ASPECTS OF NOCTURNAL PHYSIOLOGY AND BEHAVIOUR IN MALACHITE SUNBIRDS (*NECTARINIA FAMOSA*)

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PREFACE

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

These studies represent original work by the author and have not otherwise been submitted in any form for any degree or diploma to any other University. Where use has been made of the work of others, it is duly acknowledged in the text. The thesis is structured with an introductory chapter and then each subsequent chapter written in manuscript format with the aim to publish in relevant scientific journals. Any repetition was unavoidable.

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December 2007
There are so many people who have helped and supported me in my studies these last few years.

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ABSTRACT

Although sleep forms an important part of an animal’s life, there is a paucity of knowledge about sleep behaviour. The function of sleep in birds is poorly understood, even though birds spend a large part of their lives sleeping. Sleep behaviour in passerine birds has not been looked at as extensively as that of non-passerine birds. I looked at the sleep behaviour of three relatively common passerine birds occurring in southern Africa, namely the Malachite Sunbird (*Nectarinia famosa*), the Cape White-eye (*Zosterops pallidus*) and the Fan-tailed Widowbird (*Euplectes axillaris*). By using an infra-red sensitive camera I described basic sleep behaviours at various ambient temperatures, of all three species, such as sleep position and eye closure, and also investigated the incidence of unihemispheric sleep. Individuals of all three species spent most of the night asleep and kept on waking up intermittently throughout the night, with no significant differences between temperatures. Cape White-eyes and Malachite Sunbirds showed an increase in back sleep and a decrease in front sleep at 5°C. Little evidence of unihemispheric sleep was found, suggesting that it is more likely to occur in non-passerines, especially ground dwelling birds.

Diurnal birds generally sleep during the hours of darkness. Most male southern African sunbirds have pectoral tufts, although the function of these is not always understood. In male Malachite Sunbirds it has recently been found that they display their pectoral tufts almost continuously throughout the night, whilst asleep. I explored the possible function of this behaviour and suggest that these tufts might be a deterrent to predators, as they look like ‘eyes’ in the dark. A review of the use and occurrence of pectoral tufts in southern African sunbird species is also presented.

Blood glucose concentrations of most birds are much higher than those found in mammals and it is still not known how they evade the complications of such high levels. I
investigated the change in blood glucose concentrations of Malachite Sunbirds at two different ambient temperatures and at different times of the night and day and explored the possibility that gluconeogenesis might be used by birds to ‘warm up’ during arousal of torpor in the early morning, before daylight. Generally blood glucose levels were fairly high, between 13.6 and 21.4 mmol/L, which was expected. Blood glucose levels were higher at 5°C than at 25°C and generally lower in the early hours of the morning. Therefore I reject the assumption that Malachite Sunbirds use gluconeogenesis as an additional form of heat generation during torpor. It is thought that the difference in the levels of blood glucose might be a function of the cold temperature and the consumption of their nectarivorous diet.

This research clearly highlights the need for further studies to be undertaken in the sleeping behaviours and patterns of birds, especially in southern African species. It also shows that more studies need to be done on the use of pectoral tufts in sunbird species and furthermore it is suggested that more research is needed to elucidate the mechanism by which Malachite Sunbirds are able to rapidly ‘warm up’ during arousal, when in torpor.
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CHAPTER 1

General Introduction

Malachite Sunbirds (*Nectarinia famosa*) are small (15 to 20 g) (although large in terms of most Sunbird species (Cheke *et al.* 2001), nectarivorous passerines which are common to locally abundant throughout southern Africa (Fry *et al.* 2000). The sexes are dimorphic and birds are monogamous, although the pair bond only lasts for the duration of the breeding season (Wolf and Wolf 1976). In this study we wanted to determine what Malachite Sunbirds did at night, particularly in terms of their sleep type, sleep patterns, sleep behaviour and physiology, as very little about this subject is known, particularly in southern Africa. During our study we also became aware of male Malachite Sunbirds displaying their pectoral tufts at night, and so we consequently investigated why birds would do this, as this has never before been mentioned in the literature.

Every animal that has so far been studied spends some of its time sleeping (Rattenborg and Amlaner 2002). A sleep-like state includes: absence of voluntary movements, spontaneous, being part of a circadian rhythm, being reversible, being characterised by species- specific postures and/or resting places that minimizes sensory stimulation, an increased arousal threshold, usually being regulated by a homeostatic mechanism that is modulated by circadian regulation, and having state-related changes in neural function including those leading to decreased sensory input to the central nervous system (Hendricks *et al.* 2000).

Diurnal birds generally sleep during the hours of darkness (Maclean 1990). Sleeping activity is shown as four recorded sleep postures, namely: bill tucked under
or on the scapula feathers (hereafter known as back sleep), head and neck held stationary with bill pointing forward and eyes usually closed (hereafter known as front sleep) (Amlaner and Ball 1983, 1994); bill on the back sleep, where the bill lies on the birds back, or head on ground sleep, where the bird lies on the ground with its neck extended along the ground (Amlaner and Ball 1983). The most common form of bird sleep postures in the reported literature are back and front sleep (Amlaner and Ball 1983). In the latter birds are classified as only resting if the eyes are open (Christe et al. 1996). When the eyelids are closed, birds generally are in sleep as shown by electrocephalographic recordings and behavioural studies (Rattenborg et al. 1999). Sleep and eyelid closure in birds are so closely related that is thought that eyelids only close in sleep, which means that eyelid closure is the most reliable index of sleep (Kavanau 1998). The time between eyelid closure and opening is known as a sleep bout (Amlaner and Ball 1983). The most common form of sleep in birds is called vigilant sleep and this is characterized by an alteration of longer periods of eye closure and shorter periods of eye-opening (Amlaner and Ball 1983). The benefit of vigilant sleep, also known as ‘peeking’, is predator detection, but this occurs at the expense of eye-closure (Lendrem 1983).

Despite recent research (Rattenborg et al. 2000), the function of sleep in birds is poorly understood, even though a large part of a bird’s life is spent sleeping (Toates 1980). Even though three general hypotheses for the function of sleep have been put forward (Meddis 1975, Amlaner and Ball 1983), most evidence suggests that during sleep, the brain functions in memory consolidation, which is crucial for brain functioning during wakefulness (Rattenborg 2006, Tononi and Cirelli 2006).

Sleep in birds and mammals are made up of two distinct sleep types. These are slow-wave sleep (SWS) or quiet sleep and rapid-eye movement sleep (REM) or
active sleep (Rattenborg et al. 2000). During SWS, an electroencephalographic (EEG) reading shows low-frequency, high-amplitude activity, otherwise known as delta or theta activity, whereas during REM sleep EEG activity is similar to wakefulness, with low-amplitude, high-frequency activity (Rattenborg et al. 2000). REM sleep is also characterised by a relaxed muscle tone, rapid-eye movements and suspended thermoregulation (Rattenborg et al. 2000). Mammals generally show more REM sleep than birds do. Most birds, with the exception of some species of shorebirds and waterfowl (Amlaner & Ball 1983) are classified as monophasic sleepers. Monophasic sleep is when animals sleep during a defined period of the day (Ball 1992), whereas the opposite of this is polyphasic sleep, where animals sleep during several episodes that can occur at any time of the day (Lima et al. 2005).

