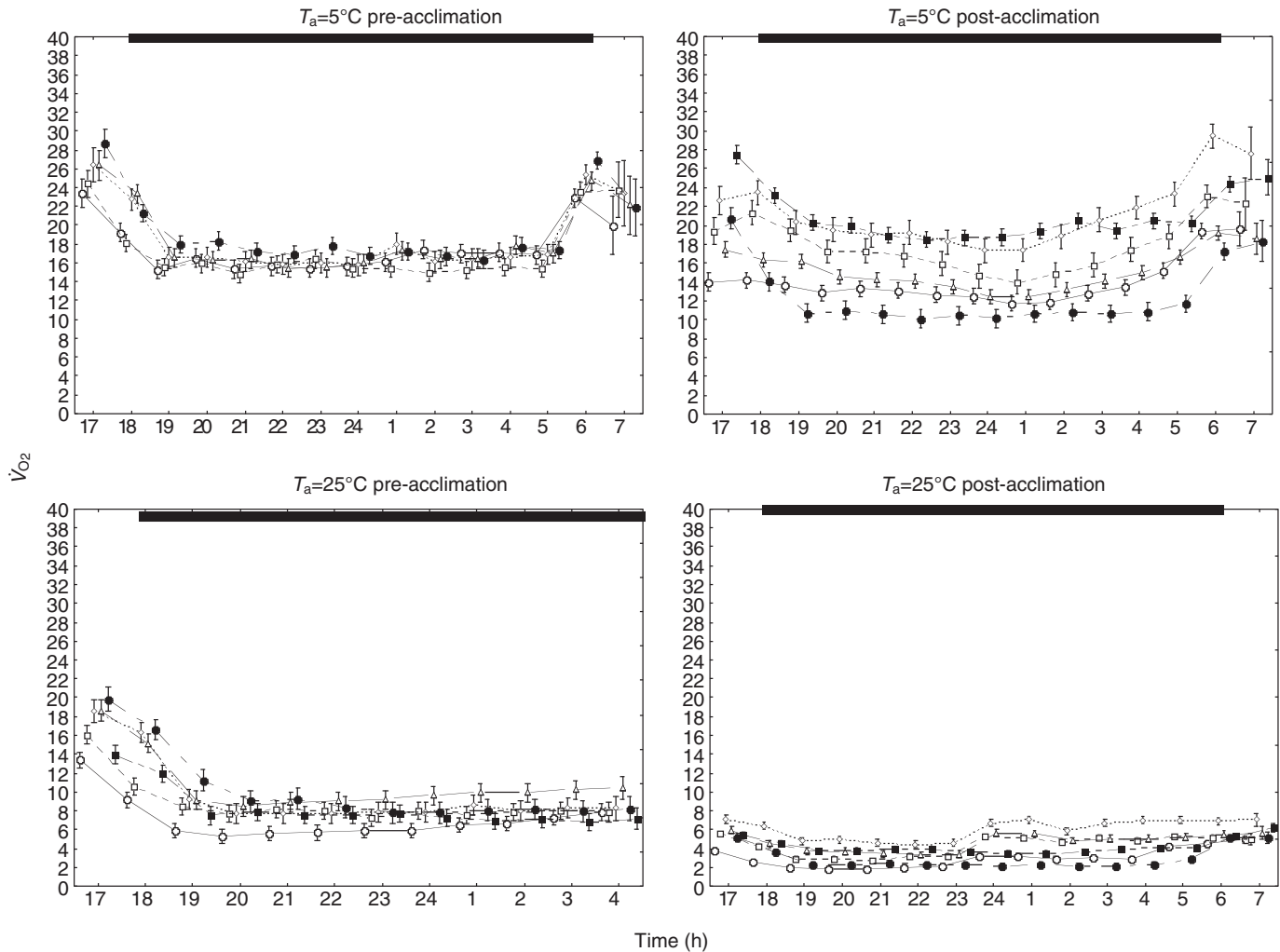


Fig. 3. Pre- versus post-acclimation  $\dot{V}_{O_2}$  ( $\text{ml O}_2 \text{g}^{-1} \text{h}^{-1}$ ) of the Howick subpopulation of amethyst sunbirds in winter, 17:00–07:00 h at 5 and 25°C (mean  $\pm$  s.e.m.,  $N=6$ ).

of ambient temperatures. Howick sunbirds displayed a high inter-individual variation in  $\dot{V}_{O_2}$  at  $T_a$ s of 5–20 and 33°C (Fig. 6), but a decrease in individual variation between  $T_a=25$ –30°C, thus corresponding to the TNZ. Inter-individual variation increased as altitude increased.

