# Food quality, fasting periods and temperature stress: effects of energy challenges on the feeding patterns of avian nectarivores

by

Angela Köhler

Submitted in partial fulfilment of the requirements for the degree

**Doctor of Philosophy**

in the Faculty of Natural and Agricultural Sciences

**University of Pretoria**

Pretoria

February 2009

The whitebellied sunbird (*Cinnyris talatala*)

The amethyst sunbird (*Chalcomitra amethystina*)

The brown honeyeater (*Lichmera indistincta*)

# Table of Contents

**CHANGES IN NECTAR CONCENTRATION: HOW QUICKLY DO WHITEBELLIED SUNBIRDS (*CINNYRIS TALATALA*) ADJUST FEEDING PATTERNS AND FOOD INTAKE?**

**CHAPTER 3 ............................................................................................ 75**

**THE RESPONSE OF TWO AVIAN NECTARIVORES TO INTERRUPTIONS IN FOOD AVAILABILITY**

**TEMPERATURE CHALLENGES IN BROWN HONEYEATERS (*LICHMERA INDISTINCTA*): ACUTE COLD EXPOSURE AND POSSIBLE EFFECTS OF ACCLIMATION**

# List of Tables

CHAPTER 2

CHAPTER 4

CHAPTER 5

CHAPTER 6

# List of Figures

CHAPTER 1

CHAPTER 2

CHAPTER 3

CHAPTER 4

CHAPTER 5

CHAPTER 6

# Acknowledgements

I would like to thank my supervisor, Prof Sue W. Nicolson, who believed in me and supported me throughout my postgraduate career, providing excellent academic supervision. I would like to thank her for her guidance and encouragement, and for constructive criticism during the drafting of this thesis. I am also grateful to Luke Verburgt, from whom I learnt so much; major parts of my project would not have been possible without his programming skills, constructive ideas and endless support.

This work would also not have been completed successfully without the constant love, encouragement and support of my family. Special thanks to my mum, Gabriele Köhler, and Peter Zedler for their advice to come to South Africa to study at the University of Pretoria. My sincere thanks to Maciej Rossudowski for his unfailing support in everyday situations, and for proof reading chapters of this thesis.

Many people have been essential for the successful completion of parts of my study. Special thanks to Dr Patricia A. Fleming and Dr Todd J. McWhorter for their enthusiastic collaboration and guidance, and for being kind hosts during my research at Murdoch University, Perth, Australia. Dr Todd J. McWhorter is further thanked for catching honeyeaters for my experiments, providing data of gut capacity and constructive criticism on drafts of individual chapters. I would also like to thank Wimpie Nortje and Hennie Johnson for assistance with the electronic equipment and Dr Craig T. Symes and Darren W. Pietersen for mist-netting sunbirds for my study. Fellow students and staff of the Department of Zoology & Entomology, University of Pretoria, as well as the School of Veterinary and Biomedical Sciences, Murdoch University, are acknowledged for their unselfish rendered assistance during my study.

My research was funded by the South African National Research Foundation and the Australian Research Council. I thank the University of Pretoria for the postgraduate bursary and the postgraduate study abroad bursary that allowed me to conduct research at

Murdoch University. The South African National Research Foundation is thanked for providing my PhD bursary.

I am grateful to Jan Cilliers Park and the Gauteng Directorate of Nature Conservation, South Africa, for permission to mist-net sunbirds. Murdoch University and the Department of Environment and Conservation, Western Australia, are acknowledged for permission to capture honeyeaters for my study.

Last, but not least, I would like to thank all 52 birds I worked with for being patient and cooperative subjects of my research.

# Declaration

The experimental work described in this thesis was carried out in the Department of Zoology and Entomology, University of Pretoria, South Africa, and in the School of Veterinary and Biomedical Sciences, Murdoch University, Western Australia, from 2006 to 2008. I, Angela Köhler, declare that the thesis, which I hereby submit for the degree Doctor of Philosophy (Zoology) at the University of Pretoria, is my own work and has not previously been submitted by me for a degree at this or any other tertiary institution.

Angela Köhler

Date

# Disclaimer

This PhD thesis consists of chapters that have been prepared as stand-alone manuscripts. These manuscripts have either been published or are being prepared for future submission. As a consequence, there may be some repetition between chapters.

# Publications and manuscripts in preparation

In the course of this research, several manuscripts were published or are currently in preparation. A list of these manuscripts follows in chronological order:

Journal publications

Köhler A., Verburgt L. and Nicolson S.W. 2008. Nectar intake of whitebellied sunbirds (*Cinnyris talatala*): can meal size be inferred from feeding duration? Physiological and Biochemical Zoology 81: 682–687.

Köhler A., Verburgt L., Fleming P.A. and Nicolson S.W. 2008. Changes in nectar concentration: how quickly do whitebellied sunbirds (*Cinnyris talatala*) adjust feeding patterns and food intake? Journal of Comparative Physiology B 178: 785–793.

Journal articles in preparation

Köhler A., Verburgt L., Fleming P.A., McWhorter T.J. and Nicolson S.W. The responses of two avian nectarivores to interruptions in food availability.

Köhler A., Verburgt L., McWhorter T.J. and Nicolson S.W. Low temperature challenges in sunbirds: effects on food intake, feeding patterns and body mass of *Cinnyris talatala* and *Chalcomitra amethystina*.

McWhorter T.J., Fleming P.A., Köhler A. and Nicolson S.W. Does digestive spare capacity in honeyeaters vary with body size? An experimental test and a model.

Köhler A., Verburgt L. and Nicolson S.W. Nectar extraction by sunbirds: does licking behaviour depend on nectar concentration or viscosity?

# SUMMARY

The small size of nectarivorous birds is associated with high mass-specific metabolic rates and energetic lifestyles. Their energy balance is likely to be strongly influenced by environmental factors. Firstly, nectar varies in sugar concentration between different food plants and birds must adjust their consumption to maintain a constant energy intake. Secondly, unfavourable weather conditions, such as storms and heavy rains, may prevent birds from feeding, and they must increase their energy intake to compensate for the loss in foraging time. Low ambient temperature, as a third energetic challenge, results in higher energy demands for thermoregulation, which leads to increased food intake. However, these compensatory feeding responses may be constrained by physiological limitations to nectar ingestion, digestion and osmoregulatory processes.

My research focused on the behavioural and physiological responses of captive sunbirds (Nectariniidae) and honeyeaters (Meliphagidae) to energetic challenges, namely variations in nectar quality and availability and in ambient temperature. For sunbirds, I also investigated on a novel short-term scale how feeding patterns are adjusted in order to compensate for alterations in energy intake or requirements. Feeding events were recorded using a photodetection system, and body mass was monitored continuously by connecting the perches to electronic balances, interfaced to a computer.

Whitebellied sunbirds (*Cinnyris talatala*) were fed various nectar sugar concentrations. Their feeding durations were found to provide an estimate of meal size on all food concentrations. When exposed to a decrease in sugar concentration, birds generally demonstrated an increased feeding frequency and food intake within 10 min. The number and duration of meals increased in the first few minutes after return of a more concentrated diet. When whitebellied sunbirds and brown honeyeaters (*Lichmera indistincta*) were exposed to a 2 h fasting period during the day, they increased their nectar intake and energy accumulation after the fast. Sunbirds achieved this by increasing meal size but not meal frequency. However, both species weighed less in the evening following the fast than the previous evening, indicating that the compensation for lost foraging time was incomplete. During acute cold exposure, whitebellied sunbirds, amethyst sunbirds (*Chalcomitra amethystina*) and brown honeyeaters increased their nectar intake, but lost body mass irrespective of nectar sugar concentration. Honeyeaters ingested more food at subsequent cold exposure, suggesting physiological adaptation to high feeding rates. A chemical reactor model of digestive capacity, which assumes sucrose hydrolysis to be the limiting step in nectar digestion, accurately predicted maximal food intake in honeyeaters, but mostly underestimated it in sunbirds. Sugar assimilation efficiency was higher than 99% in whitebellied sunbirds and brown honeyeaters. Lastly, licking frequencies and tongue loads of whitebellied and amethyst sunbirds were investigated. In both species, tongue lick duration increased, and licking frequency and consumption per lick decreased, with increasing nectar concentration. Birds did not adjust their licking behaviour after a fasting period.

In conclusion, the response to varied energy challenges is shaped by both compensatory feeding and physiological constraints. Although unrelated, sunbirds and honeyeaters showed convergence in their responses, probably due to their similar nectar-feeding lifestyle.

# GENERAL INTRODUCTION AND OUTLINE OF THE STUDY

Energy is essential for metabolic processes, activity, growth and reproduction of all animals. Thus, animal survival and fitness greatly depend on the regulation of energy intake. It is therefore not surprising that a comprehensive literature has focused on the foraging behaviour of animals that underlies energy and nutrient intake. Optimal foraging theories predict that animal fitness depends on the efficiency of foraging, and that animals forage so as to maximize their fitness (Pyke et al. 1977).

When the nutrient or energy content of a food source is low, animals ingest larger amounts. This is commonly known as compensatory feeding, and can be observed in various animal taxa (Karasov and Martínez del Rio 2007). Animals also increase their food intake after periods of food deprivation to compensate for an energy deficit (Zubair and Leeson 1996). When energy demands increase, such as during exercise, reproduction or cold exposure, food intake is also increased (Starck 1999). This compensation for low food quality, food deprivation or high energy requirements has been demonstrated in insects, fish, reptiles, birds and mammals. The widespread distribution of the compensatory feeding response demonstrates the importance of the regulation of energy intake for animal survival and fitness.

The energy that animals gain from their food, however, is determined not only by their foraging decisions, but also by the digestive processing of food. The macronutrients (carbohydrates, proteins and lipids) present in ingested food need to be broken down into their smallest components, a process that involves numerous enzymatic pathways. The breakdown products are subsequently absorbed by the epithelium of the gastrointestinal tract, either passively by diffusion or actively via carrier-mediated mechanisms (Karasov and Martínez del Rio 2007). The rate at which nutrients are ingested and digested under maintenance conditions can be increased when the animal experiences energetically challenging conditions. The difference between nutrient intake rate for maintenance and maximal nutrient intake rate is termed spare digestive capacity (Karasov and McWilliams 2005). However, the increase in food intake is limited by the volumetric capacity of the intestine or the rate at which food is broken down (Karasov and McWilliams 2005). To overcome these physiological constraints, the gastrointestinal tract of many vertebrates shows morphological flexibility: its size and structure can be adjusted according to food quality and availability, and energy requirements (Starck 1999). Animals that experience increased energy demands and unpredictable variations in food supply, such as birds during migration, can increase their energy intake by increasing their intestinal size (McWilliams and Karasov 2001). Partial atrophy of the intestine, on the other hand, may save energy during periods of food restriction (McWilliams and Karasov 2001; Ott and Secor 2007). Such changes in gut size can occur rapidly, especially in small animals, and one must carefully distinguish between acute and long-term spare capacities of an animal (Karasov and McWilliams 2005).

In addition to experimental studies of animal digestive performances, mathematical models have provided insight into the efficiency of energy and nutrient extraction from food. Penry and Jumars (1986) were the first to compare digestive processes to principles of chemical-reactor theory. As in a chemical reactor, the performance of animal intestines can be estimated from nutrient conversion efficiency, reaction time, digesta retention time, intestinal volume and the flow rate of digesta (Karasov and Martínez del Rio 2007). After early models of digestion were proposed (Penry and Jumars 1987), later work incorporated nutrient breakdown and absorption into the models to predict the ingestion rate that maximizes an animal’s net rate of nutrient absorption (for a review see McWhorter 2005).

Experimental and theoretical studies of animal foraging behaviour and energy assimilation demonstrate the interplay between the behavioural regulation of food intake and digestive efficiency (Martínez del Rio and Karasov 1990). My research focuses on the interaction between compensatory feeding and physiological constraints in nectarivorous birds exposed to energetically challenging conditions. I investigated whether these birds can match energy intake to their energy expenditure when experiencing variations in food quality and availability and metabolic requirements. In the following sections, I will introduce nectar as a food source and the avian nectarivores that consume it, before moving on to energetic challenges in these birds and the outline of my research.

## *Nectar as food source*

Nectar is one of the most common foods, produced by plants as a reward for pollinators or defenders against herbivores (Nicolson 2007a). Nectar consumers come from a wide taxonomic range. A wide variety of insects feeds on nectar, including beetles (Coleoptera), true flies (Diptera), butterflies and moths (Lepidoptera) and bees, wasps and ants (Hymenoptera) (Nicolson 2007a). Vertebrate nectarivores embrace various bird and bat species (Nicolson and Fleming 2003a; Nicolson 2007a). Many non-flying mammals, including rodents, marsupials and primates, also visit flowers to feed on nectar, and play a role in plant pollination (Wiens et al. 1983; Carthew and Goldingay 1997). Lizards have also been identified as common nectar consumers, especially on islands (Olesen and Valido 2003). In addition, birds and mammals that are specialized on other diets also feed on nectar occasionally (Garber 1988; Symes et al. 2008). Not all nectar consumers benefit the plants: unwanted visitors include nectar thieves, which are morphologically unsuited to pollinate flowers, and nectar robbers, which puncture the base of flowers to access nectar (Nicolson 2007a).

What makes floral nectar such a desirable food source? Nectar is an easily digested food and rich in energy. It contains sugars predominantly in the form of the disaccharide sucrose or the monosaccharides glucose and fructose (Nicolson and Fleming 2003a). Nectar may also contain other sugars, such as xylose, which remains puzzling because pollinators are averse to this sugar (Jackson and Nicolson 2002). Besides sugar and water as major components, nectar further contains inorganic ions, enzymes, amino acids and lipids (Nicolson and Fleming 2003a; Nicolson 2007b; Nicolson and Thornburg 2007). Secondary compounds found in nectar, such as phenolics, alkaloids and terpenoids, may be toxic or repellent to some nectar consumers, while they attract others (Nicolson and Thornburg 2007).

Nectar sugars are synthesized in the nectary of flowers or derive from sucrose transported in the phloem sap (Nicolson and Thornburg 2007). The enzyme invertase, which is found in the nectary, hydrolyzes sucrose to its components glucose and fructose, thus determining the relative amount of each main nectar sugar (Pate et al. 1985). A dichotomy between sucrose and hexose nectars is evident in bird-pollinated plant species. In a large data set of 112 plant species in Costa Rica, sucrose was found to be the dominant nectar sugar in plants pollinated by hummingbirds (Stiles and Freeman 1993). The literature review by Nicolson and Fleming (2003a) supports this finding, as sucrose was the dominant nectar sugar found in most of the 278 hummingbird-pollinated plant species investigated in America. Plants pollinated by sunbirds and honeyeaters, on the other hand, showed a bimodal pattern, with about half of the nectars being hexose-dominant, whereas sucrose is the dominant sugar in the other half (Nicolson and Fleming 2003a). This dichotomy is not seen as a consequence of bird physiology, as specialist nectarivorous birds assimilate both sucrose and hexose sugars equally well (Lotz and Nicolson 1996). However, some occasional avian nectarivores lack the enzyme sucrase and can not hydrolyze sucrose, which appears to lead to aversion of sucrose nectars (Fleming et al. 2008).

Plants that are pollinated by birds or mammals produce large volumes of dilute nectar, compared to the smaller volumes of concentrated nectar of insect-pollinated plants (Pyke and Waser 1981; Nicolson and Thornburg 2007). In bird-pollinated flowers, the concentration of nectar ranges mainly from 15–30% w/w sugar (Nicolson and Fleming 2003a; Johnson and Nicolson 2008). However, nectar sugar concentration varies greatly both within and between food plants (Pyke and Waser 1981; Stiles and Freeman 1993; Nicolson and Thornburg 2007). Nectar of southern African passerine-pollinated flowers, for instance, ranges from 6.5% w/w (*Aloe speciosa*) to 36.7% (*Liparia splendens*) (Nicolson 2002). Nectar viscosity increases exponentially with concentration, which may affect the extraction of nectar from flowers (Nicolson and Thornburg 2007). Tongue licking frequencies and tongue loads of hummingbirds are influenced by high viscosities of the food source (Hainsworth 1973; Roberts 1995). A biophysical model of hummingbird feeding predicted optimal licking behaviour at nectar concentrations of 20–25% (Kingsolver and Daniel 1983).

## *Nectarivorous birds*

Nectarivory has evolved independently in three major radiations of birds: sunbirds (Nectariniidae) in Africa and Asia, honeyeaters (Meliphagidae) in Australasia, and hummingbirds (Trochilidae) of the Americas. These avian nectarivores show morphological and physiological adaptations to their nectar-feeding lifestyle, such as brightly coloured plumages (Johnsgard 1983; Longmore 1991; Hockey et al. 2005), long straight or curved bills (Temeles and Kress 2003), specialized tongues (Hainsworth 1973; Schlamowitz et al. 1976; Downs 2004) and a gut adapted to nectar digestion (Richardson and Wooller 1986; Mbatha et al. 2002). Sunbirds, honeyeaters and hummingbirds are equally efficient in sugar uptake, assimilating between 95 and almost 100% of ingested sugar (Lotz and Schondube 2006). However, the ingestion of copious amounts of characteristically dilute nectar results in high energy costs for food warming (Lotz et al. 2003). Nectarivorous birds are also challenged by the elimination of excess water, and chronic diuresis is an inevitable consequence of their dilute food (Martínez del Rio et al. 2001; Nicolson 2007a).

Avian nectarivores have remarkably low nitrogen requirements, much lower than predicted by their body mass (Paton 1982; Roxburgh and Pinshow 2000; McWhorter et al. 2003). Their rates of endogenous protein turnover and loss of nitrogen in excreta are low (see McWhorter et al. 2003 for a review). It has been suggested that this is an evolutionary adaptation to their diet, which is low in protein (Tsahar et al. 2005). The amino acid content of floral nectar, although sometimes high (Nicolson 2007b), is insufficient to meet the nitrogen needs of nectarivorous birds; they rely on additional protein sources, such as pollen and arthropods (Paton 1982; Roxburgh and Pinshow 2000; Van Tets and Nicolson 2000). Their simple gut structure, adapted to the nectar diet, appears to make avian nectarivores less efficient at extracting protein than insectivorous birds (Roxburgh and Pinshow 2002): although the transit time of insects is longer in sunbirds than in similar-sized insectivores, only 60% of nitrogen is extracted by the sunbirds.

Nectarivorous birds are generally smaller than non-nectarivorous birds, with the family Trochilidae being the smallest sized birds in the world (Pyke 1980). Hummingbirds weigh 2–20 g, sunbirds 5–22 g and honeyeaters, being the largest of the nectar-feeding birds, weigh 8–250 g (Nicolson and Fleming 2003a). The small size of avian nectarivores is often associated with predicted low capacities for energy storage (e.g. Brown et al. 1978; Nicolson and Fleming 2003a). However, small hummingbirds may store considerable amounts of fat to provide energy for migration (Hiebert 1993). At the same time, fat storage implies higher flight costs, while a lower body mass reduces energy requirements (Calder et al. 1990; Chai et al. 1999). Small body size further entails energetic lifestyles and high mass-specific metabolic rates (Nicolson and Fleming 2003a). The energy balance of avian nectarivores is therefore likely to be affected by adverse environmental conditions, which makes them ideal subjects for investigating responses to energy stress.

## *Energetic challenges in avian nectarivores*

Avian nectarivores have to feed frequently to meet their high energy requirements. Actively feeding hummingbirds use recently ingested sugars to fuel up to 95% of their metabolism, thus using essentially no stored fat (Carleton et al. 2006; Welch and Suarez 2007). Under energetically mild conditions and with food available *ad libitum*, nectarivorous birds accumulate energy steadily throughout the day (Wolf and Hainsworth 1977; Collins and Morellini 1979; Collins et al. 1980; Köhler et al. 2006). This energy accumulation over the entire day is necessary to sustain the birds at night when they do not feed (Fleming et al. 2004a; Köhler et al. 2006).

The energy balance of nectarivorous birds is strongly influenced by environmental factors. Firstly, wild birds encounter nectar of varying sugar concentrations while foraging on different food plants (Nicolson and Fleming 2003a). In addition, nectar can be drastically diluted by rain and high humidity (Tadey and Aizen 2001; Aizen 2003; Nicolson and Thornburg 2007). Despite varying nectar concentrations, birds are able to maintain a constant energy intake by adjusting their volumetric intake, which is commonly known as compensatory feeding (Nicolson and Fleming 2003b; see Martínez del Rio et al. 2001 for a review). However, if the sugar concentration is too low, birds are not able to compensate, as shown for whitebellied sunbirds (*Cinnyris talatala*), which lose body mass on a 3.6% w/w sucrose diet (Nicolson and Fleming 2003b). As nectar sugar concentration declines, sunbirds and hummingbirds increase the number of feeding events, while a nearly constant feeding event length is maintained (Wolf and Hainsworth 1977; Köhler et al. 2006). To my knowledge, only one study to date has investigated how quickly the feeding pattern is adjusted after changes in nectar concentration (Gass 1978): rufous hummingbirds (*Selasphorus rufus*) responded to an increased concentration within 10 min, primarily via adjusting their meal frequency, while their response to decreased concentration was preceded by a lag.

A second energetically stressful condition is the interruption of food intake, which birds may experience during migration, reproduction, unfavourable weather conditions and chases by predators or competitors (Glück 1987; Carpenter and Hixon 1988; Robin et al. 1988; Moore and Yong 1991). The loss of foraging time affects energy balance, especially of small birds, and can lead to depletion of energy stores. When the feeding of a wild rufous hummingbird was interrupted by a storm, it lost body mass but replenished its energy reserves within one day after feeding recommenced (Carpenter and Hixon 1988). Hummingbirds, exposed to fasting periods in the laboratory, were unable to increase their food intake after a fast to compensate for the loss in foraging time, and used torpor to compensate for the energy deficit (Hainsworth et al. 1981; Tooze and Gass 1985). Hainsworth et al. (1981) hypothesized that hummingbirds regulate feeding to maintain rates of energy accumulation which are set at dawn each day, and that the accumulated energy is not monitored during the day. Whitebellied sunbirds, on the other hand, were able to increase their rate of food intake following a fast (Nicolson et al. 2005).

Low ambient temperature is a third energetic challenge because it increases the costs of temperature regulation. Birds are expected to increase their dietary intake at low ambient temperatures in order to meet higher energetic requirements. Earlier studies suggest inter-specific differences in the ability of nectarivorous birds to compensate for low dietary energy content and high energy demands. Southern double-collared sunbirds (*Cinnyris chalybeus*), kept at 10–30˚C for two days and fed 0.4 or 1.2 M sucrose diets, increased their food intake in the cold and maintained energy balance on both diets (Lotz 1999). As examples for hummingbirds, Anna's hummingbirds (*Calypte anna*) nearly doubled their food intake over a 30˚C decrease in ambient temperature, whereas rufous hummingbirds showed only a slight increase in food intake (Beuchat et al. 1979; also see Beuchat et al. 1990 for a review). Different studies have revealed contradictory results for broadtailed hummingbirds (*Selasphorus platycercus*) that were rapidly exposed to 10˚C and fed dilute (0.25 and 0.29 M sucrose) and concentrated diets (1 M): in one study they did not increase their sugar intake and lost body mass (McWhorter and Martínez del Rio 2000), but in another they increased their intake and lost no more body mass in the cold than at moderate temperature (Fleming et al. 2004b).

Avian nectarivores may be able to maintain energy balance in the cold on concentrated diets, but their food intake may be restricted on low diet concentrations. When switched suddenly to low ambient temperature, rufous hummingbirds were able to meet their energy requirements on a relatively concentrated diet of 30% w/v sucrose, but not on lower concentrations (Gass et al. 1999). Whitebellied sunbirds, when exposed to 10˚C, increased their energy intake by 18% on a 1 M diet, but were not able to increase their intake on a very dilute diet of 0.1 M (Fleming et al. 2004b). These sunbirds were fed sucrose and equicaloric hexose solutions, and sugar type had an effect on their energy balance on a dilute diet (0.1 M) (Fleming et al. 2004b). Green-backed firecrowns (*Sephanoides sephanoides*), on the other hand, did not cope on any diet concentration at

15˚C, even after acclimation to the low temperature for seven days (Fernández et al. 2002).

These studies suggest that the food intake response of avian nectarivores under energetically stressful conditions is shaped by compensatory feeding and digestive constraint. When exposed to low sugar concentrations, low ambient temperature, or interruptions to feeding, birds compensate by increasing their intake. But when exposed to extremely low nectar concentrations, extremely low ambient temperature or extended losses in foraging time, birds may not be able to meet their energy demands because of physiological constraints to nectar ingestion and digestion, as well as osmoregulatory processes. Firstly, nectar ingestion may be limited by the rate at which nectar can be licked from flowers (Kingsolver and Daniel 1983). The amount of nectar that can be ingested may be restricted by the absence of a crop (Mbatha et al. 2002) or by stomach size (Bednekoff and Houston 1994). Ingesting large volumes of nectar is also energetically costly due to food warming costs (Lotz et al. 2003). Secondly, the digestive processing of nectar meals may be restricted by sucrose hydrolysis rates (McWhorter and Martínez del Rio 2000; Martínez del Rio et al. 2001) and the absorption of sugars (McWhorter et al. 2006; Napier et al. 2008). Thirdly, birds may face osmoregulatory problems when consuming large volumes, for example the disposal of excess water (McWhorter and Martínez del Rio 1999; Hartman Bakken and Sabat 2006) and loss of electrolytes (Fleming and Nicolson 2003).

## *Study species*

My research focuses on avian nectarivores of the families Nectariniidae and Meliphagidae. I studied two sunbird species, namely the whitebellied sunbird, *Cinnyris* (*Nectarinia*) *talatala*, and the amethyst sunbird, *Chalcomitra* (*Nectarinia*) *amethystina*, and one honeyeater species, the brown honeyeater, *Lichmera indistincta*.

The whitebellied sunbird occurs widely in sub-Saharan Africa and is common in the north and north-east of South Africa (Cheke et al. 2001). It prefers semi-arid savannas and woodland, but can also be found in cities, particularly in gardens and parks (Hockey et al. 2005). It is resident or locally migrant and lives alone as well as in pairs or small groups (Hockey et al. 2005). Its average body mass is 7 g and the sexes are dimorphic in their plumage colouration, with males being brilliant iridescent green on the head and back, with a royal blue band on their chest, yellow pectoral tufts and an off-white belly; and females being greyish-olive (Skead 1967; Hockey et al. 2005). Whitebellied sunbirds forage on flowers of *Acacia*, *Eucalyptus*, *Strelitzia*, *Jacaranda*, *Protea*, *Leonotis* and other plants (Skead 1967).

The amethyst sunbird is endemic to central and sub-Saharan Africa (Cheke et al. 2001). In South Africa, it occurs in the northern region and along the whole east and south coast, preferring open woodland or bush, hillsides with *Aloe* species and suburban gardens and parks (Hockey et al. 2005). It is sedentary or locally migratory and lives alone, in pairs or groups (Cheke et al. 2001). The body mass averages 15 g and the sexes are dimorphic; males being black with an iridescent reddish-purple throat and a malachite green forehead, and females being brown with an off-white, streaked brown belly and blackish throat (Skead 1967; Hockey et al. 2005). Both whitebellied and amethyst sunbirds are characterized by a long, slender and curved bill (Downs 2004; Hockey et al. 2005). Food plants of amethyst sunbirds are species of *Strelitzia*, *Aloe*, *Protea*, *Erythrina* and *Eucalyptus*, among others (Skead 1967).

The brown honeyeater occurs in eastern, western and northern Australia and throughout New Guinea (Longmore 1991). It can be found in semi-arid shrub lands, forests and woodlands, as well as in suburban parks and gardens. This honeyeater species is locally resident and lives alone, in pairs or in small groups (Longmore 1991). The brown honeyeater is one of the smallest honeyeater species, weighing on average 10 g (Richardson and Wooller 1986). This species was chosen for my experiments as it is of comparable size to whitebellied and amethyst sunbirds. Male and female brown honeyeaters are both of dull olive-brown colour with a lighter belly and a characteristic small wedge-shaped patch of yellow or white behind the eye (Longmore 1991). Their bill is shorter than that of sunbirds and only slightly curved. Examples of food plants of brown honeyeaters include species of *Calothamnus*, *Eucalyptus*, *Grevillea*, *Hakea*, *Lysiana* and others (Hopper 1981).

## *Objectives of my research*

My research focuses on the effects of nectar sugar concentration, fasting periods and ambient temperature on the feeding patterns, food intake and energy balance of sunbirds and honeyeaters. I aimed to compare the two main lineages of passerine nectar feeders with regards to their response to similar energetically challenging conditions.

In the first chapter of my thesis, I investigated whether the feeding duration of whitebellied sunbirds can provide an estimate of their meal size over a range of nectar sugar concentrations. This knowledge was essential for subsequent experiments, in which I needed to be able to use meal size as a surrogate for feeding duration of these birds. The custom-designed experimental equipment and software used in my study allowed for remarkably precise data collection. All feeding events of the bird were recorded with an infrared photodetection system, which was interfaced to a computer. In addition, nectar consumption and the body mass of the bird were recorded continuously (every 0.2 s) by electronic balances which were also interfaced to the computer. Food intake and body masses of whitebellied sunbirds have been recorded at hourly or daily intervals in previous studies (e.g. Nicolson et al. 2005), and my study is the first to provide information on a much finer scale. I predicted that meal size can be used as estimate of feeding duration on all diet concentrations.

Chapter 2 focuses on the response of whitebellied sunbirds to sudden changes in nectar concentration. My aim was to determine how rapidly these birds adjust their volumetric intake and feeding patterns after changes in diet concentration, when fed various sugar concentrations, ranging from 2.5–30% w/w sucrose, for 1.5 h each. Using the same experimental equipment as in Chapter 1, I continuously recorded feeding events and body mass in order to investigate whether the birds maintain a constant energy intake on various nectar concentrations and whether this is achieved by altering meal frequency and/or meal size. Nothing is known of how quickly sunbirds respond to a change in nectar concentration. I hypothesized that whitebellied sunbirds adjust their feeding frequency, and thus their food intake, within minutes after a switch in diet concentration.

In Chapter 3, the response of whitebellied sunbirds and brown honeyeaters to a fasting period during the photophase was investigated. I used a similar experimental design to those in previous studies (Tooze and Gass 1985; Nicolson et al. 2005) to allow for comparison. I predicted that, like whitebellied sunbirds (Nicolson et al. 2005), brown honeyeaters would adjust their food intake to compensate for the fasting period. However, in the case of the sunbirds, my aim was to collect data at a very fine temporal resolution to investigate the feeding patterns following the fasting period. I hypothesized that sunbirds increase their feeding frequency, rather than meal size, to adjust their food intake after the fasting period.

In another experiment, whitebellied sunbirds and amethyst sunbirds were exposed to low ambient temperature and varying nectar sugar concentrations (Chapter 4). Their food intake and body mass were recorded daily to determine whether the birds are able to adjust their food intake and maintain energy balance. I further recorded their feeding events using a photodetection system. As ambient temperature and nectar concentration decrease, birds were expected to increase the number of feeding events, rather than meal size. On moderate and concentrated diets, I predicted that the sunbirds would be able to increase their food intake sufficiently to maintain energy balance at low ambient temperature. When birds are fed dilute diets, however, I hypothesized that food intake might be limited by physiological constraints, leading to body mass loss.

In Chapter 5, brown honeyeaters were exposed to a similar temperature challenge. As in the case of sunbirds, I hypothesized that the honeyeaters would increase their food intake in the cold and maintain body mass on moderate and concentrated diets, but not on dilute diets. My experiment was conducted using both main sugar types in nectar (sucrose and hexoses). However, I did not expect differences in food intake and body mass between sucrose and hexose diets. My findings from the temperature challenge experiments were compared to the digestive capacities of brown honeyeaters as well as whitebellied and amethyst sunbirds predicted by a chemical reactor model, which assumes sucrose hydrolysis to be the limiting step in nectar digestion, and relies on knowledge of gut morphometrics, sucrase activity and sugar assimilation efficiency (McWhorter and Martínez del Rio 2000; Martínez del Rio et al. 2001).

The investigation of feeding patterns of sunbirds included the determination of feeding frequencies, feeding durations and meal sizes. However, one last aspect remains unidentified: tongue licking frequencies, durations of licks and food consumption per lick may change with changing viscosity, such as when nectar concentration (or ambient temperature) varies. Birds may also adjust these licking parameters after a fasting period, to maximize their food intake. My last thesis chapter (Chapter 6) describes the licking behaviour of whitebellied and amethyst sunbirds feeding on varying nectar concentrations with varying viscosities, and on days with and without a fasting period. No study to date has investigated licking frequencies and consumption per lick of avian nectarivores following a fast. My study is also the first to investigate licking behaviour in these two species. Early studies have recorded licking behaviour of avian nectarivores with video cameras (Hainsworth 1973; Collins et al. 1980), which may provide insufficient resolution. In my experiments, a photodetection system, providing remarkable fine-scale data recording (every 1 ms), was used to record tongue licks of the sunbirds. I hypothesized that licking frequencies and consumption per lick would decline, while lick duration would increase, with increasing sugar concentration due to increased viscosity of the solution. I further predicted that these licking parameters would not depend on the degree of starvation.

In summary, my research focuses on the behavioural and physiological responses of avian nectarivores to energetic challenges. I aimed to determine whether the response of sunbirds and honeyeaters to variations in nectar quality and availability, and ambient temperature, are shaped by compensatory feeding or physiological constraints. Furthermore, I investigated on a novel short-term scale how sunbirds adjust their feeding patterns in an attempt to compensate for alterations in energy intake or energy requirements.

## References

Aizen M.A. 2003. Down-facing flowers, hummingbirds and rain. Taxon 52: 675–680.

Bednekoff P.A. and Houston A.I. 1994. Avian daily foraging patterns: effects of digestive constraints and variability. Evolutionary Ecology 8: 36–52.

Beuchat C.A., Chaplin S.B. and Morton M.L. 1979. Ambient temperature and the daily energetics of two species of hummingbirds, *Calypte anna* and *Selasphorus rufus*. Physiological Zoology 53: 280–295.

Beuchat C.A., Calder W.A. and Braun E.J. 1990. The integration of osmoregulation and energy balance in hummingbirds. Physiological Zoology 63: 1059–1081.

Brown J.H., Calder W.A. and Kodric-Brown A. 1978. Correlates and consequences of body size in nectar-feeding birds. American Zoologist 18: 687–738.

Calder W.A., Calder L.L. and Fraizer T.D. 1990. The hummingbird’s restraint: a natural model for weight control. Cellular and Molecular Life Sciences 46: 999–1002.

Carleton S.A., Hartman Bakken B. and Martínez del Rio C. 2006. Metabolic substrate use and the turnover of endogenous energy reserves in broad-tailed hummingbirds (*Selasphorus platycercus*). Journal of Experimental Biology 209: 2622–2627.

Carpenter F.L. and Hixon M.A. 1988. A new function for torpor: fat conservation in a wild migrant hummingbird. Condor 90: 373–378.

Carthew S.M. and Goldingay R.L. 1997. Non-flying mammals as pollinators. Trends in Ecology and Evolution 12: 104–108.

Chai P., Altshuler D.L., Stephens D.B. and Dillon M.E. 1999. Maximal horizontal flight performance of hummingbirds: effects of body mass and molt. Physiological and Biochemical Zoology 72: 145–155.

Cheke R.A., Mann C.F. and Allen R. 2001. Sunbirds: A guide to the sunbirds, flowerpeckers, spiderhunters and sugarbirds of the world. Christopher Helm, London, UK.

Collins B.G. and Morellini P.C. 1979. The influence of nectar concentration and time of day upon energy intake and expenditure by the singing honeyeater, *Meliphaga virescens*. Physiological Zoology 52: 165–175.

Collins B.G., Cary G. and Packard G. 1980. Energy assimilation, expenditure and storage by the brown honeyeater, *Lichmera indistincta*. Journal of Comparative Physiology B 137: 157–163.

Downs C.T. 2004. Some preliminary results of studies on the bill and tongue morphology of Gurney's Sugarbird and some southern African sunbirds. Ostrich 75: 169–175.

Fernández M.J., López-Calleja M.V. and Bozinovic F. 2002. Interplay between the energetics of foraging and thermoregulatory costs in the green-backed firecrown hummingbird *Sephanoides sephanoides*. Journal of Zoology 258: 319–326.

Fleming P.A. and Nicolson S.W. 2003. Osmoregulation in an avian nectarivore, the whitebellied sunbird *Nectarinia talatala*: response to extremes of diet concentration. Journal of Experimental Biology 206: 1845–1854.

Fleming P.A., Gray D.A. and Nicolson S.W. 2004a. Circadian rhythm of water balance and aldosterone excretion in the whitebellied sunbird *Nectarinia talatala*. Journal of Comparative Physiology B 174: 341–346.

Fleming P.A., Hartmann Bakken B., Lotz C.N. and Nicolson S.W. 2004b. Concentration and temperature effects on sugar intake and preferences in a sunbird and a hummingbird. Functional Ecology 18: 223–232.

Fleming P.A., Xie S., Napier K., McWhorter T.J. and Nicolson S.W. 2008. Nectar concentration affects sugar preferences in two Australian honeyeaters and a lorikeet. Functional Ecology 22: 599–605.

Garber P.A. 1988. Foraging decisions during nectar feeding by Tamarin monkeys (*Saguinus mystax* and *Saguinus fuscicollis*, Primates) in Amazonian Peru. Biotropica 20: 100–106.

Gass C.L. 1978. Experimental studies of foraging in complex laboratory environments. American Zoologist 18: 729–738.

Gass C.L., Romich M.T. and Suarez R.K. 1999. Energetics of hummingbird foraging at low ambient temperature. Canadian Journal of Zoology 77: 314–320.

Glück E. 1987. An experimental study of feeding, vigilance and predator avoidance in a single bird. Oecologia 71: 268–272.

Hainsworth F.R. 1973. On the tongue of a hummingbird: its role in the rate and energetics of feeding. Comparative Biochemistry and Physiology A 46: 65–78.

Hainsworth F.R., Tardiff M.F. and Wolf L.L. 1981. Proportional control for daily energy regulation in hummingbirds. Physiological Zoology 54: 452–462.

Hartman Bakken B. and Sabat P. 2006. Gastrointestinal and renal responses to water intake in the green-backed firecrown (*Sephanoides sephanoides*), a South American hummingbird. American Journal of Physiology (Regulatory, Integrative and Comparative Physiology) 291: R830–R836.

Hiebert S. 1993. Seasonal changes in body mass and use of torpor in a migratory hummingbird. Auk 110: 787–797.

Hockey P.A.R., Dean W.R.J. and Ryan P.G. 2005. Roberts: Birds of Southern Africa. VII$^{th}$ edition. The Trustees of the John Voelcker Bird Book Fund, Cape Town, South Africa.

Hopper S.D. 1981. Honeyeaters and their winter food plants on granite rocks in the central wheatbelt of Western Australia. Australian Wildlife Research 8: 187–197.

Jackson S. and Nicolson S.W. 2002. Xylose as nectar sugar: from biochemistry to ecology. Comparative Biochemistry and Physiology B 131: 613–620.

Johnsgard P.A. 1983. The hummingbirds of North America. Smithsonian Institution Press, Washington D.C., USA.

Johnson S.D. and Nicolson S.W. 2008. Evolutionary associations between nectar properties and specificity in bird pollination systems. Biology Letters 4: 49–52.

Karasov W.H. and Martínez del Rio C. 2007. Physiological ecology: how animals process energy, nutrients, and toxins. Princeton University Press, New Jersey, USA.

Karasov W.H. and McWilliams S.R. 2005. Digestive constraints in mammalian and avian ecology. In: Physiological and ecological adaptations to feeding in vertebrates.

J.M. Starck and T. Wang (Eds.), Science Publishers, Enfield, New Hampshire, USA, pp. 87–112.

Kingsolver J.G. and Daniel T.L. 1983. Mechanical determinants of nectar feeding strategy in hummingbirds: energetics, tongue morphology, and licking behaviour. Oecologia 60: 214–226.

Köhler A., Verburgt L. and Nicolson S.W. 2006. Short-term feeding patterns of whitebellied sunbirds (*Nectarinia talatala*): feeding frequency, daily rhythms and individual differences. Journal of Experimental Biology 209: 2880–2887.

Longmore W. 1991. Honeyeaters and their allies of Australia. Angus and Robertson Publishers, North Ryde, Australia.

Lotz C.N. 1999. Energy and water balance in the lesser double-collared sunbird, *Nectarinia chalybea*. PhD thesis, University of Cape Town, South Africa.

Lotz C.N. and Nicolson S.W. 1996. Sugar preferences of a nectarivorous passerine bird, the Lesser Double-collared Sunbird (*Nectarinia chalybea*). Functional Ecology 10: 360–365.

Lotz C.N. and Schondube J.E. 2006. Sugar preferences in nectar- and fruit-eating birds: behavioural patterns and physiological causes. Biotropica 38: 3–15.

Lotz C.N., Martínez del Rio C. and Nicolson S.W. 2003. Hummingbirds pay a high cost for a warm drink. Journal of Comparative Physiology B 173: 455–462.

Martínez del Rio C. and Karasov W.H. 1990. Digestion strategies in nectar- and fruit-eating birds and the sugar composition of plant rewards. American Naturalist 136: 618–637.

Martínez del Rio C., Schondube J.E., McWhorter T.J. and Herrera L.G. 2001. Intake responses in nectar feeding birds: digestive and metabolic causes, osmoregulatory consequences, and coevolutionary effects. American Zoologist 41: 902–915.

Mbatha K., Downs C.T. and Penning M. 2002. Nectar passage and gut morphology in the Malachite Sunbird and the Black-capped Lory: implications for feeding in nectarivores. Ostrich 73: 138–142.

McWhorter T.J. 2005. Carbohydrate hydrolysis and absorption: lessons from modeling digestive function. In: Physiological and ecological adaptations to feeding in vertebrates. J.M. Starck and T. Wang (Eds.), Science Publishers, Enfield, New Hampshire, USA, pp. 59–86.

McWhorter T.J. and Martínez del Rio C. 1999. Food ingestion and water turnover in hummingbirds: how much dietary water is absorbed? Journal of Experimental Biology 202: 2851–2858.

McWhorter T.J. and Martínez del Rio C. 2000. Does gut function limit hummingbird food intake? Physiological and Biochemical Zoology 73: 313–324.

McWhorter T.J., Powers D.R. and Martínez del Rio C. 2003. Are hummingbirds facultatively ammonotelic? Nitrogen excretion and requirements as a function of body size. Physiological and Biochemical Zoology 76: 731–743.