However, certain species of birds and marine mammals simultaneously engage in both wakefulness and sleep known as unihemispheric sleep, with one eye open (Symczack et al. 1996, Rattenborg et al. 1999, Rattenborg et al. 2000). Birds (and aquatic mammals) that exhibit unihemispheric sleep, in which slow wave sleep in one hemisphere is associated with closure of the contralateral eye, whilst the other eye is awake and the hemisphere is active (Amlaner and Ball 1983, Rattenborg et al. 2000). Importantly, research has only shown this in northern hemisphere birds and furthermore, not many studies have looked at the function of unihemispheric sleep in passerine birds (Rattenborg et al. 2000), although the most obvious explanation is that birds use unihemispheric sleep to keep a look out for predators, whilst still acquiring some of the benefits of sleep (Rattenborg et al. 2000). There is also some evidence that migratory birds that fly long distances might use unihemispheric sleep (Rattenborg 2006). In aquatic mammals unihemispheric sleep
allows animals to surface in order to breathe and in some cases also to scan their surroundings for predators (Goley 1999, Rattenborg et al. 2000). It was predicted that Malachite Sunbirds, would not exhibit unihemispheric sleep at night. In terms of their sleeping posture, we expected Malachite Sunbirds to sleep in either back or front sleep positions and assumed that the sleeping behaviours would change at colder temperatures. We suggest that they display their pectoral tufts at night as a deterrent to predators, and review the use and occurrence of pectoral tuft display in other southern African sunbird species.

Of interest was the physiology of Malachite Sunbirds at night. Malachite Sunbirds use heterothermy, including torpor, to conserve energy, especially at night, when temperatures are low (Downs and Brown 2002). Downs and Brown (2002) indicated that these birds might have used an additional form of heat generation to warm up, as they raised their body temperature fairly quickly. We suggested that they might be using gluconeogenesis, which is the formation of glucose in the liver and leads to a rise in plasma blood glucose (Vander et al. 2004). Therefore we measured blood glucose levels in Malachite Sunbirds at different temperatures and times of the day and night. Blood glucose levels of most bird species are much higher than those found in mammals (Umminger, 1975; Pollock, 2002). Yet it is still not known how they evade the complications of such high levels (Beuchat and Chong, 1998). We predicted that the blood glucose levels in Malachite Sunbirds would be high, but that the levels would not be constant through the day and night and that blood glucose levels might be affected by their nectarivorous diet.
References


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CHAPTER 2

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A behavioural study of sleep patterns in the Malachite Sunbird, Cape White-eye, and Fantailed Widowbird

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Although sleep forms an important part of an animal’s life, there is a paucity of knowledge about sleep behaviour. The function of sleep in birds is poorly understood, even though birds spend a large part of their lives sleeping. Sleep behaviour in passerine birds has not been looked at as extensively as that of non-passerine birds. We looked at the sleep behaviour of three relatively common passerine birds occurring in southern Africa, namely the Malachite Sunbird (Nectarinia famosa), the Cape White-eye (Zosterops pallidus) and the Fan-tailed Widowbird (Euplectes axillaris). By using an infra-red sensitive camera we described basic sleep behaviour, at various ambient temperatures, of all three species, such as sleep position and eye closure. We also investigated the incidence, or lack thereof, of unihemispheric sleep. Individuals of all three species spent most of the night asleep and kept on waking up intermittently throughout the night, with no significant differences between temperatures. Cape White-eyes and Malachite Sunbirds showed an increase in back sleep and a decrease in front sleep at 5°C. Little evidence of unihemispheric sleep was found, suggesting that it is more likely to occur in non-passerines, especially ground dwelling birds.
Sleep is a universal feature in the daily cycle of vertebrates, but not much is known about sleep behaviour in birds, as it has received relatively little consideration from animal behaviourists (Lima et al. 2005). Sleeping activity is generally recorded as four different sleep postures, namely: bill tucked under or on the scapula feathers (hereafter referred to as back sleep), head and neck held stationary with bill pointing forward and eyes usually closed (hereafter referred to as front sleep) (Amlaner & Ball 1983; 1994); bill on the back sleep (where the bill lies on the birds back), or head on ground sleep, where the bird lies on the ground with its neck extended along the ground (Amlaner & Ball 1983). The most common form of bird sleep postures in the reported literature are back and front sleep. In the latter birds are considered only resting if their eyes are open (Christe et al. 1996). When the eyelids are closed, birds generally are in sleep as shown by electrocephalographic recordings and behavioural studies (Rattenborg et al. 1999). Sleep and eyelid closure in birds are so closely related that is thought that eyelids only close in sleep, which means that eyelid closure is the most reliable index of sleep (Amlaner & Ball 1983; Kavanau 1998). The time between eyelid closure and opening is known as a sleep bout (Amlaner & Ball 1983).

The most common form of sleep in birds is called vigilant sleep and this is characterized by an alteration of longer periods of eye closure and shorter periods of eye-opening (Amlaner & Ball 1983). The benefit of vigilant sleep, also known as ‘peeking’, is predator detection, but this occurs at the expense of eye-closure (Lendrem 1983).
Despite recent research (Rattenborg et al. 2000), the function of sleep in birds is poorly understood, even though a large part of a bird's life is spent sleeping (Toates 1980). Three general types or hypotheses for the function of sleep have been put forward and these are 1) Sleep conserves energy (during sleep the body temperature ($T_b$) is lowered and energy is thereby saved), 2) Sleep has a restoring function (restores physiological properties of some tissues), 3) Sleep is a state of immobilization that increases safety against predators during times that are unbeneﬁcial for the presentation of other activities (Meddis 1975; Amlaner & Ball 1983).

Sleep in birds and mammals is made up of two distinct sleep types. These are slow-wave sleep (SWS) or quiet sleep and rapid-eye movement sleep (REM) or active sleep (Rattenborg et al. 2000). During SWS, an electroencephalographic (EEG) reading shows low-frequency, high-amplitude activity, otherwise known as delta or theta activity, whereas during REM sleep EEG activity is similar to wakefulness, with low-amplitude, high-frequency activity (Rattenborg et al. 2000). REM sleep is also characterised by a relaxed muscle tone, rapid-eye movements and suspended thermoregulation (Rattenborg et al. 2000). The duration of REM sleep shown by mammals is greater in comparison to that in birds (Ayla-Guerrero et al. 2003). Data on sleep in the turkey (Meleagris gallopavo) show that birds only have minimal amounts of REM sleep, the function why this is so still remains unknown (Ayla-Guerrero et al. 2003). Most birds, with the exception of some species of shorebirds and waterfowl (Amlaner & Ball 1983), are classified as monophasic sleepers. Monophasic sleep is when animals concentrate a distinct portion of the day to sleep (Ball 1992), whereas the opposite of this is polyphasic sleep, where animals sleep during several episodes that can occur at any time of the day (Lima et al. 2005).
Certain species of birds simultaneously engage in both wakefulness and sleep known as unihemispheric sleep, with one eye open (Symczack et al. 1996; Rattenborg et al. 1999; Rattenborg et al. 2000). Unihemispheric sleep only occurs during slow-wave sleep or quiet sleep (Rattenborg et al. 2000). Unihemispheric sleep entails slow wave sleep in one hemisphere, and is associated with closure of the contralateral eye, whilst the other eye is open and the hemisphere is active (Amlaner & Ball 1983; Rattenborg et al. 2000). However, this has only been shown in a few northern hemisphere bird species.

Not many studies have looked at the function of unihemispheric sleep and sleep behaviour in passerine birds (Rattenborg et al. 2000), although the most obvious explanation is that birds use unihemispheric sleep to keep a look out for predators, whilst still acquiring some of the benefits of sleep (Rattenborg et al. 2000). There is also some evidence that migratory birds that fly long distances might use unihemispheric sleep (Rattenborg 2006). We examined the sleep type and behaviour of three southern African passerines, namely Malachite Sunbirds (*Nectarinia famosa*), Cape White-eyes (*Zosterops pallidus*) and Fan-tailed Widowbirds (*Euplectes axillaris*).