Winter mean resting metabolic rates for each of the different amethyst sunbirds subpopulations over the range of ambient temperatures are summarised in Fig. 8. There was little variation in  $\dot{V}_{O_2}$  between individuals from the same locality, whereas significant variation was observed at the same temperatures between localities and thus between altitudes. There was a significant effect of altitude and temperature when comparing  $\dot{V}_{O_2}$  values for the subpopulations of sunbirds (RM ANOVA,  $F_{(14,70)}=21.039$ ,  $P<0.001$ ). There was a significant difference between the  $\dot{V}_{O_2}$  values of the high altitude Underberg and low altitude Oribi Gorge subpopulations at 5, 10, 15, 20, 28, 30°C (*post-hoc* Sheffé,  $P<0.05$ ), and between Underberg and Howick subpopulations at 25 and 28°C (*post-hoc* Sheffé,  $P<0.05$ ), and finally between Howick and Oribi Gorge subpopulations at 5, 10 and 15°C (*post-hoc* Sheffé,  $P<0.05$ ). BMR was determined for each subpopulation by using the mean of the lowest hourly individual RMRs: Underberg [ $5.71 \pm 0.402 \text{ ml O}_2 \text{g}^{-1} \text{h}^{-1}$  (0.032 W)

at 33°C], Howick [ $2.46 \pm 0.299 \text{ ml O}_2 \text{g}^{-1} \text{h}^{-1}$  (0.014 W) at 28°C] and Oribi Gorge [ $3.49 \pm 0.312 \text{ ml O}_2 \text{g}^{-1} \text{h}^{-1}$  (0.019 W) at 30°C].

Overall there was a significant effect of altitude on winter BMR (RM ANOVA,  $F_{(2,10)}=15.183$ ,  $P=0.001$ ). BMR decreased significantly from Underberg to Howick by 56.9% (*post-hoc* Sheffé,  $P<0.05$ ), and by 38.9% from Underberg to Oribi Gorge (*post-hoc* Sheffé,  $P<0.05$ ). However, comparison of the BMR data from Howick to Oribi Gorge showed a non-significant increase of 41.7% (*post-hoc* Sheffé,  $P>0.05$ ).

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

#### Body mass

There was no significant difference between winter mean pre- and post-acclimation body mass (g) of amethyst sunbirds within sites, nor were there significant differences between winter pre- and post-acclimation body masses between altitudinal subpopulations of amethyst sunbirds (Fig. 9; RM ANOVA,  $F_{(2,22)}=0.345$ ,  $P=0.712$ ).