McWhorter T.J., Hartman Bakken B., Karasov W.H. and Martínez del Rio C. 2006. Hummingbirds rely on both paracellular and carrier-mediated intestinal glucose absorption to fuel high metabolism. Biology Letters 2: 131–134.

McWilliams S.R. and Karasov W.H. 2001. Phenotypic flexibility in digestive system structure and function in migratory birds and its ecological significance. Comparative Biochemistry and Physiology A 128: 579–593.

Moore F.R. and Yong W. 1991. Evidence of food-based competition among passerine migrants during stopover. Behavioural Ecology and Sociobiology 28: 85–90.

Napier K.R., Purchase C., McWhorter T.J., Nicolson S.W. and Fleming P.A. 2008. The sweet life: diet sugar concentration influences paracellular glucose absorption. Biology Letters 4: 530–533.

Nicolson S.W. 2002. Pollination by passerine birds: why are the nectars so dilute? Comparative Biochemistry and Physiology B 131: 645–652.

Nicolson S.W. 2007a. Nectar consumers. In: Nectaries and nectar. S.W. Nicolson, M. Nepi and E. Pacini (Eds.), Springer, Dordrecht, Netherlands, pp. 289–342.

Nicolson S.W. 2007b. Amino acid concentrations in the nectars of southern African bird-pollinated flowers, especially *Aloe* and *Erythrina*. Journal of Chemical Ecology 33: 1707–1720.

Nicolson S.W. and Fleming P.A. 2003a. Nectar as food for birds: the physiological consequences of drinking dilute sugar solutions. Plant Systematics and Evolution 238: 139–153.

Nicolson S.W. and Fleming P.A. 2003b. Energy balance in the Whitebellied Sunbird *Nectarinia talatala*: constraints on compensatory feeding, and consumption of supplementary water. Functional Ecology 17: 3–9.

Nicolson S.W. and Thornburg R.W. 2007. Nectar chemistry. In: Nectaries and nectar. S.W. Nicolson, M. Nepi and E. Pacini (Eds.), Springer, Dordrecht, Netherlands, pp. 215–264.

Nicolson S.W., Hoffmann D. and Fleming P.A. 2005. Short-term energy regulation in nectar-feeding birds: the response of Whitebellied Sunbirds (*Nectarinia talatala*) to a midday fast. Functional Ecology 19: 988–994.

Olesen J.M. and Valido A. 2003. Lizards as pollinators and seed dispersers: an island phenomenon. Trends in Ecology and Evolution 18: 177–181.

Ott B.D. and Secor S.M. 2007. Adaptive regulation of digestive performance in the genus *Python*. Journal of Experimental Biology 210: 340–356.

Pate J.S., Peoples M.B., Storer P.J. and Atkins C.A. 1985. The extrafloral nectaries of cowpea (*Vigna unguiculata* (L.) Walp.) II. Nectar composition, origin of nectar solutes, and nectary functioning. Planta 166: 28–38.

Paton D. 1982. The diet of the New Holland honeyeater, *Phyliodonyris novaehollandiae*. Australian Journal of Ecology 7: 279–298.

Penry D.L. and Jumars P.A. 1986. Chemical reactor analysis and optimal digestion. Bioscience 36: 310–315.

Penry D.L. and Jumars P.A. 1987. Modeling animal guts as chemical reactors. American Naturalist 129: 69–96.

Pyke G.H. 1980. The foraging behaviour of Australian honeyeaters: a review and some comparisons with hummingbirds. Australian Journal of Zoology 5: 343–369.

Pyke G.H. and Waser N.M. 1981. The production of dilute nectars by hummingbird and honeyeater flowers. Biotropica 13: 260–270.

Pyke G.H., Pulliam H.R. and Charnov E.L. 1977. Optimal foraging: a selective review of theory and tests. Quarterly Review of Biology 52: 137–154.

Richardson K.C. and Wooller R.D. 1986. The structures of the gastrointestinal tracts of honeyeaters and other small birds in relation to their diets. Australian Journal of Zoology 34: 119–124.

Roberts W.M. 1995. Hummingbird licking behaviour and the energetics of nectar feeding. Auk 112: 456–463.

Robin J.P., Frain M., Sardet C., Groscolas R. and Le Maho Y. 1988. Protein and lipid utilization during long-term fasting in emperor penguins. American Journal of Physiology (Regulatory, Integrative and Comparative Physiology) 254: R61–R68.

Roxburgh L. and Pinshow B. 2000. Nitrogen requirements of an Old World nectarivore, the orange-tufted sunbird *Nectarinia osea*. Physiological and Biochemical Physiology 73: 638–645.

Roxburgh L. and Pinshow B. 2002. Digestion of nectar and insects by Palestine sunbirds. Physiological and Biochemical Physiology 75: 583–589.

Schlamowitz R., Hainsworth F.R. and Wolf L.L. 1976. On the tongues of sunbirds. Condor 78: 104–107.

Skead C.J. 1967. The sunbirds of southern Africa: also the sugarbirds, the white-eyes and the spotted creeper. The Trustees of the South African Bird Book Fund, Balkema, Cape Town, South Africa.

Starck J.M. 1999. Structural flexibility of the gastro-intestinal tract of vertebrates – implications for evolutionary morphology. Zoologischer Anzeiger 238: 87–101.

Stiles F.G. and Freeman C.E. 1993. Patterns in floral nectar characteristics of some bird-visited plant species from Costa Rica. Biotropica 25: 191–205.

Symes C.T., Nicolson S.W. and McKechnie A.E. 2008. Response of avian nectarivores to the flowering of *Aloe marlothii*: a nectar oasis during dry South African winters. Journal of Ornithology 149: 13–22.

Tadey M. and Aizen M.A. 2001. Why do flowers of a hummingbird-pollinated mistletoe face down? Functional Ecology 15: 782–790.

Temeles E.J. and Kress W.J. 2003. Adaptation in a plant-hummingbird association. Science 300: 630–633.

Tooze Z.J. and Gass C.L. 1985. Responses of rufous hummingbirds to midday fasts. Canadian Journal of Zoology 63: 2249–2253.

Tsahar E., Martínez del Rio C., Arad Z., Joy J.P. and Izhaki I. 2005. Are the low protein requirements of nectarivorous birds the consequence of their sugary and watery diet? A test with an omnivore. Physiological and Biochemical Zoology 78: 239–245.

Van Tets I.G. and Nicolson S.W. 2000. Pollen and the nitrogen requirements of the lesser double-collared sunbird. Auk 117: 826–830.

Welch K.C., Jr. and Suarez R.K. 2007. Oxidation rate and turnover of ingested sugar in hovering Anna’s (*Calypte anna*) and rufous (*Selasphorus rufus*) hummingbirds. Journal of Experimental Biology 210: 2154–2162.

Wiens D., Rourke J.P., Casper B.B., Rickardt E.A., LaPine T.R., Peterson C.J. and Channing A. 1983. Non-flying mammal pollination of southern African Proteas: a non-coevolved system. Annals of the Missouri Botanical Garden 70: 1–31.

Wolf L.L. and Hainsworth F.R. 1977. Temporal patterning of feeding by hummingbirds. Animal Behaviour 25: 976–989.

Zubair A.K. and Leeson S. 1996. Compensatory growth in the broiler chicken: a review. World’s Poultry Science Journal 52: 189–201.

# CHAPTER 1

# FOOD INTAKE OF WHITEBELLIED SUNBIRDS (*CINNYRIS TALATALA*): CAN MEAL SIZE BE INFERRED FROM FEEDING DURATION?

**Angela Köhler*, Luke Verburgt and Susan W. Nicolson**

Department of Zoology and Entomology, University of Pretoria, Pretoria 0002, South Africa

*Corresponding author, e-mail: akoehler@zoology.up.ac.za.

*Physiological and Biochemical Zoology, 2008, 81: 682–687.*

## Abstract

A positive relationship between feeding duration and meal size of avian nectarivores has often been assumed in earlier studies. We investigated whether feeding duration can be used as a surrogate for the amount of sugar solution ingested by whitebellied sunbirds, *Cinnyris* (*Nectarinia*) *talatala*. Feeding durations of sunbirds consuming three sucrose concentrations (10, 20 and 40% w/w) were measured using an infrared photodetection system, and the amounts consumed were recorded simultaneously by weighing the feeder throughout the experiment. For all three diet concentrations, a positive relationship was found between the time spent feeding per 30 min and the mass consumed. Therefore, feeding duration is demonstrated to be an index of the amount ingested on a particular sugar concentration. The rate of ingestion, however, depended on the sugar concentration, being highest at the lowest concentration of 10% and lowest at 40%. Total time spent feeding was lower on 20% than on 10% sucrose, but increased on the 40% diet due to viscosity effects. There appeared to be a weak relationship between feeding patterns and sex, but this was not significant, probably due to inter-individual variation.

## Introduction

Food intake as a fundamental physiological function of animals has been extensively investigated. Feeding patterns are examined by measuring characteristic parameters such as meal size and feeding duration. Meal size can be recorded by weighing the food continuously, for example by placing food containers on scales as in experiments with rats (Blanton et al. 1998; Kahler et al. 1998). In nectar-feeding insects, meal size has been measured by weighing the insect before and after the meal (Hainsworth et al. 1991; Paul and Roces 2003). For avian nectarivores, however, food intake is commonly determined on a daily or hourly basis only. Nectar intake has been measured by weighing the feeder (Nicolson and Fleming 2003; Nicolson et al. 2005) or by reading changes in fluid meniscus levels in glass or plastic feeders, as done for honeyeaters (Collins and Clow 1978), sunbirds and sugarbirds (Jackson et al. 1998) and hummingbirds (López-Calleja and Bozinovic 2003). Feeding durations of nectar-feeding birds, on the other hand, have been recorded with a stopwatch (Collins and Clow 1978) or a video camera (Mitchell and Paton 1990). Feeding durations have also been measured using a photodetection system, connected to a timer, with a photo beam being broken when the bird feeds (Gass 1978; Garrison and Gass 1999; Köhler et al. 2006).

The relationship between feeding duration and meal size has often been assumed in previous studies on nectar-feeding birds. Mitchell and Paton (1990), for instance, recorded honeyeaters’ handling times for artificial flowers containing nectar of known volume and concentration. They calculated sugar intake rate on the assumption that handling time is positively related to the volume consumed. However, this relationship has not been sufficiently investigated in avian nectarivores. To our knowledge, only one study, on western spinebills (*Acanthorhynchus superciliosis*), has demonstrated that longer feeding durations are reflected in larger volumes of nectar ingested (Collins and Clow 1978).

Previously we have used an infrared photodetection system to investigate short-term feeding patterns of whitebellied sunbirds, *Cinnyris* (*Nectarinia*) *talatala*, fed on artificial nectar of various sugar concentrations (Köhler et al. 2006). However, the relationship between feeding duration of the sunbirds and their meal size could not be determined directly. We used feeding duration as an estimate of meal size, based on the assumption that food intake rates should be constant for birds feeding on a single sugar concentration. The present study aimed to determine whether feeding duration of whitebellied sunbirds can in fact be used as a surrogate for the amount of sugar solution ingested. Here we defined meal size as the amount of sugar solution ingested in 30 min, since the minute amounts of solution ingested in a single feeding event could not be detected. We also compared males and females to determine whether there is a sex-specific feeding pattern in this species.

# Materials and methods

## *Study animals and their maintenance*

Ten non-reproductive whitebellied sunbirds were captured with mist nets in Jan Cilliers Park, Pretoria. Birds were initially housed together in an outdoor aviary measuring 8 x 5 x 2 m. One week prior to experiments, birds were moved to individual cages of 45 x 45 x 32 cm in a climate-controlled room. They were acclimated to the experimental temperature of 20 ± 2ºC and a light : dark photoperiod of 12:12 h, with lights on at 07h00. The maintenance diet consisted of 20% weight / weight (w/w) sucrose (0.63 M) with a nutritional supplement (Ensure®, Abbott Laboratories, Johannesburg, South Africa) to provide dietary nitrogen (van Tets and Nicolson 2000). The maintenance diet and supplementary water were provided *ad libitum* in inverted, stoppered syringes. Body mass (mean ± SE) of five males and five females was 9.31 ± 0.39 g and 8.08 ± 0.18 g respectively. Our experiments were approved by the Animal Use and Care Committee of the University of Pretoria (AUCC 060515-012).

## *Experimental design*

Each sunbird received 10%, 20% and 40% w/w sucrose solutions (0.32, 0.63 and 1.35 M) in randomized order for a period of 24 h each. The experimental diet was changed every morning before lights on. To prevent mass loss on sugar-only diets (Nicolson and Fleming 2003) all experimental diets contained Ensure®. The amount of Ensure® added was adjusted according to the sugar concentration of the experimental diet, since the volumes ingested are inversely proportional to sugar concentration. This means that the 40% diet contained the largest amount of Ensure® to keep protein intake constant. Even if the carbohydrate component of the added Ensure® were completely digested by sunbirds, it would increase the highest sugar concentration to only 42.3%, and is therefore negligible.

Birds were moved sequentially to an experimental cage (Köhler et al. 2006) and were allowed to acclimate for one day before measurements commenced. The cage (50 x 40 x 45 cm) was constructed from Perspex, with ventilation holes, and contained a feeding perch and a resting perch (Fig. 1). Automated recording of the time spent feeding as well as the number of feeding events was achieved by an infrared photodetection system interfaced to a computer (Köhler et al. 2006). Outside the cage, the feeder with the sucrose solution was mounted on an electronic balance (Mettler Toledo PB-602S, 0.01 g, Microsep Ltd, Johannesburg), interfaced to the same computer. Feeder mass was recorded every 0.2 s to determine the mass consumed by the bird. Occasional drips were collected in a container with liquid paraffin (to avoid evaporative mass loss), which was also placed on the electronic balance (Fig. 1). Thus, the dripping of the solution did not affect the results.

## *Data processing*

For each bird we obtained the start and end times of all feeding events and their durations (± 0.001 s). Separate feeding events could not be defined by a return to the perch after feeding since sunbirds remain perched during feeding events and do not hover to feed like hummingbirds. We therefore defined feeding event duration as the time between insertion of the bill into the sucrose solution and its removal (Köhler et al. 2006). Feeding event durations of less than 0.1 s were excluded from analysis since observations revealed that they were caused by wing movements of the bird or incomplete insertion of the bill into the feeding aperture. Separate feeding events were merged into a single feeding event when the interval between them was less than 0.25 s, since turns of the bill or its incomplete removal from the feeder were recorded as two feeding events (A. Köhler, pers. observation).

The light period of the day was divided into 24 intervals of 30 min each. The first and last of these were excluded because individuals differed in the time of their first and last feeding event of the day (described in detail in Köhler et al. 2006), leaving 22 intervals that were used for analysis. For each individual and each diet concentration, the number of feeding events was calculated for each of the 30 min intervals, defined as feeding frequency. Furthermore, the total time that the bird spent feeding during each 30 min interval, defined as feeding duration, was determined by summing the durations of all feeding events. Although we recorded the feeder mass every 0.2 s, the minute amount of sugar solution consumed in a single feeding event could not be detected, since the resolution of our balance was too coarse (0.01 g). Therefore, we defined the mass consumed per 30 min interval as meal size. Meal size was calculated by subtracting the last feeder mass record of each 30 min interval from the first one. Furthermore, we determined the mass of solution consumed daily by summing the meal size of each interval. Sucrose intake per 30 min interval was calculated for all birds on all diet concentrations.

## *Statistical procedures*

### 1) Meal size and feeding behaviour

Linear regressions were calculated to determine relationships between (a) feeding duration and meal size and (b) feeding frequency and meal size on all diets. For each relationship (a and b), data were arranged for each individual (n=10) and for each diet concentration (n=3), by 30 min intervals (n=22). This meant that each regression (n=30 for each relationship) was based on 22 data points. This analysis was performed for each individual separately since we demonstrated large differences in feeding pattern between individuals in our previous study (Köhler et al. 2006). The regression slopes obtained from (a) are a measure of food intake rate and are hereafter exclusively referred to as intake rate. Intake rates of each individual were compared between the three diet concentrations by repeated-measures ANOVA (RM-ANOVA).

### 2) Differences between the sexes and body mass relationships

Data were tested for normality and homogeneity of variance; log transformations were used when data were not normally distributed. Although body mass data were normally distributed (Kolmogorov-Smirnov test: $D>0.21$; $P>0.20$), we used the more conservative non-parametric Mann-Whitney U test to test for differences in body mass between males and females because of the problems associated with distribution estimation from small sample sizes (Zar 1999). RM-ANOVAs were conducted to determine differences between the sexes in feeding duration, feeding frequency and meal size. Data were arranged so that intervals (n=22) were nested within each diet concentration (n=3), with sex being the categorical predictor (between-effect). The intake rates obtained from 1(a) were also subjected to RM-ANOVA to test for differences between the sexes. Intake rates of the 10 sunbirds on all diet concentrations (n=3) were dependent variables, with sex being the categorical predictor. Linear regressions were calculated to determine sex-independent relationships between body mass and feeding duration over the whole day and between body mass and mass of solution consumed daily for all diets. For each relationship, data were arranged for each diet concentration (n=3), by individual (n=10). This meant that each regression (n=3 for each relationship) was based on 10 data points.

### 3) Daily rhythm, feeding patterns and consumption on the different diets

Data were tested for normality and homogeneity of variance; square root transformations were used when data were heteroscedastic. RM-ANOVA was performed to determine differences in feeding duration and meal size throughout the day (between 30 min intervals) and between diet concentrations. Data were arranged as follows: intervals (n=22) were nested within sucrose concentration (n=3), yielding 66 dependent variables. The daily mass of solution consumed and sucrose intake of the 10 sunbirds were compared between the diet concentrations (n=3) by RM-ANOVA.

Linear regressions describing individual relationships between feeding behaviour and meal size were followed by a sequential Bonferroni correction to avoid Type I errors (Rice 1989). *Post-hoc* comparisons for all RM-ANOVA were conducted with Tukey's Honest Significant Difference test for equal sample sizes, followed by a Bonferroni correction for multiple comparisons. All data are presented as means ± SE. Prior to Bonferroni corrections the level of significance was $P \leq 0.05$ for all tests.

# Results

## *1) Meal size and feeding behaviour*

**1.1) Feeding duration:** The feeding duration of each sunbird was positively related to meal size for all three sucrose concentrations (all $F_{1,20}\geq4.54$, all $P\leq0.05$, all $R^2\geq0.19$), except for one individual on the 40% diet ($F_{1,20}=2.94$, $P=0.10$, $R^2=0.13$). However, this bird was not excluded from analysis since there was no obvious reason such as poor condition of the bird or equipment malfunction which would justify its exclusion. Figure 2 shows the positive relationship between meal size and feeding duration of all 10 birds for each sucrose concentration (10%: y = 0.013 [±0.0004] x + 0.125, $R^2=0.98$; 20%: y = 0.011 [±0.0012] x + 0.014, $R^2=0.82$; 40%: y = 0.005 [±0.0003] x + 0.008, $R^2=0.90$).

Intake rates for all 10 sunbirds, obtained from the regression analysis of feeding duration and meal size on the three diets, are shown in Figure 3. The intake rate decreased with increasing sucrose concentration ($F_{2,18}=27.86$, $P<0.001$). *Post-hoc* analysis revealed a significant difference in intake rates between 10 and 40% ($P<0.001$) and between 20 and 40% ($P<0.001$). The intake rates on the 10 and 20% sucrose concentrations did not differ ($P=0.67$).

**1.2) Feeding frequency:** A positive relationship was evident between meal size and feeding frequency, with 24 out of the 30 linear regression analyses of all three sucrose concentrations being significant (all $F_{1,8}\geq5.63$, all $P\leq0.03$, all $R^2\geq0.22$). However, the regression slopes varied greatly between individuals. Mean values of these slopes (mg per feeding event) ± SE (range) were: 10%: 10.2 ± 1.4 (3.2–16.0); 20%: 8.0 ± 1.5 (1.7–16.2); 40%: 4.2 ± 0.8 (0.9–8.1). Therefore, our analysis focuses exclusively on the relationship between feeding duration and meal size.

## 2) Differences between the sexes and body mass relationships

A significant sexual body mass dimorphism was evident ($U<0.01$, $P<0.01$), with males being 15.2% heavier than females. No significant difference between the sexes was found for feeding duration (10%: $F_{1,8}=0.61$, $P=0.46$; 20%: $F_{1,8}=0.26$, $P=0.62$; 40%: $F_{1,8}=0.44$, $P=0.53$) or meal size (10%: $F_{1,8}=4.61$, $P=0.06$; 20%: $F_{1,8}=1.17$, $P=0.31$; 40%: $F_{1,8}=3.84$, $P=0.09$). However, there appeared to be a weak relationship between sex and feeding duration or meal size respectively, with males feeding for longer and consuming larger amounts than females on all diets. Feeding duration over the whole day was not related to body mass on any diet concentration (all $F_{1,8}\leq0.49$, all $P\geq0.51$, all $R^2\leq0.06$). There was also no significant relationship between the mass of solution consumed daily and body mass on any of the diet concentrations (all $F_{1,8}\leq1.33$, all $P\geq0.28$, all $R^2\leq0.14$). Feeding frequency did not differ between the sexes (10%: $F_{1,8}=0.87$, $P=0.38$; 20%: $F_{1,8}=2.81$, $P=0.13$; 40%: $F_{1,8}=1.80$, $P=0.22$), but females appeared to have higher feeding frequencies than males on all diets. The intake rates on each diet concentration (Fig. 3) did not differ between the sexes ($F_{1,8}=0.11$, $P=0.75$).

## 3) Daily rhythm, feeding patterns and consumption on the different diets

Irrespective of diet concentration, birds showed a daily rhythm in their feeding patterns. Both feeding duration ($F_{2,21}=5.02$, $P<0.001$) and meal size ($F_{2,21}=5.37$, $P<0.001$) varied significantly over the course of the day. Both were lower in the last 2.5 h of the experimental period than during the rest of the day (feeding duration: $P\leq0.03$; meal size: $P\leq0.003$). In Fig. 2, these evening intervals lie at the lower end of the regression lines for all three sucrose concentrations.

Feeding duration differed between sucrose concentrations ($F_{2,21}=31.86$, $P<0.001$). Feeding duration was lower on the 20% diet than on 10%, but increased on the 40% diet (Fig. 2). *Post-hoc* analysis showed significant differences in feeding duration between all concentrations (10% and 20%: $P<0.001$; 10% and 40%: $P<0.001$; 20% and 40%: $P=0.01$). Meal size decreased with increasing diet concentration (Fig. 2; $F_{2,21}=424.30$, $P<0.001$). *Post-hoc* analysis revealed a significant difference in meal size between all concentrations ($P<0.001$). Mean masses of solution consumed daily (g) ± SE were: 10%: 19.30 ± 0.75; 20%: 10.26 ± 0.35; 40%: 5.04 ± 0.18 ($F_{2,18}=861.39$, $P<0.0001$). *Post-hoc* analysis showed a significant difference in mass of solution consumed daily between all concentrations ($P<0.001$). The daily sucrose intake did not differ between diet concentrations ($F_{2,18}=2.59$, $P=0.08$; Fig. 4). Sucrose intake was highest during the morning and decreased during the afternoon (Fig. 4).

# Discussion

## *Meal size and feeding duration*

Feeding duration of whitebellied sunbirds has been clearly demonstrated to be an index of the meal size on a particular sugar concentration. This evidence of the positive relationship between feeding duration and meal size has major implications for other studies, since it could only be assumed up to now. With the exception of one study on western spinebills, which showed that feeding durations are positively related to volumes of nectar consumed (Collins and Clow 1978), no other evidence exists for nectar-feeding birds. In hovering hawk moths (*Macroglossum stellatarum*) a relationship between feeding duration and the volume of sucrose solution ingested has also been demonstrated (Josens and Farina 2001). A study measuring the food intake of Sprague-Dawley rats (*Rattus norvegicus*), on the other hand, showed that the correlation between meal size and feeding duration is not as strong as would be needed to accurately estimate meal size from duration data (Castonguay et al. 1986). Cameron (1998) reviewed studies that have correlated time spent suckling by mammalian infants with their milk intake. Less than half of the reviewed studies found a significant positive relationship, which is insufficient evidence that time spent suckling provides an index of milk intake (Cameron 1998).

### *Viscosity effects and compensatory feeding*

The food intake rate depended on the sugar concentration of the diet, being highest at the lowest concentration of 10% and lowest at 40%. Increasing viscosity of the solution with increasing concentration might be an explanation for this observation. A study on licking rates of rufous hummingbirds (*Selasphorus rufus*) showed that both licking frequency and volume per lick decreased with increasing sugar concentration (Roberts 1995). In our study, feeding duration on the 40% solution was prolonged due to the increased viscosity, accentuated by the Ensure® which was added in adjusted quantity to the experimental diets (see Materials and methods). As a result, feeding duration was lower on 20% than on 10%, but was found to increase on the 40% diet, although meal size decreased with increasing sugar concentration due to compensatory feeding (Nicolson and Fleming 2003). Thus, birds were able to maintain a constant daily sucrose intake (Fig. 4) by adjusting meal size according to diet concentration.

### *Daily rhythm in feeding patterns*

Irrespective of diet concentration, birds showed a daily rhythm in their feeding patterns. Both feeding duration and meal size were found to be lower in the late afternoon and evening, compared to the rest of the day. Our previous study also showed the tendency for birds to spend less time feeding during the afternoon than in the late morning (Köhler et al. 2006). A rather different pattern was evident when food intake was examined on an hourly basis: whitebellied sunbirds fed steadily during the morning, followed by reduced intake during the afternoon and then an increase in food intake in the end of the day to provide energy stores for the night (Fleming et al. 2004).

### *Differences between the sexes and individual variation*

A sexual body mass dimorphism was evident for whitebellied sunbirds used in this study, which suggests possible differences in food intake between the sexes. Markman et al. (2006) demonstrated sex-specific differences in transit time of Palestine sunbirds (*Cinnyris oseus*), which might affect foraging behaviour. However, the sexual size dimorphism of their birds was exceptionally high, with males being 28% heavier than females (Markman et al. 2006). In our study the sexes did not differ in their feeding patterns, despite males being 15% heavier than females. However, males appeared to feed for longer and have larger meal sizes than females on all diets. This may indicate the possibility of a sex-specific feeding pattern, since there was no sex-independent relationship between body mass and both feeding duration over a whole day and mass of solution consumed daily. Interestingly, females appeared to have higher feeding frequencies than males. Meal size of females might be smaller due to their smaller body size (and thus greater volumetric constraint since sunbirds do not have a crop), and they might compensate by feeding more often.

Sex-specific feeding patterns were probably obscured by individual feeding patterns. Inter-individual differences were also evident in the large variation in the regression slopes obtained from the relationship between meal size and feeding frequency. Our previous work has already highlighted pronounced variation between individual sunbirds, which differ greatly in their feeding frequency and duration of feeding events (Köhler et al. 2006). Nicolson and Fleming (2003) also demonstrated large variation in sucrose intake between individual whitebellied sunbirds. Therefore, we suggest that the number of birds used in further investigations could be increased to avoid the likelihood of sex-specific feeding patterns being obscured by inter-individual variation.

## Acknowledgements

This project was funded by the South African National Research Foundation. We are grateful to Jan Cilliers Park for permission to mist-net sunbirds under permit from the Gauteng Directorate of Nature Conservation. Our experiments were approved by the Animal Use and Care Committee of the University of Pretoria.

# References

Blanton C.A., Horwitz B.A., Murtagh-Mark C., Gietzen D.W., Griffey S.M. and McDonald R.B. 1998. Meal patterns associated with the age-related decline in food intake in the Fischer 344 rat. American Journal of Physiology (Regulatory, Integrative and Comparative Physiology) 275: R1494–R1502.

Cameron E.Z. 1998. Is suckling behaviour a useful predictor of milk intake? A review. Animal Behaviour 56: 521–532.

Castonguay T.W., Kaiser L.L. and Stern J.S. 1986. Meal pattern analysis: artefacts, assumptions and implications. Brain Research Bulletin 17: 439–443.

Collins B.G. and Clow H. 1978. Feeding behaviour and energetics of the Western Spinebill, *Acanthorhynchus superciliosis* (Aves: Meliphagidae). Australian Journal of Zoology 26: 269–277.

Fleming P.A., Gray D.A. and Nicolson S.W. 2004. Circadian rhythm of water balance and aldosterone excretion in the whitebellied sunbird *Nectarinia talatala*. Journal of Comparative Physiology B 174: 341–346.

Garrison J.S.E. and Gass C.L. 1999. Response of a traplining hummingbird to changes in nectar availability. Behavioral Ecology 10: 714–725.

Gass C.L. 1978. Experimental studies of foraging in complex laboratory environments. American Zoologist 18: 729–738.

Hainsworth F.R., Precup E. and Hamill T. 1991. Feeding, energy processing rates and egg production in painted lady butterflies. Journal of Experimental Biology 156: 249–265.

Jackson S., Nicolson S.W. and Lotz C.N. 1998. Sugar preferences and “side bias” in Cape sugarbirds and lesser double-collared sunbirds. Auk 115: 156–165.

Josens R.B. and Farina W.M. 2001. Nectar feeding by the hovering hawk moth *Macroglossum stellatarum*: intake rate as a function of viscosity and concentration of sucrose solutions. Journal of Comparative Physiology A 187: 661–665.

Kahler A., Geary N., Eckel L.A., Campfield L.A., Smith F.J. and Langhans W. 1998. Chronic administration of OB protein decreases food intake by selectively reducing meal size in male rats. American Journal of Physiology (Regulatory, Integrative and Comparative Physiology) 275: R180–R185.

Köhler A., Verburgt L. And Nicolson S.W. 2006. Short-term energy regulation of whitebellied sunbirds (*Nectarinia talatala*): effects of food concentration on feeding frequency and duration. Journal of Experimental Biology 209: 2880–2887.

López-Calleja M.V. and Bozinovic F. 2003. Dynamic energy and time budgets in hummingbirds: a study in *Sephanoides sephanoides*. Comparative Biochemistry and Physiology A 134: 283–295.

Markman S., Tadmor-Melamed H., Arieli A. and Izhaki I. 2006. Sex differences in food intake and digestive constraints in a nectarivorous bird. Journal of Experimental Biology 209: 1058–1063.

Mitchell R.J. and Paton D.C. 1990. Effects of nectar volume and concentration on sugar intake rates of Australian honeyeaters (Meliphagidae). Oecologia 83: 238–246.

Nicolson S.W. and Fleming P.A. 2003. Energy balance in the Whitebellied Sunbird *Nectarinia talatala*: constraints on compensatory feeding, and consumption of supplementary water. Functional Ecology 17: 3–9.

Nicolson S.W., Hoffmann D. and Fleming P.A. 2005. Short-term energy regulation in nectar-feeding birds: the response of whitebellied sunbirds (*Nectarinia talatala*) to a midday fast. Functional Ecology 19: 988–994.

Paul J. and Roces F. 2003. Fluid intake rates in ants correlate with their feeding habits. Journal of Insect Physiology 49: 347–357.

Rice W.R. 1989. Analyzing tables of statistical tests. Evolution 43: 223–225.

Roberts W.M. 1995. Hummingbird licking behaviour and the energetics of nectar feeding. Auk 112: 456–463.

Van Tets I.G. and Nicolson S.W. 2000. Pollen and the nitrogen requirements of the lesser double-collared sunbird. Auk 117: 826–830.

Zar J.H. 1999. Biostatistical Analysis. 4th ed. Prentice Hall, Upper Saddle River, New Jersey, USA.

# Figure legends

**Fig. 1.** The experimental cage was constructed from Perspex and contained two perches. An infrared photodetection system, interfaced to a computer, was set up on either side of the feeding aperture in order to record feeding events. Next to the cage, the feeder with sugar solution was mounted on an electronic balance, interfaced to the same computer.

**Fig. 2.** Meal size (g) as a function of feeding duration (s) for 10 sunbirds each fed three sucrose concentrations (10, 20 and 40% w/w). Each data point (mean ± SE) represents one 30 min interval (n=22 per concentration). Grey lines represent 95% confidence intervals; x-axis error bars were omitted for clarity.

**Fig. 3.** Intake rates for individual birds (n=10; mean ± SE) for the three sucrose concentrations. The ranges in intake rate (g•min$^{-1}$) for each concentration were as follows: 10%: 0.61–1.15; 20%: 0.51–1.40; 40%: 0.19–0.39. Statistical results derive from the Tukey's Honest Significant Difference *post-hoc* test that followed comparison of the intake rates on the different diets by RM-ANOVA (n. s. $P>0.05$; ** $P<0.01$).

**Fig. 4.** Sucrose intake (mg•g body mass$^{-1}$) per 30 min interval of 10 sunbirds (mean ± SE) on each of the three sucrose concentrations (10, 20 and 40% w/w). Birds maintained a constant sugar intake irrespective of sucrose concentration. Error bars have been partly omitted for clarity.

# Figures

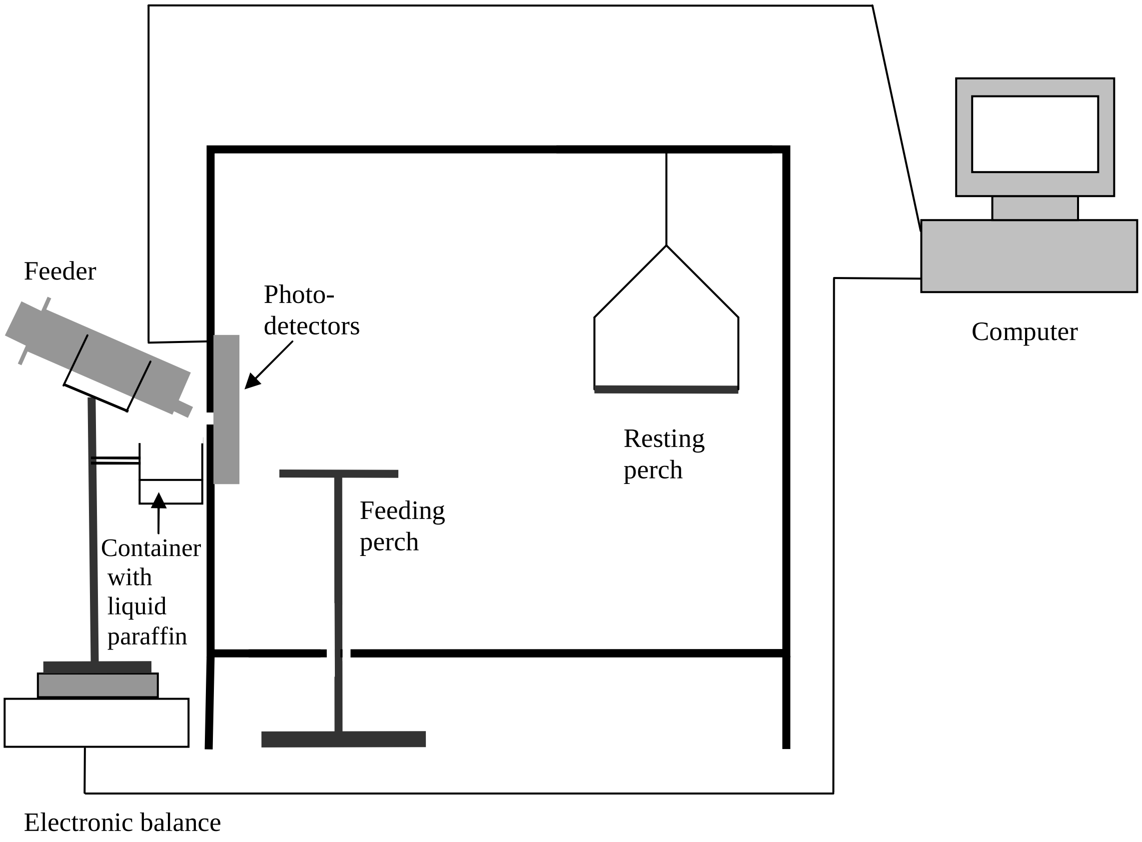


Figure 1.

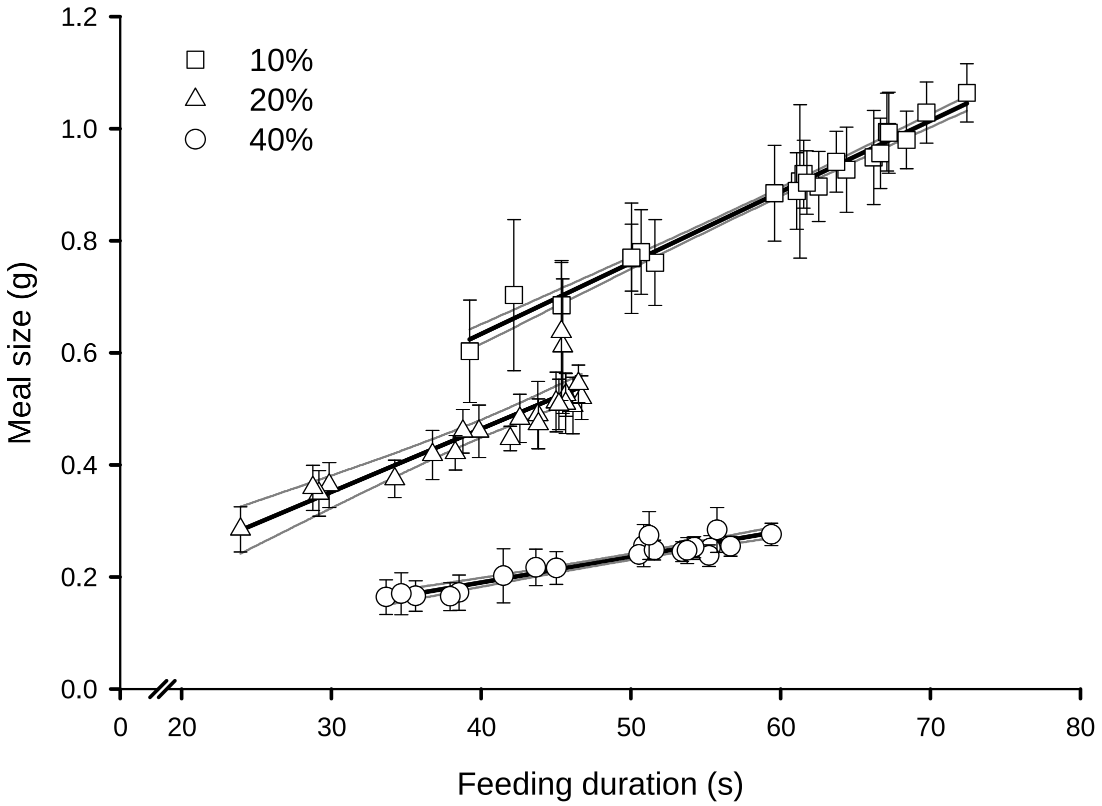

1.2
1.0
0.8
0.6
0.4
0.2
0.0
Meal size (g)
□ 10%
△ 20%
○ 40%
0
20
30
40
50
60
70
80
Feeding duration (s)

Figure 2.

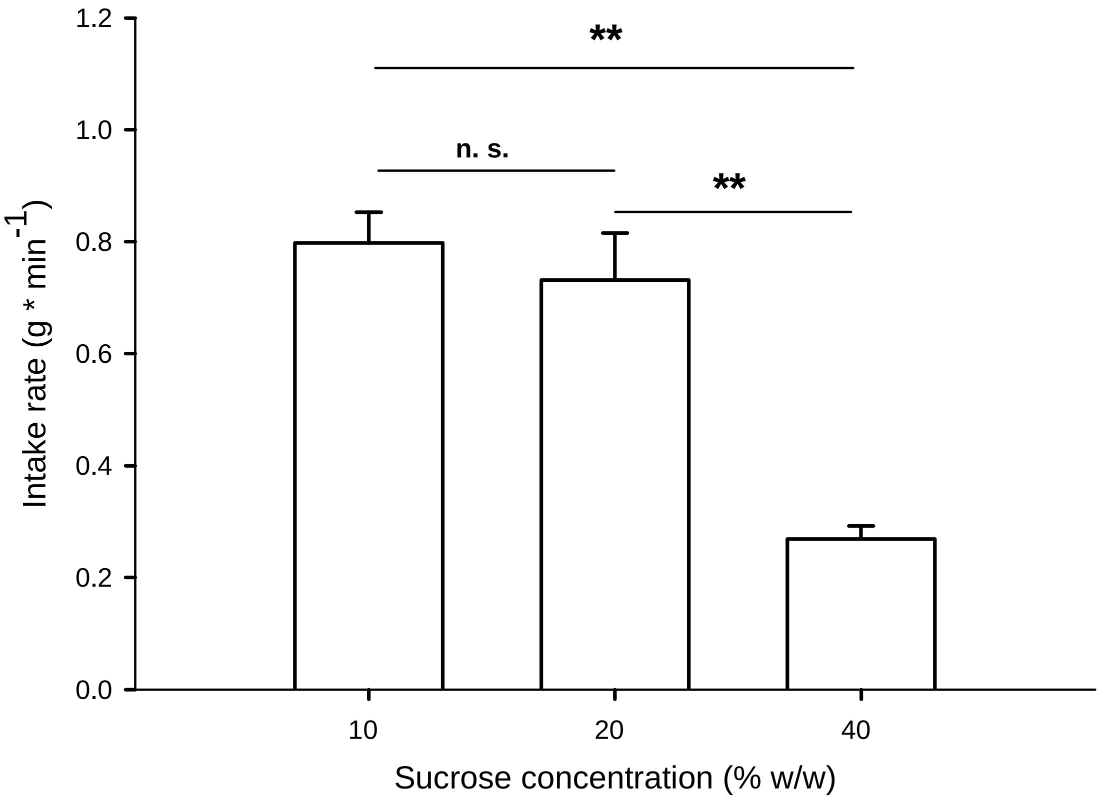

**
n. s.
**
1.2
1.0
0.8
0.6
0.4
0.2
0.0
Intake rate (g * min$^{-1}$)
10
20
40
Sucrose concentration (% w/w)

Figure 3.