Malachite Sunbirds (15-20g) are nectarivores but also occasionally feed on arthropods (Hockey et al. 2005). They use torpor at night to conserve energy, especially when temperatures are low (between 5 and 10°C), and allow their body temperature to drop significantly (Downs & Brown 2002). Cape White-eyes are small in size (13.5g) and are generalist feeders feeding mainly on insects, spiders, nectar and fleshy fruits (Skead 1967; Hockey et al. 2005). These birds roost in pairs, closely huddled together on a perch, their feathers fluffed out and displaying typical back sleep (Skead 1967). Fan-tailed Widowbirds, (22-29g) are seed eaters, feeding mainly
on different seeds of grasses (Hockey et al. 2005). These birds are highly gregarious and form large flocks, especially during the non-breeding season (Hockey et al. 2005).

We predicted that individuals of all three species, as non-ground dwelling passerines, would not exhibit unihemispheric sleep. We also predicted that these species would sleep in either back or front sleep positions. In addition, we expected them not to show vigilant sleep, characterised by an alteration of longer periods of eye closure and shorter periods of eye-opening, at low temperatures. The sleep-wake cycle is distinctly affected by stress, which we assume would lead to different sleep patterns for different stresses (Papale et al. 2005). We will assume that cold temperature would act as a stress factor that could affect sleeping behaviour. Predicted differences were that birds would fluff their feathers out at colder temperatures and assume different sleeping positions. There are some problems with studying sleep in animals (Amlaner & Ball 1983; Campbell & Tobler 1984), as it is easier to do studies in laboratory conditions, because under natural conditions they are easily disturbed and thus difficult to observe. These unnatural laboratory conditions might change certain sleep behaviours of animals.

METHODS

Capture and Maintenance

Permits from Ezemvelo/KwaZulu-Wildlife were obtained for all birds that were captured. Five male and three female Malachite Sunbirds were captured in Himeville (29° 40” S, 29° 32” E) and Underberg (29° 48” S, 29° 30” E), KwaZulu-Natal, South Africa during 2007, using mist nets. Birds were colour banded for identification and
kept in cages (90 × 30 × 45 cm³) at the Animal House, University of KwaZulu-Natal, Pietermaritzburg campus. Birds were fed a nectar diet (125 ml of sugar and 1 teaspoon of Ensure ® (Abbott Laboratories SA Ltd, Johannesburg, South Africa) per 500 ml of water. Water was provided *ad libitum*. Experiments were conducted during December 2006 – April 2007.

Eight Cape White-eyes were trapped in Howick (29° 34′ S, 30° 13′ E), KwaZulu-Natal, South Africa during 2006 and kept in aviaries at the Animal House, University of KwaZulu-Natal, Pietermaritzburg campus. Birds were fed on a diet of mealworms (*Tenebrio molitor* larvae), a choice of oranges, pears, paw-paws, apples, and a mixed nectar diet (125 ml of sugar and 1 teaspoon of Ensure ® (Abbott Laboratories SA Ltd, Johannesburg, South Africa) per 500 ml of water. Water was provided *ad libitum*. Experiments were conducted during January 2006 – December 2007.

Eight Fan-tailed Widowbirds were trapped in Pietermaritzburg, KwaZulu-Natal, South Africa (29° 36′ S, 30° 26′ E) during 2006 and kept in aviaries at the Animal House, University of KwaZulu-Natal, Pietermaritzburg campus. Birds were fed a mixed seed diet and a mixture of chopped spinach, broccoli and apple. Water was provided *ad libitum*. Experiments were conducted during September 2006 – December 2006.

Experiments were conducted in constant environment rooms (25°C, 12 L: 12D and 5°C, 12 L: 12D), in a specially made wooden cage (60 × 30 cm). The front of the cage had a glass partition, which could be removed. Inside the cage there was a wooden perch, which could be adjusted. The cage also had numerous small ‘breathing holes’ in the top. Caging conditions and experimental procedures were approved by
the Ethics Committee of the University of Kwa-Zulu-Natal, which are in accordance with South African law.

Filming and Sleep Parameters

The sleeping posture and duration of eye closure of the individual birds were recorded through the night using a remotely controlled closed circuit camera (Eagle CCTV, Model: MTV 13W1C, Taiwan) (Infrared-sensitive) mounted on a tripod opposite the cage, connected to a computer. Eagle DVR software (Eagle Appliances SA (Pty) Ltd 2005) was used for the recording. Single birds were placed in the cage for overnight recording at 18:00 and removed again at 06:00 the next morning. This process was repeated with each of the eight birds at 5°C and at 25°C, and also at 15°C for the Cape White-eyes. Fantailed Widowbirds were only filmed at 25°C. Sleep behaviour categories that were recorded included back sleep/bill under scapulars (BUS) and rest/front sleep and other behaviours such as preening, stretching and states of eye closure/opening. Eye closure was taken as a reliable indicator of sleep (Kavanau 1998). Total duration of each activity during the night was determined by summing the length of each behaviour bout that occurred during each hour through the night. Percentage of each night spent awake or asleep, as well as percentage of the night spent in a certain sleeping posture was recorded for each bird at the different temperatures. We also recorded the time it took for birds to fall asleep after lights went out, and the time it took them to wake up before lights went on.
Analyses

Comparisons between the percentage of the night in back and front sleep and the percentage of the night awake and asleep were analysed using nonparametric statistics (Friedmans analysis of variance (ANOVA)), as data were ranked (Siegel & Castellan 1988). The time it took for birds to fall asleep when lights went off, and to wake up before lights went on at different temperatures was compared using a T-test. Data was analysed with STATISTICA, Version 7, (Statsoft, Tulsa, USA).

RESULTS

Malachite Sunbirds

Malachite Sunbirds spent most of the night asleep at both 25°C (88%) and at 5°C (90%) (Fig.1). There was no significant difference in amount of sleep between the two temperatures (Friedman ANOVA, F (1, 7) = 5, p = 0.485). Birds spent 58% of the night in back sleep at 5°C and 83% of the night at 25°C, however there were no significant differences between temperatures in the amount of back sleep (Friedman ANOVA, F (1, 7) = 4.5, p = 0.340) (Fig.2). There was a significant difference at 5°C between amount of back and front sleep respectively (Friedman ANOVA, F (1, 7) = 8, p = 0.005), and similarly at 25°C (Friedman ANOVA, F (1, 7) = 4.5, p = 0.034). Greater variability was observed at the higher temperature for the percentage of the night spent in a particular sleeping position as well as for the percentage of the night spent awake and asleep. No recordings of unilateral eye-closure were made when both eyes were visible. Although it took Malachite Sunbirds longer to fall asleep after the lights
went out at 5 °C (mean ± SE) (x = 33.75 ± 6.80 min) than at 25 °C (x = 26.25 ± 3.75 min, Fig. 7), this difference was not significant (T-test, df = 7, t = 1.32, p = 0.227).

Less time was spent awake before the lights went on (x = 3.75 ± 2.45 min) at 5 °C, than at 25 °C (x = 11.25 ± 2.45 min, Table 1), with no significant differences between temperatures (T-test, df = 7, t = -1.87, p = 0.104).

**Cape White-eyes**

Cape White-eyes spent most of the night asleep and consequently less time awake for each of the three temperatures, with no significant difference in amount of time asleep between temperatures (Friedman ANOVA, F(2, 7) = 4.07, p = 0.131) (Fig. 3), although there were individuals that did wake intermittently throughout the night, mostly to stretch or preen themselves. However there were significant differences between percentage of the night spent asleep and awake at each temperature (Friedman ANOVA, F(1, 7) = 8, p=0.005).