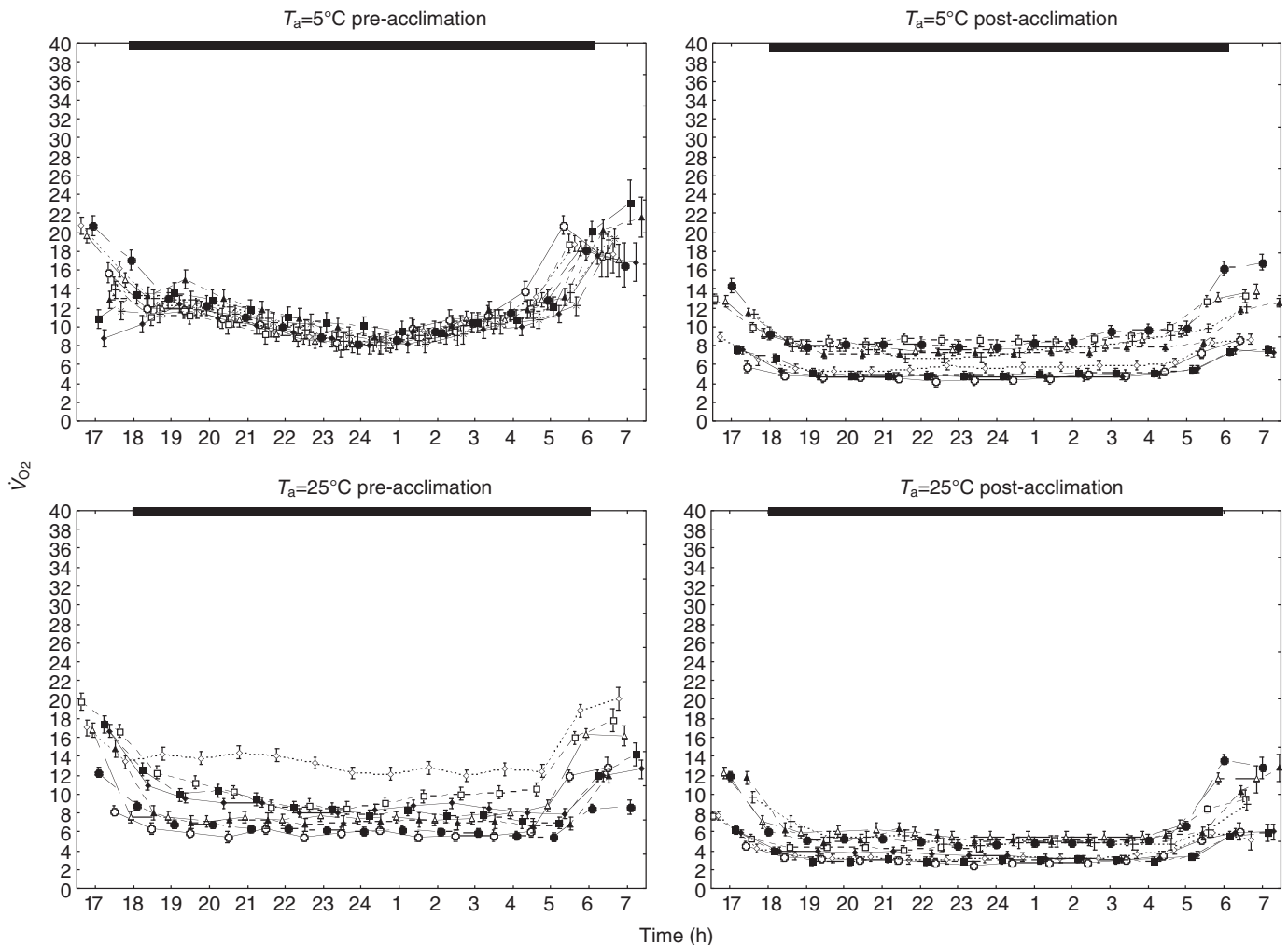


Fig. 4. Pre- versus post-acclimation  $\dot{V}_{O_2}$  (ml O<sub>2</sub> g<sup>-1</sup> h<sup>-1</sup>) of the Oribi Gorge subpopulation of amethyst sunbirds in winter, 17:00–07:00 h at 5 and 25°C (mean  $\pm$  s.e.m.,  $N=8$ ).

#### Ambient temperature

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

#### DISCUSSION

Many metabolic studies (particularly comparative studies), of both birds and other animals, have used a mean BMR for a population regardless of capture location, or alternatively have used data from one population as a representative for an entire species (e.g. Bech, 1980; Cooper and Swanson, 1994; Maddocks and Geiser, 2000; McKechnie and Lovegrove, 2001; López-Calleja and Bozinovic, 2003; Lovegrove and Smith, 2003; McKechnie et al., 2007; Smit et al., 2008). However, this winter study showed that populations respond differently to acclimation, possibly as a result of persistent underlying physiological differences, or persistent effects of altitudinal acclimatization. Recent evidence suggests that winter BMRs of species living in highly seasonal environments reflect the conditions that the animals were in immediately prior to metabolic measurements being

taken (McKechnie, 2008). As a result of this, after the 6 week acclimation period to 25°C and an altitude of 660 m, we would expect birds from all of the altitudinal subpopulations to react similarly if not uniformly to the range of ambient temperatures of the respirometry trials, and thus differently to pre-acclimation trials, thereby displaying similar phenotypic flexibility. However, differences in winter MR, and in particular winter BMR, between subpopulations in post-acclimation trials indicate that this is not necessarily the case. Altitudinal subpopulations still showed differences in MR post-acclimation, thus indicating that captive-bred populations of birds would not represent the entire species as well as might be assumed, unless altitudinal origin of the original population is known, and laboratory populations represent subpopulations over the entire altitudinal gradient occupied by the species. It is also difficult to assess the end point of thermal acclimation (Rezende et al., 2004; Bush et al., 2008a) and thus it is possible that this was merely a stage in the ability of amethyst sunbirds to change their thermal phenotype over a longer period of time.