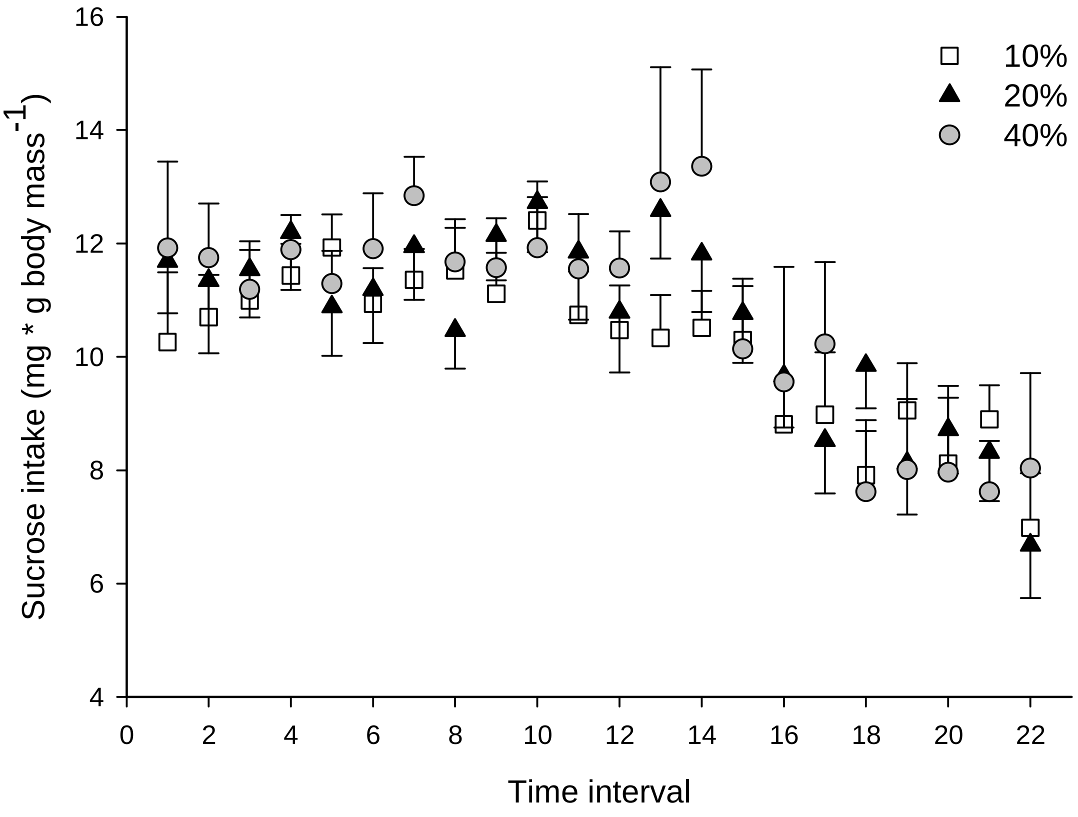

Sucrose intake (mg * g body mass$^{-1}$)
16
14
12
10
8
6
4
0
2
4
6
8
10
12
14
16
18
20
22
Time interval
□ 10%
▲ 20%
● 40%

Figure 4.

# CHAPTER 2

# CHANGES IN NECTAR CONCENTRATION: HOW QUICKLY DO WHITEBELLIED SUNBIRDS (*CINNYRIS TALATALA*) ADJUST FEEDING PATTERNS AND FOOD INTAKE?

**Angela Köhler[†]*, Luke Verburgt[†], Patricia A. Fleming[‡] and Susan W. Nicolson[†]**

[†] Department of Zoology and Entomology, University of Pretoria, Pretoria 0002, South Africa
[‡] School of Veterinary and Biomedical Sciences, Murdoch University, Murdoch WA 6150, Australia

*Corresponding author, e-mail: akoehler@zoology.up.ac.za.

*Journal of Comparative Physiology B, 2008, 178: 785–793.*

# Abstract

Nectarivorous birds encounter varying nectar concentrations while foraging on different food plants and must adjust their consumption to maintain constant energy intake. We determined how rapidly captive whitebellied sunbirds (*Cinnyris talatala*) adjust their volumetric intake and feeding patterns after changes in diet concentration. On four consecutive days, birds were fed sucrose diets alternating between a standard diet of 16% w/w and test diets of 2.5, 8.5, 16 or 30% w/w respectively for 1.5 h periods. Feeding events were recorded with an infrared photodetection system and food intake and body mass were monitored continuously by electronic balances interfaced to a computer. Generally, birds demonstrated a measurable increase in feeding frequency and food intake within 10 min after a decrease in sucrose concentration. However, individuals responded differently to the most dilute diet (2.5%): while most increased their food intake, others stopped feeding for a short while, appearing to dislike this diet. Furthermore, the number and duration of feeding events increased in the first 5 min after the switch from 2.5% back to 16%, as the birds attempted to compensate for previous reduced sugar intake. Daily sugar intake was lower when birds alternated between 2.5% and 16% diets than on other test days, but birds were able to maintain body mass, presumably through behavioural adjustments.

Key-words: compensatory feeding, feeding duration, feeding frequency, food intake, sugar concentration, sunbird, *Cinnyris talatala*

# Introduction

The nectar of bird flowers is characteristically dilute, but still varies widely in sugar concentration (Pyke and Waser 1981; Stiles and Freeman 1993). In bird-pollinated plants of southern Africa, nectar concentrations range from less than 5% up to 55% w/w within and among plant species (Nicolson and Fleming 2003a). As an example, major food plants for three sunbird and one sugarbird species are *Greyia sutherlandii* (11.4% w/w), *Aloe arborescens* (13.2%), *Halleria lucida* (21.3%) and *Leonotis leonurus* (24.4%), which flower at the same time at one site in the Drakensberg region of South Africa (Daniels 1987). Other southern African passerine-pollinated flowers provide more dilute (e.g. *Aloe speciosa*: 6.5%) or more concentrated nectar (e.g. *Leucospermum tottum*: 28.5%; *Liparia splendens*: 36.7%) (Nicolson 2002). Nectarivorous birds may therefore encounter very different sugar concentrations within a short time period while foraging on the nectar of different food plants in the wild.

Although avian nectarivores feed on nectar of varying sugar concentration, and thus varying energy content, they are able to maintain a constant energy intake by adjusting their volumetric intake according to the concentration of nectar; this is commonly known as compensatory feeding (Martínez del Rio et al. 2001). The increase in food intake in response to a decrease in sugar concentration has been shown for hummingbird, honeyeater and sunbird species (Collins and Clow 1978; Collins et al. 1980; Downs 1997; López-Calleja et al. 1997; Lotz and Nicolson 1999; McWhorter and Martínez del Rio 1999; McWhorter et al. 2004). Whitebellied sunbirds, *Cinnyris talatala*, for instance, defend a constant sugar intake on diets from 0.25–2.5 M (8.5–65% w/w) sucrose (Nicolson and Fleming 2003b). As a result of adjusting volumetric intake, nectarivorous birds may need to switch between water conservation and water excretion when they experience changes in nectar concentration (Fleming et al. 2004a; Lotz and Martínez del Rio 2004). On very low sugar concentrations, birds may face physiological constraints, such as handling large amounts of preformed water (Nicolson and Fleming 2003b). Furthermore, sucrose hydrolysis rates and hexose absorption rates in the gut may limit feeding (McWhorter and Martínez del Rio 2000; McWhorter et al. 2006).

The adjustment of food intake according to variation in nectar concentration can take place by altering meal frequency, meal size or both. Data for sunbird, hummingbird and honeyeater species demonstrate that they increase the number of feeding events as the sugar concentration is decreased, while the length of feeding events remains nearly constant (Wolf and Hainsworth 1977; Collins and Clow 1978; Collins and Cary 1980; López-Calleja et al. 1997; Köhler et al. 2006).

In earlier studies, the resolution of feeding data has been too coarse to determine how quickly the birds detect a change in sugar concentration and adjust their nectar intake (e.g. McWhorter and Martínez del Rio 2000; Fleming et al. 2004a). For whitebellied sunbirds, the rate of feeding increases within 1 h of changing from a concentrated to a dilute sucrose diet (Fleming et al. 2004a), but no detailed studies providing finer temporal resolution are available for sunbirds. Only one experiment on a single rufous hummingbird (*Selasphorus rufus*) has demonstrated that nectar intake is altered quickly after a concentration change: the bird responded to an increased concentration within 10 min, while its response to decreased concentration was preceded by a lag (unpublished data in Gass 1978). It is therefore important to collect data on a short-term basis to provide understanding of how quickly sunbirds adjust their feeding patterns and food intake according to the sugar concentration of nectar.

In the present study, we examined how rapidly whitebellied sunbirds adjust their feeding pattern and food intake following an acute change in sugar concentration. The concentration of artificial nectar was changed every 1.5 h and feeding events (frequency and duration), food intake and body mass of the birds were recorded continuously throughout the experiment. We predicted that sunbirds adjust their feeding frequency, and thus their food intake, within minutes after a switch in diet concentration.

# Materials and methods

## *Study animals and their maintenance*

Eight non-reproductive whitebellied sunbirds, *Cinnyris* (*Nectarinia*) *talatala* (A. Smith), were mist-netted in Pretoria and were initially housed together in an outdoor aviary measuring 8 x 5 x 2 m. One week prior to experiments, birds were moved to individual cages of 45 x 45 x 32 cm in a climate-controlled room. They were acclimated to the experimental temperature of 20 ± 2ºC and a 11.5:12.5 h L:D photoperiod with lights on at 07h00. The maintenance diet consisted of a 20% w/w sucrose diet (0.63 M) with a nutritional supplement (Ensure®, Abbott Laboratories, Johannesburg, South Africa) to provide dietary nitrogen (Van Tets and Nicolson 2000). All sucrose concentrations used in this study were mixed on a % weight / weight basis. A sucrose diet was used, since sunbirds are indifferent to sucrose vs. hexoses on a 20% diet and both are equally well assimilated (Lotz and Nicolson 1996). The maintenance diet and supplementary water were provided *ad libitum* in inverted, stoppered syringes. Body mass (mean ± SE) of the four males and four females was 9.09 ± 0.31 g and 7.68 ± 0.16 g respectively.

## *Experimental procedure*

The eight sunbirds were tested sequentially, since there was only one experimental cage (50 x 40 x 45 cm). Each sunbird was allowed one acclimation day on a 16% (0.50 M) sucrose diet before the measurements commenced. The experiment consisted of four treatments in randomized order, which lasted one day each. The first and last 0.5 h of the light phase were omitted from analysis because individuals differed in the timing of their first and last feeding event of the day (Köhler et al. 2006). The remaining feeding time (7h30–18h00) was divided into seven periods of 1.5 h each. During each treatment, a standard and test diet were presented for 1.5 h alternately throughout the day, commencing and ending with the standard diet of 16% sucrose. Test diet concentrations were 2.5, 8.5, 16 and 30% (0.08, 0.25, 0.50 and 0.99 M) sucrose (Table 1). Treatments are hereafter referred to by the sucrose concentration of the test diet, e.g. 2.5% treatment.

The 16% treatment served as a control since feeders were changed but sucrose concentration did not vary. This protocol enabled us to analyze the effect of daily patterns in feeding frequency and feeding duration previously recorded for this species (Köhler et al. 2006).

All experimental diets contained Ensure® to prevent mass loss on sugar-only diets (Nicolson and Fleming 2003b). The amount of Ensure® added was adjusted according to the sugar concentration of the diet, since the diet volumes ingested are inversely proportional to sugar concentration. If the carbohydrate component of Ensure® were completely assimilated by sunbirds, it would increase the highest sugar concentration (30%, which contained the most Ensure®) to only 31.7%, and is therefore negligible.

## *Data collection*

The experimental cage (Köhler et al. 2006) was constructed from Perspex. Automated recording of the time spent feeding as well as the number of feeding events was obtained by an infrared photodetection system interfaced to a computer. Feeding duration and frequency were measured simultaneously to show how rapidly birds change their feeding behaviour to compensate for the differences in sugar concentration.

The feeder was mounted on an electronic balance (Mettler Toledo PB-602S, 0.01 g, Microsep Ltd, Johannesburg) interfaced to the computer. The mass of the feeder was recorded every 0.5 s throughout the experiment to determine the mass of food consumed by the bird. Occasional drips were collected in a container with liquid paraffin (to avoid evaporative mass loss), which was also placed on the balance. Feeder dripping therefore did not affect the results.

The cage contained two perches (a feeding perch and a resting perch), both connected to electronic balances (identical to that for the feeder), interfaced to the computer. The body mass of the bird was recorded every 0.5 s. Since the cage was constructed from Perspex the bird was not able to cling to the sides, and flexible plastic bristles discouraged resting on the bottom of the cage. Holes for ventilation were placed below the plastic bristles and were therefore inaccessible to the birds.

### *Definitions and processing of feeding data*

For each bird we obtained the start and end times of every feeding event and calculated feeding event duration (± 0.001 s) as the time between insertion of the bill into the sucrose diet and its removal (Köhler et al. 2006). Each of the 1.5 h periods (n=7) was further divided into sub-periods of 5 min duration each (n=18 for each period). For all periods and sub-periods, we calculated the number of feeding events (feeding frequency), mean feeding duration, and total time spent feeding (total feeding duration) as the sum of the durations of all feeding events in the period or sub-period respectively. Although we recorded the feeder mass every 0.5 s, the minute amount of diet consumed in a single feeding event could not be detected, since the resolution of our balance was too coarse (0.01 g). Therefore, food intake of the bird (i.e. the mass of diet consumed) was calculated by subtracting the last feeder mass record of each period or sub-period from the first one. Daily sucrose intake was calculated from the food intake on the standard and test diets.

### *Definitions and processing of bird mass data*

Body mass recordings contained unreliable dynamic balance readings caused by movements of the bird. Therefore, the mean of at least three consecutive stable mass records was used for analysis (i.e. the bird had to remain stationary for at least 1.5 s). Mean body mass was calculated for each 1.5 h period. The percentage daily body mass increase was calculated as the difference in mean body mass between the first and last period of the day.

As an estimate of flight activity, the number of flights between the two perches in the cage was calculated for each sunbird and each period. A flight was defined and programmatically detected when the difference between the mass readings of the two balances in the cage changed sign (dynamic balance readings were used). Daily flight activity was calculated by summing the number of flights in each period.

### *Statistical procedures*

Data were tested for normality and homogeneity of variance (Levene's test). Log or square root transformations were used when data were heteroscedastic. Data obtained on the control treatment were subjected to repeated-measures ANOVA (RM-ANOVA) to test for a daily rhythm in feeding frequency, mean feeding duration, total feeding duration and food intake. The fact that most parameters varied over the course of the day (feeding frequency: $F_{6,42}=2.90$, $P=0.02$; mean feeding duration: $F_{6,42}=5.43$, $P<0.001$; total feeding duration: $F_{6,42}=1.33$, $P=0.26$; food intake: $F_{6,42}=3.66$, $P<0.01$) warranted the separate analysis of each of the seven periods. RM-ANOVA was therefore used to determine treatment effects for each parameter by comparing the same period on each of the four treatments. To detect how quickly the birds adjust their feeding behaviour and food intake after a diet change, feeding parameters for 5 min sub-periods of each 1.5 h period were analyzed by RM-ANOVA. RM-ANOVA were also conducted to test for differences in daily sucrose intake, daily body mass increase and daily flight activity between the four treatments.

*Post-hoc* comparisons for all RM-ANOVA were conducted with Tukey's Honest Significant Difference test for equal sample sizes, followed by a Bonferroni correction for multiple comparisons (Rice 1989). All data are presented as means ± SE. For all tests, the level of significance was $\alpha \leq 0.05$.

# Results

## *Differences between treatments*

Summarized RM-ANOVA results, comparing food intake, feeding frequency and mean feeding duration between the same periods on the four treatments are presented in Table 2. We demonstrated previously that food intake may be used as a surrogate for total feeding duration in *C. talatala* (Köhler et al. 2008). In the present study, results for total feeding duration also corresponded with those for food intake over all periods; results for total feeding duration were therefore omitted.

**Food intake:** The quantity of diet consumed by the birds increased with decreasing sugar concentration. Fig. 1A shows food intake on the four treatments, where the standard diet and a test diet (2.5, 8.5, 16 or 30%) were alternately presented for 1.5 h periods (n=7). Except for periods 2 and 4, when food intake was similar for the 2.5 and 8.5% diets ($P>0.25$), food intake differed significantly between the four test diets ($P<0.01$). When the birds were feeding on the standard diet in periods 1, 3 and 5, food intake did not differ between the treatments (Table 2). In the last period of the day, however, food intake on the standard diet was higher on the 2.5% treatment than on all other treatments ($P<0.03$).

**Feeding frequency:** There was a significant difference in feeding frequency between the test diets (Table 2; Fig. 1B), with birds having a higher feeding frequency on the 2.5% diet than on all other test diets (periods 2, 4 and 6: $P<0.02$). Feeding frequency tended to decrease with increasing sucrose concentration, but this was not significant between the 8.5, 16 and 30% diets in those periods ($P>0.07$). Birds fed at a lower feeding frequency on the standard diet on the 2.5% treatment than on the 30% treatment (period 3: $P<0.01$) or on the control and 8.5% treatment (period 7: $P<0.04$).

**Mean feeding duration:** There was no difference in mean feeding duration between the four test diets (periods 2, 4 and 6; Table 2; Fig. 1C). However, mean feeding duration was longer when the birds were offered the standard diet on the 2.5% treatment compared to all other treatments (periods 3, 5 and 7: $P<0.03$).

## *How fast do birds adjust feeding patterns and food intake?*

**2.5% treatment:** Birds started to increase the rate of feeding within 10 min after receiving the 2.5% diet (as shown for two individuals in Fig. 2A,B). When the diet was switched to 2.5%, birds significantly increased their feeding frequency ($F_{17,119}>2.00$, $P<0.02$) and food intake ($F_{17,119}>2.24$, $P<0.01$). *Post-hoc* analysis revealed that both feeding frequency and food intake were significantly higher after 75 min (period 2: $P<0.01$), after 45 min (period 4: $P<0.03$) and after 20 min (period 6: $P<0.03$) respectively when compared with the first 5 min sub-period after the concentration change. However, individual birds responded differently to the most dilute diet (2.5%): while most increased their food intake immediately, others probed the diet and then stopped feeding for a short while. These birds increased their feeding duration and feeding frequency immediately after the 2.5% diet was replaced by the standard diet and food consumption only decreased after several minutes (Fig. 2C,D). Thus, birds had a significantly higher feeding frequency ($F_{17,119}>2.34$, $P<0.01$) and higher food intake ($F_{17,119}>4.21$, $P<0.001$) in the first 5 min after the standard diet was returned on the 2.5% treatment (periods 3, 5 and 7: $P<0.03$).

**8.5% treatment:** Birds increased their feeding frequency on the 8.5% diet in period 4 ($F_{17,119}=3.15$, $P<0.01$) after 70 min ($P=0.02$) and therefore food intake was significantly increased ($F_{17,119}=3.21$, $P<0.001$) after 70 min ($P=0.02$) in the same period. When the birds were feeding on the 8.5% diet in periods 2 and 6, however, there was no significant change in number of feeding events ($F_{17,119}<1.28$, $P>0.21$) or food intake ($F_{17,119}<1.35$, $P>0.18$). Mean feeding duration did not change when birds received the 8.5% diet ($F_{17,119}<1.29$, $P>0.21$). Feeding frequency, mean feeding duration and food intake did not change on the standard diet on the 8.5% treatment ($F_{17,119}<1.53$, $P>0.09$).

**30% treatment:** There was no change in feeding frequency ($F_{17,119}$<1.57, $P$>0.08) or mean feeding duration ($F_{17,119}$<1.40, $P$>0.15) when the birds received the 30% diet. Food intake decreased significantly in period 2 ($F_{17,119}$=1.89, $P$=0.03) after 30 min ($P$<0.01), but did not change significantly when birds received the 30% diet in periods 4 and 6 ($F_{17,119}$<1.08, $P$>0.38). Feeding frequency, mean feeding duration and food intake did not change when birds were offered the standard diet on the 30% treatment ($F_{17,119}$<1.62, $P$>0.07).

**Control treatment:** On the 16% treatment, birds did not change their feeding frequency ($F_{17,119}$<1.45, $P$>0.13), mean feeding duration ($F_{17,119}$<1.08, $P$>0.38) or food intake ($F_{17,119}$<1.53, $P$>0.10) after a feeder change.

### *Sucrose intake, body mass and flight activity on the different treatments*

Daily sucrose intake differed between treatments ($F_{3,21}$=30.90, $P$<0.001), with birds consuming less sucrose on the 2.5% treatment than on all other treatments ($P$<0.001). Daily sucrose intake of the eight sunbirds (mean ± SE, g) was: 2.5% treatment: 1.38 ± 0.007; 8.5% treatment: 1.83 ± 0.011; control treatment: 1.85 ± 0.004; 30% treatment: 1.85 ± 0.007. Despite the differences in food intake, body mass increase over the day did not differ between treatments ($F_{3,21}$=0.56, $P$=0.65). Birds gained an average of 2.70 ± 0.50% of their initial body mass during the 11.5 h photophase.

Although there was no difference in daily flight activity of the birds between the different treatments ($F_{3,21}$=2.17, $P$=0.12), patterns were revealed when flight activity was assessed by time of day. Flight activity did not differ between the four treatments in the morning (periods 1 to 3: $F_{3,21}$<1.15, $P$>0.35), but did differ significantly in the afternoon and evening (periods 4 to 7: $F_{3,21}$>3.59, $P$<0.03). Birds showed higher flight activity while feeding on the 2.5% diet than on the control treatment (periods 4 and 6: $P$<0.04). Late in the day on the 2.5% treatment, birds flew less when offered the standard diet than on the standard diet of the control treatment (period 5: $P$=0.04). During the last period of the day, flight activity was lower on the 2.5% treatment than on all other treatments (period 7: *P*<0.001).

# Discussion

## *Differences between treatments*

Whitebellied sunbirds increase their food intake with decreasing sucrose concentration. Many nectarivorous birds similarly adjust food intake according to diet concentration, as shown for sunbirds (e.g. Lotz and Nicolson 1999), hummingbirds (e.g. López-Calleja et al. 1997) and honeyeaters (e.g. Collins et al. 1980). In our study, however, birds differed in their response to the 2.5% diet: when exposed to it for the first time, some individuals immediately increased their food intake, while others probed the diet and stopped feeding for a short while, appearing to dislike this very dilute diet. This resulted in a smaller food intake on the 2.5% diet compared with 8.5% upon first exposure to this experimental diet (period 2). The first response of some individuals is therefore to try to avoid the dilute diet, but with repeated exposure to the dilute diet they had to increase their food intake to avoid an energy deficit. Furthermore, on the 2.5% treatment, birds tried to compensate for reduced sugar intake on the 2.5% diet by increasing their intake of the standard diet as soon as it became available again.

The adjustment in food intake due to changes in diet concentration was predominantly caused by changes in feeding frequency, with more feeding events on the 2.5% diet than on all other test diets. This confirms earlier findings for whitebellied sunbirds (Köhler et al. 2006) and other avian nectarivores (Wolf and Hainsworth 1977; Gass 1978; López-Calleja et al. 1997). Mean feeding duration did not increase with decreasing sucrose concentration. Blue-throated hummingbirds (*Lampornis clemenciae*), on the other hand, have been shown to increase their meal size when their energy reserves are depleted (Hainsworth et al. 1981). Hummingbirds use the crop to store food but sunbirds do not have a crop and the volumetric intake at each feeding event might be limited (Mbatha et al. 2002). However, the increased food intake immediately after return of the standard diet on the 2.5% treatment was caused by longer mean feeding durations, indicating that the birds are indeed able to ingest relatively large meals and do so in order to maximize their food intake after feeding on a very low sugar concentration. This suggests that meal size of sunbirds in our study was not limited by the absence of a crop.

### *How fast do birds adjust feeding patterns and food intake?*

Following a decrease in sucrose concentration from the standard diet to the 2.5% diet, most birds started to increase their feeding frequency and food intake within 10 min, while others reduced their feeding for a short while. Feeding frequency and food intake gradually increased until a significant increase occurred after 75 min in the morning. The onset of a significant increase in feeding frequency and food intake on the test diet subsequently accelerated over the day (20 min in period 6). Following a decrease in sugar concentration from the standard diet to the 8.5% diet, birds significantly increased their feeding frequency and food intake in one period only, since the difference in sugar content between these two diets is smaller than between the 2.5% and the standard diet.

After an increase in sucrose concentration from the standard diet to the 30% diet, birds started to decrease their food intake within 10 min, although this was only statistically significant for period 2. When the sucrose concentration was increased from the 2.5% diet to the standard diet, birds increased their feeding duration and feeding frequency, and thus food intake, within the first 5 min after the switch. This shows that whitebellied sunbirds are able to adjust their feeding behaviour and food intake according to the sugar concentration of their diet within minutes.

The response of the sunbirds to a decrease in sucrose concentration was delayed compared to their response to an increase in concentration. Our findings correspond with the observation by Gass (1978), where a single rufous hummingbird showed a delayed response to a decrease in sugar concentration, while it adjusted its food intake within 10 min following an increase in concentration. It is of ecological importance for nectarivorous birds to adjust their food intake immediately when encountering high nectar sugar concentrations since this maximizes their energy gain. This is especially important if the birds previously fed on less concentrated nectar or if their energy reserves are depleted, such as when feeding has been interrupted by a storm (Carpenter and Hixon 1988). When birds face low sugar content of nectar, the increase in intake is also ecologically relevant, but birds may increase their food intake slowly at first and search for flowers that provide nectar with higher energy content.

Choice studies show that sunbirds and hummingbirds prefer more concentrated sugar solutions over dilute ones (Lotz and Nicolson 1996; Roberts 1996; Leseigneur 2008). The preference for more concentrated diets clearly had an influence on how fast the birds in our study adjusted their feeding patterns and food intake according to sucrose concentration. The lag in the response to the 2.5% diet, especially at first encounter, was caused by several individuals that disliked the most dilute diet and attempted to avoid it. Consequently, birds ingested more of the standard diet immediately after its return to compensate for their energy deficit. The adjustment of food intake according to sugar concentration and the preference for the moderate diet imply the ability of sunbirds to distinguish between sugar concentrations. To our knowledge, no studies investigating the taste sense of sunbirds are available to date. A study in whitebellied sunbirds, however, has shown that artificially increasing the viscosity of diets of low and moderate sugar concentrations leads to a decrease in energy intake rates (Leseigneur 2008).

### *Sucrose intake, body mass and flight activity on the different treatments*

Sunbirds were able to maintain a constant sugar intake on diets from 8.5–30% sucrose. This perfect compensatory feeding has been shown previously in whitebellied sunbirds (Nicolson and Fleming 2003b): on diets from 8.5–65% w/w sucrose they maintain a constant energy intake by adjusting their volumetric intake. However, when fed 2.5% or 3.5% sucrose over 24 h these sunbirds are not able to meet their energetic requirements and lose body mass (Nicolson and Fleming 2003b). The failure to maintain energy balance on low diet concentrations is also evident in frugivorous cedar waxwings (*Bombycilla cedrorum*), which reduce their volumetric intake on a 3.5% hexose diet (Levey and Martínez del Rio 1999). In our study, daily sucrose intake of the birds was reduced on the 2.5% treatment (even though they increased their food intake on both the 2.5% and standard diets) compared to all other treatments. That our sunbirds did not increase their food intake sufficiently on the 2.5% diet may reflect physiological constraints to nectar digestion. The processing of sucrose diets requires the hydrolysis of sucrose to glucose and fructose by the enzyme sucrase. Sucrose hydrolysis rates have been suggested as limiting (McWhorter and Martínez del Rio 2000). However, food intake has also been shown to be limited on hexose-only diets, indicating constraints to hexose absorption rates (Levey and Martínez del Rio 1999; Fleming et al. 2004b). In addition, the transit time for digesta decreases with decreasing dietary sugar concentration (Markman et al. 2006); thus the time available for sucrose hydrolysis and hexose absorption may be reduced on very dilute diets. Furthermore, birds may face constraints to osmoregulatory processes. When fed dilute diets, birds may be restricted by having to deal with very large volumes of preformed water (Fleming and Nicolson 2003), although Palestine sunbirds (*Cinnyris oseus*) consuming dilute diets are able to reduce the absorption of ingested water in the gut and thus the load on the kidneys (McWhorter et al. 2004). Avian nectarivores consuming dilute diets are also challenged by electrolyte and metabolite losses in the excreted fluid: the excreta produced by hummingbirds and sunbirds on electrolyte-free diets are extremely dilute but the loss of salts is still substantial (Lotz and Nicolson 1999; Fleming and Nicolson 2003; Lotz and Martínez del Rio 2004). Whitebellied sunbirds are able to maintain energy balance on very dilute sucrose solutions only when these diets are supplemented with electrolytes (C. Purchase, S.W. Nicolson and P.A. Fleming, unpublished data). Nectar-feeding birds also incur high energetic costs during the warming of large nectar volumes to body temperature: experiments on rufous hummingbirds have confirmed that the cost of warming nectar increases exponentially as nectar concentration decreases (Lotz et al. 2003).

It might have been favourable for the birds to adjust their behaviour in order to save energy on the 2.5% treatment, instead of ingesting even more of the diet. The number of flights per day did not differ between the 2.5% and control treatments but birds may have reduced hopping, vertical flights and flights with return to the same perch (A. Köhler, pers. observation) in order to save energy on the 2.5% treatment. Since we only calculated the number of flights between the two perches in the cage as a measure of flight activity, we were not able to quantify other activities or measure other energy-saving strategies that may have taken place. Behavioural adjustments to save energy on the 2.5% treatment are suggested since the birds ingested less sucrose but were able to maintain their body mass increase throughout the day. It is important that birds accumulate sufficient energy during the light period to sustain them during the night when they do not feed. Feeding frequency and flight activity on the standard diet on this 2.5% treatment were reduced. Birds may have saved energy by having less frequent but longer meals on the standard diet, instead of repeatedly visiting the feeder to consume smaller meals. The long feeding events immediately after the return of the standard diet led to an increase in body mass, which may have resulted in higher flight costs (DeBenedictis et al. 1978).

Behavioural and physiological adjustments that enable birds to maintain body mass increase over the day have been found in other nectarivorous birds. Honeyeaters and several hummingbird species reduce their flight activity under energetically challenging conditions to conserve energy (Beuchat et al. 1979; Collins and Morellini 1979; Hainsworth et al. 1981; Fernández et al. 2002). Hummingbirds also use torpor to save energy when exposed to energy stress (Hainsworth et al. 1977; Tooze and Gass 1985; Carpenter and Hixon 1988; Calder 1994). Besides avian nectarivores, zebra finches (*Taeniopygia guttata*) reduce energy expenditures when the energy content of their food is low (Wiersma and Verhulst 2005) and Pekin ducklings (*Anas platyrhyncos domesticus*) reduce their metabolic rate to save energy during phases of low food intake (Moe et al. 2005).

We conclude that sunbirds are able to adjust their feeding pattern and food intake immediately after a change in nectar concentration. Birds increased their food intake on a dilute diet and maintained their body mass increase over the day despite the lower daily sugar intake. Behavioural adjustments to conserve energy are therefore likely but could not be quantified in this study. Besides their ability to increase food intake on a dilute diet, sunbirds in the field probably look for flowers providing more concentrated nectar and would ingest very dilute nectar only if they could not find alternatives. The increase in flight activity on the 2.5% diet may reflect an increased number of trips to the feeder caused by the increase in the rate of feeding. Rufous hummingbirds are more active when energy availability is low because they spend more time foraging (Gass et al. 1999). In our study, however, the birds could have stayed on the feeding perch between meals to save energy. The increased flight activity may therefore represent a search for feeders providing more concentrated nectar. However, no equivalent field studies on wild sunbirds are available to date and future investigations are needed.

## Acknowledgements

This project was funded by the University of Pretoria, the South African National Research Foundation and the Australian Research Council. We are grateful to Jan Cilliers Park for permission to mist-net sunbirds under permit from the Gauteng Directorate of Nature Conservation. Our experiments were approved by the Animal Use and Care Committee of the University of Pretoria.

## References

Beuchat C.A., Chaplin S.B. and Morton M.L. 1979. Ambient temperature and the daily energetics of two species of hummingbirds, *Calypte anna* and *Selasphorus rufus*. Physiological Zoology 52: 280–295.

Calder W.A. 1994. When do hummingbirds use torpor in nature? Physiological Zoology 67: 1051–1076.

Carpenter F.L. and Hixon M.A. 1988. A new function for torpor: fat conservation in a wild migrant hummingbird. Condor 90: 373–378.

Collins B.G. and Cary G. 1980. Short-term regulation of food intake by the brown honeyeater, *Lichmera indistincta*. Comparative Biochemistry and Physiology A 68: 635–640.

Collins B.G. and Clow H. 1978. Feeding behaviour and energetics of the western spinebill, *Acanthorhynchus superciliosis* (Aves: Meliphagidae). Australian Journal of Zoology 26: 269–277.

Collins B.G. and Morellini P.C. 1979. The influence of nectar concentration and time of day upon energy intake and expenditure by the singing honeyeater, *Meliphaga virescens*. Physiological Zoology 52: 165–175.

Collins B.G., Cary G. and Packard G. 1980. Energy assimilation, expenditure and storage by the brown honeyeater, *Lichmera indistincta*. Journal of Comparative Physiology B 137: 157–163.

Daniels C.L. 1987. The feeding ecology of nectarivorous birds in the Natal Drakensberg. MSc Thesis, University of Natal, Pietermaritzburg, South Africa.

DeBenedictis P.A., Gill F.B., Hainsworth F.R., Pyke G.H. and Wolf L.L. 1978. Optimal meal size in hummingbirds. American Naturalist 112: 301–316.

Downs C.T. 1997. Sugar digestion efficiencies of Gurney's sugarbirds, malachite sunbirds, and black sunbirds. Physiological Zoology 70: 93–99.

Fernández M.J., López-Calleja M.V. and Bozinovic F. 2002. Interplay between the energetics of foraging and thermoregulatory costs in the green-backed firecrown hummingbird *Sephanoides sephanoides*. Journal of Zoology London 258: 319–326.

Fleming P.A. and Nicolson S.W. 2003. Osmoregulation in an avian nectarivore, the whitebellied sunbird *Nectarinia talatala*: response to extremes of diet concentration. Journal of Experimental Biology 206: 1845–1854.

Fleming P.A., Gray D.A. and Nicolson S.W. 2004a. Osmoregulatory response to acute diet change in an avian nectarivore: rapid rehydration following water shortage. Comparative Biochemistry and Physiology A 138: 321–326.

Fleming P.A., Hartman-Bakken B., Lotz C.N. and Nicolson S.W. 2004b. Concentration and temperature effects on sugar intake and preferences in a sunbird and a hummingbird. Functional Ecology 18: 223–232.

Gass C.L. 1978. Experimental studies of foraging in complex laboratory environments. American Zoologist 18: 729–738.

Gass C.L., Romich M.T. and Suarez R.K. 1999. Energetics of hummingbird foraging at low ambient temperature. Canadian Journal of Zoology 77: 314–320.

Hainsworth F.R., Collins B.G. and Wolf L.L. 1977. The function of torpor in hummingbirds. Journal of Experimental Zoology 195: 215–222.

Hainsworth F.R., Tardiff M.F. and Wolf L.L. 1981. Proportional control for daily energy regulation in hummingbirds. Physiological Zoology 54: 452–462.

Köhler A., Verburgt L. and Nicolson S.W. 2006. Short-term feeding patterns of whitebellied sunbirds (*Nectarinia talatala*): feeding frequency, daily rhythms and individual differences. Journal of Experimental Biology 209: 2880–2887.

Köhler A., Verburgt L. and Nicolson S.W. 2008. Nectar intake of whitebellied sunbirds (*Cinnyris talatala*): Can meal size be inferred from feeding duration? Physiological and Biochemical Zoology 81: 682–687.

Leseigneur C.D.C. 2008. The feeding response of whitebellied sunbirds (*Cinnyris talatala*) to sugar concentration and viscosity of artificial nectar. MSc thesis, University of Pretoria, Pretoria, South Africa.

Levey D.J. and Martínez del Rio C. 1999. Test, rejection, and reformulation of a chemical reactor-based model of gut function in a fruit-eating bird. Physiological and Biochemical Zoology 72: 369–383.

López-Calleja M.V., Bozinovic F. and Martínez del Rio C. 1997. Effects of sugar concentration on hummingbird feeding and energy use. Comparative Biochemistry and Physiology A 118: 1291–1299.

Lotz C.N. and Martínez del Rio C. 2004. The ability of rufous hummingbirds *Selasphorus rufus* to dilute and concentrate urine. Journal of Avian Biology 35: 54–62.

Lotz C.N. and Nicolson S.W. 1996. Sugar preferences of a nectarivorous passerine bird, the Lesser Double-collared Sunbird (*Nectarinia chalybea*). Functional Ecology 10: 360–365.

Lotz C.N. and Nicolson S.W. 1999. Energy and water balance in the lesser double-collared sunbird (*Nectarinia chalybea*) feeding on different nectar concentrations. Journal of Comparative Physiology B 169: 200–206.

Lotz C.N., Martínez del Rio C. and Nicolson S.W. 2003. Hummingbirds pay a high cost for a warm drink. Journal of Comparative Physiology B 173: 455–462.

Markman S., Tadmor-Melamed H., Arieli A. and Izhaki I. 2006. Sex differences in food intake and digestive constraints in a nectarivorous bird. Journal of Experimental Biology 209: 1058–1063.

Martínez del Rio C., Schondube J.E., McWhorter T.J. and Herrera L.G. 2001. Intake responses in nectar feeding birds: digestive and metabolic causes, osmoregulatory consequences, and coevolutionary effects. American Zoologist 41: 902–915.

Mbatha K., Downs C.T. and Penning M. 2002. Nectar passage and gut morphology in the Malachite Sunbird and the Black-capped Lory: implications for feeding in nectarivores. Ostrich 73: 138–142.

McWhorter T.J. and Martínez del Rio C. 1999. Food ingestion and water turnover in hummingbirds: how much dietary water is absorbed? Journal of Experimental Biology 202: 2851–2858.

McWhorter T.J. and Martínez del Rio C. 2000. Does gut function limit hummingbird food intake? Physiological and Biochemical Zoology 73: 313–324.

McWhorter T.J., Martínez del Rio C., Pinshow B. and Roxburgh L. 2004. Renal function in Palestine sunbirds: elimination of excess water does not constrain energy intake. Journal of Experimental Biology 207: 3391–3398.

McWhorter T.J., Hartman Bakken B., Karasov W.H. and Martínez del Rio C. 2006. Hummingbirds rely on both paracellular and carrier-mediated intestinal glucose absorption to fuel high metabolism. Biology Letters 2: 131–134.

Moe B., Stølevik E. and Bech C. 2005. Ducklings exhibit substantial energy-saving mechanisms as a response to short-term food shortage. Physiological and Biochemical Zoology 78: 90–104.

Nicolson S.W. 2002. Pollination by passerine birds: why are the nectars so dilute? Comparative Biochemistry and Physiology B 131: 645–652.

Nicolson S.W. and Fleming P.A. 2003a. Nectar as food for birds: the physiological consequences of drinking dilute sugar solutions. Plant Systematics and Evolution 238: 139–153.

Nicolson S.W. and Fleming P.A. 2003b. Energy balance in the Whitebellied Sunbird *Nectarinia talatala*: constraints on compensatory feeding, and consumption of supplementary water. Functional Ecology 17: 3–9.

Pyke G.H. and Waser N.M. 1981. The production of dilute nectars by hummingbird and honeyeater flowers. Biotropica 13: 260–270.

Rice W.R. 1989. Analyzing tables of statistical tests. Evolution 43: 223–225.

Roberts W.M. 1996. Hummingbirds’ nectar concentration preferences at low volume: the importance of time scale. Animal Behaviour 52: 361–370.

Stiles F.G. and Freeman C.E. 1993. Patterns in floral nectar characteristics of some bird-visited plant species from Costa Rica. Biotropica 25: 191–205.

Tooze Z.J. and Gass C.L. 1985. Responses of rufous hummingbirds to midday fasts. Canadian Journal of Zoology 63: 2249–2253.

Van Tets I.G. and Nicolson S.W. 2000. Pollen and the nitrogen requirements of the lesser double-collared sunbird. Auk 117: 826–830.

Wiersma P. and Verhulst S. 2005. Effects of intake rate on energy expenditure, somatic repair and reproduction of zebra finches. Journal of Experimental Biology 208: 4091–4098.

Wolf L.L. and Hainsworth F.R. 1977. Temporal patterning of feeding by hummingbirds. Animal Behaviour 25: 976–989.

# Tables

**Table 1.** Experimental design for subjecting whitebellied sunbirds to acute changes in sucrose concentration. On four consecutive days (treatments) in randomized order, a standard diet (16% w/w) and one test diet (2.5, 8.5, 16 or 30% w/w) were presented alternately for 1.5 h periods at a time. Light:dark phase was 11.5:12.5 h with lights on at 07h00. The first and last 0.5 h of the light phase, with birds feeding on the standard diet, was omitted from analysis (see text for explanation). Starting time of each period is given in parentheses.

| | Period | | | | | | |
|---|---|---|---|---|---|---|---|
| Treatment | 1 (7h30) | 2 (9h00) | 3 (10h30) | 4 (12h00) | 5 (13h30) | 6 (15h00) | 7 (16h30) |
| 2.5% | 16% | 2.50% | 16% | 2.50% | 16% | 2.50% | 16% |
| 8.5% | 16% | 8.50% | 16% | 8.50% | 16% | 8.50% | 16% |
| 16% (control) | 16% | 16% | 16% | 16% | 16% | 16% | 16% |
| 30% | 16% | 30% | 16% | 30% | 16% | 30% | 16% |

**Table 2.** Differences in food intake, feeding frequency and mean feeding duration between the four experimental treatments, where birds were presented alternately with a standard diet (16% w/w, grey background) and test diet (2.5, 8.5, 16 or 30% w/w, white background) for 1.5 h periods at a time. RM-ANOVA results for eight birds are given for each period. Degrees of freedom for all *F*-values were 3,21; significant results are highlighted in bold.

| | Food intake | | Feeding frequency | | Mean feeding duration | |
|---|---|---|---|---|---|---|
| Period | *F* | *P* | *F* | *P* | *F* | *P* |
| 1 | 1.32 | 0.30 | 1.98 | 0.15 | 2.53 | 0.09 |
| 2 | 29.08 | **<0.001** | 11.12 | **<0.001** | 0.80 | 0.51 |
| 3 | 0.26 | 0.85 | 4.93 | **<0.01** | 6.63 | **<0.01** |
| 4 | 78.69 | **<0.001** | 15.22 | **<0.001** | 0.37 | 0.77 |
| 5 | 2.77 | 0.07 | 3.69 | **0.03** | 6.64 | **<0.01** |
| 6 | 106.02 | **<0.001** | 37.73 | **<0.001** | 2.07 | 0.14 |
| 7 | 12.02 | **<0.001** | 4.43 | **0.02** | 13.29 | **<0.001** |

# Figure legends

**Fig. 1.** Food intake (g; A), number of feeding events (B) and mean feeding duration (s; C) per 1.5 h period for eight sunbirds (mean ± SE). The birds fed alternately on a standard diet of 16% (grey background) and test diets of 2.5, 8.5, 16 or 30% w/w sucrose (white background) over four consecutive days (treatments; see Table 1). Each feeding parameter was compared by RM-ANOVA for each period separately, followed by *post-hoc* comparison. Significant differences ($P \leq 0.05$) are indicated by different letters; correspondence of at least one letter indicates no significant difference.