Of all Cape White-eyes observed with both eyes visible, only one individual was filmed engaged in unihemispheric sleep at 25°C with its right eye closed and the left eye open for approximately two minutes, and at 15°C one bird had its right eye open and left eye closed for one minute. Occurrence of unihemispheric sleep might have been greater, but their eyes were not always visible during recording, especially when they were perched with their backs towards the camera, or were engaged in back sleep. Cape White-eyes alternated between front and back sleep positions throughout the night. Percentage of the night spent in back sleep increased and front sleep decreased with a decrease in temperature (Fig. 4). No significant difference was found between the percentage of night spent in back and front sleep at 25 °C
(Friedman ANOVA, $F_{(1, 7)} = 2, p = 0.157$), although significant differences were obtained between the front and back sleeping postures at 15 °C (Friedman ANOVA, $F_{(1, 7)} = 4.50, p = 0.034$) and at 5 °C (Friedman ANOVA, $F_{(1, 7)} = 8, p = 0.005$). At 5°C, Cape White-eyes spent most of the night in bill under scapular or back sleep mode (98%) with their feathers covering their eyes, thus making it impossible to determine the state of eye closure/opening. At the 25°C and 15°C temperatures, percentage of the night spent in either front or back sleep was highly variable amongst individuals. This was not so at 5°C. At 25°C it took birds the least amount of time to fall asleep after the lights went off ($\bar{x} = 15.00 \pm 5.67$ min), followed by the 5°C temperature ($\bar{x} = 16.88 \pm 4.43$ min) and longest at 15 °C ($\bar{x} = 31.88 \pm 6.61$ min, Fig. 7). There were no significant differences between temperatures (T-test, $df = 7, t = -1.87, p = 0.104$). No birds were awake before the lights came on (Table 1). All individuals opened their eyes as the lights came on.

**Fan-tailed Widowbirds**

Fan-tailed Widowbirds spent 89% of the whole night asleep at 25 °C and 88% of the whole night in front sleep at 25 °C, with significant differences between percentage of night spent awake and asleep and between back and front sleep at 25 °C (Friedman ANOVA, $F_{(1, 6)} = 7, p = 0.008$) (Figs. 5 and 6). Percentage of the night spent awake and asleep was highly variable for individuals at 25 °C, as some birds kept waking up intermittently throughout the night. We did not find any incidence of unilateral eye-closure of any of the birds when both eyes were visible. Fan-tailed Widowbirds took a mean of $31.88 \pm 6.61$ min to fall asleep after the lights went off and were awake for a mean of $10.00 \pm 10.00$ min before the lights went on (Table 1), with no significant difference between the two times (T-test, $df = 5, t = 0.00, p = 1.000$).
DISCUSSION

All individuals of all three species used back and front sleep positions. This was expected; as these sleep positions are the most common sleeping positions that have been reported in other species (Amlaner & Ball 1983; 1994). Cape White-eyes and Malachite Sunbirds increased their back sleep and decreased their front sleep at 5°C. Furthermore when they were sleeping under the 5°C conditions, their posture resembled that of a circular ball, with their feathers fluffed out, protecting their feet and their heads tucked under their scapular feathers (back sleep posture). A study done with Glaucous-winged Gulls (*Larus glaucescens*) has shown that sleep length and quality is reduced with an increase in thermal loading (temperatures outside their thermo-neutral zone), indicating that sleep and thermoregulation can be viewed as competing actions (Opp et al. 1987). In a study by Reebs (1986), Black-billed Magpies (*Pica pica*) that were sleeping under freezing winter conditions slept in the back sleep posture (bill tucked under the scapular feathers). This sleeping position would reduce heat loss, as it decreases volume/surface ratio (Reebs 1986).

Unilateral eye-closure was only observed in Cape White-eyes on two occasions. No observations of unilateral eye-closure were made in the other two species. This could be a behavioural indicator of unihemispheric sleep (Rattenborg et al. 2000), although an electrophysiological study should be undertaken to investigate this. In the review by Rattenborg et al. (2000) unihemispheric sleep is shown to be widespread amongst many bird families, with the exception of the Passeriformes, where only three species have thus far shown an indication of unilateral eye-closure. All three species in this study are passerines which roost in vegetation. Elevated roost sites provide protection from ground-based predators (Maclean 1990), and as
Unihemispheric sleep in birds has the function of keeping a lookout for predators (Rattenborg et al. 2000), it could be concluded that these species do not use unihemispheric sleep, as many ground dwelling birds do. Dial (2003) explains how small passerine birds benefit from a greater predator-escape feat through their better vertical and horizontal acceleration. This is because they have reduced hindlimb and increased forelimb investment for increased flight capacity (Dial 2003). As ground dwelling birds do not have this feature, we would expect them to rely more on unihemispheric sleep for predator avoidance. Unihemispheric sleep might be a function of a species habit or habitat and so they would use it accordingly. It is suggested that further research should explore the sleep patterns in species of southern African ground dwelling birds.

A typical night of bird sleep is made up of consecutive sleep cycles, which are interrupted by many awakenings (Fuchs et al. 2006). Similar observations where made in the present study with individuals of all three species waking up intermittently throughout the night, and this was highly variable for Malachite Sunbirds, Cape White-eyes and for the Fantailed Widowbird at 25°C. This could be a form of vigilant sleep which is characterized by an alteration of longer periods of eye closure and shorter periods of eye-opening (Amlaner & Ball 1983). Vigilant sleep or ‘peeking’ decreases with in increase in group size (Lendrem 1984), as flocking of birds allows an increase in eye-closure while sleeping. It is suggested for further research that pairs or small groups of the three species should be filmed in order to see if they will engage in vigilant sleep. There is a trade-off between sleep and peaking rates as shown by Lendrem (1983) in a study with Mallard Ducks (Anas platyrhynchos). Peeking helps with predator detection, but when bird’s eyes are
closed (and sleeping) they will obviously not be able to detect predators but rather benefit from the function of sleep (Lendrem 1983).

In this study individuals of all three species took longer to fall asleep after the lights went off. This could possibly be because when lights go out there is instant darkness, whereas in a natural (in the wild) setting, darkness comes about gradually and so it would take the birds more time to adjust to the instant darkness in an unnatural laboratory environment. Malachite Sunbirds and Fantailed Widowbirds spent between about three and eleven minutes awake before the lights came on again in the morning. This might be an innate behaviour as they were expecting it to become lighter as the morning approached. This is contradictory to the results of the Cape White-eyes, where no individuals were awake before the lights came on.

Unilateral eye closure might increase if the birds perceived an increased risk of predation on a particular night, for instance, following the presentation of a predator. This together with the research presented here clearly highlights the need for further studies to be undertaken in the sleeping behaviours and patterns of birds, especially in southern African species.

ACKNOWLEDGEMENTS
Mark Brown is thanked for his invaluable assistance throughout this study. We want to thank Professor and Mrs. Steven Piper and Edmund and Erika Smith for allowing us to capture Malachite Sunbirds on their property. We would also like to thank all of those that helped with the feeding of the birds. The National Research Foundation provided funding for the duration of this project (GUN 2053510).
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Legends for Figures

Fig. 1: Percentage of night spent awake or asleep of Malachite Sunbirds at 5°C and 25°C. (n = 8).

Fig. 2: Percentage of night spent in back and front sleep of Malachite Sunbirds at 25°C and 5°C. (n = 8).

Fig. 3: Percentage of night spent awake or asleep of Cape White-eyes at different temperatures. (n = 8).

Fig. 4: Percentage of night spent in back or bill under scapular sleep of Cape White-eyes at 25°C, 15°C and 5°C. (n = 8).

Fig. 5: Percentage of night spent awake and asleep of Fantailed Widowbirds at 25°C. (n = 8).

Fig. 6: Percentage of night spent in front and back sleep of Fantailed Widowbirds at 25°C. (n = 8).
Fig. 1: Percentage of night spent awake or asleep of Malachite Sunbirds at 5°C and 25°C. (n = 8).
Fig. 2: Percentage of night spent in back and front sleep of Malachite Sunbirds at 25°C and 5°C. (n = 8).
Fig. 3: Percentage of night spent awake or asleep of Cape White-eyes at different temperatures. (n = 8).
Fig. 4: Percentage of night spent in back or bill under scapular sleep of Cape White-eyes at 25°C, 15°C and 5°C. (n = 8).
Fig. 5: Percentage of night spent awake and asleep of Fantailed Widowbirds at 25°C. (n = 8).
Fig. 6: Percentage of night spent in front and back sleep of Fantailed Widowbirds at 25°C. (n = 8).
Table 1: Parameters (mean ± SE) of the time taken to fall asleep after lights went off and time spent awake before lights went on for Malachite Sunbirds, Cape White-eyes and Fantailed Widowbirds.