The fact that there was no significant difference between the winter post-acclimation masses of the three subpopulations of amethyst sunbirds showed that these differences in winter RMR and BMR were not a result of the effects of body mass and thus may indicate a difference in acclimation strategies, most likely as

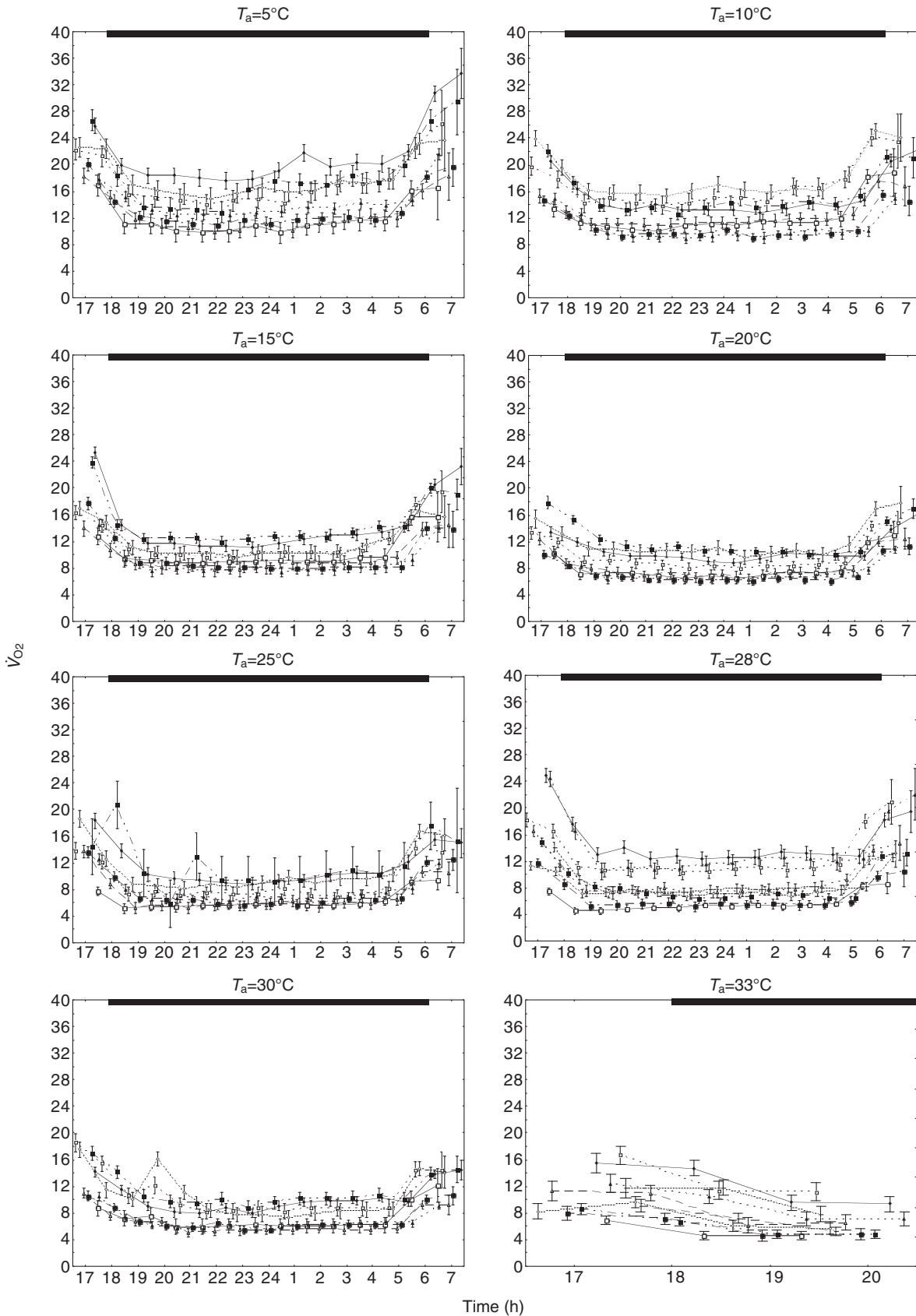


Fig. 5. Post-acclimation  $\dot{V}_{O_2}$  (ml  $O_2$  g $^{-1}$  h $^{-1}$ ) in the Underberg subpopulation of amethyst sunbirds over a range of temperatures ( $T_a$ ) in winter (mean  $\pm$  s.e.m.,  $N=8$ ).