**Fig. 2.** Cumulative feeding duration (s) as a function of time (min) for four individuals. Each graph shows 15 min before and after a concentration change from 16% to 2.5% w/w sucrose (A,B) or from 2.5% to 16% w/w sucrose (C,D). N=the number of feeding events per 15 min. Graphical presentation of the switches from 8.5 to 16% and from 30 to 16% w/w sucrose were omitted since there were no clear changes in cumulative feeding duration.

# Figures

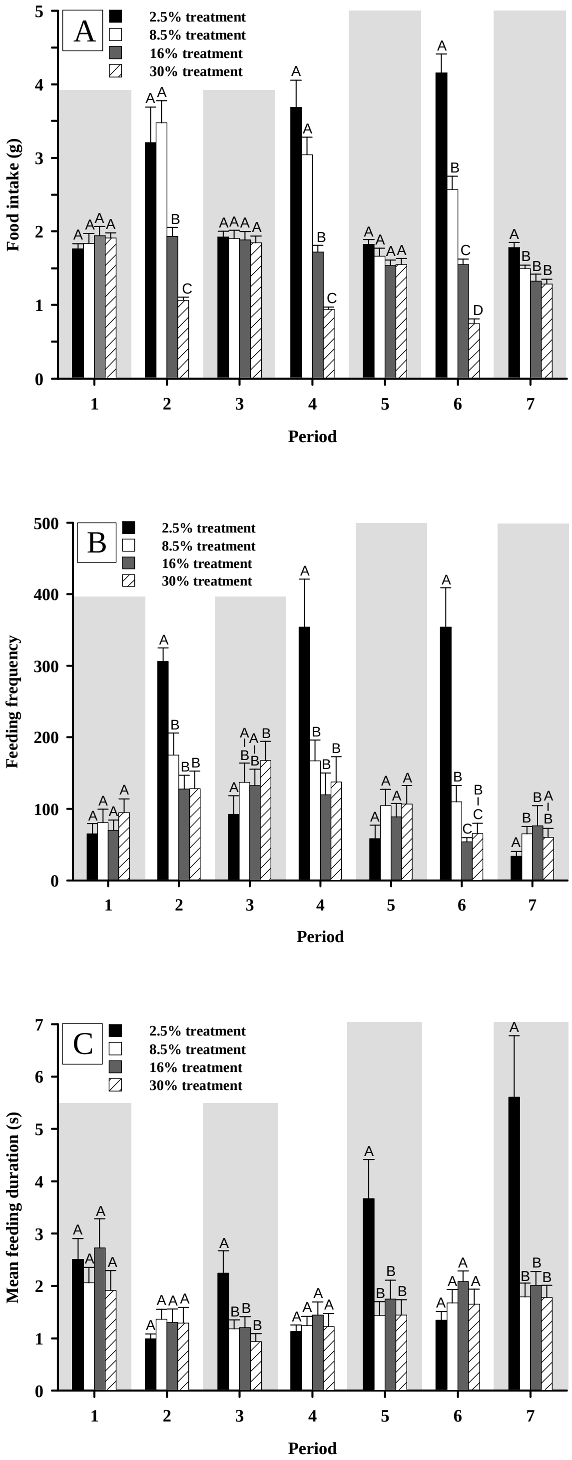


Figure 1.

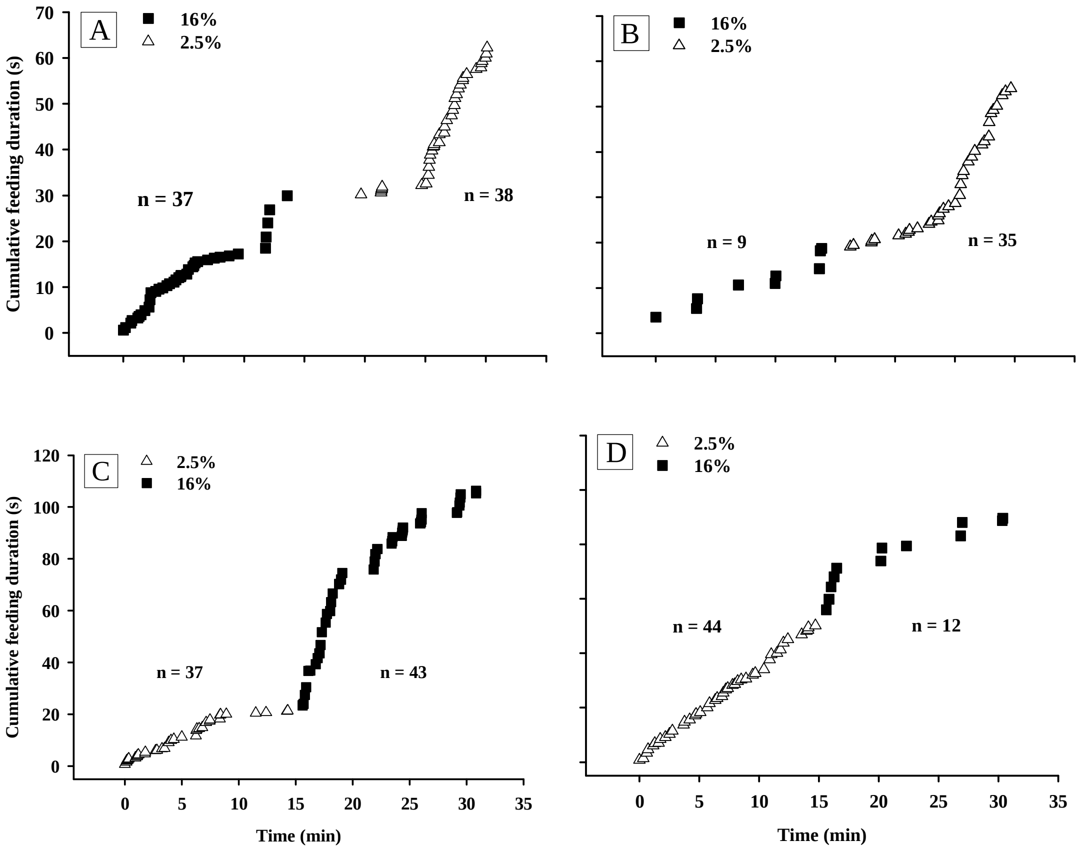

A
■ 16%
△ 2.5%
n = 37
n = 38
B
■ 16%
△ 2.5%
n = 9
n = 35
C
△ 2.5%
■ 16%
n = 37
n = 43
D
△ 2.5%
■ 16%
n = 44
n = 12
Cumulative feeding duration (s)
0
10
20
30
40
50
60
70
0
20
40
60
80
100
120
0
5
10
15
20
25
30
35
Time (min)

Figure 2.

# CHAPTER 3

# THE RESPONSE OF TWO AVIAN NECTARIVORES TO INTERRUPTIONS IN FOOD AVAILABILITY

**Angela Köhler[†]*, Luke Verburgt[†], Patricia A. Fleming[‡], Todd J. McWhorter[‡‡] and Susan W. Nicolson[†]**

[†] Department of Zoology and Entomology, University of Pretoria, Pretoria 0002, South Africa
[‡] School of Veterinary and Biomedical Sciences, Murdoch University, Murdoch WA 6150, Australia
[‡‡] School of Veterinary Science, University of Adelaide, Adelaide SA 5371, Australia

*Corresponding author, e-mail: akoehler@zoology.up.ac.za.

# Abstract

Nectarivorous birds are generally small, have high metabolic rates and have to feed frequently to meet high energy requirements. Their energy balance is therefore likely to be adversely affected by interruptions in foraging opportunities, as caused by unfavourable weather conditions for instance. To investigate how nectarivorous birds cope with a loss in foraging time, we exposed captive whitebellied sunbirds (*Cinnyris talatala*) and brown honeyeaters (*Lichmera indistincta*) to a 2 h fasting period during the day, while birds were kept at 10°C and fed a 20% w/w sucrose solution. Both species increased their food intake significantly following the fasting period, relative to uninterrupted feeding. Although the short-term feeding pattern of honeyeaters was not investigated, whitebellied sunbirds increased their food intake by increasing the length of meals immediately after the fast. Thereafter, sunbirds returned to having shorter meals during the remaining afternoon and appeared to compensate for the fasting period by increasing the number of meals, although this was not significant. In contrast to published data for hummingbirds, these two passerines accumulated energy at a higher rate after the fast compared to a control day. However, food intake over the whole day was lower on the fasting day and birds weighed less in the evening following the fast compared to the control, indicating that the compensation of energy intake and accumulation was incomplete. Although unrelated, sunbirds and honeyeaters show convergence in behavioural responses to energetic challenge, perhaps due to their similar nectar-feeding lifestyle.

# Introduction

The availability and quality of food, required for animal metabolism and activity, fluctuates both in time and space in natural habitats (e.g. Collins et al. 1990; Miles 1990; Rathcke 1992). During periods of food deprivation, animals rely on internal stores of energy to fuel their metabolism. Small-sized nectar-feeding vertebrates need to feed frequently to maintain high metabolic rates. Actively feeding nectarivorous bats and hummingbirds, for instance, use recently ingested sugars to fuel up to 95% of their metabolism, thus using essentially no stored fat (Carleton et al. 2006; Voigt and Speakman 2007; Welch and Suarez 2007; Welch et al. 2007). Avian nectarivores accumulate energy at constant rates throughout the day when food is available *ad libitum* (Wolf and Hainsworth 1977; Collins and Morellini 1979; Collins et al. 1980; Köhler et al. 2006). They feed steadily during the morning, followed by a reduced intake in the afternoon and then an increase at the end of the day to sustain the birds at night when they do not feed (e.g. Fleming et al. 2004; Köhler et al. 2006). This daily feeding pattern in small birds is greatly influenced by environmental variability (Bednekoff and Houston 1994).

Interruptions to feeding may occur during migration and reproduction and may be caused by predators, competitors or unfavourable weather conditions, such as storms and heavy rain (e.g. Glück 1987; Carpenter and Hixon 1988; Robin et al. 1988; Moore and Yong 1991). The loss of foraging time can lead to depletion of energy stores and therefore affects the energy balance of small birds. When the feeding of a free-living rufous hummingbird (*Selasphorus rufus*) was interrupted by a storm, it lost body mass but replenished its energy reserves within one day after the weather improved and feeding recommenced (Carpenter and Hixon 1988). Tooze and Gass (1985) exposed captive rufous hummingbirds to a 2 h midday fast and found that they were unable to increase their food intake to compensate for the fast, weighing less in the evening than on a day without a fasting period. These birds used torpor during the fast and the night following the fast to attempt to cope with the energy deficit. Wild broadtailed hummingbirds (*S. platycercus*) also utilize torpor to compensate for low nectar intake during storms (Calder 1994). Whitebellied sunbirds (*Cinnyris talatala*), on the other hand, increase their rate of food intake following a midday fast and their evening body mass is the same as on a day without fasting period (Nicolson et al. 2005). However, their morning body mass on the following day is lower than on previous days, indicating that the compensation for the fasting period is incomplete (Nicolson et al. 2005). When foraging of a wild incubating orange-breasted sunbird (*Anthobaphes violacea*) was interrupted by a storm a reduction in body temperature has been observed (Williams 1993). Brown honeyeaters (*Lichmera indistincta*) and singing honeyeaters (*Meliphaga virescens*) also drop their body temperature at night to conserve energy when deprived of food for 1–2 h at the end of the day (Collins and Briffa 1984).

In order to compare two major families of avian nectarivores in their response to energetic challenges, we exposed brown honeyeaters and whitebellied sunbirds, both passerines and of similar size, to a 2 h midday fast. We recorded the food intake and body mass of brown honeyeaters hourly. For whitebellied sunbirds, we aimed to determine the mechanism of short-term feeding adjustment after the fast and provide data at fine temporal resolution. We therefore recorded their feeding events (number and duration), food intake and body mass continuously throughout the experiment. Do whitebellied sunbirds ingest larger meals and/or feed more often? The mechanism by which sunbirds adjust their food intake after the fast has not yet been investigated, since in the previous study of these birds food intake was recorded hourly by weighing feeders (Nicolson et al. 2005). We predicted that both whitebellied sunbirds and brown honeyeaters would be able to adjust their food intake to compensate for the fasting period.

## Materials and methods

### Study animals and their maintenance

Eight whitebellied sunbirds were mist-netted at Jan Cilliers Park, Pretoria, South Africa, and eight brown honeyeaters on the Murdoch University campus, Perth, Western Australia. Birds were housed in individual cages at 20 ± 2ºC and a 12:12 h L:D photoperiod with lights-on at 07h00. The maintenance diet for sunbirds consisted of a 20% w/w sucrose solution (0.63 M) with a nutritional supplement (Ensure®, Abbott Laboratories, Johannesburg, South Africa) to provide dietary nitrogen (Van Tets and

Nicolson 2000). Brown honeyeaters were fed a maintenance diet consisting of commercially available honeyeater and lorikeet nectar (Wombaroo® Food products, Adelaide, South Australia), which contains sucrose as main sugar type, supplemented with additional sucrose for a total content of 25% w/w. The nectar substitute and supplementary water were provided *ad libitum* in inverted, stoppered syringes. Body mass (mean ± SE) of the four male and four female whitebellied sunbirds was 9.19 ± 0.19 g and 7.67 ± 0.23 g respectively. The sexes of brown honeyeaters could not be distinguished; body mass (mean ± SE) of the eight individuals was 10.10 ± 0.42 g.

## *Experimental procedure and processing of data*

Each bird was moved to an experimental cage and acclimated for one day to the experimental temperature of 10°C and a 20% w/w (0.63 M) sucrose-only solution. This acclimation day was followed by one control day with uninterrupted feeding, defined as CONTROL. On the second day feeding was interrupted for 2 h (10h00–12h00) by turning off the lights, which is defined as FAST. The interval from 10h00–12h00 was chosen for the fast as the food intake rate of whitebellied sunbirds is most stable in the morning until 13h00 (Köhler et al. 2006). Brown honeyeaters also feed most frequently during the morning (Collins and Briffa 1983). Our protocol was similar to those of Tooze and Gass (1985) and Nicolson et al. (2005). The order of CONTROL and FAST was not randomized, as the fasting period may lead to an energy deficit that may affect the feeding behaviour of the birds on the following day.

Four brown honeyeaters were tested simultaneously in experimental Perspex cages, where the only perch available was suspended from a balance (Scout Pro SP 402, 0.01 g, Ohaus Corp., Pine Brook, NJ USA). The body mass was recorded manually every hour during the photophase. The feeder was placed on the outside of the cage, allowing for hourly weighing of the syringe with minimal disturbance to the honeyeaters. Dripping solution was collected in a tray with liquid paraffin (to avoid evaporative mass loss), which was similarly weighed hourly and food intake was corrected accordingly.

The eight sunbirds were tested sequentially, since there was only one experimental cage (Köhler et al. 2006). Automated recording of the time spent feeding as well as the number of feeding events was obtained by an infrared photodetection system interfaced to a computer. For each sunbird we obtained the start and end times of every feeding event and calculated feeding duration (± 0.001 s) as the time between insertion of the bill into the sucrose solution and its removal (Köhler et al. 2006). The feeder was mounted on an electronic balance (Mettler Toledo PB-602S, 0.01 g, Microsep Ltd, Johannesburg, South Africa) interfaced to the computer. Although the mass of the feeder was recorded every 0.5 s throughout the experiment, the minute amount of solution consumed in a single feeding event could not be detected, since the resolution of our balance was too coarse (0.01 g). Therefore, food intake of the sunbird (i.e. the mass of solution consumed) was calculated for each hour by subtracting the last feeder mass record of each hour from the first one. Occasional drips were collected in a container with liquid paraffin, which was also placed on the balance. Feeder dripping therefore did not affect food intake measurements. The cage contained two perches (a feeding perch and a resting perch), both connected to electronic balances (identical to that for the feeder), interfaced to the computer. The body mass of the sunbird was recorded every 0.5 s. Since the cage was constructed from Perspex the bird was not able to cling to the sides, and flexible plastic bristles discouraged resting on the bottom of the cage. Ventilation holes were situated below the plastic bristles so that birds could not cling to them. Mean body mass of each sunbird was calculated for each hour, using stable balance readings only (dynamic balance readings, caused by movements of the bird, were filtered automatically).

We calculated (a) mean feeding duration and (b) number of feeding events of whitebellied sunbirds for 0.5 h time intervals to determine whether sunbirds adjust the duration and/or number of feeding events to compensate for the fast. For both whitebellied sunbirds and brown honeyeaters, we calculated mass-specific food intake rates (mg•h$^{-1}$•g body mass$^{-1}$) for CONTROL and FAST. For further analysis we calculated (c) mass-specific food intake (mg•g body mass$^{-1}$) for the morning (07h00–10h00) and afternoon (12h00–19h00), referred to as morning and afternoon food intake;

(d) mass-specific afternoon (12h00–19h00) food intake rate (mg•h$^{-1}$•g body mass$^{-1}$), referred to as afternoon food intake rate; and (e) mass-specific food intake of the whole day (07h00–19h00; mg•g body mass$^{-1}$), referred to as daily consumption.

## *Statistical analysis*

All data were tested for normality (Kolmogorov-Smirnov) and homogeneity of variance (Levene's test). Log transformation was used when data were heteroscedastic. Repeated-measures ANOVA (RM-ANOVA) was conducted to compare the control day (CONTROL) and the day with interrupted feeding (FAST) in terms of (c) morning and afternoon food intake, (d) afternoon food intake rate, (e) daily consumption and (f) morning (07h00) and evening (19h00) body mass. For each bird we calculated (g) rate of mass gain (slope of mass vs. time regression) during the afternoon (12h00–19h00) and compared these slopes between CONTROL and FAST by RM-ANOVA. For the above RM-ANOVA analyses, species was the categorical predictor (between-effects) (c–g) and treatment and time (c, d, f) or treatment only (e, g) were used as within-effects. Initially, separate RM-ANOVA for each species were performed, which yielded very similar results to the combined analysis and the results from the latter are therefore presented. For whitebellied sunbirds, mean feeding duration (a) and number of feeding events (b) for 0.5 h intervals were subjected to RM-ANOVA, with treatment and time being within-effects. *Post-hoc* comparisons for all RM-ANOVA were conducted with Tukey's Honest Significant Difference test for equal sample sizes, followed by a Bonferroni correction for multiple comparisons. All data are presented as mean ± SE. For all tests, the level of significance was $\alpha \leq 0.05$.

# Results

## *Food intake*

Mass-specific food intake rates (mg•$h^{-1}$•g body $mass^{-1}$) of whitebellied sunbirds and brown honeyeaters for CONTROL and FAST are shown in Fig. 1. Sunbirds and honeyeaters, which have a similar body mass, did not differ in (c) their morning (07h00–10h00) and afternoon (12h00–19h00) food intake (mg•g body $mass^{-1}$; $F_{1,14}$=0.33, $P$=0.58), (d) their afternoon (12h00–19h00) food intake rate (mg•$h^{-1}$•g body $mass^{-1}$; $F_{1,14}$=0.83, $P$=0.38) and (e) their daily consumption (07h00–19h00; mg•g body $mass^{-1}$; $F_{1,14}$=0.31, $P$=0.59). Morning and afternoon food intake (c) of both species differed between treatments (CONTROL vs. FAST) ($F_{1,14}$=60.94, $P$<0.001) and with time of day ($F_{1,14}$=276.72, $P$<0.001). *Post-hoc* analysis revealed that morning food intake of both species did not differ between CONTROL and FAST (sunbirds: $P$=0.99; honeyeaters: $P$=1.00). Both sunbirds and honeyeaters increased their food intake after the 2 h fasting period, resulting in a significantly higher afternoon food intake on FAST than on CONTROL (sunbirds and honeyeaters: $P$<0.001). Sunbirds consumed 19.21 ± 2.90% more sugar solution during the afternoon of FAST than of CONTROL; honeyeaters drank 13.39 ± 3.09% more. The afternoon food intake rate (d) of both species differed between CONTROL and FAST ($F_{1,14}$=63.97, $P$<0.001) and between hours ($F_{6,84}$=12.99, $P$<0.001). *Post-hoc* analysis confirmed that the sunbirds consumed significantly more sugar solution in the second hour following the fast (13h00–14h00) than at the same time on CONTROL ($P$=0.01; Fig. 1). Food intake rate for the remaining afternoon tended to be higher on FAST than on CONTROL, but did not differ significantly ($P$>0.05). Brown honeyeaters, on the other hand, showed a higher food intake rate in the first hour after the fast compared to CONTROL ($P$<0.001; Fig. 1). Food intake rate of the honeyeaters did not differ between CONTROL and FAST in other time intervals of the afternoon ($P$>0.99). Daily consumption (e) differed between CONTROL and FAST ($F_{1,14}$=49.93, $P$<0.001), with sunbirds consuming 7.81 ± 1.74% less and honeyeaters 11.21 ± 1.91% less on FAST than on CONTROL (sunbirds: $P$<0.01; honeyeaters: $P$<0.001).

## *Adjustment of feeding behaviour in sunbirds*

The mean feeding duration (a) of whitebellied sunbirds did not differ between treatments ($F_{1,7}$=0.71, $P$=0.43), but differed between 0.5 h time intervals ($F_{19,133}$=6.73, $P$<0.001). *Post-hoc* analysis revealed that the mean feeding duration of sunbirds was higher in the first 0.5 h after the fast (12h00–12h30) than at the same time on CONTROL ($P$=0.01; Fig. 2A), while the remaining 0.5 h time intervals did not differ between the treatments ($P$>0.99). The number of feeding events (b) also did not differ between CONTROL and FAST ($F_{1,7}$=1.71, $P$=0.23), but differed between 0.5 h time intervals ($F_{19,133}$=2.69, $P$<0.001). However, none of the relevant 0.5 h time intervals in the afternoon differed significantly between treatments ($P$>0.95), although sunbirds appeared to feed more often in most of the afternoon intervals of FAST than of CONTROL (Fig. 2B).

## *Body mass*

Body mass (f) of both sunbirds and honeyeaters differed between CONTROL and FAST ($F_{1,14}$=16.85, $P$<0.01) and with time of day ($F_{1,14}$=106.29, $P$<0.001). As expected there was no difference in morning body mass (07h00) of both species between CONTROL and FAST (sunbirds: $P$=0.99; honeyeaters: $P$=0.11), although some birds tended to weigh less at the beginning of FAST than of CONTROL since the experimental diet was not supplemented with protein. Both sunbirds and honeyeaters lost body mass during the 2 h fasting period (Fig. 3) and weighed less in the evening of FAST than of CONTROL (sunbirds: $P$=0.02; honeyeaters: $P$<0.01).

Whitebellied sunbirds and brown honeyeaters accumulated body mass at a steady rate throughout the 12 h light period of CONTROL (Fig. 3): sunbirds increased their body mass by 7.93 ± 0.78%, whereas honeyeaters showed a slightly lower increase in mass of 5.82 ± 1.36%. Birds accumulated less mass over the entire FAST than on CONTROL, with sunbirds gaining 6.44 ± 0.78% of their morning body mass and brown honeyeaters gaining 4.71 ± 0.87%. The rates of body mass gain in the afternoon (g; 12h00–19h00) did not differ between the two species ($F_{1,14}$=1.07, $P$=0.32), but differed between the treatments ($F_{1,14}$=38.60, $P$<0.001; Fig. 3), being greater on FAST than on CONTROL (sunbirds: $P$=0.01; honeyeaters: $P$<0.001).

# Discussion

## *Adjustment of food intake after the fast*

Small nectarivorous birds must feed frequently due to high energy requirements and food deprivation is therefore likely to affect their energy balance. Can short-term adjustment of food intake lead to an increased energy accumulation rate after a period of food deprivation? In the present study, both whitebellied sunbirds and brown honeyeaters demonstrated an immediate response at the end of an imposed fasting period. They increased their food intake within the first hour after the fast and accelerated their rate of energy accumulation. The body mass of the birds increased at a higher rate in the 7 h after the fasting period compared to a control day. This confirms earlier findings for whitebellied sunbirds (Nicolson et al. 2005), and shows the same pattern in the brown honeyeater. Besides avian nectarivores, nectar-feeding bats (*Glossophaga longirostris*) are also able to accelerate their rate of feeding and body mass increase when exposed to short nights, i.e. limited time for feeding (Winter 1998). The compensation for fasting periods via an increase in food intake has been demonstrated in various animal taxa, including insects, fish, birds and mammals (McLean and Kinsey 1969; Tempel et al. 1989; Zubair and Leeson 1996; Qian et al. 2000).

Unlike sunbirds and honeyeaters in the present study, hummingbirds are apparently unable to adjust their food intake according to changing energy reserves within one day. Rufous hummingbirds do not increase their food intake after a 2 h fast, resulting in energy deficits at the end of the day (Tooze and Gass 1985). Hainsworth et al. (1981) hypothesized that hummingbirds do not monitor their energy reserves during the day, but rather set the regulation of food intake by the extent of the energy deficit at the beginning of a day and then maintain constant energy accumulation rates. These authors found that magnificent (*Eugenes fulgens*) and blue-throated (*Lampornis clemenciae*) hummingbirds had an energy deficit in the evenings after 4–5 h fasting periods on successive days. Continuous access to food for 8 h following the fast was not sufficient for these birds to compensate for their energy deficit. They adjusted their food intake only in the longer term, leading to a progressive increase in energy accumulation on successive fasting days (Hainsworth et al. 1981; Hainsworth 1983). The immediate adjustment of food intake after the fast that we found in the present study demonstrates that both sunbirds and honeyeaters are able to monitor their energy reserves continuously during the day and estimate required energy accumulation rates. However, the physiological mechanisms underlying this process are unknown.

### *Does the fast lead to an energy deficit?*

The loss of 2 h foraging time led to a lower daily consumption, with sunbirds ingesting 8% less, and honeyeaters 11% less, sugar solution over the entire fasting day compared to the control day. Even though both species increased their food intake after the fast, they did not perfectly compensate for the loss in foraging time. To avoid an energy deficit at the end of the day, birds could have exhibited behavioural or physiological energy-saving mechanisms, such as reduced activity or reduced metabolic rate (for a review see Wang et al. 2006). It is not likely, however, that our birds saved adequate energy in such a way because their body mass was lower in the evening after the fast than in the evening of the control day, despite the increased energy accumulation rate after the fast.

In contrast to our findings, rufous hummingbirds became torpid during a comparable 2 h fast and during the following night (Tooze and Gass 1985). Hummingbirds have been shown to use torpor at a minimum threshold of energy reserves (Hainsworth et al. 1977). Whitebellied sunbirds and brown honeyeaters are about three times the size of rufous hummingbirds and therefore have a lower mass-specific metabolic rate and their capacity to store energy may be larger; the use of torpor during and after the 2 h fasting period may therefore be unnecessary. However, reduction in body temperature as a response to energy stress does occur in several species of hummingbirds, honeyeaters and sunbirds (Collins and Briffa 1984; Williams 1993; for review see McKechnie and Lovegrove 2002). Other avian taxa and mammals also decrease their body temperature or use torpor to save energy when deprived of food (Lovegrove et al. 2001; McKechnie and Lovegrove 2003; Ehrhardt et al. 2005; Moe et al. 2005; also see Wang et al. 2006 for review). Among nectarivorous bats, *Glossophaga soricina* becomes torpid during the day at low to moderate ambient temperatures (Cruz-Neto and Abe 1997) and Queensland blossom bats (*Syconycteris australis*) frequently enter daily torpor when deprived of food and water (Coburn and Geiser 1998).

The lower body mass of our birds in the evening after the fast confirms the earlier study by Nicolson et al. (2005): sunbirds are lighter on a day when their feeding has been interrupted, which was suggested in the earlier dataset (Nicolson et al. 2005: $P$=0.07) and is now confirmed statistically (present study: $P$=0.02). The difference in statistical significance might be due to the different methods used to obtain body mass data. In the earlier study, feeding events or excretions immediately before weighing could not be accounted for. Both sunbirds and honeyeaters ended the fasting day with a lower body mass which indicates that the compensation of food intake was incomplete. This may be due to physiological limitations, as discussed below.

## *Physiological constraints to food intake*

The behavioural response of our birds to the fasting period may have been influenced by constraints on nectar ingestion and digestion, as well as osmoregulatory processes, resulting in limitations on food intake and thus energy balance. Nectar ingestion may be limited by the absence of a crop for food storage in sunbirds and honeyeaters (Collins et al. 1980; Mbatha et al. 2002) and/or by stomach size (Bednekoff and Houston 1994). However, sunbirds are indeed able to ingest relatively large meals (as discussed below). The ingested food also needs to be warmed to body temperature, which results in substantial energetic costs for large nectar volumes (Lotz et al. 2003).

The digestive processing of nectar meals requires hydrolysis of the sucrose component to glucose and fructose, then absorption of the latter. Sucrose hydrolysis rates may be limiting in birds feeding on low nectar concentrations (McWhorter and Martínez del Rio 2000), and the passive component of hexose absorption may also be affected (McWhorter et al. 2006; Napier et al. 2008) as may carrier-mediated hexose absorption (Martínez del Rio and Karasov 1990). If our study were repeated on a hexose diet and birds were to increase their food intake after a fast sufficiently to maintain their evening body mass, it would suggest that the rate of sucrose hydrolysis is the limiting step in the digestive process.

When hummingbirds ingest large volumes of dilute nectar, they may be constrained by the elimination of excess water because water regulation appears to depend mainly on the kidneys (McWhorter and Martínez del Rio 1999; Hartman Bakken and Sabat 2006). Palestine sunbirds (*Cinnyris oseus*), on the other hand, reduce intestinal water absorption when consuming dilute diets, thus relieving the kidneys (McWhorter et al. 2004). High throughput of water may also lead to difficulties in regulating ion levels, and thus electrolyte balance may be a problem. When whitebellied sunbirds feed on extremely dilute sucrose-only diets, on which they cannot maintain energy balance, their excreted ion levels are relatively high (Fleming and Nicolson 2003). Adding ions to the sugar diet in a future study may facilitate active absorption of hexose sugars, helping the birds to cope with large volumes ingested after a fasting period. If birds maintained their evening body mass after the fast on a diet supplemented with ions, the rate of hexose absorption would be identified as the limiting step and not the handling of large volumes of ingested water.

### *How do whitebellied sunbirds adjust their food intake?*

Whitebellied sunbirds increase their food intake by increasing feeding duration in the first 0.5 h following the fast. Feeding duration of these birds is positively related to meal size (Köhler et al. 2008a), i.e. the longer the birds feed the more they ingest at one feeding event. The observed increase in meal size corresponds with an earlier study of whitebellied sunbirds: when they were fed alternating dilute and moderate sucrose concentrations, meal size increased immediately after the return of the moderate diet (Köhler et al. 2008b). An increase in meal size has also been demonstrated for blue-throated hummingbirds with depleted energy reserves (Hainsworth et al. 1981). Sunbirds might save energy immediately after the fast by ingesting fewer but larger meals, instead of a higher number of smaller meals, because this may reduce the number of visits to the feeder. The increase in body mass after a meal is associated with higher flight costs in hummingbirds, which hover to feed: DeBenedictis et al. (1978) proposed that it is an energetic disadvantage to maximize meal size due to increased energy expenditures resulting from the weight of a meal. Sunbirds and honeyeaters in our study, however, were able to remain perched next to the feeder.

Our sunbirds showed an increased meal size only in the first 0.5 h after the fast. For the rest of the afternoon after the fast, the length of feeding events did not differ from the control afternoon. Instead, birds appeared to increase their food intake by feeding more often compared to the control day, although this was not statistically significant. This confirms the general finding that short-term adjustment of food intake in avian nectarivores takes place through regulation of feeding frequency (Wolf and Hainsworth 1977; Collins and Clow 1978; Gass 1978; López-Calleja et al. 1997; Köhler et al. 2006).

In summary, both whitebellied sunbirds and brown honeyeaters showed a similar physiological response to the energetic challenge of a fasting period: they responded immediately after the fast by increasing their food intake, which resulted in an accelerated energy accumulation rate in the following hours. In whitebellied sunbirds, this short-term adjustment of food intake took place via a significant increase in meal size immediately after the fast and a non-significant increase in the number of feeding events thereafter. For both species, all birds weighed less in the evening following the fast, indicating that the compensation of energy intake and accumulation was adequate but not perfect. It therefore remains a mystery how nectarivorous birds cope with prolonged interruptions to feeding, such as several days of rain, if a fasting period of only 2 h already results in a lower evening body mass.

## Acknowledgements

This project was funded by the South African National Research Foundation and the Australian Research Council (DP0665730). AK's stay at Murdoch University, Perth, was funded by the University of Pretoria. We are grateful to Jan Cilliers Park for permission to mist-net sunbirds under permit from the Gauteng Directorate of Nature Conservation. We also thank the Department of Environment and Conservation (Western Australia) and Murdoch University for permission to mist-net honeyeaters. Craig Symes is thanked for catching sunbirds for our study. Our experiments were approved by the Animal Use and Care Committee of the University of Pretoria and the Animal Ethics Committee of Murdoch University.

## References

Bednekoff P.A. and Houston A.I. 1994. Avian daily foraging patterns: effects of digestive constraints and variability. Evolutionary Ecology 8: 36–52.

Calder W.A. 1994. When do hummingbirds use torpor in nature? Physiological Zoology 67: 1051–1076.

Carleton S.A., Hartman Bakken B. and Martínez del Rio C. 2006. Metabolic substrate use and the turnover of endogenous energy reserves in broad-tailed hummingbirds (*Selasphorus platycercus*). Journal of Experimental Biology 209: 2622–2627.

Carpenter F.L. and Hixon M.A. 1988. A new function for torpor: fat conservation in a wild migrant hummingbird. Condor 90: 373–378.

Coburn D.K. and Geiser F. 1998. Seasonal changes in energetics and torpor patterns in the subtropical blossom-bat *Syconycteris australis* (Megachiroptera). Oecologia 113: 467–473.

Collins B.G. and Briffa P. 1983. Seasonal and diurnal variations in the energetics and foraging activities of the brown honeyeater, *Lichmera indistincta*. Australian Journal of Ecology 8: 103–111.

Collins B.G. and Briffa P. 1984. Nocturnal energy expenditure by honeyeaters experiencing food shortage and low environmental temperatures. Comparative Biochemistry and Physiology A 78: 77–81.

Collins B.G. and Clow H. 1978. Feeding behaviour and energetics of the western spinebill, *Acanthorhynchus superciliosis* (Aves: Meliphagidae). Australian Journal of Zoology 26: 269–277.

Collins B.G. and Morellini P.C. 1979. The influence of nectar concentration and time of day upon energy intake and expenditure by the singing honeyeater, *Meliphaga virescens*. Physiological Zoology 52: 165–175.

Collins B.G., Cary G. and Packard G. 1980. Energy assimilation, expenditure and storage by the brown honeyeater, *Lichmera indistincta*. Journal of Comparative Physiology B 137: 157–163.

Collins B.G., Grey J. and McNee S. 1990. Foraging and nectar use in nectarivorous bird communities. Studies in Avian Biology 13: 110–121.

Cruz-Neto A.P. and Abe A.S. 1997. Metabolic rate and thermoregulation in the nectarivorous bat, *Glossophaga soricina* (Chiroptera, Phyllostomatidae). Revista Brasileira de Biologia 57: 203–209.

DeBenedictis P.A., Gill F.B., Hainsworth F.R., Pyke G.H. and Wolf L.L. 1978. Optimal meal size in hummingbirds. American Naturalist 112: 301–316.

Ehrhardt N., Heldmaier G. and Exner C. 2005. Adaptive mechanisms during food restriction in *Acomys russatus*: the use of torpor for desert survival. Journal of Comparative Physiology B 175: 193–200.

Fleming P.A. and Nicolson S.W. 2003. Osmoregulation in an avian nectarivore, the whitebellied sunbird *Nectarinia talatala*: response to extremes of diet concentration. Journal of Experimental Biology 206: 1845–1854.

Fleming P.A., Gray D.A. and Nicolson S.W. 2004. Circadian rhythm of water balance and aldosterone excretion in the whitebellied sunbird *Nectarinia talatala*. Journal of Comparative Physiology B 174: 341–346.

Gass C.L. 1978. Experimental studies of foraging in complex laboratory environments. American Zoologist 18: 729–738.

Glück E. 1987. An experimental study of feeding, vigilance and predator avoidance in a single bird. Oecologia 71: 268–272.

Hainsworth F.R. 1983. Models and evidence for feeding control of energy. American Zoologist 23: 261–272.

Hainsworth F.R., Collins B.G. and Wolf L.L. 1977. The function of torpor in hummingbirds. Journal of Experimental Zoology 195: 215–222.

Hainsworth F.R., Tardiff M.F. and Wolf L.L. 1981. Proportional control for daily energy regulation in hummingbirds. Physiological Zoology 54: 452–462.

Hartman Bakken B. and Sabat P. 2006. Gastrointestinal and renal responses to water intake in the green-backed firecrown (*Sephanoides sephanoides*), a South American hummingbird. American Journal of Physiology (Regulatory, Integrative and Comparative Physiology) 291: R830–R836.

Köhler A., Verburgt L. and Nicolson S.W. 2006. Short-term feeding patterns of whitebellied sunbirds (*Nectarinia talatala*): feeding frequency, daily rhythms and individual differences. Journal of Experimental Biology 209: 2880–2887.

Köhler A., Verburgt L. and Nicolson S.W. 2008a. Nectar intake of whitebellied sunbirds (*Cinnyris talatala*): Can meal size be inferred from feeding duration? Physiological and Biochemical Zoology 81: 682–687.

Köhler A., Verburgt L., Fleming P.A. and Nicolson S.W. 2008b. Changes in nectar concentration: how quickly do whitebellied sunbirds (*Cinnyris talatala*) adjust feeding patterns and food intake? Journal of Comparative Physiology B 178: 785–793.

López-Calleja M.V., Bozinovic F. and Martínez del Rio C. 1997. Effects of sugar concentration on hummingbird feeding and energy use. Comparative Biochemistry and Physiology A 118: 1291–1299.

Lotz C.N., Martínez del Rio C. and Nicolson S.W. 2003. Hummingbirds pay a high cost for a warm drink. Journal of Comparative Physiology B 173: 455–462.

Lovegrove B.G., Raman J. and Perrin M.R. 2001. Daily torpor in elephant shrews (*Macroscelidea: Elephantulus spp.*) in response to food deprivation. Journal of Comparative Physiology B 171: 11–21.

Martínez del Rio C. and Karasov W.H. 1990. Digestion strategies in nectar- and fruit-eating birds and the sugar composition of plant rewards. American Naturalist 136: 618–637.

Mbatha K., Downs C.T. and Penning M. 2002. Nectar passage and gut morphology in the Malachite Sunbird and the Black-capped Lory: implications for feeding in nectarivores. Ostrich 73: 138–142.

McKechnie A.E. and Lovegrove B.G. 2002. Avian facultative hypothermic responses: a review. Condor 104: 705–724.

McKechnie A.E. and Lovegrove B.G. 2003. Facultative hypothermic responses in an Afrotropical arid-zone passerine, the red-headed finch (*Amadina erythrocephala*). Journal of Comparative Physiology B 173: 339–346.

McLean P.L. and Kinsey M.G. 1969. Probing behaviour of the pea aphid, *Acyrthosiphon pisum*. IV. Effects of starvation on certain probing activities. Annals of the Entomological Society of America 62: 987–994.

McWhorter T.J. and Martínez del Rio C. 1999. Food ingestion and water turnover in hummingbirds: how much dietary water is absorbed? Journal of Experimental Biology 202: 2851–2858.

McWhorter T.J. and Martínez del Rio C. 2000. Does gut function limit hummingbird food intake? Physiological and Biochemical Zoology 73: 313–324.

McWhorter T.J., Martínez del Rio C., Pinshow B. and Roxburgh L. 2004. Renal function in Palestine sunbirds: elimination of excess water does not constrain energy intake. Journal of Experimental Biology 207: 3391–3398.

McWhorter T.J., Hartman Bakken B., Karasov W.H. and Martínez del Rio C. 2006. Hummingbirds rely on both paracellular and carrier-mediated intestinal glucose absorption to fuel high metabolism. Biology Letters 2: 131–134.

Miles D.B. 1990. The importance and consequences of temporal variation in avian foraging behaviour. Studies in Avian Biology 13: 210–217.

Moe B., Stølevik E. and Bech C. 2005. Ducklings exhibit substantial energy-saving mechanisms as a response to short-term food shortage. Physiological and Biochemical Zoology 78: 90–104.

Moore F.R. and Yong W. 1991. Evidence of food-based competition among passerine migrants during stopover. Behavioural Ecology and Sociobiology 28: 85–90.

Napier K.R., Purchase C., McWhorter T.J., Nicolson S.W. and Fleming P.A. 2008. The sweet life: diet sugar concentration influences paracellular glucose absorption. Biology Letters 4: 530–533.

Nicolson S.W., Hoffmann D. and Fleming P.A. 2005. Short-term energy regulation in nectar-feeding birds: the response of whitebellied sunbirds (*Nectarinia talatala*) to a midday fast. Functional Ecology 19: 988–994.

Qian X., Cui Y., Xiong B. and Yang Y. 2000. Compensatory growth, feed utilization and activity in gibel carp, following feed deprivation. Journal of Fish Biology 56: 228–232.

Rathcke B.J. 1992. Nectar distributions, pollinator behaviour, and plant reproductive success. In: Effects of resource distribution on animal-plant interactions. M.D. Hunter, T. Ohgushi and P.W. Price (Eds.), Academic Press, New York, USA, pp. 113–137.

Robin J.P., Frain M., Sardet C., Groscolas R. and Le Maho Y. 1988. Protein and lipid utilization during long-term fasting in emperor penguins. American Journal of Physiology (Regulatory, Integrative and Comparative Physiology) 254: R61–R68.

Tempel D.L., Shor-Posner G., Dwyer D. and Leibowitz S.F. 1989. Nocturnal patterns of macronutrient intake in freely feeding and food-deprived rats. American Journal of Physiology (Regulatory, Integrative and Comparative Physiology) 256: R541–R548.

Tooze Z.J. and Gass C.L. 1985. Responses of rufous hummingbirds to midday fasts. Canadian Journal of Zoology 63: 2249–2253.

Van Tets I.G. and Nicolson S.W. 2000. Pollen and the nitrogen requirements of the lesser double-collared sunbird. Auk 117: 826–830.

Voigt C.C. and Speakman J.R. 2007. Nectar-feeding bats fuel their high metabolism directly with exogenous carbohydrates. Functional Ecology 21: 913–921.