<table>
<thead>
<tr>
<th>Malachite Sunbirds</th>
<th>Time taken to fall asleep (min)</th>
<th>Time awake before lights went on (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5°C</td>
<td>33.75 ± 6.80</td>
<td>3.75 ± 2.45</td>
</tr>
<tr>
<td>25°C</td>
<td>26.25 ± 3.75</td>
<td>11.25 ± 2.45</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Cape White-eyes</th>
<th>Time taken to fall asleep (min)</th>
<th>Time awake before lights went on (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5°C</td>
<td>16.88 ± 4.43</td>
<td>0</td>
</tr>
<tr>
<td>15°C</td>
<td>31.88 ± 6.61</td>
<td>0</td>
</tr>
<tr>
<td>25°C</td>
<td>15.00 ± 5.67</td>
<td>0</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Fantailed Widowbirds</th>
<th>Time taken to fall asleep (min)</th>
<th>Time awake before lights went on (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature</td>
<td></td>
<td></td>
</tr>
<tr>
<td>25°C</td>
<td>22.5 ± 9.29</td>
<td>10.00 ± 10.00</td>
</tr>
</tbody>
</table>
Diurnal birds generally sleep during the hours of darkness. Most male southern African sunbirds have pectoral tufts, although the function of these is not always understood. In male Malachite Sunbirds it has recently been found that they display their pectoral tufts almost continuously throughout the night, whilst asleep. We explored the possible function of this behaviour and suggest that these tufts might be a deterrent to predators, as they look like ‘eyes’ in the dark. A review of the use and occurrence of pectoral tufts in southern African sunbird species is also discussed.

*Keywords:* sleep; pectoral tuft; Malachite Sunbird (*Nectarinia famosa*)
Every animal that has so far been studied spends some of its time sleeping (Rattenborg & Amlaner 2002), and the closure of the eyelids is the most consistent indicator of sleep (Amlaner & Ball 1983; Kavanau 1998).

Diurnal birds generally sleep during the hours of darkness (Maclean 1990). When sleeping, many birds will find a place to roost for the night with some species roosting communally in elevated vegetation which can provide added vigilance against predators (Maclean 1990).

Behavioural adaptations contribute to the gene-copying success of an individual (Alcock 1979). In addition to being important in mate choice, reproductive behavioural adaptations such as extravagant plumage in birds may be used to display territory ownership (Peek 1972; Andersson 1982; Evans 1991; Petrie et al. 1991). Scarlet-tufted Malachite Sunbirds (Nectarinia johnstoni) use their pectoral tufts to mediate male-male competition for territories and their long tails are also important in mate choice (Evans 1991). The size of pectoral tufts and tail length of Scarlet-tufted Malachite Sunbirds indicate increased fitness and this increases with an increase in food resources (Evans 1991). Most sunbird species in southern Africa have pectoral tufts, although the function of these is poorly understood (Maclean 1990). Male Malachite Sunbirds (Nectarinia famosa) moult from a green-light brown into an iridescent green plumage for the summer breeding months (Maclean 1985). They also have bright yellow tufts all year round that are not commonly displayed. These tufts are not visible when the birds are perched during the daytime. Even though sexes are dimorphic in colour, females lack pectoral tufts (Cheke et al. 2001; Hockey et al. 2005). A study of the nectarivorous community in the KwaZulu-Natal Drakensberg, has shown that the overall occurrence of interspecific aggression seemed to be low, except in large stands of flowering Protea caffra (Daniels 1987). Most of the
aggression observed was intraspecific, with much of the aggression being by males and directed at females (Daniels 1987). Recently though, an interesting observation was made where male Malachite Sunbirds displayed their yellow pectoral tufts during the night, whilst perched and resting with their eyes closed (Downs, pers. observation). The function of this is difficult to show. It is definitely not for territorial defence or mate recognition as birds were housed individually, but rather their tufts appeared as ‘eyes’ in the dark. We investigated the occurrence of pectoral tuft display during sleep in male Malachite Sunbirds. We predicted birds would display their pectoral tufts all night irrespective of temperature. We also reviewed the occurrence and use of pectoral tufts of southern African Sunbird species (Table 1) (Cheke et al. 2001; Hockey et al. 2005).

METHODS

Five male Malachite Sunbirds were captured in Underberg (29° 48’ S, 29° 30’ E) and Himeville (29° 40’ S, 29° 32’ E), KwaZulu-Natal, South Africa under permit from Ezemvelo KwaZulu-Wildlife during 2006 and 2007, using mist nets. Birds were colour banded for identification and kept in cages (90 × 30 × 45 cm³) at the Animal House, University of KwaZulu-Natal, Pietermaritzburg campus. Birds were fed a nectar diet (125 ml of sugar and 1 teaspoon of Ensure ® (Abbott Laboratories SA Ltd, Johannesburg, South Africa) per 500 ml of water. Water was provided ad libitum. Experiments were conducted in constant environment rooms (25°C, 12 L: 12D and 5°C, 12 L: 12D), in a specially made wooden cage (60 × 30 cm), with a glass front which could be removed. Inside the cage there was a wooden perch, which could be adjusted. The box also had numerous small ‘breathing holes’ in the top. Experiments were conducted during December 2006 – April 2007.
The sleep patterns and display of pectoral tufts of the birds were recorded through the night using a remotely controlled close circuit camera (Eagle CCTV, Model: MTV 13W1C, Taiwan) (Infrared-sensitive) mounted on a tripod opposite the cages connected to a computer. Eagle DVR software (Eagle Appliances SA (Pty) Ltd 2005) was used for the recording. Single birds were placed in the recording cage overnight at 18:00 and removed again at 06:00 the next morning. Sleep patterns were observed at 5°C and 25°C, 12L: 12D for each bird and all recordings of birds were examined on a computer. Sleep patterns and total duration of pectoral display during the night was determined by summing the length of each behaviour bout that occurred during each hour through the night. Caging conditions and experimental procedures were approved by the Ethics Committee of the University of Kwa-Zulu-Natal, which are in accordance with South African law. At the end of the study all birds were released back at the sites that they were captured at.

Analyses
Comparisons of the two temperatures were analysed using nonparametric statistics (Friedman’s analysis of variance (ANOVA), as data were not normally distributed (Siegel & Castellan 1988). Data was analysed with STATISTICA, Version 7, (Statsoft, Tulsa, USA).

RESULTS
Male Malachite Sunbirds displayed their pectoral tufts in two different sleeping positions, whilst their eyes were closed, namely as shown in Figs. 1a and b. The colour of these tufts is a bright yellow. Most of the Malachite Sunbirds displayed their
pectoral tufts continuously during the night. Pectoral tufts were displayed for 97% and 92% of the night, at 5°C and 25°C respectively (Fig. 2), with significant differences between the two temperatures (Friedman ANOVA, \( F_{(4, 1)} = 5, p = 0.025 \)). At the 25°C temperature the results were more variable as a consequence of one individual that did not display pectoral tufts. Malachite Sunbirds spent most of the night asleep at 25°C (88%) and at 5°C (90%) (Fig. 3). Significant differences were found between the percentage of night spent awake and asleep at 25°C and at 5°C (Friedman ANOVA, \( F_{(1, 7)} = 8, p = 0.005 \)). It appeared that birds were “puffed up” irrespective of temperature. It is difficult to define “puffed up”, however birds fluffed their feathers at night possibly to reduce energy loss.