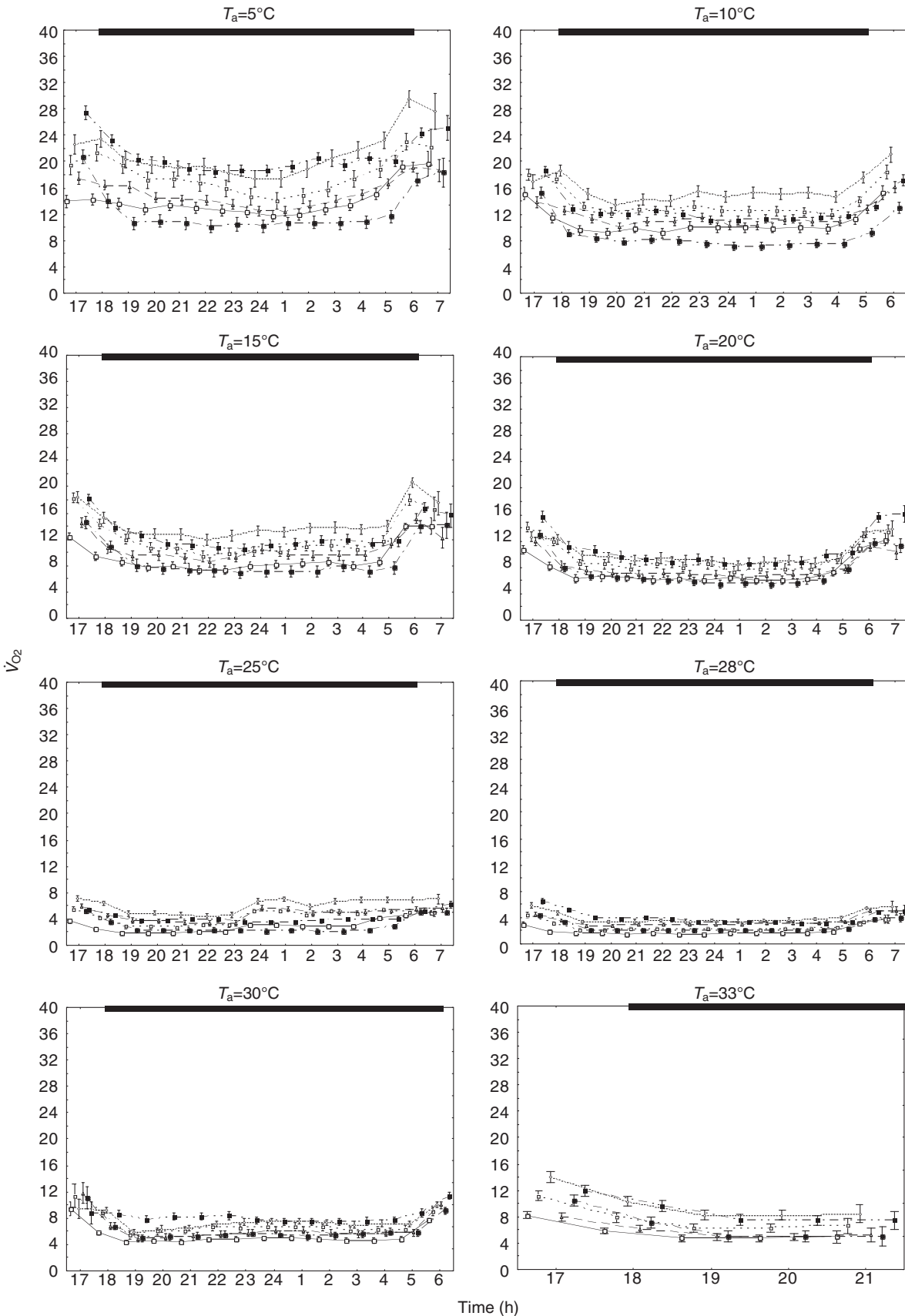


Fig. 6. Post-acclimation  $\dot{V}_{O_2}$  ( $\text{ml O}_2 \text{ g}^{-1} \text{ h}^{-1}$ ) in the Howick subpopulation of amethyst subbirds over a range of temperatures ( $T_a$ ) in winter (mean  $\pm$  s.e.m.,  $N=6$ ).