Wang T., Hung C.C.Y. and Randall D.J. 2006. The comparative physiology of food deprivation: from feast to famine. Annual Review of Physiology 68: 223–251.

Welch K.C., Jr. and Suarez R.K. 2007. Oxidation rate and turnover of ingested sugar in hovering Anna’s (*Calypte anna*) and rufous (*Selasphorus rufus*) hummingbirds. Journal of Experimental Biology 210: 2154–2162.

Welch K.C., Jr., Herrera L.G. and Suarez R.K. 2007. Dietary sugar as a direct fuel for flight in the nectarivorous bat *Glossophaga soricina*. Journal of Experimental Biology 211: 310–316.

Williams J.B. 1993. Energetics of incubation in free-living orange-breasted sunbirds in South Africa. Condor 95: 115–126.

Winter Y. 1998. *In vivo* measurement of near maximal rates of nutrient absorption in a mammal. Comparative Biochemistry and Physiology A 119: 853–859.

Wolf L.L. and Hainsworth F.R. 1977. Temporal patterning of feeding by hummingbirds. Animal Behaviour 25: 976–989.

Zubair A.K. and Leeson S. 1996. Compensatory growth in the broiler chicken: a review. World's Poultry Science Journal 52: 189–201.

## Figure legends

**Fig. 1.** Mass-specific food intake rate (mg•$h^{-1}$•g body $mass^{-1}$; mean ± SE; SE partly omitted for clarity) of eight whitebellied sunbirds (above) and eight brown honeyeaters (below). Birds fed continuously on CONTROL, whereas feeding was interrupted for 2 h on FAST (10h00–12h00). Statistical significance derives from the Tukey's Honest Significant Difference *post-hoc* test that followed comparison of the mass-specific food intake rate of both species between CONTROL and FAST by RM-ANOVA (* $P \leq 0.05$; *** $P \leq 0.001$).

**Fig. 2.** Feeding duration of eight whitebellied sunbirds (s; A) and the number of feeding events (B) (mean ± SE; SE partly omitted for clarity). Birds fed continuously on CONTROL, whereas feeding was interrupted for 2 h on FAST (10h00–12h00). Statistical significance derives from the Tukey's Honest Significant Difference *post-hoc* test that followed comparison of the feeding duration between CONTROL and FAST by RM-ANOVA (* $P \leq 0.05$).

**Fig. 3.** Body mass (g) throughout the day for eight whitebellied sunbirds (above) and eight brown honeyeaters (below) (mean ± SE; SE partly omitted for clarity). Birds fed continuously on CONTROL, whereas feeding was interrupted for 2 h on FAST (10h00–12h00). The slopes of the regression lines (body mass vs. time) are given as m. Slopes of body mass vs. time regressions for the afternoon (12h00–19h00) differed between CONTROL and FAST in both species ($F_{1,14}=38.60$, $P<0.001$).

# Figures

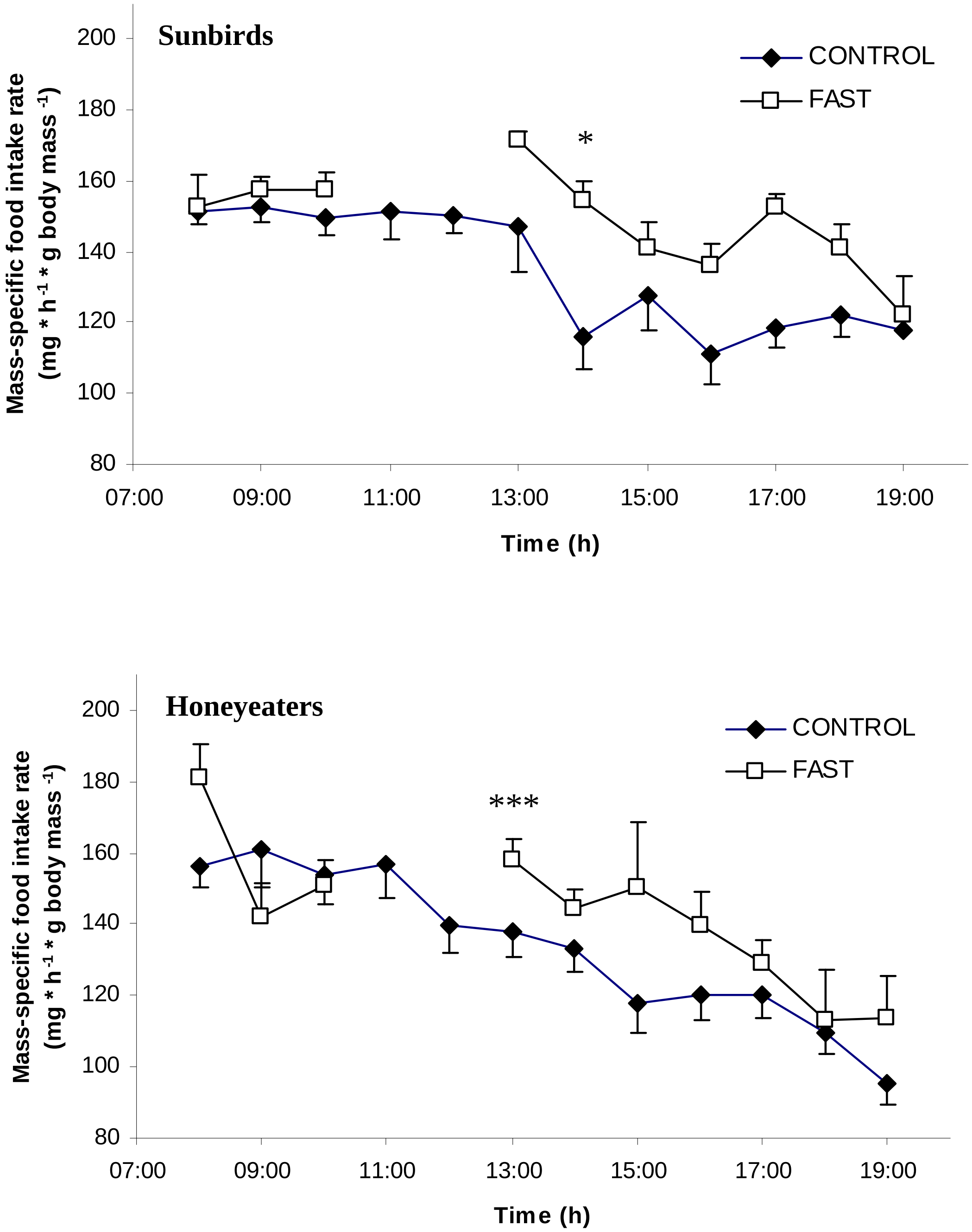


Figure 1.

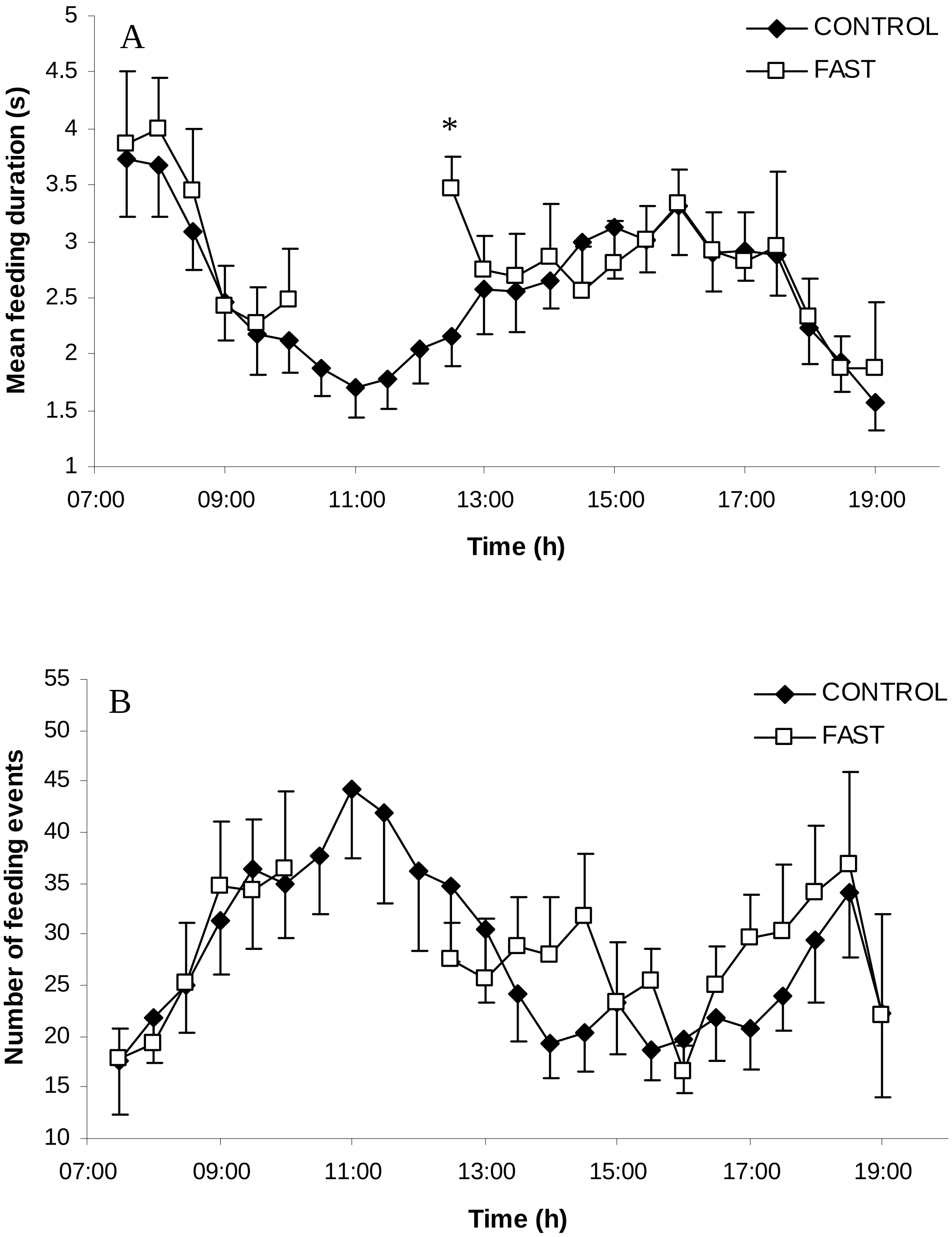
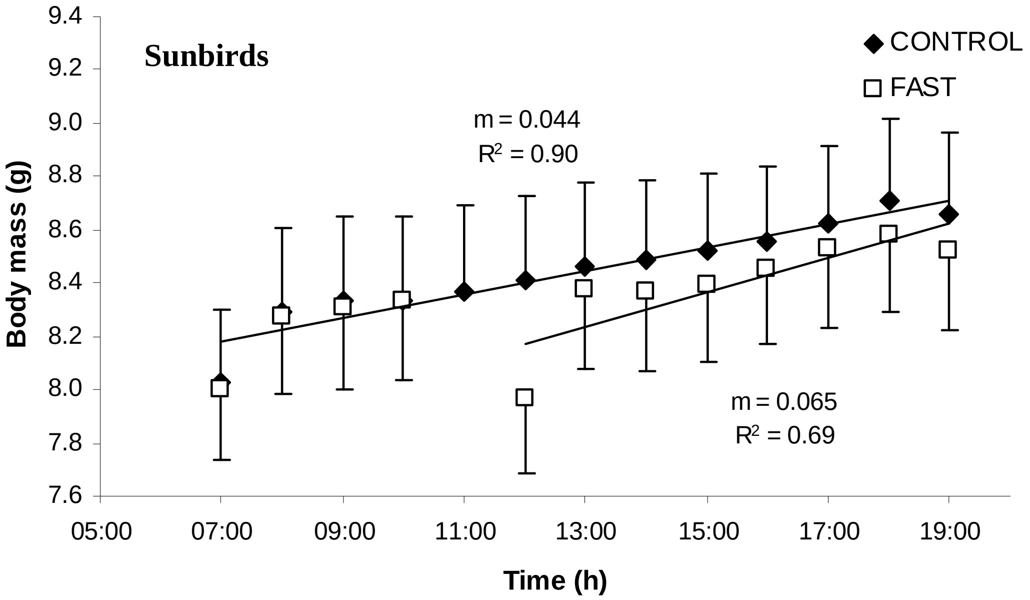

A
CONTROL
FAST
*
Mean feeding duration (s)
5
4.5
4
3.5
3
2.5
2
1.5
1
07:00
09:00
11:00
13:00
15:00
17:00
19:00
Time (h)
B
CONTROL
FAST
Number of feeding events
55
50
45
40
35
30
25
20
15
10
07:00
09:00
11:00
13:00
15:00
17:00
19:00
Time (h)

Figure 2.


Sunbirds
CONTROL
FAST
m = 0.044
R2 = 0.90
m = 0.065
R2 = 0.69
Body mass (g)
9.4
9.2
9.0
8.8
8.6
8.4
8.2
8.0
7.8
7.6
05:00
07:00
09:00
11:00
13:00
15:00
17:00
19:00
Time (h)

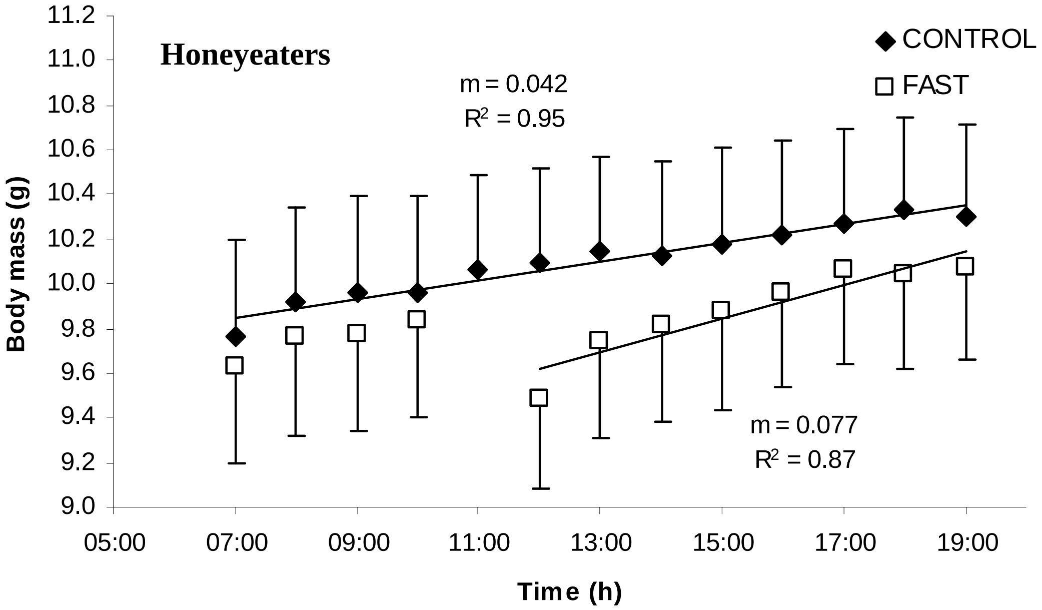

Honeyeaters
CONTROL
FAST
m = 0.042
R2 = 0.95
m = 0.077
R2 = 0.87
Body mass (g)
11.2
11.0
10.8
10.6
10.4
10.2
10.0
9.8
9.6
9.4
9.2
9.0
05:00
07:00
09:00
11:00
13:00
15:00
17:00
19:00
Time (h)

Figure 3.

# CHAPTER 4

# LOW TEMPERATURE CHALLENGES IN SUNBIRDS: EFFECTS ON FOOD INTAKE, FEEDING PATTERNS AND BODY MASS OF *CINNYRIS TALATALA* AND *CHALCOMITRA AMETHYSTINA*

**Angela Köhler[†]*, Luke Verburgt[†], Todd J. McWhorter[‡] and Susan W. Nicolson[†]**

[†] Department of Zoology and Entomology, University of Pretoria, Pretoria 0002, South Africa
[‡] School of Veterinary Science, University of Adelaide, Adelaide SA 5371, Australia

*Corresponding author, e-mail: akoehler@zoology.up.ac.za.

# Abstract

Animals commonly experience variation in both food quality and metabolic requirements, and regulate their food intake to maintain energy balance. We exposed whitebellied (*Cinnyris talatala*) and amethyst (*Chalcomitra amethystina*) sunbirds (Nectariniidae) to varying nectar sugar concentrations (0.25, 0.5 and 1 M sucrose), i.e. food qualities, and ambient temperatures (5, 15 and 25˚C), i.e. energy requirements, to examine the effects on food intake, feeding patterns and body mass of the birds. Birds of both species attempted to compensate for a decrease in sugar concentration by increasing their food intake, but still ingested less sugar on the 0.25 M diet than on the 0.5 M diet. During acute short-term exposure to 5˚C, birds increased their food and thus sugar intake on all sugar concentrations by almost 27%, compared to 15˚C, while intake at 15 and 25˚C was similar. The adjustment of food intake in whitebellied and amethyst sunbirds took place via changes in feeding frequency, with duration of feeding events staying the same. Apparent sugar assimilation in whitebellied sunbirds was >99%, irrespective of sugar concentration and temperature. The maximal food intake of both sunbird species in the cold was mostly underestimated by a chemical reactor model of digestive capacity, which used measurements of intestinal hydrolytic capacity to calculate maximal food intake rates in these birds. Despite the increased food consumption, all birds lost more body mass at 5 and 15˚C than at the highest temperature. It is therefore concluded that the intake response of whitebellied and amethyst sunbirds is shaped by both compensatory feeding and physiological constraint.

# Introduction

The balance between energy intake and expenditure is of major importance for animal survival and fitness. When the energy content of the food source is decreased, various animals compensate by ingesting larger amounts (e.g. Montgomery and Baumgardt 1965; Yang and Joern 1994; McCauley 1999; Cruz-Rivera and Hay 2000). Animals also increase their food consumption in response to high energetic demands, such as during reproduction, exercise and cold exposure (Nance et al. 1977; Goymann et al. 2006; Valencak et al. 2009; for a review see Starck 1999). Endothermic animals, for instance, ingest more food when they need to maintain a constant body temperature at low ambient temperatures (Kleiber and Dougherty 1934; Bozinovic and Nespolo 1997; Liu et al. 2002).

These two kinds of compensation, for food quality and increased energy demands, may be influenced by physiological constraints. Food intake in birds and mammals can be limited by the volumetric capacity of the intestine or the rate at which food is broken down (for a review see Karasov and McWilliams 2005). Mammalian and avian gastrointestinal tracts can adjust to high feeding rates by an increase in size, and thus an increase in amounts of nutrient transporters and digestive enzymes (Lee and Houston 1993; Hammond et al. 1994; McWilliams and Karasov 2001). This adjustment of the digestive system occurs faster in small animals than in larger ones, and leads to a long-term spare capacity of 100–125% above routine rates (Starck 1999; Karasov and McWilliams 2005). When energy demands increase suddenly, i.e. without sufficient time for physiological changes, animals can increase their food intake by only 9–50% compared to their maintenance intake rates (Karasov and McWilliams 2005), which may not be sufficient to maintain energy balance.

Nectarivorous birds are particularly interesting subjects for investigating the effect of temperature stress and varying dietary energy content on energy balance. For these small birds it is energetically expensive to maintain a constant body temperature in the cold due to their unfavourable surface area to volume ratio. When amethyst sunbirds (*Chalcomitra amethystina*) were kept at 5 and 25˚C overnight, their resting metabolic rate doubled with the 20˚C decrease in temperature (Lindsay et al. 2009a and b). Southern double-collared sunbirds (*Cinnyris chalybeus*) also doubled their resting metabolic rate when ambient temperature was decreased by 20˚C (Leon and Nicolson 1997). In addition, avian nectarivores often ingest large volumes of dilute nectar to meet high metabolic requirements. The cost of warming nectar to body temperature increases substantially with decreasing nectar temperature and decreasing nectar concentration, as demonstrated in rufous hummingbirds (*Selasphorus rufus*) (Lotz et al. 2003). As a result of food-warming costs, southern double-collared sunbirds showed a 15% increase in metabolic rate on a dilute diet (0.2 M sucrose) compared to a concentrated diet of 1.2 M (Lotz and Nicolson 2002).

Earlier studies in which avian nectarivores were exposed to low ambient temperatures and varying nectar concentrations have revealed differences in the birds' abilities to compensate for low dietary energy content and high energy demands. Southern double-collared sunbirds, kept at temperatures ranging from 10–30˚C for two days and fed 0.4 or 1.2 M sucrose diets, increased their food intake in the cold and maintained energy balance on both diets (Lotz 1999). Whitebellied sunbirds (*Cinnyris talatala*), when exposed to 10˚C for two days, increased their energy intake by 18% on 1 M sucrose and hexose diets, but were not able to increase their intake on very dilute diets of 0.1 M (Fleming et al. 2004). In the case of hummingbirds, green-backed firecrowns (*Sephanoides sephanoides*) showed a lower energy intake on 0.5 and 0.75 M sucrose diets at 15 than at 25˚C, even though they were acclimated to the low temperature for seven days (Fernández et al. 2002). After similar acclimation to low ambient temperatures, Anna's hummingbirds (*Calypte anna*) nearly doubled their food intake over a 30˚C decrease in ambient temperature, whereas rufous hummingbirds showed only a slight increase in food intake (Beuchat et al. 1979; also see Beuchat et al. 1990 for a review). Contradictory results have been obtained in broadtailed hummingbirds (*Selasphorus platycercus*) that were rapidly exposed to 10˚C and fed dilute (0.25 and 0.29 M sucrose) and concentrated diets (1 M): they did not increase their sugar intake and lost body mass in the study of McWhorter and Martínez del Rio (2000), but increased their intake and did not lose more body mass in the cold in the study of Fleming et al. (2004).

During these temperature studies, the food intake of avian nectarivores was recorded hourly or daily (Beuchat et al. 1979; Lotz 1999; McWhorter and Martínez del Rio 2000; Fleming et al. 2004). Only few studies have investigated the short-term feeding patterns at different ambient temperatures. Fernández et al. (2002) used video recorders to determine feeding frequencies of green-backed firecrowns at two temperatures and found that they feed more often at the high temperatures. Feeder visits of rufous hummingbirds kept at 5˚C for 4 h have been monitored by a computer via photocells: they visited the feeder more frequently when fed low sucrose concentrations compared to higher concentrations (Gass et al. 1999). The body mass was recorded continuously by balances interfaced to the computer and it was found that these hummingbirds are able to maintain energy balance on a 1 M sucrose diet, but lose mass on lower diet concentrations (Gass et al. 1999).

Several physiological constraints to food intake in nectarivorous birds have been proposed. Sucrose hydrolysis rates and hexose absorption may be limiting in birds ingesting large amounts of nectar (Martínez del Rio and Karasov 1990; McWhorter and Martínez del Rio 2000; McWhorter et al. 2006; Napier et al. 2008). Birds may further be constrained by the elimination of excess water (McWhorter and Martínez del Rio 1999; Hartman Bakken and Sabat 2006) and regulation of ion levels when consuming large diet volumes (Fleming and Nicolson 2003). If birds can not adequately increase their food intake in response to increased energy requirements, they may reduce their activity to save energy, as shown for green-backed firecrowns (Fernández et al. 2002). Birds may also reduce their body temperature to save energy, as demonstrated in cold-stressed southern double-collared sunbirds (Leon and Nicolson 1997), or even go into torpor, such as food-deprived rufous hummingbirds (Tooze and Gass 1985).

The present study was aimed at investigating the interplay between compensatory feeding and physiological constraints in two species from the family Nectariniidae, whitebellied (*Cinnyris talatala*) and amethyst (*Chalcomitra amethystina*) sunbirds. We varied both food quality and ambient temperature to examine the effect on food intake, feeding patterns and body mass of the sunbirds. We predicted that birds of both species would compensate for a decrease in sugar concentration by increasing their food intake irrespective of ambient temperature. We further hypothesized that birds would increase their food intake at low ambient temperature on all sugar concentrations. Based on findings of previous studies, the metabolic rate of sunbirds should be twice as high at 5ºC as at 25ºC (Leon and Nicolson 1997; Lindsay et al. 2009a and b); therefore food intake must double to meet the energy requirements of the birds in the cold. The adjustment of food intake was expected to take place through regulation of feeding frequency rather than meal size, as shown previously for whitebellied sunbirds and hummingbirds (Wolf and Hainsworth 1977; Köhler et al. 2006). Despite increases in food intake, birds were expected to lose body mass in the cold due to physiological limitations. The food intake of the sunbirds in the cold was compared with their maximal intake predicted by a mathematical model, using measurements of intestinal hydrolytic capacity (McWhorter and Martínez del Rio 2000). We hypothesized that the birds, when challenged by high metabolic demands, would ingest amounts close to their maximal intake, especially when encountering low energy content of food at the same time.

## Materials and methods

### *Study animals and their maintenance*

Nine whitebellied sunbirds (*Cinnyris talatala*) and nine amethyst sunbirds (*Chalcomitra amethystina*) were mist-netted at Jan Cilliers Park, Pretoria, South Africa. At the time of capture, body mass of the seven male whitebellied sunbirds was 8.56 ± 0.14 g (mean ± SE) and of the two females was 6.85 and 7.63 g. The four male and five female amethyst sunbirds weighed 14.59 ± 0.26 g and 14.12 ± 0.50 g (mean ± SE) respectively. Birds were housed in individual cages (45 x 45 x 32 cm) in a climate-controlled room at 20 ± 2ºC. The photoperiod was 12:12 h L:D, with lights on at 07h00. Dawn and dusk were simulated by an additional 0.5 h dimmed light at the beginning and end of each day. The maintenance diet consisted of a 0.63 M sucrose solution with a nutritional supplement (Ensure®, Abbott Laboratories, Johannesburg, South Africa) to provide dietary nitrogen (Van Tets and Nicolson 2000). This maintenance diet and supplementary water were provided *ad libitum* in inverted, stoppered syringes. Birds were acclimated to these laboratory conditions for three weeks before the commencement of the study.

## *Experimental procedure*

Whitebellied and amethyst sunbirds were exposed to three ambient temperatures of 5, 15 and 25˚C, while feeding on one of three sugar-only diets of 0.25, 0.5 and 1 M sucrose (8.5, 16 and 30% w/w). These dietary sugar concentrations are within the range of nectar concentrations of sunbird-pollinated flowers, commonly from 0.15–1.35 M (Nicolson and Fleming 2003a). Each bird received all three diet sugar concentrations at all three ambient temperatures, i.e. the nine birds of each species were exposed to each temperature three times. Three birds received the same sugar concentration at a time. The sequence of temperature and sugar concentration was randomized. The photoperiod remained the same as during maintenance, but dawn and dusk periods were omitted. Each part of the experiment consisted of one day during which the birds could get used to the experimental cages, ambient temperature and experimental diet, followed by one test day. Birds were given four maintenance days between the different parts of the experiment to regain energy balance. The birds generally lost body mass on the experimental diets since their diet was not supplemented with protein (Nicolson and Fleming 2003b).

In addition to the three sucrose concentrations, whitebellied and amethyst sunbirds were also fed energetically equivalent glucose:fructose (1:1) mixtures, once at 5 and once at 25˚C, i.e. three birds per hexose concentration. This was done to allow for a comparison between the different sugar types found in nectar of sunbird-pollinated plants (Nicolson and Fleming 2003a).

## *Data collection*

Birds were captured, placed in a cloth bag and weighed before lights-on on each test day and the day after, using an electronic balance (Denver Instrument PK-352, 0.01 g, Denver, Colorado). At the same time, feeders were weighed to determine the mass of food consumed on the test day. Any drips from feeders were collected in containers with liquid paraffin (to avoid evaporative mass loss), which were weighed at the same time as the feeders. Diet evaporation was determined using additional feeders with all diet concentrations and both sugar types. These feeders were weighed before and after one test day at each ambient temperature, and the mass of food consumed was corrected accordingly.

The densities of all experimental test diets and distilled water were determined by weighing six 1 ml samples each to the nearest 0.1 mg (Mettler Toledo AG 64, Microsep Ltd, Johannesburg). The density of distilled water was divided by the expected density of distilled water (1 $g•ml^{-1}$), resulting in the dimensionless correction factor q. Diet densities were then divided by q to correct for pipette errors.

On all test days, automated recording of the time spent feeding as well as the number of feeding events was obtained by an infrared photodetection system interfaced to a computer. For each sunbird we obtained the start and end times of every feeding event and calculated feeding duration (± 0.001 s) as the time between insertion of the bill into the sucrose solution and its removal (Köhler et al. 2006).

Excreta of whitebellied sunbirds produced over 24 h were collected on plastic trays which were placed underneath the cages on one test day at each ambient temperature on sucrose diets and on both test days on hexose diets (5 and 25˚C). The excreta samples were allowed to evaporate and later re-diluted with distilled water of known volume. The samples were then assayed for sucrose content (when birds were fed sucrose diets), and glucose and fructose content (for both sucrose and hexose diets) respectively, using Sigma-Aldrich (Munich, Germany) colorimetric / enzymatic kits and a spectrophotometer (Biowave S2100 UV/Vis, Biochrom Ltd., Cambridge, UK). Data on sugar assimilation of amethyst sunbirds were obtained from Downs (1997).

Four additional whitebellied sunbirds and five amethyst sunbirds were caught at Jan Cilliers Park, Pretoria, and euthanased by a halothane overdose. The small intestine was immediately removed from each bird and flushed clean with ice cold saline. It was then cut into sections and dissected lengthwise. The length and nominal surface area of each section were measured and used to calculate the volume of each section, and later the total volume of the small intestine. The sections of the small intestine were then weighed to the nearest 0.1 mg (Mettler Toledo AG 64, Microsep Ltd, Johannesburg) and thereafter stored in liquid nitrogen. Samples were thawed and homogenized (Ultra-Turrax T25, Janke & Kunkel GmbH + Co.KG, Staufen, Germany) in 300 mM mannitol in 1 mM Hepes/KOH buffer (pH 7.5). Disaccharidase activities were measured according to Dahlquist (1984) as modified by Martínez del Rio et al. (1995) and Fassbinder-Orth and Karasov (2006). In short, 30 µl tissue homogenates were diluted with 300 mM mannitol in 1 mM Hepes/KOH and incubated with 30 µl of 56 mM maltose in 0.1 M maleate/NaOH buffer (pH 6.5) at 40˚C for 20 min. Reactions were stopped by adding 400 µl of a stop-develop reagent (glucose assay kit, Sigma Aldrich, Munich, Germany). After 30 min incubation at 40˚C, 400 µl of 12 N $H_2SO_4$ were added and the absorbance was read at 540 nm (Novaspec II, Pharmacia Biotech, Cambridge, UK). Apparent Michaelis constant ($K_m$) and pH optima for intestinal sucrase activity were 15.40 ± 2.24 mM (mean ± SE) and 5.5 for whitebellied sunbirds, and 14.08 ± 2.46 mM (mean ± SE) and 5.5 for amethyst sunbirds.

## Data processing

Body mass change ($\%\bullet 24\ h^{-1}$) was calculated for each bird and each test day from the body mass (g) before lights-on on the test day and the day after. Mass-specific food intake per day ($g\bullet 24\ h^{-1}\bullet g$ body mass$^{-1}$) was calculated using the morning body mass of each bird on the test day. Mass-specific daily sugar intake ($mg\bullet 24\ h^{-1}\bullet g$ body mass$^{-1}$), i.e. sucrose and hexose (sum of glucose and fructose) intake, was calculated as the product of volumetric food intake (determined by dividing the food intake by the relative density of the diet), diet concentration and molar mass of each sugar and is hereafter referred to as sugar intake. For all birds, we calculated mean feeding duration, number of feeding events, and total time spent feeding during the 12 h light period on the test day. Our aim was to determine whether sunbirds adjust the duration and/or number of feeding events to compensate for changes in diet sugar concentration and ambient temperature.

For simplicity, the three sugars will hereafter be summarized as {SUGAR}, with {SUGAR} being sucrose, glucose or fructose respectively. The amount of {SUGAR} excreted (mg•24 $h^{-1}$) by whitebellied sunbirds was calculated as the product of the content of each sugar per ml sample and the volume of the sample after re-dilution. The total amount of excreted sugar was determined as the sum of all three sugars on sucrose diets, or the sum of glucose and fructose on hexose diets. The apparent sucrose, glucose and fructose assimilation coefficients (SucrAC*, GlucAC* and FrucAC*, in short {SUGAR}AC*) were calculated for each bird on each test day as the percentage of sugar ingested that was not excreted:

$$\{\text{SUGAR}\}\text{AC}^{*}=100 \bullet \frac{\left[\{\text{SUGAR}\}\text{ ingested (mg}\bullet 24\text{h}^{-1}) - \{\text{SUGAR}\}\text{ excreted (mg}\bullet 24\text{h}^{-1})\right]}{\{\text{SUGAR}\}\text{ ingested (mg}\bullet 24\text{h}^{-1})}$$

SucrAC* were calculated for sucrose diets; GlucAC* and FrucAC* were calculated for hexose diets.

The maximal volumetric food intake rates and maximal sugar intake rates of whitebellied and amethyst sunbirds were predicted using a chemical reactor model of digestive capacity. This model is described in detail by McWhorter and Martínez del Rio (2000) and Martínez del Rio et al. (2001) and in the Appendix. The model assumes that sucrose hydrolysis is the limiting factor in sugar digestion of nectar-feeding birds when they are feeding on sucrose-rich nectars and relies on the sucrose assimilation efficiency as well as the data on gut morphology and intestinal sucrase activity of each sunbird species. The daily maximal volumetric and sucrose intake was calculated for the 12 h light period used in this experiment.

## *Statistical analysis*

All data were tested for homogeneity of variance (Levene's test) and normality (Kolmogorov-Smirnov test). Data were log transformed when heteroscedastic or not normally distributed. Only data obtained from the test days on sucrose diets were used for analysis, since data on hexose diets were insufficient for statistical tests and only served comparative purposes. Food intake and sugar intake data on hexose diets were very similar to sucrose diets, and we therefore present only results for the sucrose diets. Food intake (g•24 $h^{-1}$•g body $mass^{-1}$) of whitebellied and amethyst sunbirds at the different test days at 5˚C was subjected to Spearman rank correlation to determine whether food intake was positively related to the number of exposures to 5˚C, i.e. whether sunbirds show physiological adjustments to high feeding rates after repeated cold exposure. Since there was no relationship between food intake of both species and the number of cold exposures ($R_s > -0.37$, $P > 0.33$), data obtained at first and subsequent cold exposures were pooled for further analysis. Food intake, sugar intake (mg•24 $h^{-1}$•g body $mass^{-1}$), number of feeding events (per 12 h), mean feeding duration (ms), total time spent feeding (min•12 $h^{-1}$) and body mass change (%•24 $h^{-1}$) were separately subjected to repeated measures ANOVA (RM-ANOVA), with ambient temperature and sugar concentration being within-effects, and species being the categorical predictor. As an exploratory data analysis, we initially performed separate RM-ANOVA for each species but these results did not differ from the combined analysis and the results from the latter are therefore presented.

The amounts of sucrose, glucose and fructose excreted and the total amounts of sugar excreted (mg•24 $h^{-1}$) were subjected to Spearman rank correlations to test for differences between diet sugar concentrations (for each ambient temperature and sucrose and hexose diets separately; n=9 each). RM-ANOVA was used to determine differences in total sugar excreted between ambient temperatures (for sucrose and hexose diets separately; n=9 each), with temperature being within-effect. RM-ANOVA was further used to test for differences between amounts of sucrose, glucose and fructose excreted (for each temperature separately; n=9 each), with type of sugar being within-effect. *Post-hoc* comparisons for all RM-ANOVA were conducted with Tukey’s Honest Significant Difference test for equal sample sizes, followed by a Bonferroni correction for multiple comparisons. Spearman rank correlations were followed by sequential Bonferroni corrections to avoid Type I errors (Rice 1989). All data are presented as mean ± SE.

# Results

## *Food and sugar intake*

Food intake (g•24 $h^{-1}$•g body $mass^{-1}$) of the nine whitebellied and nine amethyst sunbirds on sucrose diets of three sugar concentrations and at three ambient temperatures is shown in Fig. 1. Food intake differed significantly between temperatures ($F_{2,32}$=21.29, $P$<0.001) and sugar concentrations ($F_{2,32}$=1196.41, $P$<0.001). *Post-hoc* analysis showed that food intake was higher at 5˚C than at 15 and 25˚C ($P$<0.001), but did not differ between 15 and 25˚C ($P$=0.82). Whitebellied and amethyst sunbirds showed a similar increase in food intake in the cold; compared to 15˚C, they increased their intake by 26.75 ± 2.93% at 5˚C, irrespective of diet sugar concentration. Food intake decreased with increasing sugar concentration and was found to be significantly different between all three concentrations ($P$<0.001). The two species differed in their mass-specific food intake ($F_{1,16}$=10.83, $P$<0.01), with whitebellied sunbirds consuming more than amethyst sunbirds ($P$<0.01).

Sugar intake (mg•24 $h^{-1}$•g body $mass^{-1}$) was found to differ between temperatures ($F_{2,32}$=20.03, $P$<0.001; Fig. 2). Similar to food intake, sugar intake was higher at 5˚C than at 15 and 25˚C ($P$<0.001), but did not differ between 15 and 25˚C ($P$=0.87). A significant difference in sugar intake was found between sugar concentrations ($F_{2,32}$=8.11, $P$<0.001). *Post-hoc* analysis revealed that birds ingested less sugar on the dilute diet (0.25 M) than on the 0.5 M diet ($P$<0.01), but not less than on the 1 M diet ($P$=0.13). Sugar intake did not differ between the 0.5 and 1 M diets ($P$=0.12). The two species differed in their mass-specific sugar intake ($F_{1,16}$=9.39, $P$<0.01), with whitebellied sunbirds ingesting more sugar than amethyst sunbirds ($P$<0.01).

## *Feeding patterns*

The number of feeding events of whitebellied and amethyst sunbirds during the 12 h light period of the test day is presented in Fig. 3. The number of feeding events differed significantly between temperatures ($F_{2,32}$=13.20, $P$<0.001) and sugar concentrations ($F_{2,32}$=28.51, $P$<0.001). *Post-hoc* analysis revealed that birds fed more often at 5˚C than at 15 and 25˚C ($P$<0.01), but there was no difference between 15 and 25˚C ($P$=0.38). Birds also increased the number of feeding events with decreasing sugar concentration, with all sugar concentrations being significantly different from one another ($P$<0.03). The number of feeding events did not differ between whitebellied and amethyst sunbirds ($F_{1,16}$=0.09, $P$=0.77).

Mean feeding duration of the birds differed significantly between temperatures ($F_{2,32}$=3.36, $P$=0.05). However, mean feeding duration only differed between 5 and 15˚C ($P$=0.05), and this became non-significant after Bonferroni correction ($P$>0.05). Mean feeding duration did not differ between the remaining temperatures ($P$>0.16). There was also no significant difference in mean feeding duration between sugar concentrations ($F_{2,32}$=1.48, $P$=0.24) and between the two species ($F_{1,16}$=3.52, $P$=0.08). The mean feeding durations of all sunbirds on the three sugar concentrations were 2.62 ± 0.21 s at 5˚C, 1.85 ± 0.11 s at 15˚C and 2.24 ± 0.28 s at 25˚C.

The two species differed in total time they spent feeding ($F_{1,16}$=4.84, $P$=0.04), with whitebellied sunbirds feeding for longer than amethyst sunbirds ($P$=0.04). Total time spent feeding differed significantly between temperatures ($F_{2,32}$=25.76, $P$<0.001) and sugar concentrations ($F_{2,32}$=18.40, $P$<0.001). According to p*ost-hoc* analysis, birds spent more time feeding at 5˚C than at 15 and 25˚C ($P$<0.001), but there was no difference between 15 and 25˚C ($P$=0.67). There was a decrease in total time spent feeding with increasing sugar concentration, with all three concentrations being significantly different ($P$<0.02). Thus, the total time spent feeding was highest at 5˚C and on the lowest sugar concentration, with whitebellied sunbirds feeding for 46.68 ± 8.84 min, and amethyst sunbirds 34.55 ± 8.56 min per day. Birds spent the least time feeding at 25˚C and on the highest sugar concentration: whitebellied sunbirds fed for only 9.41 ± 1.61 min, and amethyst sunbirds only 9.62 ± 1.62 min over the entire day.

### *Body mass*

Birds generally lost body mass during test days (Fig. 4) because the experimental diets were not supplemented with protein. The change in body mass (%•24 $h^{-1}$) differed significantly between temperatures ($F_{2,32}$=14.93, $P$<0.001). Body mass change was the same at 5 and 15˚C ($P$=0.93), but birds lost more body mass at these two temperatures than at 25˚C ($P$<0.001). Change in body mass further differed between sugar concentrations ($F_{2,32}$=13.16, $P$<0.001), with birds losing more mass on the 0.25 M diet than on the 0.5 and 1 M diets ($P$<0.001). The two more concentrated diets did not differ in terms of body mass change ($P$=0.78). Body mass change differed between the two species ($F_{1,16}$=5.19, $P$=0.04), with whitebellied sunbirds losing less mass than amethyst sunbirds ($P$=0.04 after Bonferroni correction).

### *Sugar assimilation in whitebellied sunbirds*

The total amounts of sugar excreted by whitebellied sunbirds averaged 13.75 ± 0.94 mg•24 $h^{-1}$ on sucrose diets, and 8.82 ± 0.83 mg•24 $h^{-1}$ on hexose diets respectively, on all sugar concentrations and all temperatures. Total amounts of sugar excreted did not differ between diet sugar concentrations at both temperatures and on both sucrose and hexose diets ($R_s$>-0.53, $P$>0.14). Data for all concentrations were therefore pooled for each dietary sugar type to determine differences between temperatures. Total amounts of sugar excreted were independent of temperature on both sucrose ($F_{2,16}$=0.38, $P$=0.69) and hexose ($F_{1,8}$=2.84, $P$=0.13) diets. The amounts of sucrose, glucose and fructose excreted (mg•24 $h^{-1}$) on sucrose diets were independent of sugar concentration ($R_s$>-0.74, $P$>0.02; sequential Bonferroni: $P$>0.006, n.s.). Data of all concentrations at a given temperature were pooled to determine differences between sugar types. The amounts of sucrose, glucose and fructose excreted were found to differ significantly at all temperatures ($F_{2,16}$>4.94, $P$<0.02). *Post-hoc* analysis showed that the amount of excreted fructose was significantly lower than the amounts of both glucose and sucrose ($P$<0.02), while the amounts of sucrose and glucose did not differ ($P$>0.43). However, the apparent sucrose, glucose and fructose assimilation of whitebellied sunbirds was exceptionally high, irrespective of sugar concentration, dietary sugar type and temperature (SucAC*=99.81 ± 0.01%, n=27; GlucAC*=99.66 ± 0.05%, n=18; FrucAC*=99.74 ± 0.02%, n=18).