**DISCUSSION**

The function of sleep in birds is poorly understood, (Rattenborg et al. 2000), even though a large part of a bird’s life is spent sleeping (Toates 1980). Sleep is a state of immobility that increases safety against predators during times that are unprofitable for the presentation of other activities (Meddis 1975; Amlaner & Ball 1983). At night it is usually unprofitable for birds to perform other activities and additionally the stillness may increase safety against predators (Meddis 1975). In some bird species, particularly those that rest during the day, there is a sleep-vigilance trade-off (Gauthier-Clerc et al. 1998), where sleep is interrupted by short periods of eye-opening referred to as peeks, and birds are able to mobilize quickly if threatened (Lendrem 1983).

In male Malachite Sunbirds it appears that the pectoral tuft display, observed while they were sleeping, irrespective of ambient temperature has a function. It is
possible that the pectoral tufts may act as a deterrent to predators, as the tufts appear as ‘eyes’ in the dark. Furthermore, birds are also puffed up while asleep which may also act as a deterrent as it gives the illusion of a larger animal and so may act as a line of defence against any nocturnal mammalian predators. Fluffing themselves up to increase insulation at night, could have initiated the pectoral tuft behaviour. In our review of pectoral tuft use and occurrence in southern African Sunbird species, it generally shows that males have pectoral tufts and females do not, with a few exceptions (Table 1) (Cheke et al. 2001; Hockey et al. 2005). This is also true for the Malachite Sunbird, where the female does not have any tufts. Of the 22 species of male sunbirds reviewed, 15 have yellow or a type of yellow coloured tufts.

The Scarlet-tufted Malachite Sunbird, which occurs outside of southern Africa, is closely related to and looks very similar to the Malachite Sunbird. The pectoral tufts of this species are scarlet in colour and are used as condition dependant sexual signals (Evans 1991; Cheke et al. 2001). What is interesting is that the female of this species also has scarlet pectoral tufts, which are smaller in size (Cheke et al. 2001). The function of the tufts in the female Scarlet-tufted Malachite Sunbird is not known, but may be for display in female-female interactions. Further research into the display of pectoral tufts in these female sunbirds is needed, particularly at night to determine if these are displayed during sleep.

Most of the male sunbirds found in southern Africa use their tufts in aggressive or breeding displays, but the pectoral tuft use in other species is either not known or has never been recorded (Table 1). Even at different temperatures, male Malachite Sunbirds were displaying their pectoral tufts; therefore they could not be displaying them only because they were fluffing themselves up, as at 25 °C it is quite warm.
Male Malachite sunbirds use their pectoral tufts for display purposes during breeding, but why they display them at night still remains unclear. An additional study, using other sunbird species should be undertaken to determine if they also display their pectoral tufts nocturnally and if so for what purpose.

ACKNOWLEDGEMENTS

We want to thank Professor and Mrs. Steven Piper and Edmund and Erika Smith for allowing us to capture Malachite Sunbirds on their property and Mark Brown for his assistance in catching birds. Numerous people are also thanked for their assistance in feeding the birds. The National Research Foundation (GUN 2053510) provided funding for the duration of this project.

REFERENCES


Legends for Figures

Fig. 1. A male Malachite Sunbird sleeping in a) front position and displaying pectoral tufts at night and b) in back position and displaying pectoral tufts at night.

Fig. 2: Percentage of night that male Malachite Sunbirds displayed pectoral tufts at two different temperatures. (n = 5).

Fig. 3: Percentage of night spent awake or asleep of Malachite Sunbirds at different temperatures. (n = 5).
Fig. 1. A male Malachite Sunbird sleeping in a) front position and displaying pectoral tufts at night and b) in back position and displaying pectoral tufts at night.
Fig. 2: Percentage of night that male Malachite Sunbirds displayed pectoral tufts at two different temperatures. (n = 5).
Fig. 3: Percentage of night spent awake or asleep of Malachite Sunbirds at different temperatures. (n = 5).
Table 1: The occurrence and use of pectoral tufts in southern African Sunbird species (after Cheke et al. 2001, Hockey et al. 2005).

<table>
<thead>
<tr>
<th>Species</th>
<th>Common Name</th>
<th>Occurrence of tufts</th>
<th>Occurrence of tufts</th>
<th>Tuft Use</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Nectarinia famosa</em></td>
<td>Malachite Sunbird</td>
<td>Bright yellow tufts</td>
<td>None</td>
<td>Sometimes shown during courtship/aggressive displays</td>
</tr>
<tr>
<td><em>Nectarinia johnstonii</em></td>
<td>Red-tufted Sunbird</td>
<td>Bright scarlet tufts</td>
<td>Scarlet/orangey, smaller tufts</td>
<td>Tufts are condition dependent sexual signals in males</td>
</tr>
<tr>
<td><em>Nectarinia kilimensis</em></td>
<td>Bronzy Sunbird</td>
<td>None</td>
<td>None</td>
<td></td>
</tr>
<tr>
<td><em>Anthreptes reichenowi</em></td>
<td>Plain-backed Sunbird</td>
<td>Lemon-yellow tufts</td>
<td>As male?</td>
<td></td>
</tr>
<tr>
<td><em>Anthreptes longuemarei</em></td>
<td>Western Violet-backed Sunbird</td>
<td>Yellow tufts</td>
<td>None</td>
<td>Breeding display, male displaying tufts to female</td>
</tr>
<tr>
<td><em>Anthobaphes violacea</em></td>
<td>Orange-breasted Sunbird</td>
<td>Bright yellow tufts</td>
<td>None</td>
<td>Tufts may/may not be displayed during courtship</td>
</tr>
<tr>
<td><em>Cyanomitra olivacea</em></td>
<td>Eastern Olive Sunbird</td>
<td>Yellow/pale orange tufts</td>
<td>Yellow/pale orange tufts</td>
<td>Tufts displayed during courtship by male</td>
</tr>
<tr>
<td><em>Cyanomitra veroxii</em></td>
<td>Grey Sunbird</td>
<td>Scarlet tufts, sometimes yellow</td>
<td>Scarlet tufts</td>
<td>Sometimes flicks wings to show tufts during flight</td>
</tr>
<tr>
<td><em>Chalcomitra amethystina</em></td>
<td>Amethyst Sunbird</td>
<td>Indications of yellow tufts</td>
<td>None</td>
<td>?</td>
</tr>
<tr>
<td><em>Chalcomitra senegalensis</em></td>
<td>Scarlet-chested Sunbird</td>
<td>None</td>
<td>None</td>
<td></td>
</tr>
<tr>
<td><em>Hedydipna collaris</em></td>
<td>Collared Sunbird</td>
<td>Lemon-yellow tufts</td>
<td>As male?</td>
<td>?</td>
</tr>
<tr>
<td><em>Cinnyris manoenisi</em></td>
<td>Miombo Double-collared Sunbird</td>
<td>Yellow tufts</td>
<td>None</td>
<td>?</td>
</tr>
<tr>
<td><em>Cinnyris chalybeus</em></td>
<td>Southern Double-collared Sunbird</td>
<td>Yellow tufts</td>
<td>None</td>
<td>Shown during aggressive displays against other males</td>
</tr>
<tr>
<td><em>Cinnyris afer</em></td>
<td>Greater Double-collared Sunbird</td>
<td>Yellow tufts</td>
<td>None</td>
<td>Sometimes shown during courtship/aggressive displays</td>
</tr>
<tr>
<td><em>Cinnyris neergardi</em></td>
<td>Neergards Sunbird</td>
<td>Yellow tufts</td>
<td>None</td>
<td>?</td>
</tr>
<tr>
<td><em>Cinnyris venustus</em></td>
<td>Variable Sunbird</td>
<td>Variable. Yellow, orange, red tufts</td>
<td>None</td>
<td>Breeding display, male displaying tufts to female</td>
</tr>
<tr>
<td><em>Cinnyris talatala</em></td>
<td>White-bellied Sunbird</td>
<td>Yellow tufts</td>
<td>None</td>
<td>Seldom shown, sometimes flashed when singing for female</td>
</tr>
<tr>
<td><em>Cinnyris cupreus</em></td>
<td>Copper Sunbird</td>
<td>Yellow tufts</td>
<td>None</td>
<td>?</td>
</tr>
<tr>
<td><em>Cinnyris fuscus</em></td>
<td>Dusky Sunbird</td>
<td>Yellow-orange</td>
<td>None</td>
<td>?</td>
</tr>
<tr>
<td><em>Cinnyris shelleyi</em></td>
<td>Shelles Sunbird</td>
<td>None</td>
<td>None</td>
<td>?</td>
</tr>
<tr>
<td>Cinnyris mariquensis</td>
<td>Marico Sunbird</td>
<td>None</td>
<td>None</td>
<td></td>
</tr>
<tr>
<td>----------------------</td>
<td>----------------</td>
<td>------</td>
<td>------</td>
<td></td>
</tr>
<tr>
<td>Cinnyris bifasciatus</td>
<td>Purple-banded Sunbird</td>
<td>None</td>
<td>None</td>
<td></td>
</tr>
</tbody>
</table>