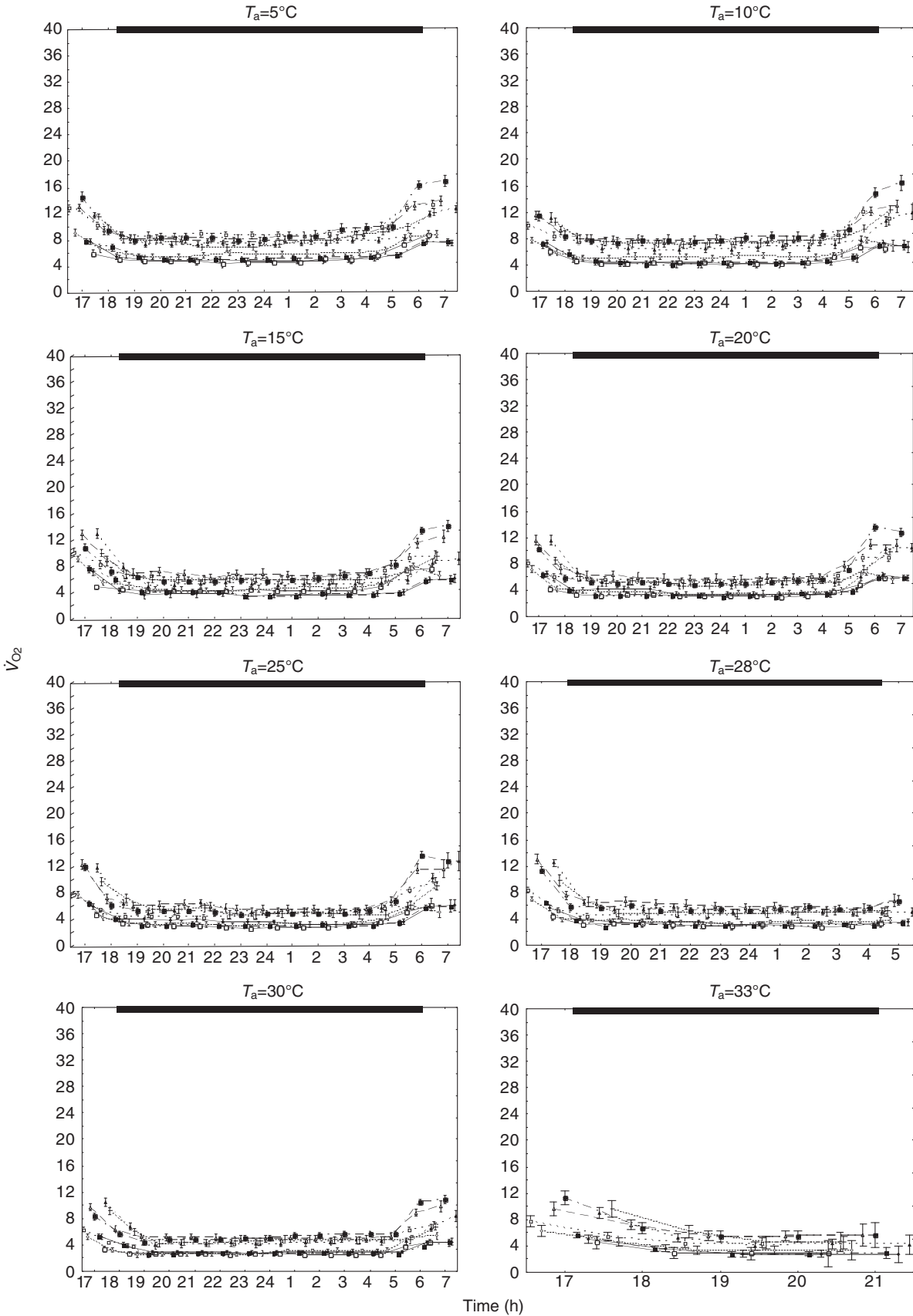


Fig. 7. Post-acclimation  $\dot{V}_{O_2}$  ( $\text{ml O}_2 \text{ g}^{-1} \text{ h}^{-1}$ ) in the Oribi Gorge subpopulation of amethyst sunbirds over a range of temperatures ( $T_a$ ) in winter (mean  $\pm$  s.e.m.,  $N=9$ ).