### *Gut morphology, sucrase activity and predicted maximal intake*

Data on gut morphology and sucrase activity were derived from four whitebellied sunbirds (body mass 8.98 ± 0.70 g), which were slightly heavier than the individuals used in our experiment, and from five amethyst sunbirds (body mass 14.17 ± 0.51 g), which had a very similar body mass to the individuals in our experiment. Total length of the intestine was 7.02 ± 0.30 cm in whitebellied and 8.40 ± 0.28 cm in amethyst sunbirds. The total intestinal volume equalled 133.33 ± 17.06 µl in whitebellied and 164.87 ± 10.65 µl in amethyst sunbirds. Maximal total intestinal sucrase activity was 8.31 ± 1.09 µmol•min$^{-1}$ in whitebellied and 13.30 ± 0.94 µmol•min$^{-1}$ in amethyst sunbirds.

The maximal daily volumetric food intake of whitebellied sunbirds predicted by the chemical reactor model of digestive capacity was lower than their daily volumetric food intake on the three sugar concentrations at 5˚C (Table 1). Consequently, the predicted maximal daily sugar intake of whitebellied sunbirds was lower than the observed sugar intake on all sugar concentrations in the cold. For amethyst sunbirds, the predicted maximal daily food intake and sugar intake were higher than the observed intakes at 5˚C, except on the 0.5 M diet where birds ingested slightly more than predicted (Table 1).

## Discussion

### *Compensatory feeding*

Nectarivorous birds regulate their energy intake in order to meet their metabolic requirements. When food quality is decreased, birds typically compensate by increasing their food intake. Whitebellied and amethyst sunbirds in our study responded to decreasing nectar sugar concentrations by increasing their consumption. This compensatory feeding response has previously been demonstrated in various avian nectarivores, including sunbirds (Downs 1997; Lotz and Nicolson 1999; McWhorter et al. 2004; Köhler et al. 2006), hummingbirds (López-Calleja et al. 1997; McWhorter and Martínez del Rio 1999) and honeyeaters (Collins and Clow 1978; Collins et al. 1980). Nectarivorous bats also compensate for low nectar concentrations by increasing their intake (Herrera and Mancina 2007; Ayala-Berdon et al. 2008).

When their energy demands are increased, birds also have to ingest more food. Ambient temperature determines the thermoregulatory costs of endothermic animals. The energetic costs of maintaining a constant body temperature increase with decreasing environmental temperature. Whitebellied and amethyst sunbirds from southern Africa experience large seasonal and daily fluctuations in ambient temperature. In winter, temperature often reaches 20˚C during the day, while it can drop to 5˚C or less at night. Rapid exposure to the cold in our study caused birds of both species to increase their nectar intake. This result coincides with findings in Anna’s hummingbirds, which also increase their nectar intake when exposed to low ambient temperatures for several days under laboratory conditions (Beuchat et al. 1979). Besides avian nectarivores, European stonechats (*Saxicola rubicola*) and young chickens (*Gallus gallus*) also increase their food intake in the cold (Kleiber and Dougherty 1934; Goymann et al. 2006). As examples for small mammals, house mice (*Mus musculus*), leaf-eared mice (*Phyllotis darwini*), Mongolian gerbils (*Meriones unguiculatus*) and Siberian hamsters (*Phodopus sungorus*) feed more in the cold than at moderate temperatures (Hammond et al. 1994; Konarzewski and Diamond 1994; Bozinovic and Nespolo 1997; Kauffman et al. 2001; Liu et al. 2002; Naya et al. 2005). Despite this increase in food intake in the cold, animals may experience physiological limitations and may be unable to maintain energy balance.

## *Physiological constraints to food intake*

The results of our study suggest that food intake in whitebellied and amethyst sunbirds, although increased in the cold, may be limited by physiological constraints. Resting metabolic rates of southern double-collared sunbirds (which are of comparable size to whitebellied sunbirds) and amethyst sunbirds increase linearly with decreasing ambient temperature and double over a 20˚C decline in temperature (Leon and Nicolson 1997; Lindsay et al. 2009a and b). Assuming that there is a similar increase in metabolic rate of our sunbirds as temperature decreases, we predicted that they would double their food intake at 5˚C compared to 25˚C. However, birds ingested only 27% more of the sugar solution at 5˚C than at 25˚C. Furthermore, there was no difference in food, and thus sugar, intake between 15 and 25˚C, although metabolic rate should have increased by approximately 25%. Consequently, birds lost more body mass at 5 and 15˚C than at 25˚C. Comparing the different dietary sugar concentrations, sunbirds ingested less sugar on the most dilute diet than on the more concentrated diets, despite the increase in nectar intake with decreasing sugar concentration. These findings indicate that the compensation for low food energy content and increased energy demands in the cold was incomplete.

The food intake of sunbirds may be limited by constraints on nectar digestion and osmoregulatory processes. The disaccharide sucrose needs to be hydrolyzed to glucose and fructose. Sucrose hydrolysis rates have been proposed to be a limiting factor when large amounts of nectar are ingested (McWhorter and Martínez del Rio 2000). Birds may also be constrained by the passive and carrier-mediated absorption of glucose and fructose (Martínez del Rio and Karasov 1990; McWhorter et al. 2006; Napier et al. 2008). Whitebellied sunbirds in our experiment, however, assimilated more than 99% of the ingested sugar, irrespective of nectar sugar concentration and ambient temperature. This confirms previous studies in sunbirds, sugarbirds, hummingbirds and honeycreepers where sugar assimilation efficiency has always been found to be 95% or higher (Downs 1997; McWhorter and Martínez del Rio 2000; Roxburgh and Pinshow 2002; Mata and Bosque 2004).

Besides possible constraints to nectar digestion, avian nectarivores ingesting large nectar volumes may be limited by the elimination of excess water (McWhorter and Martínez del Rio 1999; Hartman Bakken and Sabat 2006). However, sunbirds may be able to reduce intestinal water absorption and relieve their kidneys, as shown in Palestine sunbirds (*Cinnyris oseus*) (McWhorter et al. 2004). The regulation of ion levels may also be challenging for birds when the throughput of water is high. Whitebellied sunbirds excrete large amounts of ions on extremely dilute sucrose-only diets, on which they can not maintain energy balance (Fleming and Nicolson 2003).

Despite these possible physiological constraints, whitebellied and amethyst sunbirds were able to increase their food intake during acute cold exposure by almost 27%. When whitebellied sunbirds were exposed to 10˚C and an extremely dilute diet (0.1 M) for one acclimation and one test day, they increased their consumption by only 1% compared to 21˚C (Fleming et al. 2004). Rufous hummingbirds, kept at temperatures ranging from 38 to -1˚C for four to eight days each, increased their nectar intake by only 20% over this 39˚C decline in temperature (Beuchat et al. 1979 and 1990). Frugivorous yellow-vented bulbuls (*Pycnonotus xanthopygos*), acclimated to 10˚C and 28˚C for seven days each, did not increase their food intake at the low temperature (Van Tets et al. 2001).

Caution must be exercised in comparing the food intake of animals under acute and long-term cold exposures, since animals are able to ingest more food when they are acclimated to high feeding rates (Karasov and McWilliams 2005). McWilliams and Karasov (2001) demonstrated digestive adjustments in migratory birds, including increased gut size and thus larger quantities of digestive enzymes and nutrient transporters. Increases in the size of digestive organs caused by high energetic demands or changes in food quality have been shown in a variety of bird species (for a review see Starck 1999). This adjustment of the digestive system can occur within a few days or weeks (Karasov and McWilliams 2005). To avoid these physiological adjustments to high feeding rates, our sunbirds were exposed to low ambient temperature under acute, short-term conditions, with sufficient maintenance days between cold exposures. There was no evidence of physiological adjustments, since food intake did not increase with repeated cold exposure.

## *Maximal food intake in sunbirds*

Assuming that sucrose hydrolysis rates are limiting the food intake of the sunbirds in our study, we predicted their maximal food and sugar intake using a chemical reactor model of digestive capacity (McWhorter and Martínez del Rio 2000; Martínez del Rio et al. 2001). The observed values of food and sugar intake of whitebellied sunbirds, challenged by low ambient temperature, exceeded the predicted values on all diet sugar concentrations. For amethyst sunbirds, the predicted values of food and sugar intake were slightly higher than the observed intake, except on the intermediate sugar concentration.

In an earlier study, the model accurately predicted maximal food intake rates of broadtailed hummingbirds challenged by low ambient temperature (McWhorter and Martínez del Rio 2000). Predicted maximal food intake values also closely matched observed intake values of magnificent hummingbirds (*Eugenes fulgens*) with experimentally altered energy expenditures, although the exponent of the relationship between sugar concentration and intake predicted by the model was lower than that of the observed data (Martínez del Rio et al. 2001). In Pallas’s long-tongued bats (*Glossophaga soricina*) fed various nectar concentrations, the predicted maximal food intake exceeded the observed intake by 40–70%, indicating that the bats did not feed at their maximal rates (Ramírez et al. 2005). However, when *G. longirostris* were forced to maximize their food intake by limiting the time available for foraging (Winter 1998), the maximal intake rates predicted by the sucrose hydrolysis model were remarkably similar to the observed intake rates of the bats (Ramírez et al. 2005).

There are two possible explanations why the model may have underestimated the maximal food intake of our sunbirds, especially of whitebellied sunbirds. The individuals used for gut morphology and sucrase analysis differed from the ones used in the experiment. Firstly, these two groups of sunbirds were caught at slightly different times of the year. Birds that were euthanased were captured early in April, while birds used in the experiment were caught between late April and the beginning of May (South African autumn) the following year. It is possible that the gut volume and/or sucrase activity increase with decreasing environmental temperature. Thus, our experimental birds, caught later in autumn, may have had a higher maximal food intake due to larger guts or increased enzyme activity. Cedar waxwings (*Bombycilla cedrorum*), when acclimated to low ambient temperature, showed an increase in intestine size, while rates of enzyme activity and nutrient uptake stayed the same as at moderate temperature (McWilliams et al. 1999). Secondly, sunbirds used for morphological analysis were euthanased a few days after capture, while experimental birds were acclimated to a sucrose maintenance diet for several weeks. Sucrase levels of house sparrows (*Passer domesticus*) tended to be higher after 10 days on a high-carbohydrate diet than on a high-lipid diet, although this was not statistically significant (Caviedes-Vidal et al. 2000). Pine warblers (*Dendroica pinus*) were found to increase their intestinal sucrase activity on a fruit diet compared to insect or seed diets (Levey et al. 1999), although it must be noted that insects and seeds have a higher fat content than fruits and fat can depress sucrase activity. Several species of non-passerine birds also increase their carbohydrase activity in response to high carbohydrate content of their diet (Sell et al. 1989; Biviano et al. 1993). However, it is not known whether an exclusive sucrose diet influences intestinal sucrase activity in sunbirds. Future studies should therefore investigate the effect of season, acclimation to maintenance diets and constant laboratory conditions on gut morphology and digestive enzyme activity of nectarivorous birds. This might explain conflicting findings in a single species, such as broadtailed hummingbirds, which were able to increase their nectar intake at low ambient temperatures in one study (Fleming et al. 2004), but not in another (McWhorter and Martínez del Rio 2000).

### ***Energy-saving mechanisms***

If sunbirds experience physiological constraints, as assumed above, they may not be able to increase their food intake sufficiently to compensate for a decrease in diet sugar concentration or an increase in energy demands. To avoid an energy deficit, birds could reduce their activity and/or metabolic rate (for a review see Wang et al. 2006). Green-backed firecrowns reduce their activity to save energy at low ambient temperature (Fernández et al. 2002). As an example for small mammals, mice spend most of the time motionless when exposed to low temperatures (Konarzewski and Diamond 1994). The activity of the sunbirds could not be quantified in our study. However, at 5˚C, sunbirds were sitting quietly and with feathers ptiloerected to increase the insulating layer of warmer air around the body, while they were much more active at 15 and 25˚C, including jumping from branch to branch, flying around in the cages and singing (A. Köhler, pers. observation). Birds may have attempted to compensate for increased energetic costs in the cold by both increasing their energy intake and adjusting their behaviour to reduce energy expenditure and maintain body heat.

Several species of sunbirds, hummingbirds and honeyeaters reduce their body temperature as a response to energy stress (for review see McKechnie and Lovegrove 2002). When exposed to 10˚C, southern double-collared sunbirds decreased their body temperature by 4–5˚C compared to their body temperature when kept at 35˚C (Leon and Nicolson 1997). Broadtailed hummingbirds become torpid during the night when exposed to 10˚C and similar diet concentrations to those used in our study (McWhorter and Martínez del Rio 2000). When brown honeyeaters (*Lichmera indistincta*) and singing honeyeaters (*Meliphaga virescens*) were deprived of food for 1–2 h at the end of the day, they reduced their body temperature by 9˚C during the following night to conserve energy (Collins and Briffa 1984). In orange-breasted sunbirds (*Anthobaphes violacea*), a reduction in body temperature has been observed in a wild incubating female, whose foraging was interrupted by a storm (Williams 1993). Many species of birds and nectarivorous bats have been shown to save energy under unfavourable environmental conditions by slightly decreasing their body temperature or going into torpor (e.g. Cruz-Neto and Abe 1997; Coburn and Geiser 1998; McKechnie and Lovegrove 2003; Moe et al. 2005). In the present study, sunbirds did not become torpid during the night; they were active when caught for weighing in the morning irrespective of ambient temperature and diet sugar concentration. However, birds may still have slightly reduced their body temperature in order to save energy in the cold. We therefore recommend that future studies should measure the body temperature of birds during similar temperature challenges.

### *Feeding patterns*

Whitebellied and amethyst sunbirds compensated for a decrease in nectar sugar concentration by increasing the number of feeding events, while the duration of feeding events stayed constant. Thus, sunbirds adjusted their food intake via the number of feeding events, rather than mean feeding duration. This result confirms our earlier work on whitebellied sunbirds, where feeding frequency increased with decreasing nectar sugar concentration (Köhler et al. 2006 and 2008a). When ambient temperature was decreased, birds also increased their food intake by increasing the number of feeding events, instead of their durations. This was expected, since the amount of nectar that can be ingested at once may be limited by the absence of a crop (Mbatha et al. 2002) or stomach size (Bednekoff and Houston 1994).

Results for the total time spent feeding were similar to results for food intake in whitebellied and amethyst sunbirds. Both feeding parameters increased with decreasing nectar concentration and were higher at 5 than at 15˚C, but did not differ between 15 and 25˚C. These coincident findings confirm that total time spent feeding can be used as a surrogate for food intake, as we have previously demonstrated for whitebellied sunbirds (Köhler et al. 2008b).

## Conclusion

In summary, our sunbirds attempted to compensate for a decrease in nectar sugar concentration and ambient temperature by increasing their food intake. However, their sugar intake was still lower on the most dilute diet and birds lost body mass at the two lower temperatures. We therefore conclude that the intake response of captive whitebellied and amethyst sunbirds is shaped by both compensatory feeding and physiological constraint. It is likely that wild sunbirds face similar limitations to food intake when ambient temperature is low or bad weather disrupts foraging or dilutes nectar. Further studies are needed to investigate whether digestive constraints affect these sunbirds under natural conditions, and whether a reduction in energy expenditures, in addition to an increase in energy intake, helps the sunbirds to cope with adverse environmental conditions.

## Acknowledgements

This project was funded by the University of Pretoria and the South African National Research Foundation. We are grateful to Jan Cilliers Park for permission to mist-net sunbirds under permit from the Gauteng Directorate of Nature Conservation. Craig Symes and Darren W. Pietersen are thanked for catching sunbirds for our study. Our experiments were approved by the Animal Use and Care Committee of the University of Pretoria.

## References

Ayala-Berdon J., Schondube J.E., Stoner K.E., Rodriguez-Peña N. and Martínez del Rio C. 2008. The intake responses of three species of leaf-nosed Neotropical bats. Journal of Comparative Physiology B 178: 477–485.

Bednekoff P.A. and Houston A.I. 1994. Avian daily foraging patterns: effects of digestive constraints and variability. Evolutionary Ecology 8: 36–52.

Beuchat C.A., Chaplin S.B. and Morton M.L. 1979. Ambient temperature and the daily energetics of two species of hummingbirds, *Calypte anna* and *Selasphorus rufus*. Physiological Zoology 53: 280–295.

Beuchat C.A., Calder W.A. and Braun E.J. 1990. The integration of osmoregulation and energy balance in hummingbirds. Physiological Zoology 63: 1059–1081.

Biviano A.B., Martínez del Rio C. and Phillips D.L. 1993. Ontogenesis of intestine morphology and intestinal disaccharidases in chickens (*Gallus gallus*) fed contrasting purified diets. Journal of Comparative Physiology B 163: 508–518.

Bozinovic F. and Nespolo R.F. 1997. Effect of ambient temperature and energy demands on digestive functions in leaf-eared mice (*Phyllotis darwini*) from central Chile. International Journal of Biometeorology 41: 23–25.

Caviedes-Vidal E., Afik D., Martínez del Rio C. and Karasov W.H. 2000. Dietary modulation of intestinal enzymes of the house sparrow (*Passer domesticus*): testing an adaptive hypothesis. Comparative Biochemistry and Physiology A 125: 11–24.

Coburn D.K. and Geiser F. 1998. Seasonal changes in energetics and torpor patterns in the subtropical blossom-bat *Syconycteris australis* (Megachiroptera). Oecologia 113: 467–473.

Collins B.G. and Briffa P. 1984. Nocturnal energy expenditure by honeyeaters experiencing food shortage and low environmental temperatures. Comparative Biochemistry and Physiology A 78: 77–81.

Collins B.G. and Clow H. 1978. Feeding behaviour and energetics of the western spinebill, *Acanthorhynchus superciliosis* (Aves: Meliphagidae). Australian Journal of Zoology 26: 269–277.

Collins B.G., Cary G. and Packard G. 1980. Energy assimilation, expenditure and storage by the brown honeyeater, *Lichmera indistincta*. Journal of Comparative Physiology B 137: 157–163.

Cruz-Neto A.P. and Abe A.S. 1997. Metabolic rate and thermoregulation in the nectarivorous bat, *Glossophaga soricina* (Chiroptera, Phyllostomatidae). Revista Brasileira de Biologia 57: 203–209.

Cruz-Rivera E. and Hay M.E. 2000. Can quantity replace quality? Food choice, compensatory feeding, and fitness of marine mesograzers. Ecology 81: 201–219.

Dahlquist A. 1984. Assay of intestinal disaccharidases. Scandinavian Journal of Clinical and Laboratory Investigation 44: 69–72.

Downs C.T. 1997. Sugar digestion efficiencies of Gurney’s sugarbirds, malachite sunbirds, and black sunbirds. Physiological Zoology 70: 93–99.

Fassbinder-Orth C.A. and Karasov W.H. 2006. Effects of feeding restriction and realimentation on digestive and immune function in the Leghorn chick. Poultry Science 85: 1449–1456.

Fernández M.J., López-Calleja M.V. and Bozinovic F. 2002. Interplay between the energetics of foraging and thermoregulatory costs in the green-backed firecrown hummingbird *Sephanoides sephanoides*. Journal of Zoology 258: 319–326.

Fleming P.A. and Nicolson S.W. 2003. Osmoregulation in an avian nectarivore, the whitebellied sunbird *Nectarinia talatala*: response to extremes of diet concentration. Journal of Experimental Biology 206: 1845–1854.

Fleming P.A., Hartman Bakken B., Lotz C.N. and Nicolson S.W. 2004. Concentration and temperature effects on sugar intake and preferences in a sunbird and a hummingbird. Functional Ecology 18: 223–232.

Gass C.L., Romich M.T. and Suarez R.K. 1999. Energetics of hummingbird foraging at low ambient temperature. Canadian Journal of Zoology 77: 314–320.

Goymann W., Trappschuh M., Jensen W. and Schwabl I. 2006. Low ambient temperature increases food intake and dropping production, leading to incorrect estimates of hormone metabolite concentrations in European stonechats. Hormones and Behavior 49: 644–653.

Hammond K.A., Konarzewski M., Torres R.M. and Diamond J. 1994. Metabolic ceilings under a combination of peak energy demands. Physiological Zoology 67: 1479–1506.

Hartman Bakken B. and Sabat P. 2006. Gastrointestinal and renal responses to water intake in the green-backed firecrown (*Sephanoides sephanoides*), a South American hummingbird. American Journal of Physiology (Regulatory, Integrative and Comparative Physiology) 291: R830–R836.

Herrera L.G. and Mancina C.A. 2007. Sucrose hydrolysis does not limit food intake by Pallas’s long-tongued bats. Physiological and Biochemical Zoology 81: 119–124.

Karasov W.H. and McWilliams S.R. 2005. Digestive constraints in mammalian and avian ecology. In: Physiological and ecological adaptations to feeding in vertebrates. J.M. Starck and T. Wang (Eds.), Science Publishers, Enfield, New Hampshire, USA, pp. 87–112.

Kauffman A.S., Cabrera A. and Zucker I. 2001. Energy intake and fur in summer- and winter-acclimated Siberian hamsters (*Phodopus sungorus*). American Journal of

Physiology (Regulatory, Integrative and Comparative Physiology) 281: R519–R527.

Kleiber M. and Dougherty J.E. 1934. The influence of environmental temperature on the utilization of food energy in baby chicks. Journal of General Physiology 17: 701–726.

Köhler A., Verburgt L. and Nicolson S.W. 2006. Short-term feeding patterns of whitebellied sunbirds (*Nectarinia talatala*): feeding frequency, daily rhythms and individual differences. Journal of Experimental Biology 209: 2880–2887.

Köhler A., Verburgt L., Fleming P.A. and Nicolson S.W. 2008a. Changes in nectar concentration: how quickly do whitebellied sunbirds (*Cinnyris talatala*) adjust feeding patterns and food intake? Journal of Comparative Physiology B 178: 785–793.

Köhler A., Verburgt L. and Nicolson S.W. 2008b. Nectar intake of whitebellied sunbirds (*Cinnyris talatala*): Can meal size be inferred from feeding duration? Physiological and Biochemical Zoology 81: 682–687.

Konarzewski M. and Diamond J. 1994. Peak sustained metabolic rate and its individual variation in cold-stressed mice. Physiological Zoology 67: 1186–1212.

Lee W.B. and Houston D.C. 1993. The effect of diet quality on gut anatomy in British voles (Microtinae). Journal of Comparative Physiology B 163: 337–339.

Leon B. and Nicolson S.W. 1997. Metabolic rate and body temperature of an African sunbird, *Nectarinia chalybea*: daily rhythm and the effect of ambient temperature. South African Journal of Zoology 32: 31–36.

Levey D.J., Place A.R., Rey P.J. and Martínez del Rio C. 1999. An experimental test of dietary enzyme modulation in pine warblers *Dendroica pinus*. Physiological and Biochemical Zoology 72: 576–587.

Lindsay C.V., Downs C.T. and Brown M. 2009a. Physiological variation in Amethyst Sunbirds (*Chalcomitra amethystina*) over an altitudinal gradient in winter. Journal of Experimental Biology 212: 483–493.

Lindsay C.V., Downs C.T. and Brown M. 2009b. Physiological variation in Amethyst Sunbirds (*Chalcomitra amethystina*) over an altitudinal gradient in summer. Journal of Thermal Biology 34: 190–199.

Liu H., Wang D.H. and Wang Z.W. 2002. Maximum metabolizable energy intake in the Mongolian gerbil (*Meriones unguiculatus*). Journal of Arid Environments 52: 405–411.

López-Calleja M.V., Bozinovic F. and Martínez del Rio C. 1997. Effects of sugar concentration on hummingbird feeding and energy use. Comparative Biochemistry and Physiology A 118: 1291–1299.

Lotz C.N. 1999. Energy and water balance in the lesser double-collared sunbird, *Nectarinia chalybea*. PhD thesis, University of Cape Town, South Africa.

Lotz C.N. and Nicolson S.W. 1999. Energy and water balance in the lesser double-collared sunbird (*Nectarinia chalybea*) feeding on different nectar concentrations. Journal of Comparative Physiology B 169: 200–206.

Lotz C.N. and Nicolson S.W. 2002. Nectar dilution increases metabolic rate in the lesser double-collared sunbird. Condor 104: 672–675.

Lotz C.N., Martínez del Rio C. and Nicolson S.W. 2003. Hummingbirds pay a high cost for a warm drink. Journal of Comparative Physiology B 173: 455–462.

Martínez del Rio C. and Karasov W.H. 1990. Digestion strategies in nectar- and fruit-eating birds and the sugar composition of plant rewards. American Naturalist 136: 618–637.

Martínez del Rio C., Brugger K.E., Rios J.L., Vergara M.E. and Witmer M. 1995. An experimental and comparative study of dietary modulation of intestinal enzymes in European starlings (*Sturnus vulgaris*). Physiological Zoology 68: 490–511.

Martínez del Rio C., Schondube J.E., McWhorter T.J. and Herrera L.G. 2001. Intake responses in nectar feeding birds: digestive and metabolic causes, osmoregulatory consequences, and coevolutionary effects. American Zoologist 41: 902–915.

Mata A. and Bosque C. 2004. Sugar preferences, absorption efficiency and water influx in a Neotropical nectarivorous passerine, the Bananaquit (*Coereba flaveola*). Comparative Biochemistry and Physiology A 139: 395–404.

Mbatha K., Downs C.T. and Penning M. 2002. Nectar passage and gut morphology in the Malachite Sunbird and the Black-capped Lory: implications for feeding in nectarivores. Ostrich 73: 138–142.

McCauley S.J. 1999. Response to dietary dilution in an omnivorous freshwater turtle: implications for ontogenetic dietary shifts. Physiological and Biochemical Zoology 72: 101–108.

McKechnie A.E. and Lovegrove B.G. 2002. Avian facultative hypothermic responses: a review. Condor 104: 705–724.

McKechnie A.E. and Lovegrove B.G. 2003. Facultative hypothermic responses in an Afrotropical arid-zone passerine, the red-headed finch (*Amadina erythrocephala*). Journal of Comparative Physiology B 173: 339–346.

McWhorter T.J. and Martínez del Rio C. 1999. Food ingestion and water turnover in hummingbirds: how much dietary water is absorbed? Journal of Experimental Biology 202: 2851–2858.

McWhorter T.J. and Martínez del Rio C. 2000. Does gut function limit hummingbird food intake? Physiological and Biochemical Zoology 73: 313–324.

McWhorter T.J., Martínez del Rio C., Pinshow B. and Roxburgh L. 2004. Renal function in Palestine sunbirds: elimination of excess water does not constrain energy intake. Journal of Experimental Biology 207: 3391–3398.

McWhorter T.J., Hartman Bakken B., Karasov W.H. and Martínez del Rio C. 2006. Hummingbirds rely on both paracellular and carrier-mediated intestinal glucose absorption to fuel high metabolism. Biology Letters 2: 131–134.

McWilliams S.R. and Karasov W.H. 2001. Phenotypic flexibility in digestive system structure and function in migratory birds and its ecological significance. Comparative Biochemistry and Physiology A 128: 579–593.

McWilliams S.R., Caviedes-Vidal E. and Karasov W.H. 1999. Digestive adjustments in cedar waxwings to high feeding rate. Journal of Experimental Zoology 283: 394–407.

Moe B., Stølevik E. and Bech C. 2005. Ducklings exhibit substantial energy-saving mechanisms as a response to short-term food shortage. Physiological and Biochemical Zoology 78: 90–104.

Montgomery M.J. and Baumgardt B.R. 1965. Regulation of food intake in ruminants. 1. Pelleted rations varying in energy concentration. Journal of Dairy Science 48: 569–574.

Nance D.M., Bromley B., Barnard R.J. and Gorski R.A. 1977. Sexually dimorphic effects of forced exercise on food intake and body weight in the rat. Physiology and Behavior 19: 155–158.

Napier K.R., Purchase C., McWhorter T.J., Nicolson S.W. and Fleming P.A. 2008. The sweet life: diet sugar concentration influences paracellular glucose absorption. Biology Letters 4: 530–533.

Naya D.E., Bacigalupe L.D., Bustamante D.M. and Bozinovic F. 2005. Dynamic digestive responses to increased energy demands in the leaf-eared mouse (*Phyllotis darwini*). Journal of Comparative Physiology B 175: 31–36.

Nicolson S.W. and Fleming P.A. 2003a. Nectar as food for birds: the physiological consequences of drinking dilute sugar solutions. Plant Systematics and Evolution 238: 139–153.

Nicolson S.W. and Fleming P.A. 2003b. Energy balance in the Whitebellied Sunbird *Nectarinia talatala*: constraints on compensatory feeding, and consumption of supplementary water. Functional Ecology 17: 3–9.

Ramírez N., Herrera L.G. and Mirón L. 2005. Physiological constraint to food ingestion in a New World nectarivorous bat. Physiological and Biochemical Zoology 78: 1032–1038.

Rice W.R. 1989. Analyzing tables of statistical tests. Evolution 43: 223–225.

Roxburgh L. and Pinshow B. 2002. Digestion of nectar and insects by Palestine sunbirds. Physiological and Biochemical Physiology 75: 583–589.

Sell J.L., Koldovsky O. and Reid B.L. 1989. Intestinal disaccharidases of young turkeys: temporal development and influence of diet composition. Poultry Science 68: 265–277.

Starck J.M. 1999. Structural flexibility of the gastro-intestinal tract of vertebrates – implications for evolutionary morphology. Zoologischer Anzeiger 238: 87–101.

Tooze Z.J. and Gass C.L. 1985. Responses of rufous hummingbirds to midday fasts. Canadian Journal of Zoology 63: 2249–2253.

Valencak T.G., Tataruch F. and Ruf T. 2009. Peak energy turnover in lactating European hares: the role of fat reserves. Journal of Experimental Biology 212: 231–237.

Van Tets I.G. and Nicolson S.W. 2000. Pollen and the nitrogen requirements of the lesser double-collared sunbird. Auk 117: 826–830.

Van Tets I.G., Korine C., Roxburgh L. and Pinshow B. 2001. Changes in the composition of the urine of yellow-vented bulbuls (*Pycnonotus xanthopygos*): the effects of ambient temperature, nitrogen, and water intake. Physiological and Biochemical Zoology 74: 853–857.

Wang T., Hung C.C.Y. and Randall D.J. 2006. The comparative physiology of food deprivation: from feast to famine. Annual Review of Physiology 68: 223–251.

Williams J.B. 1993. Energetics of incubation in free-living orange-breasted sunbirds in South Africa. Condor 95: 115–126.

Winter Y. 1998. *In vivo* measurement of near maximal rates of nutrient absorption in a mammal. Comparative Biochemistry and Physiology A 119: 853–859.

Wolf L.L. and Hainsworth F.R. 1977. Temporal patterning of feeding by hummingbirds. Animal Behaviour 25: 976–989.

Yang Y. and Joern A. 1994. Compensatory feeding in response to variable food quality by *Melanoplus differentialis*. Physiological Entomology 19: 75–82.

# Table

**Table 1.** Observed daily food and sugar intake of nine whitebellied (WBSB) and nine amethyst (ASB) sunbirds on three sucrose concentrations at 5°C (mean ± SE) and maximal daily food and sugar intake predicted by a chemical reactor model of digestive capacity (McWhorter and Martínez del Rio 2000; see Appendix for a description of the model).

| Species | Sucrose concentration (M) | Volumetric food intake ($ml \cdot 12\ h^{-1}$) | | Sugar intake ($g \cdot 12\ h^{-1}$) | |
|---|---|---|---|---|---|
| | | Observed (mean ± SE) | Predicted | Observed (mean ± SE) | Predicted |
| WBSB | 0.25 | 36.131 ± 0.809 | 30.154 | 3.092 ± 0.069 | 2.580 |
| WBSB | 0.5 | 19.249 ± 0.690 | 16.967 | 3.295 ± 0.118 | 2.904 |
| WBSB | 1 | 9.582 ± 0.271 | 9.051 | 3.280 ± 0.093 | 3.098 |
| ASB | 0.25 | 41.250 ± 1.559 | 46.670 | 3.530 ± 0.133 | 3.994 |
| ASB | 0.5 | 26.273 ± 1.558 | 26.037 | 4.497 ± 0.267 | 4.456 |
| ASB | 1 | 12.288 ± 0.456 | 13.819 | 4.206 ± 0.156 | 4.730 |

# Figure legends

**Fig. 1.** Daily mass-specific food intake (g•24 $h^{-1}$•g body $mass^{-1}$) of nine whitebellied sunbirds (above) and nine amethyst sunbirds (below) feeding on sucrose diets of three sugar concentrations and at three ambient temperatures (mean ± SE). Trendlines are power functions.

**Fig. 2.** Daily mass-specific sugar intake (g•24 $h^{-1}$•g body $mass^{-1}$) of nine whitebellied sunbirds (above) and nine amethyst sunbirds (below) feeding on sucrose diets of three sugar concentrations and at three ambient temperatures (mean ± SE).

**Fig. 3.** Number of feeding events during the 12 h light period for nine whitebellied sunbirds (above) and nine amethyst sunbirds (below) feeding on sucrose diets of three sugar concentrations and at three ambient temperatures (mean ± SE).

**Fig. 4.** Daily body mass change (%•24 $h^{-1}$) of nine whitebellied sunbirds (above) and nine amethyst sunbirds (below) feeding on sucrose diets of three sugar concentrations and at three ambient temperatures (mean ± SE).

# Figures

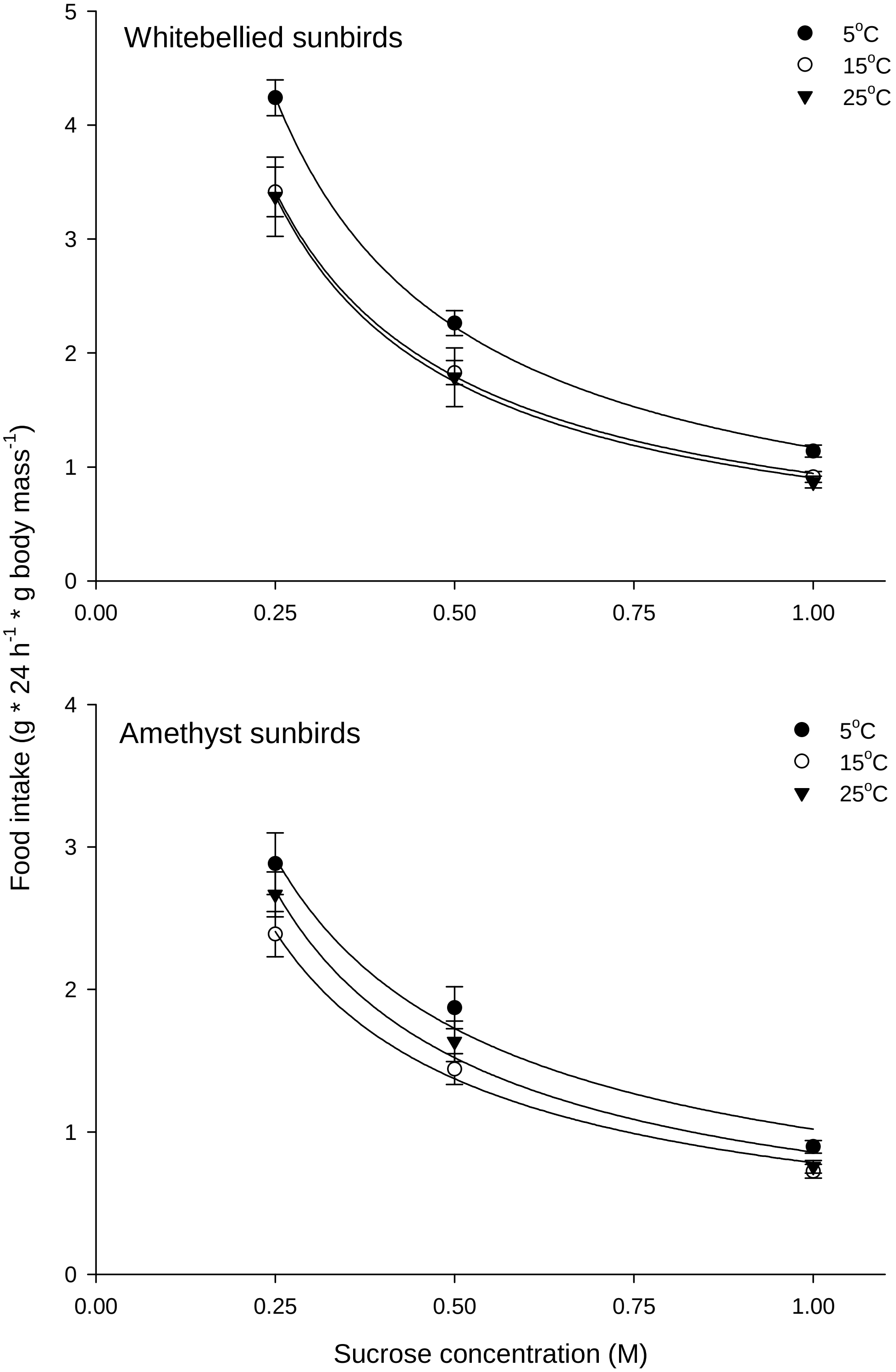


Figure 1.

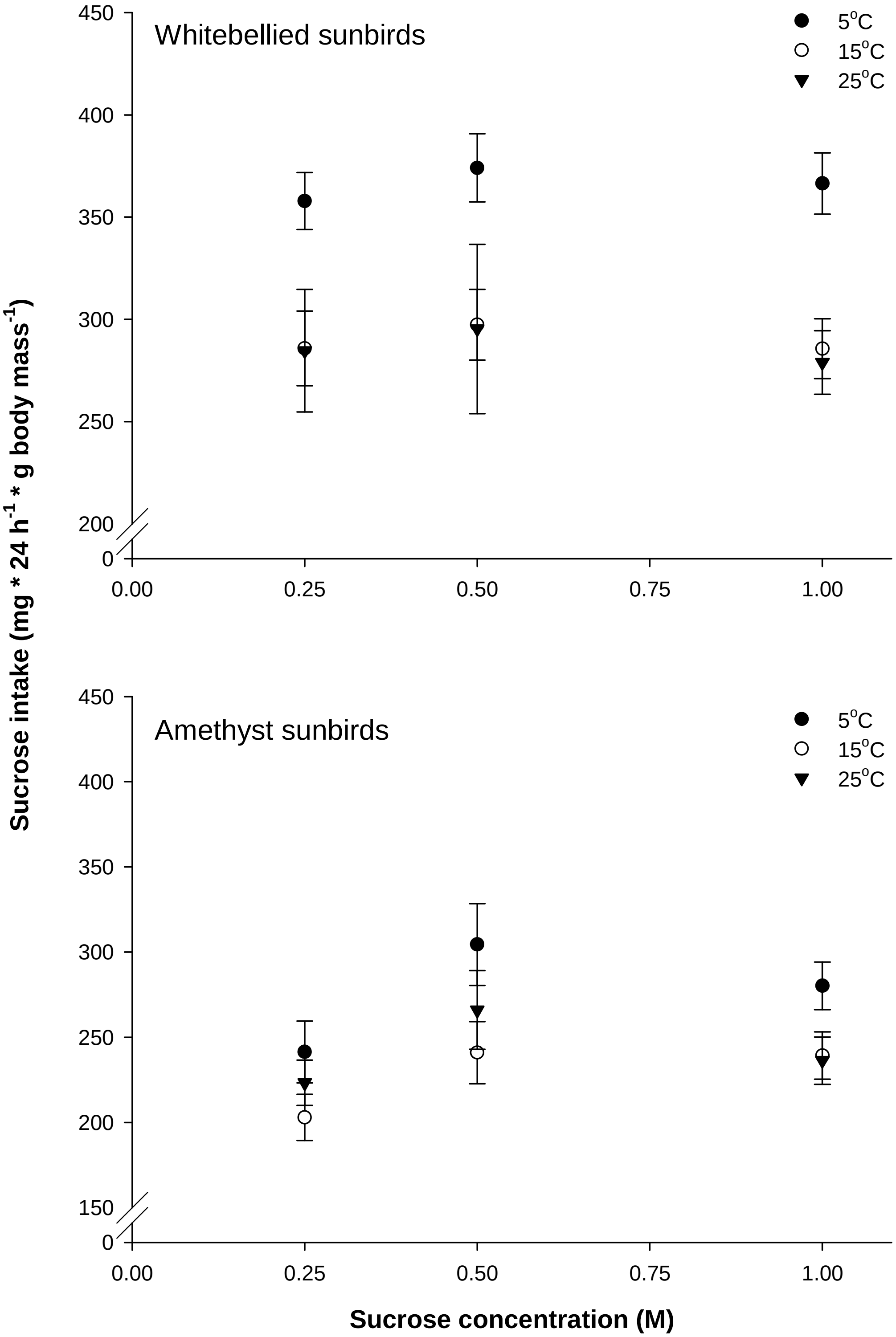

Whitebellied sunbirds
● 5°C
○ 15°C
▼ 25°C
450
400
350
300
250
200
0
0.00
0.25
0.50
0.75
1.00
Amethyst sunbirds
● 5°C
○ 15°C
▼ 25°C
450
400
350
300
250
200
150
0
0.00
0.25
0.50
0.75
1.00
Sucrose intake (mg * 24 h-1 * g body mass-1)
Sucrose concentration (M)

Figure 2.