* Occurs outside southern Africa
CHAPTER 4

Format followed is for submission to the *Journal of Experimental Biology*

**Diel variations in blood glucose levels in Malachite Sunbirds at different temperatures**

*Andrea E. Wellmann, Colleen T. Downs & Mark Brown*

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**Summary**

Blood glucose concentrations of most birds are much higher than those found in mammals and it is still not known how they evade the complications of such high levels. We investigated the change in blood glucose concentrations of Malachite Sunbirds at two different ambient temperatures and at different times of the night and day, and explored the possibility that gluconeogenesis might be used by birds to ‘warm up’ during arousal of torpor in the early morning, before daylight. Generally blood glucose levels were fairly high, between 13.6 and 21.4 mmol/L, which we expected. Blood glucose levels were higher at 5°C than at 25°C and generally lower in the early hours of the morning. Therefore we reject our assumption that Malachite Sunbirds use gluconeogenesis as an additional form of heat generation during torpor. It is thought that the difference in the levels of blood glucose might be a function of the cold temperature and the consumption of their nectarivorous diet.
Introduction

The pancreas in most birds and mammals secretes the sugar-regulating hormones, glucagon and insulin, which both act on the liver to insure stable blood glucose levels, which are critical for the functioning of the brain (Beuchat and Chong, 1998; Willmer et al., 2005).

Blood glucose levels of most bird species are 150 to 300% higher than in mammals of similar body mass (Umminger, 1975; Pollock, 2002). Yet it is still not known how they evade the complications of such high levels (Beuchat and Chong, 1998). Nectarivores, such as hummingbirds, which feed almost exclusively on simple sugars, have some of the highest fasting plasma glucose levels (17mM) ever measured in an animal (Beuchat and Chong, 1998). These blood glucose levels increase after birds have fed on carbohydrate rich nectar (Beuchat and Chong, 1998) as the ability to absorb glucose in birds generally matches their dietary glucose content (Pollock, 2002). Because of their relatively small size and limited blood volume, substantial changes in blood glucose levels in hummingbirds are difficult to document (Beuchat and Chong, 1998).

Glucocorticoids are steroid hormones which have developmental and metabolic effects, such as gluconeogenesis (Withers, 1992). The main corticosteroid produced by birds is the glucocorticoid corticosterone (Carsia and Harvey, 2000). Stress in birds is characterized by physiological changes such as increased respiratory frequency, arterial blood pressure, glycaemia and circulating glucocorticoids (Sapolsky 1992). Stress causes increases in catecholamine secretion which can result in hyperglycaemia (Bell, 1971). Glucocorticoids are involved in glucose metabolism, as blood glucose is increased by glucocorticoids, which brings about the breakdown of glycogen to glucose in the liver (Assenmacher, 1973). The transition to gluconeogenesis occurs rapidly in birds (Pollock,
2002), and more than 70 % of all glucose released into the bloodstream is made available by the liver (Hazelwood, 2000). Blood glucose concentrations in birds are also affected by circadian rhythms (Hess, 2002) as higher glucose values are obtained in diurnal species, during the day (Ferrer et al., 1994).

Malachite Sunbirds are small (15-20g) nectarivores (Hockey et al. 2005) and use heterothermy at night, especially when temperatures are low, as they go into torpor between 5 and 10°C (Downs and Brown, 2002). They are one of the few species of passerines in which torpor has been described in detail (Downs and Brown, 2002). Torpor is characterized as a drop in body temperature, accompanied by a reduction in circulation, respiration and metabolic rate (Willmer et al., 2005), so that energy loss is reduced (Downs and Brown, 2002). Torpor also involves the energetically expensive occurrence of arousal in which the body temperature is raised back to normal values to permit consequent activity (Willmer et al., 2005). Arousal in birds or ‘warming up’ is achieved through endogenous heat production (Geiser, 1998). Malachite Sunbirds raise their body temperature in a short period of time and it is suggested that they use an additional form of heat generation to do so (Downs and Brown 2002).

We explored the possibility that the rapid warming up in these birds in the early hours of morning, before daylight, might be because of gluconeogenesis, which leads to a rise in blood glucose (Vander et al., 1994) and so the level of blood glucose might reflect this in the early morning at colder temperatures, with an increase in blood glucose. Furthermore we expected Malachite Sunbirds to have high plasma glucose levels, but that these levels would not be constant through the day and night and at very different temperatures. We predicted that blood glucose levels in Malachite Sunbirds would be higher at colder temperatures and suggest that blood glucose levels might be affected by their nectarivorous diet.
Materials and methods

Five male and three female Malachite Sunbirds were mist-netted in Himeville (29° 40" S, 29° 32" E) and Underberg (29° 48" S, 29° 30" E), KwaZulu-Natal, South Africa under permit from Ezemvelo KwaZulu-Wildlife during 2006 and kept in cages (90 × 30 × 45 cm³) at the Animal House, University of KwaZulu-Natal, Pietermaritzburg campus. Birds were fed a nectar diet (125 ml of sugar and 1 teaspoon of Ensure® (Abbott Laboratories SA Ltd, Johannesburg, South Africa) per 500 ml of water. Water was provided ad libitum. Experiments were conducted in constant environment rooms (25°C, 12 L: 12D and 5°C, 12 L: 12D), during January 2007 – December 2007.

Blood from each bird was taken from the brachial vein and a drop of blood was then placed on an Asencia Elite® Blood Glucose Test Strip (Bayer HealthCare) and glucose concentrations (mmol/L) were measured by using a Glucometer Elite® Blood Glucose Meter (Bayer Diagnostics Manufacturing Limited), which is usually used to monitor blood glucose levels in humans. Birds were sampled in this way at 10h00, 22h00, 24h00 am and 4h00 at 5°C, to monitor differences in glucose levels. Sampling at each different time was done every four to five days, to avoid unnecessary stress and for the birds to replenish their blood supplies. Birds were weighed at the end of sampling. This process was repeated again after a week, when birds were kept at 25°C.