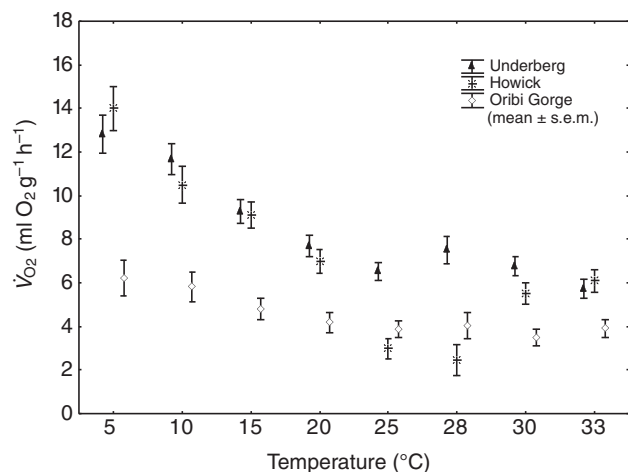


Fig. 8. Post-acclimation mean resting metabolic rates ( $\dot{V}_{O_2}$ , mean  $\pm$  s.e.m.) for the three winter acclimatized subpopulations of amethyst sunbirds (Underberg, Howick and Oribi Gorge) at  $T_a=5, 10, 15, 20, 28, 30, 33^\circ\text{C}$ .

a result of adapting to life in different altitudinal and thus thermal environments. This further emphasizes the need for knowledge of the origin of study populations, and not just in terms of captive *versus* wild-caught populations. In recent comparative avian reviews, much focus is placed on whether study birds were captive bred or wild caught (e.g. Weathers et al., 1983; McKechnie et al., 2007; McKechnie, 2008) and on phenotypic flexibility. However, changing views in avian physiology as yet omit to recognize the altitudinal origin of the study population. A review by McKechnie (McKechnie, 2008) recognized that the data in the literature often uses a single BMR value per species and is assumed to represent a fixed species-specific value. Indeed, most studies of avian thermoregulatory abilities have used a mean BMR from one population as a representative for the entire species, which does not take into consideration the altitudinal origin of the study animals, seasonal effects, nor does it acknowledge the fact that phenotypic flexibility, with respect to physiological parameters, may not be consistent throughout a species.

Amethyst sunbirds exhibited significant differences in winter pre-acclimation metabolic rates at both 5 and 25°C, indicating that altitudinal acclimatization plays a big role in sunbird physiology at any point in time. The results also indicate that different

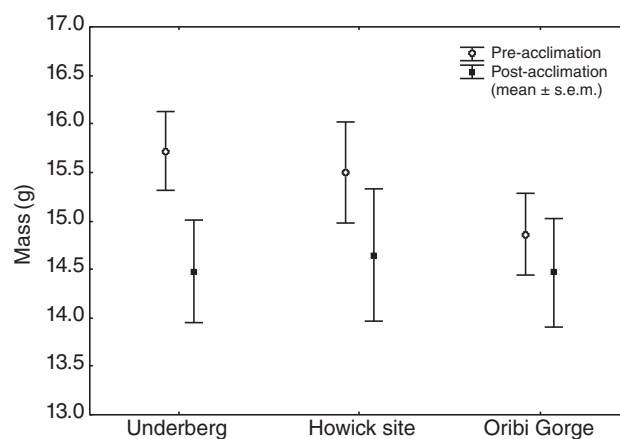


Fig. 9. Change in body mass (g) of amethyst sunbirds between pre- and post-acclimation trials in winter.