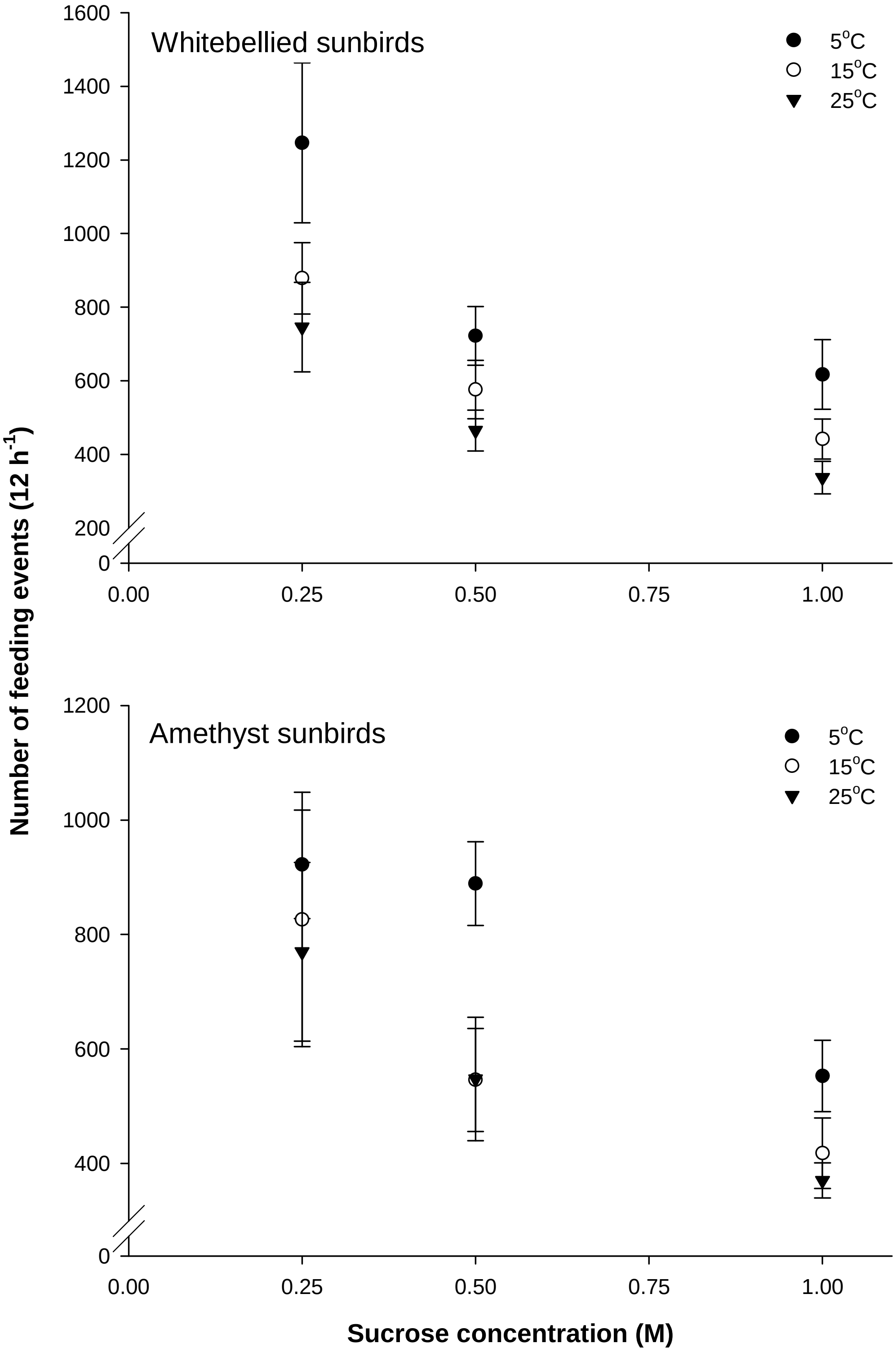

Whitebellied sunbirds
Amethyst sunbirds
● 5°C
○ 15°C
▼ 25°C
1600
1400
1200
1000
800
600
400
200
0
0.00
0.25
0.50
0.75
1.00
Number of feeding events (12 h-1)
Sucrose concentration (M)

Figure 3.

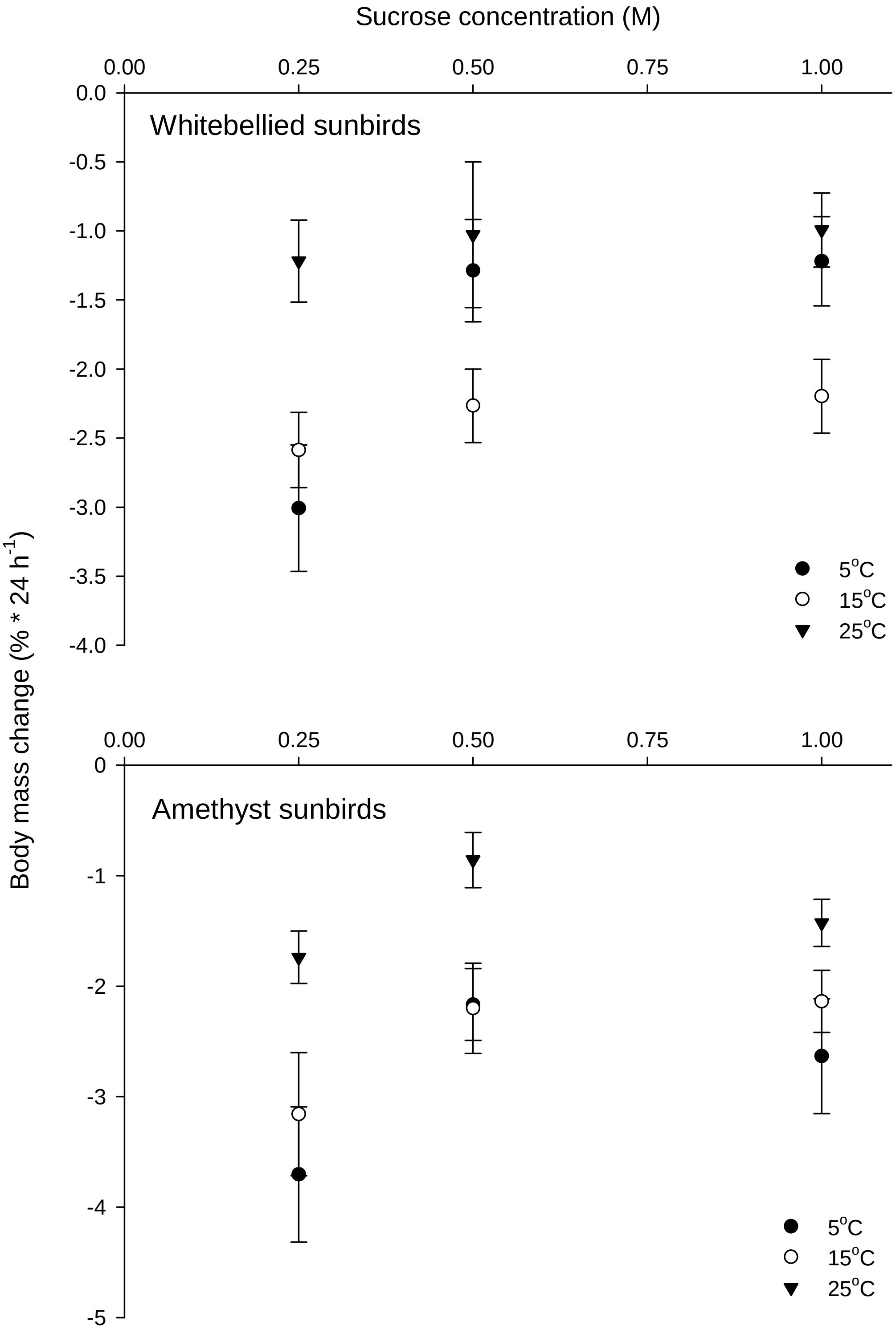

Sucrose concentration (M)
0.00
0.25
0.50
0.75
1.00
Whitebellied sunbirds
0.0
-0.5
-1.0
-1.5
-2.0
-2.5
-3.0
-3.5
-4.0
● 5°C
○ 15°C
▼ 25°C
Body mass change (% * 24 h-1)
Amethyst sunbirds
0
-1
-2
-3
-4
-5
● 5°C
○ 15°C
▼ 25°C

Figure 4.

# CHAPTER 5

# TEMPERATURE CHALLENGES IN BROWN HONEYEATERS (*LICHMERA INDISTINCTA*): ACUTE COLD EXPOSURE AND POSSIBLE EFFECTS OF ACCLIMATION

## Abstract

The food consumption of endothermic animals typically increases with decreasing ambient temperature due to the higher energetic costs of maintaining a constant body temperature. In the present study, captive brown honeyeaters (*Lichmera indistincta*) were exposed to two ambient temperatures (5˚C and 22˚C), while feeding on four diet sugar concentrations (0.25, 0.5, 0.75 and 1 M) and two dietary sugar types (sucrose and an energetically equivalent glucose:fructose (1:1) mixture). Birds increased their food intake with decreasing diet sugar concentration at both temperatures. During acute short-term cold exposure, birds increased their food intake by 18%, compared to the moderate temperature, on all sugar concentrations. Food intake was the same for both sucrose and hexose diets. Birds lost more body mass in the cold than at 22˚C on sucrose diets, but not on hexose diets, indicating physiological constraints. Apparent sugar assimilation in these honeyeaters was >99% on all diet sugar concentrations and both sugar types and at both ambient temperatures. During the second exposure to 5˚C, birds showed similar compensatory feeding over the range of sugar concentrations used and increased their food intake by 21%, compared to 22˚C. In this second experiment, birds ate more on the most dilute diet at both ambient temperatures and on both sucrose and hexose diets.

These findings suggest that physiological adjustments to high feeding rates may have already taken place after a few days of cold exposure. The maximal food and sucrose intake predicted by a chemical reactor model of digestive capacity closely matched the observed intake of cold-stressed brown honeyeaters in this experiment.

## Introduction

Ambient temperature determines the thermoregulatory costs of endothermic animals. The energetic costs of maintaining a constant body temperature increase with decreasing ambient temperature, which results in higher energy requirements at low temperatures (McNab 2002). Birds compensate for these increased energetic needs by increasing their food consumption at low environmental temperature (e.g. Goymann et al. 2006; Salvante et al. 2007). Mammals, such as gerbils, mice and voles also ingest more food in the cold (Mele 1972; Bozinovic and Nespolo 1997; Zhang and Wang 2007). At low ambient temperature, small nectar-feeding birds face particularly high energetic costs to defend a constant body temperature because of their unfavourable surface area to volume ratio (López-Calleja and Bozinovic 1995). Increased energy demands are associated with an increase in nectar intake, which results in high food warming costs (Lotz and Nicolson 2002; Lotz et al. 2003).

When energy demands increase suddenly, animals can increase their food intake only within the limit of their acute spare digestive capacity, which is the difference between the rate of digestion at maintenance level and the maximum rate of digestion, and ranges from 9–50% above routine rates in different species (Karasov and McWilliams 2005). At acute cold exposure, rufous hummingbirds (*Selasphorus rufus*) are able to increase their food intake sufficiently to maintain energy balance on concentrated nectar diets, but not on dilute diets (Gass et al. 1999). Whitebellied sunbirds (*Cinnyris talatala*) increased their intake on a moderate diet (1 M sucrose and hexoses) by 18% at 10˚C, compared to 21˚C, but on a dilute diet (0.1 M) food consumption was increased by only 1% in the cold (Fleming et al. 2004). These studies suggest that the compensatory feeding response of nectar-feeding birds may be influenced by constraints to digestive and osmoregulatory processes (e.g. McWhorter and Martínez del Rio 1999 and 2000; Fleming and Nicolson 2003; Napier et al. 2008; for more details see Chapter 4). Instead of matching high energy requirements at low ambient temperature with increased food intake, birds may reduce their activity and/or body temperature, or even go into torpor to save energy (Bicudo 1996; Fernández et al. 2002).

The ability of birds to increase their food intake increases when they are acclimated to high feeding rates. McWilliams and Karasov (2001) demonstrated digestive adjustments in migratory birds, including increased gut size and thus increased amounts of nutrient transporters and digestive enzymes. Increases in the size of digestive organs caused by high energetic demands or changes in food quality have been demonstrated for a variety of bird species (for a review see Starck 1999a). This adjustment of the digestive system leads to a long-term spare capacity of 100–125% above routine rates (Karasov and McWilliams 2005). White-throated sparrows (*Zonotrichia albicollis*) were able to increase their feeding rate by 45% when switched rapidly from 21˚C to -20˚C, but their food intake increased even more, by 83%, when they were gradually acclimated to this low temperature over 50 days (Karasov and McWilliams 2005). Anna's hummingbirds (*Calypte anna*), when kept at ambient temperatures ranging from 38 to -1˚C for 4–8 days each, nearly doubled their food intake over a 30˚C decline in ambient temperature (Beuchat et al. 1990). Physiological changes that lead to increased digestive capacity can take place within few days or weeks of exposure to high feeding rates, and occur faster in small animals than in larger ones (Starck 1999a; Karasov and McWilliams 2005).

The aim of this study was to investigate the effect of acute exposure to low ambient temperature on food intake and energy balance of brown honeyeaters (*Lichmera indistincta*, Meliphagidae), as well as the effect of various diet sugar concentrations and the two main sugar types found in nectar (sucrose and hexoses). I predicted (a) that honeyeaters would increase their food intake with decreasing sugar concentration irrespective of ambient temperature, which is commonly known as compensatory feeding

(Martínez del Rio et al. 2001) and has been demonstrated in various avian nectarivores (e.g. Collins and Clow 1978; McWhorter and Martínez del Rio 1999; Köhler et al. 2006). I further predicted (b) that birds would increase their food intake at low ambient temperature on all sugar concentrations, (c) that birds would lose more body mass in the cold than at moderate ambient temperature and (d) that there would be no difference in food intake and body mass changes between sugar types, as nectarivorous birds assimilate both sucrose and hexose diets equally well (Lotz and Schondube 2006). When birds are repeatedly exposed to low ambient temperature, I expected (e) that food intake and energy balance of the birds would not differ from the first acute cold exposure since the acclimation period is expected to be too short to result in physiological adjustments.

# Materials and methods

## *Study animals and their maintenance*

Eight brown honeyeaters were captured with mist-nets on the Murdoch University campus, Perth, Western Australia. Birds were housed in individual cages at 20 ± 2ºC and a 12:12 h L:D photoperiod with lights-on at 07h00. The maintenance diet consisted of commercially available honeyeater and lorikeet nectar (Wombaroo® Food products, Adelaide, South Australia), which contains sucrose as the main sugar type, with additional sucrose, resulting in a total sugar concentration of 0.8 M. The nectar substitute and supplementary water were provided *ad libitum* in inverted, stoppered syringes. The sexes of brown honeyeaters can not be distinguished morphologically; body mass (mean ± SE) of the eight individuals was 10.10 ± 0.42 g.

## *Experimental procedure*

### Part I: Acute cold exposure

Honeyeaters were exposed to two ambient temperatures (5 and 22˚C), two dietary sugar types (sucrose and an energetically equivalent glucose:fructose (1:1) mixture) and four diet sugar concentrations (0.25, 0.5, 0.75 and 1 M, which are equivalent to 8.5, 16, 24 and 30% w/w). These experimental diets were chosen because the floral nectar of honeyeater-pollinated plants contains both sugar types and measured sugar concentrations range from 0.15–1.35 M (Nicolson and Fleming 2003a). Each bird was randomly assigned to one of the four sugar concentrations, which it received at both temperatures and using both sugar types, i.e. each sugar concentration was consumed by two birds. The sequence of temperature and sugar type exposure was randomized. The photoperiod remained the same as during maintenance. Each part of the experiment, hereafter referred to as trial, consisted of one day during which the birds were acclimated to the experimental temperature and the test diet, followed by one test day. Note that honeyeaters were exposed to 5˚C for the first time and under acute, short-term conditions. Without sufficient time to acclimate, the increase in their food intake in the cold represents the birds' acute spare digestive capacity (Karasov and McWilliams 2005). Birds were given at least two maintenance days between the trials to regain energy balance. They generally lost body mass during the trials because experimental diets were not supplemented with protein (Nicolson and Fleming 2003b). The next trial commenced when birds regained their original body mass, as measured before the start of the experiment.

Birds were captured, placed in a cloth bag and weighed before lights-on on each test day and the day after, using an electronic balance (Scout Pro SP 402, 0.01 g, Ohaus Corp., Pine Brook, NJ USA). At the same time, feeders were weighed to determine the mass of food consumed on the test day. Any food dripping from feeders was collected in trays with liquid paraffin (to prevent evaporative mass loss), which were weighed at the same time as the feeders. Diet evaporation was determined using additional feeders with all diet concentrations and both sugar types. These feeders were weighed before and after one test day at 5˚C, and one at 22˚C, and the mass of food consumed was corrected accordingly.

## Part II: Repeated cold exposure

The experiment described in Part I was repeated one week after the birds had been exposed to energetically challenging conditions. During Part I of this experiment, they were kept at low ambient temperature (5˚C) twice for two days each. In addition, some birds received low diet sugar concentrations. All birds were further exposed to 10˚C once for three consecutive days, including a 2 h fasting period, during another experiment (compare Chapter 3). Thus, the birds were not naïve to the cold any longer and acclimation to energetically challenging conditions, resulting in physiological adjustments (Starck 1999a; McWilliams and Karasov 2001), may have occurred. Consequently, the increase in food intake may no longer represent the birds' acute spare digestive capacity. Each bird was randomly assigned to one of the four sugar concentrations it did not receive in Part I, and was fed this concentration at both temperatures and both sugar types. The experimental conditions and procedures were identical to Part I.

## *Diet density, sugar assimilation and gut physiology measurements*

The densities of all experimental test diets and distilled water were determined by weighing six 1 ml samples each to the nearest 0.1 mg (BP221S, Sartorius AG, Göttingen, Germany). The density of distilled water was divided by the expected density of distilled water (1 g•ml$^{-1}$), resulting in the dimensionless correction factor q. Diet densities were then divided by q to correct for pipette errors. On each test day of Part I, 24 h excreta from all birds were collected on plastic trays placed underneath the cages. The excreta samples were allowed to evaporate and later re-diluted with distilled water of known volume. The samples were then assayed for sucrose content (when birds were fed sucrose diets), and glucose and fructose content (for both sucrose and hexose diets) respectively, using Sigma-Aldrich (Munich, Germany) colorimetric/enzymatic kits and a spectrophotometer (UV mini 1240 UV-VIS, Shimadzu Scientific, Balcatta, Western Australia).

After completion of the experiment, five birds (three males and two females; mean body mass ± SE: 10.62 ± 0.51 g) were euthanased by a halothane overdose and data on gut morphology and intestinal sucrase activity were obtained by Dr T.J. McWhorter (Murdoch University, Perth, Western Australia). The small intestine of each bird was removed instantly after euthanasia, flushed clean with ice cold saline, cut into sections and dissected lengthwise. The length and nominal surface area of each section were measured and used to calculate the volume of each section and later the total volume of the small intestine. All sections of the small intestine were weighed to the nearest 0.1 mg (BP221S Sartorius AG, Göttingen, Germany) before being stored in liquid nitrogen. Samples were later thawed and homogenized (Heidolph Diax 600, Heidolph, Germany) in 300 mM mannitol in 1 mM Hepes/KOH buffer (pH 7.5). Disaccharidase activities were measured according to Dahlquist (1984) as modified by Martínez del Rio et al. (1995) and Fassbinder-Orth and Karasov (2006). In short, 30 μl tissue homogenates were diluted with 300 mM mannitol in 1 mM Hepes/KOH and incubated with 30 μl of 56 mM maltose in 0.1 M maleate/NaOH buffer (pH 6.5) at 40˚C for 20 min. Reactions were stopped by adding 400 μl of a stop-develop reagent (glucose assay kit, Sigma Aldrich, Munich, Germany). After 30 min incubation at 40˚C, 400 μl of 12 N $H_2SO_4$ were added and the absorbance was read at 540 nm (UV mini 1240 UV-VIS, Shimadzu Scientific, Balcatta, Western Australia). Apparent Michaelis constant ($K_m$) was 37.81 ± 6.20 mM (mean ± SE), and the pH optimum for intestinal sucrase activity was 6.

## *Data processing*

The daily proportional body mass change ($\%\bullet 24\ h^{-1}$) was calculated for each bird and each test day from the body mass (g) before lights-on on the test day and the day after. Daily mass-specific food intake ($g\bullet 24\ h^{-1}\bullet g$ body $mass^{-1}$) was calculated using the morning body mass of each bird on the test day. Daily mass-specific volumetric food intake ($ml\bullet 24\ h^{-1}\bullet g$ body $mass^{-1}$), hereafter referred to as volumetric food intake only, was calculated by dividing the food intake (g•24 $h^{-1}$•g body $mass^{-1}$) by the relative density of the diet. Mass-specific daily sugar intake (mg•24 $h^{-1}$•g body $mass^{-1}$), i.e. sucrose and hexose (sum of glucose and fructose) intake, was calculated as the product of volumetric food intake, diet concentration and molar mass of each sugar and is referred to as sugar intake. For simplicity, the three sugars will hereafter be summarized as {SUGAR}, with {SUGAR} being sucrose, glucose or fructose respectively. The total amount of {SUGAR} excreted (mg•24 $h^{-1}$) was calculated as the product of {SUGAR} concentration and total volume of each sample after re-dilution. The apparent sucrose, glucose and fructose assimilation coefficients (SucAC*, GlucAC* and FrucAC*, in short {SUGAR}AC*) were calculated for each bird on each test day as the proportion (%) of sugar ingested that was not excreted:

$$\{\text{SUGAR}\}\text{AC}^{*}=100 \bullet \frac{\left[\{\text{SUGAR}\}\text{ ingested }(\text{mg}\bullet 24\text{h}^{-1}) - \{\text{SUGAR}\}\text{ excreted }(\text{mg}\bullet 24\text{h}^{-1})\right]}{\{\text{SUGAR}\}\text{ ingested }(\text{mg}\bullet 24\text{h}^{-1})}$$

SucAC* were calculated for sucrose diets; GlucAC* and FrucAC* were calculated for hexose diets. The apparent overall sugar assimilation coefficients (SAC*) were calculated for each bird on each test day using the formula above, with {SUGAR} ingested being sucrose for sucrose diets and the sum of glucose and fructose for hexose diets; and {SUGAR} excreted being the sum of sucrose, glucose and fructose for sucrose diets and the sum of glucose and fructose for hexose diets.

The maximal volumetric food intake rate, and thus maximal sucrose intake rate, was predicted using a chemical reactor model of digestive capacity (McWhorter and Martínez del Rio 2000; see Appendix for a description of the model). This model assumes that sucrose hydrolysis is the limiting factor in sugar digestion of nectar-feeding birds when they are feeding on sucrose-rich nectars (McWhorter and Martínez del Rio 2000; Martínez del Rio et al. 2001) and relies on the sucrose assimilation efficiency as well as the data on gut morphology and intestinal sucrase activity of the brown honeyeaters. The daily maximal volumetric and sucrose intake was calculated for the 12 h light period used in this experiment. Mass-specific maximal intake was calculated by dividing the daily maximal volumetric and sucrose intake by the mean body mass of the five euthanased birds.

### *Statistical analysis*

All data were tested for normality (Kolmogorov-Smirnov test) and homogeneity of variance (Levene’s test). Log transformation was used when data were heteroscedastic. Volumetric food intake (ml•24 $h^{-1}$•g body $mass^{-1}$), sugar intake (mg•24 $h^{-1}$•g body $mass^{-1}$) and body mass change (%•24 $h^{-1}$) of Part I and Part II were separately subjected to ANCOVA. ANCOVA on volumetric food intake was performed on log transformed data since the relationship between intake and diet sugar concentration was best described by a power function. Ambient temperature was the categorical predictor, diet sugar concentration the continuous predictor and the dependent variable was either volumetric food intake, sugar intake or body mass change. Linear regressions were calculated to determine relationships between diet sugar concentration and volumetric food intake (performed on log transformed data) at each ambient temperature and each sugar type (sucrose and hexose diets), for each Part of the experiment (eight regressions, each based on eight data points deriving from the eight birds). The slopes from the regressions were then compared to a value of -1 (i.e. perfect compensatory feeding) using two-tailed Student’s t-distribution.

The apparent overall sugar assimilation coefficients (SAC*) and the total amounts of sucrose, glucose and fructose excreted (mg•24 $h^{-1}$; on sucrose diets only) were subjected to Spearman rank correlations to test for differences between diet sugar concentrations (for each ambient temperature and sucrose and hexose diets separately; n=8 each). Repeated measures ANOVA was used to determine differences in SAC* between ambient temperatures (for sucrose and hexose diets separately; n=8 each; ambient temperature being the within-effect) and to test for differences between amounts of sugars excreted (n=8; type of sugar being the within-effect). *Post-hoc* comparisons for all statistical tests were conducted with Tukey’s Honest Significant Difference test for equal sample sizes and/or Bonferroni corrections (Rice 1989). All data are presented as mean ± SE.

# Results

## *Food and sugar intake*

### Part I: Acute cold exposure

The volumetric food intake (ml•24 $h^{-1}$•g body $mass^{-1}$) of the eight brown honeyeaters on sucrose and hexose diets of four sugar concentrations and at two ambient temperatures is shown in Fig. 1A,B. In this Part I of the experiment, birds were exposed to the low temperature for the first time and under acute, short-term conditions. Volumetric food and sugar intake were the same for sucrose and hexose diets; statistical results are therefore summarized for both sugar types in this section. The volumetric food intake differed significantly between temperatures ($F_{1,13}>5.55$, $P<0.03$; Fig. 1A,B). *Post-hoc* analysis showed that volumetric intake was higher at 5˚C than at 22˚C ($P<0.03$). Birds increased their volumetric intake on average 18.43 ± 1.42% in the cold, irrespective of diet sugar concentration. Consequently, sugar intake (mg•24 $h^{-1}$•g body $mass^{-1}$) was found to differ between temperatures ($F_{1,13}>12.11$, $P<0.01$; Fig. 2A,B), being higher at 5˚C than at 22˚C ($P<0.01$). At both temperatures, the relationship between volumetric food intake and diet sugar concentration was well described by a power function (Fig. 1A,B). In all cases, birds significantly increased their volumetric food intake with decreasing diet sugar concentration ($F_{1,13}>118.76$, $P<0.001$). Because of this adjustment in volumetric intake, sugar intake appeared similar over all diet sugar concentrations at a given temperature (Fig. 2A,B). However, there was a significant difference in sugar intake between diet sugar concentrations ($F_{1,13}>4.86$, $P<0.05$).

## Part II: Repeated cold exposure

In Part II of the experiment, at repeated cold exposure, the volumetric food intake of the brown honeyeaters (Fig. 1C,D) was similar to that in Part I for higher diet sugar concentrations but, interestingly, intake was higher on the dilute diet (0.25 M) compared to Part I at both ambient temperatures and on both sucrose and hexose diets. When birds were fed the most dilute sucrose diet at 5 and 22˚C, for instance, mass-specific daily volumetric food intake was between 0.8–1.5 ml higher in Part II than in Part I, i.e. birds ingested about 10 ml more per day. Results for volumetric food and sugar intake were the same for sucrose and hexose diets, as in Part I, and are therefore presented together. The volumetric food intake differed significantly between temperatures ($F_{1,13}>6.51$, $P<0.02$; Fig. 1C,D), being higher at 5˚C than at 22˚C ($P<0.02$). The increase in volumetric intake in the cold was slightly higher than in Part I, averaging 21.04 ± 2.78%, and was similar on all diet sugar concentrations. As in Part I, this resulted in a significant difference in sugar intake between temperatures ($F_{1,13}>13.55$, $P<0.01$; Fig. 2C,D), intake being higher at 5˚C than at 22˚C ($P<0.01$). The relationship between volumetric food intake and diet sugar concentration at both temperatures was again well described by a power function (Fig. 1C,D), with birds increasing their volumetric food intake with decreasing diet sugar concentration ($F_{1,13}>214.68$, $P<0.001$). In contrast to Part I, sugar intake did not differ between diet sugar concentrations ($F_{1,13}<1.18$, $P>0.30$; Fig. 2C,D), indicating perfect compensatory feeding by the birds irrespective of temperature.

Linear regression results derived from the relationship between diet sugar concentration and volumetric food intake in Part I and II are presented in Table 1. A slope of -1 indicates perfect compensatory feeding. Slopes were always greater in Part II than in Part I (at both temperatures and both dietary sugar types). However, slopes were not significantly different from -1 ($t_6<1.92$, $P>0.10$), apart from one test day in Part I (22˚C, 0.5 M hexoses; $t_6=2.56$, $P=0.04$), that also became non-significant after sequential Bonferroni correction ($P>0.01$; n.s.).

## *Body mass*

### Part I: Acute cold exposure

Birds generally lost body mass during test days (Fig. 3) because the experimental diets were not supplemented with protein. The change in body mass (%•24 $h^{-1}$) differed significantly between temperatures on sucrose diets ($F_{1,13}$=7.43, $P$=0.02; Fig. 3A), but not on hexose diets ($F_{1,13}$=2.93, $P$=0.11; Fig. 3B). When fed sucrose diets, birds lost more body mass at 5˚C than at 22˚C ($P$<0.02). Change in body mass on sucrose and hexose diets did not differ between diet sugar concentrations ($F_{1,13}$<1.94, $P$>0.19; Fig. 3A,B).

### Part II: Repeated cold exposure

There was a significant difference in body mass change between temperatures for both dietary sugar types ($F_{1,13}$>9.63, $P$<0.01; Fig. 3C,D). Surprisingly, birds lost more body mass at 22˚C than at 5˚C when fed sucrose diets ($P$<0.01). On hexose diets, on the other hand, they lost more mass at 5˚C compared to 22˚C ($P$<0.001). As in Part I, change in body mass on both dietary sugar types did not differ between diet sugar concentrations ($F_{1,13}$<0.24, $P$>0.64; Fig. 3C,D).

## *Sugar assimilation*

Apparent sucrose, glucose and fructose assimilation was exceptionally high on all diet sugar concentrations, both dietary sugar types and at both temperatures (SucAC*=99.77 ± 0.02%, GlucAC*=99.79 ± 0.05%, FrucAC*=99.94 ± 0.02%; n=16 each). The apparent overall sugar assimilation coefficients (SAC*) did not differ between diet sugar concentrations at both temperatures and on both sucrose and hexose diets ($R_s$<0.68, $P$>0.06). Data of all sugar concentrations were therefore pooled to determine differences between temperatures. SAC* on the hexose diets were independent of temperature ($F_{1,7}$=0.08, $P$=0.78). However, SAC* on the sucrose diets were found to differ between the two temperatures ($F_{1,7}$=11.40, $P$=0.01), being lower at 5˚C than at 22˚C ($P$=0.01).

The total amounts of sucrose, glucose and fructose excreted (mg•24 $h^{-1}$) on sucrose diets did not differ between diet sugar concentrations at 22˚C ($R_s$<0.49, $P$>0.64). At 5˚C, total amounts of sucrose and glucose excreted were also independent of diet sugar concentration ($R_s$>-1.37, $P$>0.22), while the total amounts of fructose excreted increased with diet sugar concentration ($R_s$=10.95, $P$<0.001). Therefore, data for all sugar concentrations were only pooled at 22˚C and total amounts of sucrose, glucose and fructose excreted were found to differ significantly ($F_{2,14}$=30.95, $P$<0.001). *Post-hoc* analysis showed that the amounts of excreted fructose were significantly lower than the amounts of both glucose and sucrose ($P$<0.01), while the amounts of sucrose and glucose did not differ ($P$=0.32). Sucrose was the most abundant sugar in excreta and fructose the least abundant.

### *Gut morphology, sucrase activity and predicted maximal intake*

Total length of the small intestine of five honeyeaters was 8.74 ± 0.52 cm and the total intestinal volume equalled 187.93 ± 25.28 µl. Maximal total intestinal sucrase activity was 11.52 ± 3.98 µmol•$min^{-1}$. On three of the four sucrose concentrations (0.5, 0.75, 1 M), the observed daily volumetric food intake of brown honeyeaters at 5˚C was only slightly lower than the maximal daily volumetric intake predicted by the chemical reactor model of digestive capacity (Fig. 1A,C). Consequently, the observed sucrose intake on these three sugar concentrations in the cold was only slightly below the predicted maximal daily sucrose intake (Fig. 2A,C). On the very dilute diet (0.25 M), however, cold-stressed honeyeaters ingested slightly more than predicted (Figs. 1 and 2A,C).

## Discussion

Brown honeyeaters responded to a decrease in ambient temperature with an increase in food intake irrespective of diet sugar concentration. This was expected since the energetic costs of maintaining a constant body temperature increase with decreasing temperature of the environment. Similar increases in food intake in the cold have been shown in hummingbirds (Beuchat et al. 1990; Fleming et al. 2004), non-nectarivorous birds (Goymann et al. 2006; Salvante 2007), and small mammals (Mele 1972; Naya et al. 2005; Zhang and Wang 2007). My results, however, suggest that food intake in brown honeyeaters, although increased in the cold, may be limited by physiological constraints. In the following section, I will therefore address possible physiological limitations that may restrict compensatory feeding. I will then suggest behavioural energy-saving mechanisms that may have occurred in this experiment. Finally, the evidence for acclimation of honeyeaters to low ambient temperature is discussed.

### *Compensatory feeding and physiological constraints*

When exposed to low ambient temperature under acute conditions, brown honeyeaters compensated by increasing their food intake, and thus energy intake, by 18% over a range in sugar concentration from 0.25–1 M. Birds also increased their food intake with decreasing sugar concentration at a given ambient temperature. This behavioural intake response has been shown previously in brown honeyeaters (Collins et al. 1980) as well as in a variety of other nectarivorous birds (e.g. Collins and Clow 1978; McWhorter and Martínez del Rio 1999; Köhler et al. 2006) and bats (Herrera and Mancina 2007; Ayala-Berdon et al. 2008). The compensation for low energy or nutrient content of a food source occurs in a variety of animals, ranging from insects (Lavoie and Oberhauser 2004; Berner et al. 2005) to mammals (Loeb et al. 1991; Castle and Wunder 1995).

Despite the increase in volumetric intake with decreasing diet sugar concentration, the sugar intake in Part I differed between sugar concentrations on both sucrose and hexose diets. The slopes of the linear regressions between volumetric intake and sugar concentration were shallower in Part I than in Part II and were, although not statistically significant, smaller than -1 (perfect compensatory feeding). Furthermore, birds lost more body mass at 5˚C than at 22˚C when fed sucrose diets in Part I. These results indicate that the compensation for low food energy content and higher energy demands during acute cold exposure was incomplete.

The food intake of brown honeyeaters may be limited by constraints on nectar ingestion and digestion, as well as osmoregulatory processes. Avian nectarivores lick nectar from flowers (Hainsworth 1973; Schlamowitz et al. 1976; Collins et al. 1980) and energy intake may be limited by the rate at which nectar can be licked by the birds (Gass and Roberts 1992). Honeyeaters do not have a crop, which may restrict the amount of nectar that can be ingested at once (Collins et al. 1980). The size of the stomach has also been suggested as limiting ingestion (Bednekoff and Houston 1994).

As described in the previous chapter, nectar digestion may be limited by sucrose hydrolysis rates (McWhorter and Martínez del Rio 2000). Cold-stressed honeyeaters in the present study were feeding close to the maximal intake rates predicted by the mathematical model of digestive capacity. This suggests that intestinal sucrose hydrolysis rates were at near-maximal levels and may have limited sucrose digestion, and thus food intake. On the most dilute diet (0.25 M sucrose), these birds ingested even more than predicted by the maximal intake rates. In Part I, honeyeaters lost more body mass at 5˚C than at 22˚C on sucrose diets, but not on hexose diets, despite the higher food intake in the cold on both sucrose and hexose diets. Sugar assimilation was lower at 5˚C than at 22˚C on sucrose diets, but not on hexose diets. Sucrose was further found to be the most abundant sugar in excreta, suggesting that sucrose hydrolysis may indeed have been a limiting factor in my study. The biological relevance of these differences, however, is questionable because more than 99% of the ingested sugar was assimilated in all cases. This confirms previous studies in nectarivorous birds where sugar assimilation efficiency has always been found to be 95% or higher (McWhorter and Martínez del Rio 2000; Mata and Bosque 2004; also see Chapter 4). Although cold-stressed birds are equally efficient in sugar uptake, it has been shown for Palestine sunbirds (*Cinnyris oseus*) that secondary compounds in nectar decrease sugar assimilation efficiency (Tadmor-Melamed et al. 2004).

The passive and carrier-mediated absorption of glucose and fructose may also be a limiting step in nectar digestion (Napier et al. 2008). Honeyeaters in my study did not lose more body mass on hexose diets in Part I in the cold than at 22˚C, which shows that they were able to absorb sufficient sugar to maintain energy balance, despite increased energy requirements at low ambient temperature. However, birds lost more body mass on hexose diets in Part II in the cold than at 22˚C, which may indicate constraints in hexose absorption. Besides possible limitations to nectar digestion, avian nectarivores ingesting large nectar volumes may experience difficulties in maintaining ion levels (Fleming and Nicolson 2003) or in eliminating excess water (Gass et al. 1999; Suarez and Gass 2002).

When avian nectarivores are exposed to extreme energetically challenging conditions, these physiological constraints to nectar digestion and osmoregulation may limit their nectar intake, thus influencing their energy balance. Honeyeaters in this study were able to increase their food intake by 18% under the first acute cold exposure, even on a dilute diet of 0.25 M. A similar acute temperature challenge in whitebellied and amethyst (*Chalcomitra amethystina*) sunbirds even showed a slightly higher increase in food intake in the cold (Chapter 4). However, whitebellied sunbirds did not increase their nectar intake in the cold on an extremely dilute diet (Fleming et al. 2004), and cold-stressed rufous hummingbirds could not maintain energy balance on nectar concentrations lower than 1 M (Gass et al. 1999). Frugivorous yellow-vented bulbuls (*Pycnonotus xanthopygos*), when kept at 10˚C, did not increase their food intake, although they were kept at low temperatures for several days (Van Tets et al. 2001). If birds can not increase their food intake sufficiently to maintain energy balance under challenging conditions, they may exhibit behavioural or physiological energy-saving mechanisms in order to compensate, as discussed below.

### *Did honeyeaters exhibit energy-saving mechanisms?*

In Part I of the experiment, the sugar intake of the brown honeyeaters differed significantly between diet sugar concentrations. However, the change in body mass did not differ between the sugar concentrations. Birds also did not lose more mass at 5˚C than at 22˚C on hexose diets (Part I) and sucrose diets (Part II). Birds could have avoided an energy deficit by reducing their flight activity, and thus reducing their energy expenditure. Hovering ruby-throated hummingbirds (*Archilochus colubris*) further conserve energy during flight by modulating their wingbeat kinematics in a way to generate more heat, which contributes to thermoregulatory requirements (Chai et al. 1998). A reduction in flight activity at low ambient temperatures or under food deprivation has been shown in green-backed firecrowns (*Sephanoides sephanoides*) and zebra finches (*Taeniopygia guttata*) (Dall and Witter 1998; Fernández et al. 2002). The activity of the honeyeaters could not be quantified in this study. I did, however, observe that the birds were sitting quietly and with feathers ptiloerected to increase the insulating layer of still air around the body at 5˚C. At 22˚C, on the other hand, they were much more active, jumped from branch to branch, flew around in their cages and sang. I can therefore conclude that they attempted to compensate for increased energetic costs in the cold by increasing their energy intake as well as adjusting their behaviour to reduce energy expenditure and maintain body heat. Birds lost more body mass at 5˚C than at 22˚C, when fed sucrose diets in Part I and hexose diets in Part II, indicating that the compensation was incomplete. Body mass data, however, must be interpreted with caution, since excretions before or during catching of the birds could not be accounted for. For more reliable body mass values I therefore suggest continuous recording in future studies, by connecting the perch to an electronic balance interfaced to a computer (Köhler et al. 2006).

Several species of hummingbirds, honeyeaters and sunbirds reduce their body temperature as a response to energy stress, or even go into torpor (for review see McKechnie and Lovegrove 2002). When broadtailed hummingbirds were exposed to 10˚C and similar diet concentrations used in my study, they became torpid during the night (McWhorter and Martínez del Rio 2000). Honeyeaters in my study did not go into torpor at night. As the sunbirds in the previous chapter, they were active when caught for weighing in the morning irrespective of ambient temperature and diet sugar concentration. However, birds may have become hypothermic in order to save energy in the cold. Such a reduction in body temperature has been observed earlier in brown honeyeaters that were deprived of food at the end of the day (Collins and Briffa 1984). Future studies should measure the body temperature of the birds during similar temperature challenges.

## *Did honeyeaters acclimate to the cold?*

Contrary to my prediction that the results would be similar for the acute and repeated exposures to low temperature, I found that food intake of the birds did differ between the two. For both the acute and the repeated exposure to low temperature, volumetric food intake, and thus sugar intake, was higher than at 22˚C. The increase in food intake from 22 to 5˚C was slightly higher in Part II (21%) than in Part I (18%). Thus, the acute spare digestive capacity (Karasov and McWilliams 2005) of the brown honeyeaters in my study was 18%, while spare capacity increased with repeated cold exposure. The slopes of the linear regressions deriving from the relationship between diet sugar concentration and volumetric food intake were shallower in Part I than in Part II for all experimental days. In Part II, birds showed perfect compensatory feeding, with the slopes being almost -1 or even steeper. In terms of compensation for varying sugar content of the diet, sugar intake differed between concentrations in Part I for both sucrose and hexose diets, but not in Part II, indicating perfect compensatory feeding irrespective of diet sugar concentration in Part II only. Interestingly, birds ingested about 10 ml more nectar daily on the most dilute diet at both ambient temperatures in Part II compared to Part I. The individuals receiving a particular diet sugar concentration differed between Part I and II. Food intake was corrected for body mass, but different activity levels of the individual birds may account for differences in food intake. However, it is unlikely that birds fed dilute diets in Part II were more active than those in Part I.

These food intake results suggest that a few days of cold exposure are sufficient for digestive adjustments to occur. However, a repetition of my study using a larger sample size is needed to test this. The higher food intake in Part II may explain why birds lost less body mass at 5˚C than at 22˚C on sucrose diets. An increase in intestine size, resulting in increased amounts of nutrient transporters and digestive enzymes, may take place in small animals within days of acclimation to high feeding rates (McWilliams and Karasov 2001; Karasov and McWilliams 2005). Enzyme and nutrient transporter expressions (i.e. number per unit area) may also increase when feeding rates are high. Starck (1999a) summarized studies of 31 bird and nine mammal species, in which size and structure of intestines were rapidly affected by energetic demands and food quality.

When dietary fibre content of Japanese quails (*Coturnix japonica*) is altered, an increase or reduction in gizzard size can be measured within 24–48 h (Starck 1999b). It was also found that gizzard size does not return to the original size after the first dietary challenge, but remains enlarged (Starck 1999b). An increase in the size of digestive organs was also found in rodents that were exposed to low ambient temperatures (Hammond et al. 1994; Naya et al. 2005).

The rapid adjustment of intestine size in order to meet energy demands is ecologically important for an animal. If the physiological response to environmental fluctuations is delayed, it may reduce fitness or even lead to death. Wild brown honeyeaters may experience acute fluctuations in ambient temperature within one day, such as a sudden decrease in temperature on a summer day caused by a storm. They also face longer-term changes in temperature, such as a cold front or seasonal temperature differences. Their major food source is nectar, which varies in availability and sugar concentration within the natural habitat. The high metabolic rates of such a small honeyeater may require a rapid physiological adjustment to allow for sufficient energy intake.