Blood glucose levels of birds were compared over time and between the two temperatures using a Repeat MANOVA (RMANOVA) followed by a Post-hoc Tukey HSD test. Data was analysed with STATISTICA, Version 7, (Statsoft, Tulsa, USA).
Results

Blood glucose levels of Malachite Sunbirds were generally higher at 5°C than at the 25°C temperature (Fig. 1). When taking temperature and time into account there were significant differences between blood glucose levels (RMANOVA, F_{(3, 18)} = 4.28, p = 0.02). A Post Hoc Tukey HSD test gave the most significant value at 5°C and 10h00 (p = 0.0002). The highest blood glucose value was obtained at 5°C at 10h00 (x̄ = 21.4 ± 1.3 mmol\text{L}^{-1}) and the lowest value at 25 °C at 04h00 (x̄ = 13.6 ± 3.6 mmol\text{L}^{-1}). There was a significant difference in body mass of Malachite Sunbirds (RMANOVA, F_{(7,42)} = 5.54, p = 0.00), when taking temperature and time into account, although a Post Hoc Tukey HSD test only showed a significant difference in mass at 5°C at 04h00 (p = 0.03). Mean (± SE) body mass varied between 17.54 ± 0.64 g and 18.70 ± 0.61g during the study.

Discussion

Blood glucose levels in Malachite Sunbirds were higher at the colder temperature. At colder temperatures, birds need to increase their metabolic thermogenesis, as they need more energy to keep warm. One would therefore assume that they ingested more nectar and this would in turn increase their glucose levels, as glucose levels are to a certain extent influenced by diet (Beuchat and Chong, 1998). This is similar to hummingbirds, where glucose levels increased after feeding (Beuchat and Chong, 1998).

Gluconeogenesis could not have been involved in the warming up of Malachite Sunbirds during torpor as blood glucose levels decreased during the early morning at 5°C, even though Malachite Sunbirds start to ‘warm up’ during this period (Downs and Brown, 2002). Nonshivering thermogenesis is used by many mammals to ‘warm-up’, but it is rare
in birds (Willmer, et al., 2005), as they usually use endogenous heat production to warm-up (Geiser, 1998). It therefore still remains unknown which form of additional heat production, if any, is used by Malachite Sunbirds when warming up rapidly from torpor.

Malachite Sunbirds have relatively high blood glucose levels (between 16.3 and 21.4 mmol/L), as is common for most bird species (Umminger, 1975). Birds in general, also have much higher metabolic rates and body temperatures in comparison to mammals of similar size, and it remains unknown as to why they are longer lived than similar sized mammals (Holmes and Austed, 1995). This is because conventional theory suggests that these factors with high blood glucose levels lead to tissue damage and eventual death in other animals (Beuchat and Chong, 1998).

Beuchat and Chong (1998) suggested that birds that feed frequently, such as nectarivores, would have high glucose levels, because of their crops always containing some nectar. In contrast, birds that feed less frequently or at long, irregular intervals would have considerable more variation in their blood glucose levels, depending on their feeding rate and size of meal (Work, 1996; Beuchat and Chong, 1998). Malachite Sunbirds do not have a crop to store nectar (Mbatha and Downs, 2002); therefore this influenced levels of blood glucose, as these were not constant in Malachite Sunbirds. Malachite Sunbirds were also fed in the mornings, so their intake of nectar would be higher, in comparison to the evening, when it was dark and birds were sleeping and not feeding. This in turn would have affected their blood glucose levels as these were higher in the mornings at 10h00, compared to the evening and early morning. The higher levels during the daytime could also be influenced by their circadian rhythms, as these are higher in diurnal species during the day (Ferrer, et al., 1994). European Starlings blood glucose levels are highest during the day, when these birds are most active and feeding, indicating that energy stores are
activated during the day times when birds need them most and stored during restful periods (Remage-Healy and Romero, 2000), as demonstrated by this study.

In a study of the influence of stress from transport and handling on blood parameters in Racing Pigeons (*Columba livia domesticus*); it was shown that the blood glucose of stressed birds increased significantly, by about 10 percent (Scope et al., 2002). In this study the stress of catching the birds in their cages and then subsequently taking blood from them could have resulted in changes in their blood glucose levels. We believe that changes were insignificant, as the results showed a general trend of decreasing blood glucose levels at both temperatures, although sampling was done a week apart for each temperature.

It seems that most studies which have looked at blood glucose in birds have focused on the high levels of blood glucose, but the mechanisms by which they evade the complications of such high levels, as typically found in diabetic humans, remains a mystery. We suggest that further research is needed in this field, particularly amongst nectarivorous birds, as they almost exclusively feed on sugars, which may provide insight into how they are able to regulate their blood glucose. Furthermore it is suggested that more research is needed to elucidate the mechanism by which Malachite Sunbirds are able to rapidly ‘warm up’ during arousal, when in torpor.

**Acknowledgements**

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Edmund and Erika Smith for allowing us to capture birds on their property and all those that helped with the capture and maintenance of the birds.

References


Legend for Figure

Fig.1. Diel blood glucose levels (mmol/L) in Malachite Sunbirds at two temperatures. (n = 8).
This dissertation investigated various nocturnal behavioural and physiological aspects of passerine birds, in particular Malachite Sunbirds. Sleep behaviour and function in birds still remains a relatively poor researched topic, even though birds spend a large part of their lives sleeping (Toates 1980). This is particularly true of unihemispheric sleep (Rattenborg, et al. 2000). What is particularly interesting is that most research thus far has only focused on the study of sleep in birds in the northern hemisphere. The present research on several passerine birds clearly highlights the need for further studies to be undertaken in the sleeping behaviours and patterns of birds, especially in southern African passerines (Chapter 2). I suggest that further research explore the sleep patterns in species of southern African ground dwelling birds, in particular to determine whether unihemispheric sleep is more prevalent in these birds than in arboreal passerines.

Further research into the display of pectoral tufts in female sunbirds is needed, as this highlights the fact that they are not always used in courtship or territorial display purposes. Male Malachite Sunbirds use their pectoral tufts for display purposes during breeding, but why they display them at night still remains unclear (Chapter 3). An additional study, using other sunbird species should be undertaken to determine if they also display their pectoral tufts nocturnally and if so for what purpose.

Blood glucose levels in birds are much higher than those found in mammals of similar body mass (Pollock, 2002). Blood glucose levels are also to a certain extent affected by diet (Beuchat and Chong, 1998) as glucose absorption in birds generally matches their dietary glucose content (Pollock, 2002). I suggest that further research is
needed in this field, particularly amongst nectarivorous birds (Chapter 4), as they almost exclusively feed on sugars, which creates a fascinating insight into how they are able to regulate their blood glucose. Furthermore it is suggested that more research is needed to elucidate the mechanism by which Malachite Sunbirds are able to rapidly ‘warm up’ during arousal when in torpor.

References


Microwave Sample Preparation Note: XprOP-1

Oil

Category: Oils

Sample Type: Oil
Application Type: Acid Digestion
Vessel Type: 55 mL
Number of Vessels: 12
Reagents: Nitric Acid (70%)
Method Sample Type: Organic
Sample Weight: 0.5 gram

Step 1:

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Heating Program: Ramp to Temperature Control

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<th>% Power</th>
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<th>Pressure (psi)</th>
<th>Temperature (°C)</th>
<th>Hold (min.)</th>
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<td>-</td>
<td>200</td>
<td>15:00</td>
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NOTE A: This procedure is a reference point for sample digestion using the CEM Microwave Sample Preparation System and may need to be modified or changed to obtain the required results on your sample.

NOTE B: Manual venting of CEM closed vessels should only be performed when wearing head, eye and body protection and only when the vessel contents are at or below room temperature to avoid the potential for chemical burns. Always point the vent hole away from the operator and toward the back of the fume hood.

NOTE C: Power should be adjusted up or down with respect to the number of vessels. General guidelines are as follows:
- 8-12 vessels (50% power), 13-20 vessels (75% power), >20 vessels (100% power).

NOTE D: "Organic Method Sample Type" should be used for most sample types. Choose "Inorganic" for samples with more than 1 gram of solid material remaining at the bottom of the vessel at the end of the digest (ex: leach methods). Choose "Water" for samples that are largely aqueous prior to digestion.

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