subpopulations show different responses to acclimation, and that differences in TNZ were evident post-acclimation, which indicated that physiological differences were not just a result of acclimation to the temperature and altitude of the acclimation site. Post-acclimation results also showed significant differences in winter  $\dot{V}_{O_2}$  between Underberg and Oribi Gorge subpopulations at 5, 10, 15, 20, 28, 30°C, between Underberg and Howick amethyst sunbird subpopulations at 25, 28°C and between Howick and Oribi Gorge subpopulations at 5, 10 and 15°C. Similarly, Soobramoney et al. (Soobramoney et al., 2003) found that there was a difference in metabolic rates of the common fiscal (*Lanius collaris*) over an altitudinal temperature gradient as colder temperatures at high altitudes require an increase in metabolic heat production in homeotherms. However, common fiscals showed higher metabolic rates in subpopulations from the warmer altitudes, whereas amethyst sunbirds subpopulations from the warmer location (Oribi Gorge, lowest altitude) showed lower winter metabolic rates. As winter temperatures vary dramatically between the two habitats it would be expected that individuals that could survive in that range of ambient temperatures would be selected for over the generations and thus we would expect underlying physiological differences between altitudinal subpopulations.

Table 1. Monthly average, minimum and maximum temperatures (°C), for Underberg (Shaleburn), Howick (Cedara) and Oribi Gorge (Paddock), January 2004–May 2007

Month	Temperature (°C)								
	Underberg (Shaleburn)			Howick (Cedara)			Oribi Gorge (Paddock)		
	Mean $\pm$ s.e.m.	Min.	Max.	Mean $\pm$ s.e.m.	Min.	Max.	Mean $\pm$ s.e.m.	Min.	Max.
1	18.27 $\pm$ 0.12	6.70	31.70	20.06 $\pm$ 0.12	9.80	34.70	21.81 $\pm$ 0.07	12.80	35.80
2	18.07 $\pm$ 0.13	3.70	31.90	20.44 $\pm$ 0.12	8.60	36.60	22.64 $\pm$ 0.06	14.00	37.30
3	15.51 $\pm$ 0.13	0.80	29.90	17.87 $\pm$ 0.12	6.90	35.40	20.49 $\pm$ 0.06	12.20	34.80
4	13.24 $\pm$ 0.16	-4.80	28.00	16.30 $\pm$ 0.13	1.00	31.00	19.47 $\pm$ 0.07	9.20	34.60
5	8.36 $\pm$ 0.21	-11.80	26.20	12.54 $\pm$ 0.16	-3.60	30.40	17.55 $\pm$ 0.08	6.60	34.00
6	5.91 $\pm$ 0.30	-8.50	24.50	10.06 $\pm$ 0.22	-1.40	26.40	15.72 $\pm$ 0.09	6.00	29.90
7	7.73 $\pm$ 0.28	-8.00	26.20	11.62 $\pm$ 0.22	-1.80	29.20	15.67 $\pm$ 0.10	6.40	34.70
8	9.86 $\pm$ 0.23	-6.90	29.00	12.31 $\pm$ 0.19	-1.70	30.70	16.86 $\pm$ 0.10	6.90	35.40
9	12.57 $\pm$ 0.24	-7.00	30.20	15.26 $\pm$ 0.16	0.20	34.10	17.30 $\pm$ 0.10	6.10	37.80
10	14.98 $\pm$ 0.23	3.30	30.80	16.79 $\pm$ 0.14	6.70	35.60	18.49 $\pm$ 0.08	9.70	36.30
11	15.77 $\pm$ 0.15	1.60	30.90	17.48 $\pm$ 0.13	5.60	35.00	19.88 $\pm$ 0.08	10.60	41.20
12	16.64 $\pm$ 0.14	5.60	31.90	18.03 $\pm$ 0.13	5.20	35.60	20.70 $\pm$ 0.08	12.10	35.20

Our data, and that of other altitudinal studies, emphasizes the need to acknowledge altitudinal differences between populations and not just use species means, as species means do not fully incorporate the effect of phenotypic plasticity/flexibility. In this study variation in MR was only examined during winter.

Amethyst sunbirds exhibited high levels of phenotypic flexibility between the three different subpopulations studied. This variation reflects acclimatization at the population level, and indicates that geographic conditions (altitude and ambient temperature) play a major role in influencing animal physiology. It also indicates that amethyst sunbirds have the capacity to adapt to a range of climatic conditions, suggesting that the impact of climate change may not be as severe on bird distributional ranges as previously thought.

### Conclusion

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

### LIST OF ABBREVIATIONS

BMR	basal metabolic rate
EWL	evaporative water loss
RMR	resting metabolic rate
TNZ	thermal neutral zone
$\dot{V}O_2$	oxygen consumption

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