In conclusion, further studies of temperature challenges in nectarivorous birds are needed to address the physiological adjustments occurring during acclimation. Birds should be exposed to low ambient temperature for several days up to weeks to investigate how long it takes for digestive adjustments to take place and to determine the long-term spare capacity of these birds. Intestine length/volume and the amounts of nutrient transporters and digestive enzymes should be compared between naïve and long-term cold-acclimated birds. My study suggests that one should distinguish between short-term acute cold exposure and repeated cold exposure, since digestive capacities of brown honeyeaters may increase after a few days of acclimation.

# Acknowledgements

This project was funded by the Australian Research Council (DP0665730), and my stay at Murdoch University, Perth, was funded by the University of Pretoria. I would like to thank the Department of Environment and Conservation (Western Australia) and Murdoch University for permission to mist-net honeyeaters. Dr T.J. McWhorter is thanked for catching honeyeaters for this study and providing data on gut morphology and enzyme activity as well as theoretical maximum food intake rates. The experiments were approved by the Animal Ethics Committee of Murdoch University.

# References

Ayala-Berdon J., Schondube J.E., Stoner K.E., Rodriguez-Peña N. and Martínez del Rio C. 2008. The intake responses of three species of leaf-nosed Neotropical bats. Journal of Comparative Physiology B 178: 477–485.

Bednekoff P.A. and Houston A.I. 1994. Avian daily foraging patterns: effects of digestive constraints and variability. Evolutionary Ecology 8: 36–52.

Berner D., Blanckenhorn W.U. and Körner C. 2005. Grasshoppers cope with low host plant quality by compensatory feeding and food selection: N limitation challenged. Oikos 111: 525–533.

Beuchat C.A., Calder W.A. and Braun E.J. 1990. The integration of osmoregulation and energy balance in hummingbirds. Physiological Zoology 63: 1059–1081.

Bicudo J.E.P.W. 1996. Physiological correlates of daily torpor in hummingbirds. In: Animals and temperature: phenotypic and evolutionary adaptation. I.A. Johnston and A.F. Bennett (Eds.), Cambridge University Press, Cambridge, UK, pp. 293–311.

Bozinovic F. and Nespolo R.F. 1997. Effect of ambient temperature and energy demands on digestive functions in leaf-eared mice (*Phyllotis darwini*) from central Chile. International Journal of Biometeorology 41: 23–25.

Castle K.T. and Wunder B.A. 1995. Limits of food intake and fiber utilization in the prairie vole, *Microtus ochragaster*. Journal of Comparative Physiology B 164: 609–617.

Chai P., Chang A.C. and Dudley R. 1998. Flight thermogenesis and energy conservation in hovering hummingbirds. Journal of Experimental Biology 201: 963–968.

Collins B.G. and Briffa P. 1984. Nocturnal energy expenditure by honeyeaters experiencing food shortage and low environmental temperatures. Comparative Biochemistry and Physiology A 78: 77–81.

Collins B.G. and Clow H. 1978. Feeding behaviour and energetics of the western spinebill, *Acanthorhynchus superciliosis* (Aves: Meliphagidae). Australian Journal of Zoology 26: 269–277.

Collins B.G., Cary G. and Packard G. 1980. Energy assimilation, expenditure and storage by the brown honeyeater, *Lichmera indistincta*. Journal of Comparative Physiology B 137: 157–163.

Dahlquist A. 1984. Assay of intestinal disaccharidases. Scandinavian Journal of Clinical and Laboratory Investigation 44: 69–72.

Dall S.R.X. and Witter M.S. 1998. Feeding interruptions, diurnal mass changes and daily routines of behaviour in the zebra finch. Animal Behaviour 55: 715–725.

Fassbinder-Orth C.A. and Karasov W.H. 2006. Effects of feeding restriction and realimentation on digestive and immune function in the Leghorn chick. Poultry Science 85: 1449–1456.

Fernández M.J., López-Calleja M.V. and Bozinovic F. 2002. Interplay between the energetics of foraging and thermoregulatory costs in the green-backed firecrown hummingbird *Sephanoides sephanoides*. Journal of Zoology 258: 319–326.

Fleming P.A. and Nicolson S.W. 2003. Osmoregulation in an avian nectarivore, the whitebellied sunbird *Nectarinia talatala*: response to extremes of diet concentration. Journal of Experimental Biology 206: 1845–1854.

Fleming P.A., Hartman Bakken B., Lotz C.N. and Nicolson S.W. 2004. Concentration and temperature effects on sugar intake and preferences in a sunbird and a hummingbird. Functional Ecology 18: 223–232.

Gass C.L. and Roberts W.M. 1992. The problem of temporal scale in optimization: three contrasting views of hummingbird visits to flowers. American Naturalist 140: 829–853.

Gass C.L., Romich M.T. and Suarez R.K. 1999. Energetics of hummingbird foraging at low ambient temperature. Canadian Journal of Zoology 77: 314–320.

Goymann W., Trappschuh M., Jensen W. and Schwabl I. 2006. Low ambient temperature increases food intake and dropping production, leading to incorrect estimates of hormone metabolite concentrations in European stonechats. Hormones and Behavior 49: 644–653.

Hainsworth F.R. 1973. On the tongue of a hummingbird: its role in the rate and energetics of feeding. Comparative Biochemistry and Physiology A 46: 65–78.

Hammond K.A., Konarzewski M., Torres R.M. and Diamond J. 1994. Metabolic ceilings under a combination of peak energy demands. Physiological Zoology 67: 1479–1506.

Herrera L.G. and Mancina C.A. 2007. Sucrose hydrolysis does not limit food intake by Pallas’s long-tongued bats. Physiological and Biochemical Zoology 81: 119–124.

Karasov W.H. and McWilliams S.R. 2005. Digestive constraints in mammalian and avian ecology. In: Physiological and ecological adaptations to feeding in vertebrates. J.M. Starck and T. Wang (Eds.), Science Publishers, Enfield, New Hampshire, USA, pp. 87–112.

Köhler A., Verburgt L. and Nicolson S.W. 2006. Short-term feeding patterns of whitebellied sunbirds (*Nectarinia talatala*): feeding frequency, daily rhythms and individual differences. Journal of Experimental Biology 209: 2880–2887.

Lavoie B. and Oberhauser K.S. 2004. Compensatory feeding in *Danaus plexippus* (Lepidoptera: Nymphalidae) in response to variation in host plant quality. Environmental Entomology 33: 1062–1069.

Loeb S.C., Schwab R.G. and Demment M.W. 1991. Responses of pocket gophers (*Thomomys bottae*) to changes in diet quality. Oecologia 86: 542–551.

López-Calleja M.V. and Bozinovic F. 1995. Maximum metabolic rate, thermal insulation and aerobic scope in a small-sized Chilean hummingbird (*Sephanoides sephanoides*). Auk 112: 1034–1036.

Lotz C.N. and Nicolson S.W. 2002. Nectar dilution increases metabolic rate in the lesser double-collared sunbird. Condor 104: 672–675.

Lotz C.N. and Schondube J.E. 2006. Sugar preferences in nectar- and fruit-eating birds: behavioural patterns and physiological causes. Biotropica 38: 3–15.

Lotz C.N., Martínez del Rio C. and Nicolson S.W. 2003. Hummingbirds pay a high cost for a warm drink. Journal of Comparative Physiology B 173: 455–462.

Martínez del Rio C., Brugger K.E., Rios J.L., Vergara M.E. and Witmer M. 1995. An experimental and comparative study of dietary modulation of intestinal enzymes in European starlings (*Sturnus vulgaris*). Physiological Zoology 68: 490–511.

Martínez del Rio C., Schondube J.E., McWhorter T.J. and Herrera L.G. 2001. Intake responses in nectar feeding birds: digestive and metabolic causes, osmoregulatory consequences, and coevolutionary effects. American Zoologist 41: 902–915.

Mata A. and Bosque C. 2004. Sugar preferences, absorption efficiency and water influx in a Neotropical nectarivorous passerine, the Bananaquit (*Coereba flaveola*). Comparative Biochemistry and Physiology A 139: 395–404.

McKechnie A.E. and Lovegrove B.G. 2002. Avian facultative hypothermic responses: a review. Condor 104: 705–724.

McNab B.K. 2002. Adaptation to temperature variation: homeothermy – endothermy. In: The physiological ecology of vertebrates: a view from energetics. B.K. McNab, Cornell University Press, New York, USA, pp. 85–130.

McWhorter T.J. and Martínez del Rio C. 1999. Food ingestion and water turnover in hummingbirds: how much dietary water is absorbed? Journal of Experimental Biology 202: 2851–2858.

McWhorter T.J. and Martínez del Rio C. 2000. Does gut function limit hummingbird food intake? Physiological and Biochemical Zoology 73: 313–324.

McWilliams S.R. and Karasov W.H. 2001. Phenotypic flexibility in digestive system structure and function in migratory birds and its ecological significance. Comparative Biochemistry and Physiology A 128: 579–593.

Mele J.A. 1972. Temperature regulation and bioenergetics of the Mongolian gerbil *Meriones unguiculatus*. American Midland Naturalist 87: 272–282.

Napier K.R., Purchase C., McWhorter T.J., Nicolson S.W. and Fleming P.A. 2008. The sweet life: diet sugar concentration influences paracellular glucose absorption. Biology Letters 4: 530–533.

Naya D.E., Bacigalupe L.D., Bustamante D.M. and Bozinovic F. 2005. Dynamic digestive responses to increased energy demands in the leaf-eared mouse (*Phyllotis darwini*). Journal of Comparative Physiology B 175: 31–36.

Nicolson S.W. and Fleming P.A. 2003a. Nectar as food for birds: the physiological consequences of drinking dilute sugar solutions. Plant Systematics and Evolution 238: 139–153.

Nicolson S.W. and Fleming P.A. 2003b. Energy balance in the Whitebellied Sunbird *Nectarinia talatala*: constraints on compensatory feeding, and consumption of supplementary water. Functional Ecology 17: 3–9.

Rice W.R. 1989. Analyzing tables of statistical tests. Evolution 43: 223–225.

Salvante K.G., Walzem R.L. and Williams T.D. 2007. What comes first, the zebra finch or the egg: temperature-dependent reproductive, physiological and behavioural plasticity in egg-laying zebra finches. Journal of Experimental Biology 210: 1325–1334.

Schlamowitz R., Hainsworth F.R. and Wolf L.L. 1976. On the tongues of sunbirds. Condor 78: 104–107.

Starck J.M. 1999a. Structural flexibility of the gastro-intestinal tract of vertebrates – implications for evolutionary morphology. Zoologischer Anzeiger 238: 87–101.

Starck J.M. 1999b. Phenotypic flexibility of the avian gizzard: rapid, reversible and repeated changes of organ size in response to changes in dietary fibre content. Journal of Experimental Biology 202: 3171–3179.

Suarez R.K. and Gass C.L. 2002. Hummingbird foraging and the relation between bioenergetics and behaviour. Comparative Biochemistry and Physiology A 133: 335–343.

Tadmor-Melamed H., Markman S., Arieli A., Distl M., Wink M. and Izhaki I. 2004. Limited ability of Palestine sunbirds *Nectarinia osea* to cope with pyridine alkaloids in nectar of Tree Tobacco *Nicotiana glauca*. Functional Ecology 18: 844–850.

Van Tets I.G., Korine C., Roxburgh L. and Pinshow B. 2001. Changes in the composition of the urine of yellow-vented bulbuls (*Pycnonotus xanthopygos*): the effects of ambient temperature, nitrogen, and water intake. Physiological and Biochemical Zoology 74: 853–857.

Zhang X.Y. and Wang D.H. 2007. Thermogenesis, food intake and serum leptin in cold-exposed lactating Brandt’s voles *Lasiopodomys brandtii*. Journal of Experimental Biology 210: 512–521.

# Table

**Table 1.** Linear regression results derived from the relationship between logarithmic diet sugar concentration (0.25, 0.5, 0.75 and 1 M) and logarithmic daily mass-specific volumetric food intake (ml•24 $h^{-1}$•g body $mass^{-1}$) of eight brown honeyeaters feeding on sucrose and energetically equivalent hexose diets (glucose:fructose 1:1) at two ambient temperatures (5 and 22˚C). Birds were acutely exposed to the low ambient temperature for the first time in Part I, while they had previously been exposed to low temperature in Part II. The slopes of the regressions are given as m (with SE); the intercepts are given as c. $R^2$-, *F*- and *P*-values are presented for each regression; degrees of freedom are 1,6. Note that the slopes were always steeper in Part II than in Part I.

| Dietary Sugar | Temperature (˚C) | Part | m | SE of m | c | $R^2$ | *F* | *P* |
|---|---|---|---|---|---|---|---|---|
| Sucrose | 5 | I | -0.910 | 0.047 | 0.073 | 0.985 | 382.63 | <0.001 |
| Sucrose | 22 | I | -0.992 | 0.145 | -0.034 | 0.887 | 46.93 | <0.001 |
| Hexoses | 5 | I | -0.923 | 0.139 | 0.104 | 0.880 | 44.11 | <0.001 |
| Hexoses | 22 | I | -0.818 | 0.071 | 0.027 | 0.956 | 131.22 | <0.001 |
| Sucrose | 5 | II | -1.007 | 0.060 | 0.108 | 0.979 | 278.10 | <0.001 |
| Sucrose | 22 | II | -1.074 | 0.078 | -0.022 | 0.969 | 188.59 | <0.001 |
| Hexoses | 5 | II | -0.976 | 0.117 | 0.0754 | 0.921 | 69.66 | <0.001 |
| Hexoses | 22 | II | -1.188 | 0.070 | -0.049 | 0.980 | 289.82 | <0.001 |

# Figure legends

**Fig. 1.** Daily mass-specific volumetric food intake (ml•24 $h^{-1}$•g body $mass^{-1}$) of eight brown honeyeaters feeding on sucrose (A,C) and energetically equivalent hexose diets (glucose:fructose 1:1; B,D) of four sugar concentrations and at two ambient temperatures. Each circle represents one bird. Birds were acutely exposed to the low ambient temperature for the first time in Part I (A,B), while they had previously been exposed to low temperature in Part II (C,D). Note that intake is higher at the low sugar concentration in Part II than in Part I. The equations and $R^2$-values are given for the power functions. Maximal volumetric food intake (ml•24 $h^{-1}$•g body $mass^{-1}$) of brown honeyeaters predicted by a chemical reactor model of digestive capacity (McWhorter and Martínez del Rio 2000; see Appendix) is also shown for sucrose concentrations (A,C).

**Fig. 2.** Daily mass-specific sugar intake (g•24 $h^{-1}$•g body $mass^{-1}$) of eight brown honeyeaters feeding on sucrose (A,C) and energetically equivalent hexose diets (glucose:fructose 1:1; B,D) of four sugar concentrations and at two ambient temperatures. Each circle represents one bird. Birds were acutely exposed to the low ambient temperature for the first time in Part I (A,B), while they had previously been exposed to low temperature in Part II (C,D). Note that sucrose intake is higher at the low ambient temperature in Part II than in Part I. Maximal sucrose intake (ml•24 $h^{-1}$•g body $mass^{-1}$) of brown honeyeaters predicted by a chemical reactor model of digestive capacity (McWhorter and Martínez del Rio 2000; see Appendix) is also shown (A,C).

**Fig. 3.** Daily body mass change (%•24 $h^{-1}$) of eight brown honeyeaters feeding on sucrose (A,C) and energetically equivalent hexose diets (glucose:fructose 1:1; B,D) of four sugar concentrations and at two ambient temperatures. Each circle represents one bird. Birds were acutely exposed to the low ambient temperature for the first time in Part I (A,B), while they had previously been exposed to low temperature in Part II (C,D). Birds generally lost body mass because experimental diets were not supplemented with protein.

# Figures

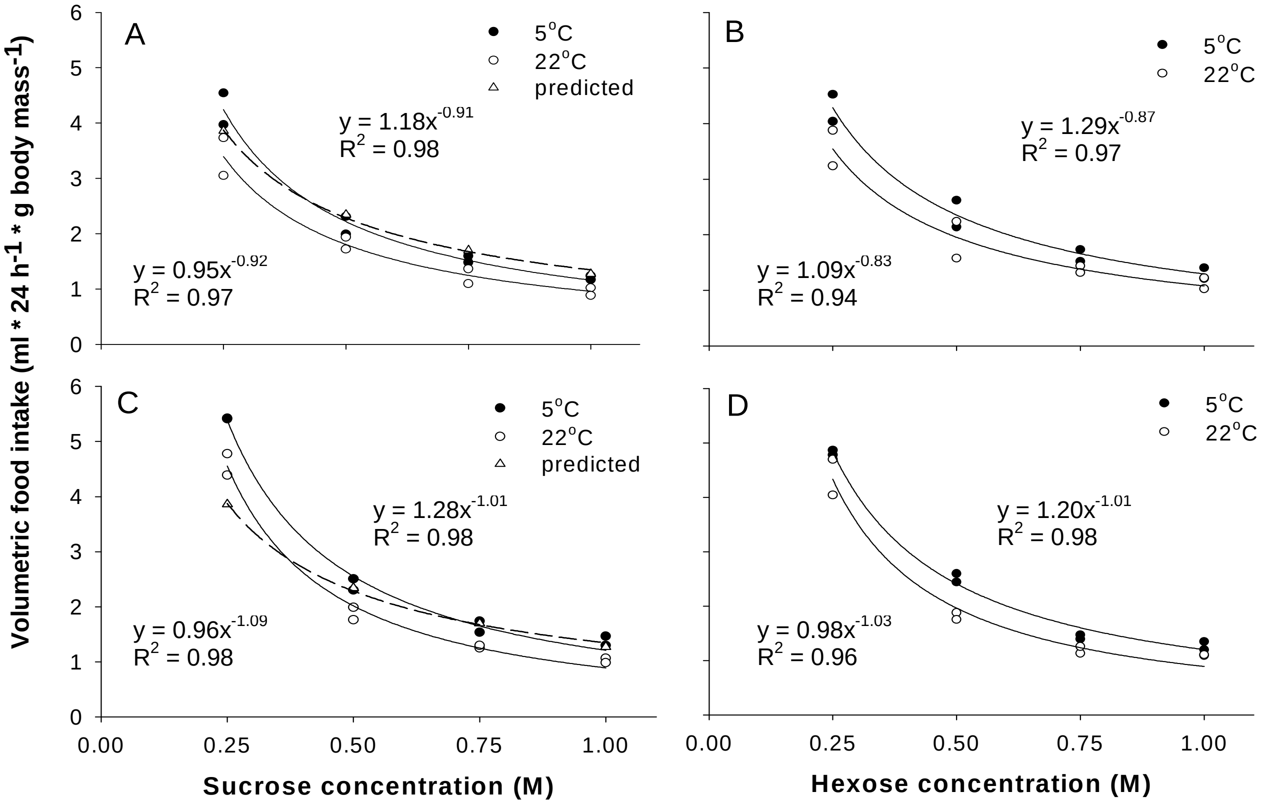


Figure 1.

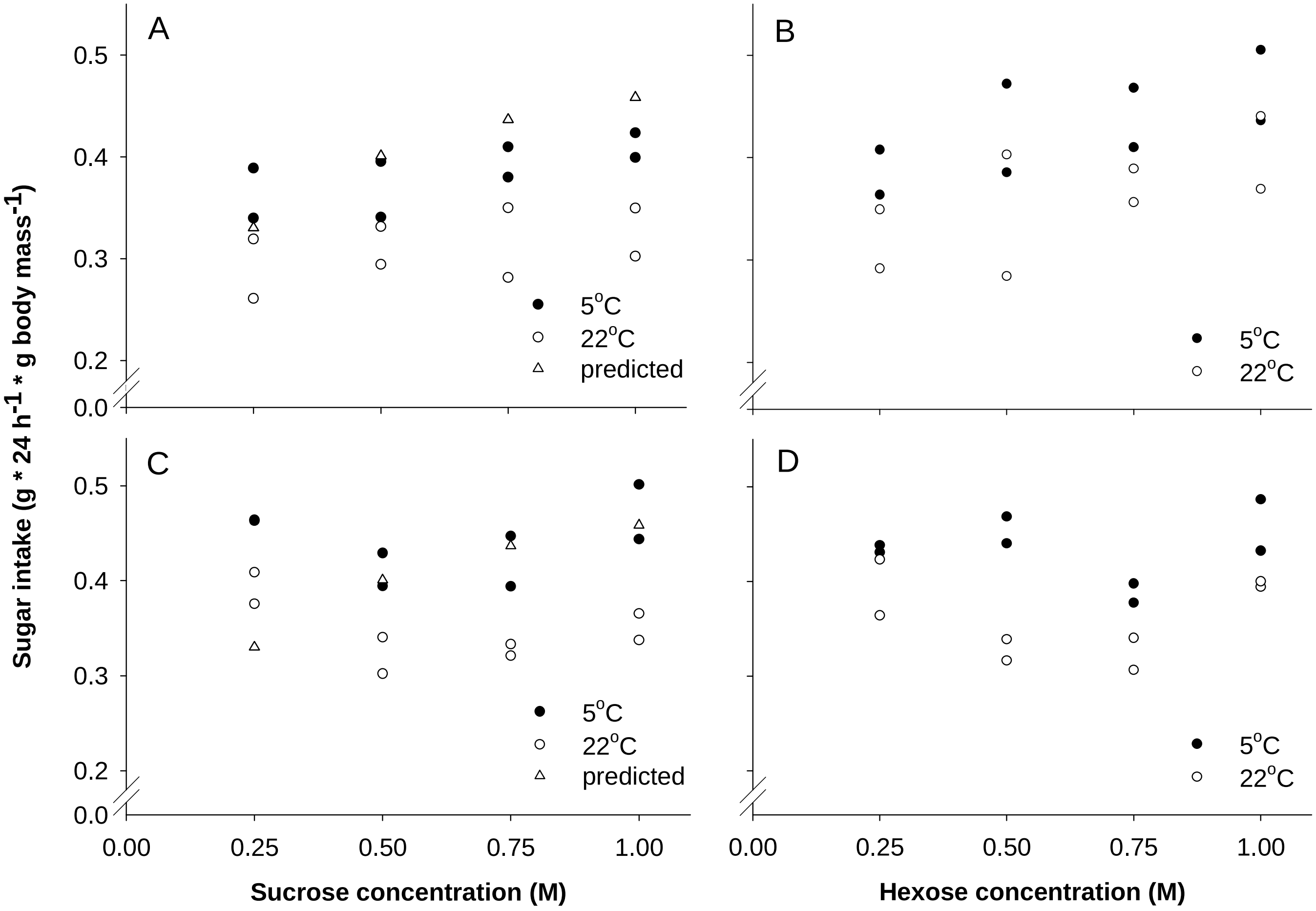

A
B
C
D
Sugar intake (g * 24 h-1 * g body mass-1)
0.5
0.4
0.3
0.2
0.0
● 5°C
○ 22°C
△ predicted
0.00
0.25
0.50
0.75
1.00
Sucrose concentration (M)
Hexose concentration (M)

Figure 2.

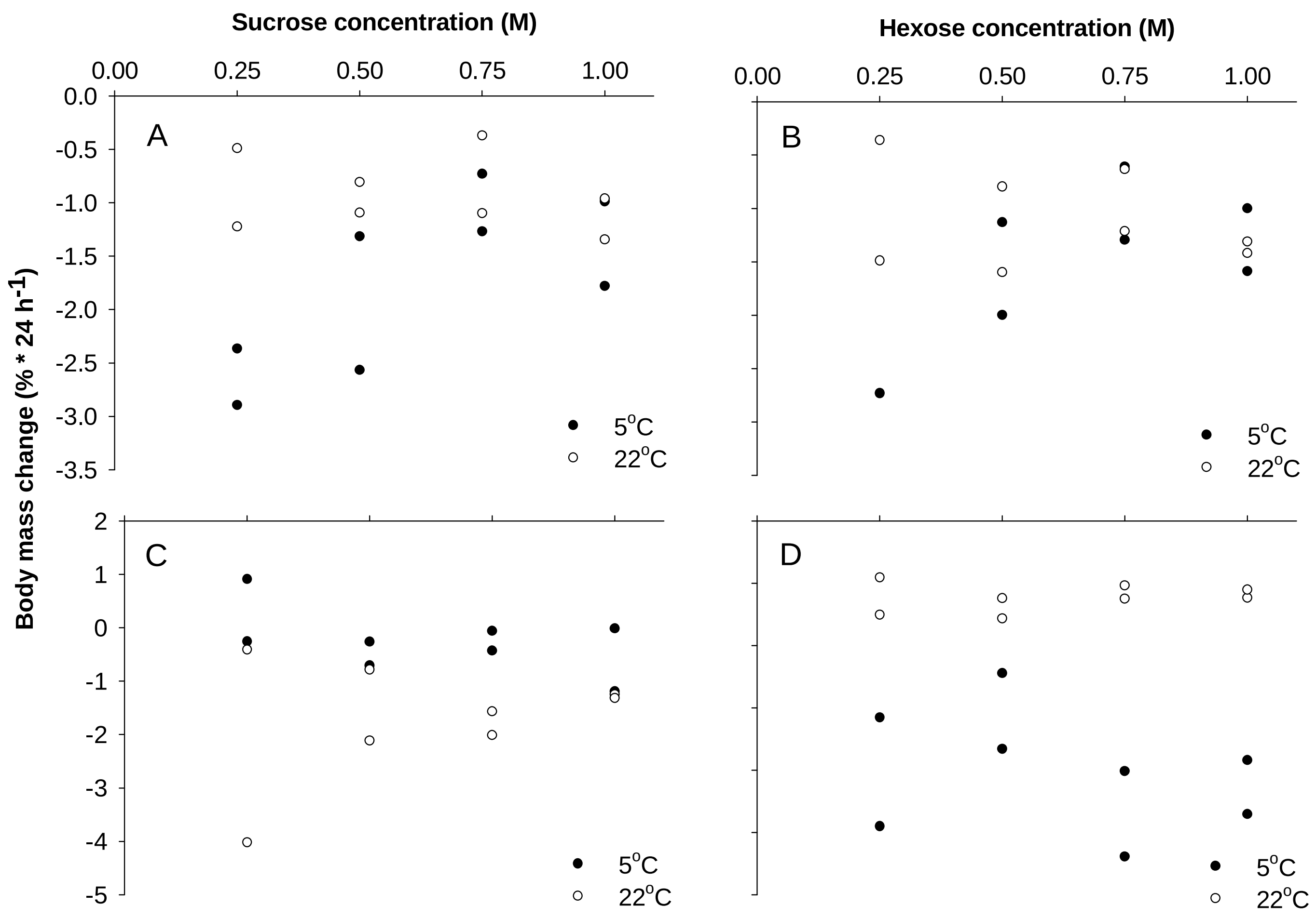

Sucrose concentration (M)
Hexose concentration (M)
0.00
0.25
0.50
0.75
1.00
Body mass change (% * 24 h-1)
0.0
-0.5
-1.0
-1.5
-2.0
-2.5
-3.0
-3.5
2
1
0
-1
-2
-3
-4
-5
A
B
C
D
● 5°C
○ 22°C

Figure 3.

# CHAPTER 6

# NECTAR EXTRACTION BY SUNBIRDS: DOES LICKING BEHAVIOUR CHANGE WITH NECTAR CONCENTRATION AND AFTER A FASTING PERIOD?

## Abstract

Nectarivorous birds lick nectar from flowers, with nectar being loaded onto their grooved tongues by capillary action. In the present study, the licking behaviour of whitebellied (*Cinnyris talatala*) and amethyst (*Chalcomitra amethystina*) sunbirds (Nectariniidae) was investigated. The number and durations of tongue licks over a 3 h period were recorded on a short-term scale (every 1 ms) using a photodetection system, and consumption per lick was calculated from the number of licks and the mass of artificial nectar consumed during the 3 h. Birds were fed various sucrose concentrations (0.25, 0.5, 1 and 1.5 M) on consecutive days. With increasing sugar concentration, lick duration increased and licking frequency decreased, indicating that tongue loading takes longer on more concentrated solutions. Birds also consumed less food per lick on the highest sugar concentration. In the second part of the experiment, licking behaviour was recorded on one control day and on a second day after a 2 h fasting period, but no differences in licking parameters were found between the treatments. The amount of food consumed per lick did not differ between the two sunbird species. However, the species differed in their licking frequencies and durations at various nectar concentrations, with smaller whitebellied sunbirds licking faster and having shorter licks than amethyst sunbirds. Licking frequencies in the present study were higher than those previously reported for

avian nectarivores: early studies may have underestimated licking rates due to insufficient resolution of video recordings. It is concluded that the concentration of nectar determines nectar ingestion rates and high viscosities presumably impede the capillarity of tongue loading, but licking behaviour is not adjusted in order to compensate for a loss in foraging time.

## Introduction

Nectarivorous birds show morphological adaptations to their nectar-feeding lifestyle. They have long, straight or curved bills which facilitate nectar extraction from tubular flowers (Temeles and Kress 2003). The tongues of hummingbirds and sunbirds have a bifurcated tip, the edges coiling inwards to form open tubes (Hainsworth 1973; Downs 2004). In hummingbirds, the two tubes continue to the proximal end of the tongue (Hainsworth 1973), while the tubes fuse to a single, open groove in the sunbird tongue (Skead 1967; Schlamowitz et al. 1976). Honeyeaters have broader, brush-tipped tongues with multiple grooves at the tip, which then join into a single channel (Collins 2008). These trough-like tongue morphologies of avian nectarivores make suction feeding impossible; nectar is licked from flowers instead (Ewald and Williams 1982; Kingsolver and Daniel 1983). When the tip of the tongue penetrates the nectar, the fluid flows onto the tongue by capillary action (Kingsolver and Daniel 1983; Cheke et al. 2001). Once the nectar is loaded, the tongue is retracted inside the bill and fluid is removed from the grooves due to the constriction of the tongue by the closing bill (Ewald and Williams 1982; Kingsolver and Daniel 1983). The brush-tipped tongues of nectarivorous bats also extract nectar by capillary action (Howell and Hodgkin 1976), but may additionally act as a spoon (Winter and von Helversen 2003). Many nectar-feeding insects, such as most bee species, also lick or lap and draw fluid by capillarity (Kingsolver and Daniel 1995; Krenn et al. 2005). This is in contrast to suction-feeding insect species like butterflies and moths, where the proboscis remains motionless during feeding and muscles generate a pressure difference to drive nectar through the food canal (Kingsolver and Daniel 1979 and 1995; Krenn et al. 2005).

For nectarivorous birds, the total nectar volume that the tongues can hold ranges from 0.4–2.8 µl in hummingbirds (Hainsworth 1973; Ewald and Williams 1982), 0.6–2.1 µl in sunbirds (Schlamowitz et al. 1976), and 1.2–20 µl in honeyeaters (Paton and Collins 1989). Despite differences in tongue structure, hummingbirds, sunbirds and honeyeaters extract nectar from flowers and artificial feeders at comparable rates. Six hummingbird species, weighing 3–9 g, were found to lick 10–72 µl•s$^{-1}$ of 0.5–1.0 M sucrose solutions, and three honeyeater species, weighing 10–25 g, ingested 20–74 µl•s$^{-1}$ of a 0.8 M sucrose solution (for a review see Paton and Collins 1989). Nectar removal rates depend on body size, as within each family larger species ingest nectar faster than smaller ones (Paton and Collins 1989). To date, nectar extraction rates have been recorded for only a single species of sunbird, the bronzy sunbirds (*Nectarinia kilimensis*; 16 g), which was shown to lick at a rate of 71 µl•s$^{-1}$ when feeding on a 0.5 M sucrose diet (Schlamowitz et al. 1976).

The two main parameters of licking behaviour recorded in previous studies are licking frequency and lick volume. At comparable nectar concentrations, early studies recorded licking frequencies of bronzy sunbirds and black-chinned (*Archilochus alexandri*) and blue-throated (*Lampornis clemenciae*) hummingbirds ranging from 3–5 licks•s$^{-1}$ (Hainsworth 1973; Schlamowitz et al. 1976). Brown (*Lichmera indistincta*) and singing (*Meliphaga virescens*) honeyeaters licked at a speed of 8–10 licks•s$^{-1}$ (Collins and Morellini 1979; Collins et al. 1980). When licking frequencies were filmed at higher resolutions, maximum licking rates above 17 licks•s$^{-1}$ were recorded in Anna's hummingbirds (*Calypte anna*) (Ewald and Williams 1982). Tongue licking behaviour is expected to be influenced by various floral features, such as corolla length and nectar volume (Paton and Collins 1989), but different studies have shown considerable inter-specific variation in how these floral features affect licking behaviour. Ewald and Williams (1982) found that the licking frequency in Anna's hummingbirds decreased with increasing corolla length. Black-chinned hummingbirds showed a higher licking frequency when a corolla was added compared to feeders without corolla, while volume per lick decreased at the same time (Hainsworth 1973). Volume per lick also decreased with increasing corolla length in bronzy sunbirds, but licking frequencies were unaltered (Schlamowitz et al. 1976). As an example for nectar-feeding bats, *Glossophaga soricina* demonstrated 12 licks•s$^{-1}$ when the food solution was close to its mouth, but licking frequency decreased when the tongue needed to be extended further (Winter and von Helversen 2003). The licking behaviour of nectarivorous animals is also affected by the volume of nectar. Collins (2008) reports that volumes per lick increased with increasing nectar volume, while licking frequencies stayed constant, in several hummingbird and honeyeater species.

Besides flower length and nectar volume, nectar ingestion rates are affected by the concentration of nectar (Paton and Collins 1989). Among insects, the intake rates of hovering hawk moths (*Macroglossum stellatarum*) and orchid bees (*Euglossa imperialis*) decreased with increasing concentration of sucrose solutions (Josens and Farina 2001; Borrell 2006). In black-chinned hummingbirds and blue-throated hummingbirds fed sucrose concentrations from 0.25–2 M, licking frequency tended to increase, and volume per lick tended to decrease, with increasing sugar concentration, however this was only statistically significant for female and juvenile black-chinned hummingbirds, as the sample sizes of the remaining birds were insufficient for statistical analysis (Hainsworth 1973). Licking frequency in brown honeyeaters was not altered over the range of sucrose concentrations from 0.8–1.6 M (Collins et al. 1980). In a later study, a rufous hummingbird (*Selasphorus rufus*) was found to decrease its licking frequency and volume per lick with increasing sucrose concentration from 0.8–2.0 M (Roberts 1995). These different results may have been caused by differences in methodology, as the older studies used video cameras to record licks (Hainsworth 1973; Collins et al. 1980), and these may have provided insufficient resolution (18–24 frames•s$^{-1}$), while the more recent study used a photodetection system (Roberts 1995).

In the present study I aimed to investigate how changing dietary sucrose concentration affects the frequency of tongue licks, lick duration and food consumption per lick of whitebellied (*Cinnyris talatala*) and amethyst (*Chalcomitra amethystina*) sunbirds. To my knowledge, this study is the first to investigate the effect of sugar concentration on the licking behaviour of sunbirds. Tongue licks were recorded on a novel short-term scale (every 1 ms) using a photodetection system, and consumption per lick was calculated from the number of licks and the mass of solution consumed over 3 h. The frequency of licks and consumption per lick were expected to decrease, while lick duration should increase, with increasing sugar concentration. In a second part of the experiment, I determined the licking behaviour of the sunbirds after a 2 h fasting period, and compared these data to a control day. I have shown earlier that whitebellied sunbirds increase their meal duration after a fast (Chapter 3), and the aim was to investigate whether the sunbirds also adjust their licking behaviour to compensate for the loss in foraging time. The licking behaviour of avian nectarivores following food deprivation has not been investigated to date, but starved nectarivorous ants (*Camponotus mus*) were found to increase their food ingestion rate (Falibene and Josens 2008). I hypothesized that the number of licks, lick duration and consumption per lick would not differ between a day without fasting and following a fasting period, since I expect these licking parameters to depend on nectar concentration rather than on the degree of starvation.

## Materials and methods

### *Study animals and their maintenance*

Nine whitebellied sunbirds (*Cinnyris talatala*) and nine amethyst sunbirds (*Chalcomitra amethystina*) were mist-netted in Jan Cilliers Park, Pretoria, South Africa. At the time of capture, body mass (mean ± SE) of the seven male whitebellied sunbirds was 8.56 ± 0.14 g and of the two females was 6.85 and 7.63 g; body mass of the four male and five female amethyst sunbirds was 14.59 ± 0.26 g and 14.12 ± 0.50 g respectively. Birds were housed in individual cages (45 x 45 x 32 cm) in a climate-controlled room at 20 ± 2ºC. The photoperiod was 12:12 h L:D, with lights on at 07h00. Dawn and dusk were simulated by an additional 0.5 h dimmed light at the beginning and end of each day. The maintenance diet consisted of a 0.63 M sucrose solution with a nutritional supplement (Ensure®, Abbott Laboratories, Johannesburg, South Africa) to provide dietary nitrogen (Van Tets and Nicolson 2000). This maintenance diet and supplementary water were provided *ad libitum* in inverted, stoppered syringes. Birds were kept under laboratory conditions for nine weeks before the commencement of the study, and were released into an outdoor aviary (8 x 5 x 2 m) after completion of the experiment.

## *Experimental procedure*

Each bird was moved to an experimental cage and trained to feed from the feeding device (Fig. 1), consisting of a hole (1.2 mm in diameter) through a 3 mm thick Perspex plate, highlighted by red nail varnish to attract the bird. A black pipe of 7 mm diameter was mounted on the other side, between the Perspex and the feeder. A phototransmitter and a photoreceptor were inserted in the pipe, 6 cm apart, on either side of the feeding hole. The hole was big enough to allow for normal food intake, but small enough to force the bird to extend its tongue to feed, since the bill could not be inserted. Nine identical feeding devices were constructed and all nine birds of one species were tested simultaneously. The photodetection systems were interfaced to a computer and tongue licks were recorded every 1 ms using custom-designed software (L. Verburgt). Data were recorded for a 3 h period, and feeders were weighed before and after this period to determine the food intake of the birds ($g \cdot 3\ h^{-1}$). Any drips from the feeders were collected in trays filled with paraffin and placed underneath the feeders. These trays were weighed at the same time as the feeders and food intake was corrected accordingly.

### Part I: Licking behaviour and sugar concentration

Birds were fed four sucrose-only test diets (0.25, 0.5, 1 and 1.5 M, which are equivalent to 8.5, 16, 30 and 43% w/w) on consecutive days to investigate whether the frequency and duration of tongue licks and food consumption per lick depend on sugar concentration. The order of the test diets was randomized for individual birds. Ambient temperature and light period were the same as during maintenance, but the dawn and dusk periods were omitted. Tongue licks were recorded from 09h00 to 12h00, since the food intake rate of nectarivorous birds is most stable in the morning (Collins and Briffa 1983; Köhler et al. 2006). Birds were fed their maintenance diet outside the test period, to prevent body mass loss on sugar-only diets (Nicolson and Fleming 2003a).

## Part II: Licking behaviour and a fasting period

The same experimental protocol as in Chapter 3 was followed to be able to compare the results obtained for licking and feeding behaviour. In short, the light period was the same as during maintenance, without the dawn and dusk periods. Birds were acclimated to the experimental temperature of 10°C for one day. This acclimation day was followed by a control day with uninterrupted feeding, then a fasting day, when feeding was interrupted for 2 h (10h00–12h00) by turning off the lights. On both the control and the fasting day, tongue licks were recorded from 12h00 to 15h00, starting immediately after the fast on the second day. Birds were fed a 0.63 M (20% w/w) sucrose-only diet during data capture. They received the maintenance diet in the mornings and after 15h00 to provide protein.

### *Data processing*

The start and end time of each tongue lick enabled calculation of the duration of the individual licks. Only photoreceptor detections longer than 2 ms were included in the analysis, since preliminary video recordings, which were conducted to detect how far the tongue extends into the sugar solution, revealed longer durations for tongue licks. Individual tongue licks within a feeding event were generally 2–50 ms apart. Licks that were more than 250 ms apart were defined as separate feeding events. The number of licks and the number of feeding events were calculated for each bird for the 3 h test period. The mean number of licks per feeding event was determined by dividing the number of licks by the number of feeding events in the 3 h period. The mean duration of licks (ms) and the total time that the bird spent licking (min•3 $h^{-1}$) were also determined. Licking frequency (licks•$s^{-1}$) was calculated by dividing the number of licks during the 3 h period by the total time that the bird spent licking (s•3 $h^{-1}$). Food consumed per lick (mg) was calculated by dividing the food intake in the 3 h period by the number of licks.

### *Statistical analysis*

All data were tested for homogeneity of variance (Levene's test) and normality (Kolmogorov-Smirnov test), and were log transformed when heteroscedastic or not normally distributed. Licking frequency (licks•$s^{-1}$), mean number of licks per feeding event, mean lick duration (ms), total time spent licking (min•3 $h^{-1}$) and food consumed per lick (mg) were separately subjected to repeated measures ANOVA (RM-ANOVA). Sugar concentration or day (control vs. fast) respectively were within-effects, and species was the categorical predictor (between-effect). Separate RM-ANOVA for each species were performed as an exploratory data analysis, but these results did not differ from the combined analysis and the results from the latter are therefore presented. *Post-hoc* comparisons for all RM-ANOVA were conducted with Tukey's Honest Significant Difference test for equal sample sizes, followed by a Bonferroni correction for multiple comparisons. All data are presented as mean ± SE.

## Results

### Part I: Licking behaviour and sugar concentration

The frequency of tongue licks (licks•$s^{-1}$) differed between the four sucrose concentrations ($F_{3,48}$=4.48, $P$<0.01; Fig. 2). *Post-hoc* analysis showed that birds licked significantly more slowly on the 1 and 1.5 M sucrose diets than on the 0.25 M diet ($P$<0.02), while there was no difference in licking frequency between the remaining sucrose concentrations ($P$>0.29). The two species differed in their licking frequency ($F_{1,16}$=24.19, $P$<0.001; Fig. 2), with whitebellied licking faster than amethyst sunbirds ($P$<0.001). The mean number of licks per feeding event did not differ between sucrose concentrations ($F_{3,48}$=0.26, $P$=0.85), but differed between the two species ($F_{1,16}$=8.58, $P$<0.01). *Post-hoc* analysis revealed that whitebellied sunbirds licked more often per feeding event than amethyst sunbirds ($P<0.01$; Fig. 2). When data were pooled for all sugar concentrations, whitebellied sunbirds averaged 9.13 ± 0.68 licks, and amethyst sunbirds 6.29 ± 0.37 licks per feeding event.

The mean duration of tongue licks increased significantly with increasing sucrose concentration ($F_{3,48}=5.01$, $P<0.01$; Fig. 3). According to *post-hoc* analysis, birds showed longer mean lick durations on the 1 and 1.5 M sucrose diets than on the 0.25 M diet ($P<0.02$), while lick durations did not differ significantly between the other sugar concentrations ($P>0.17$). Mean lick durations differed between the species ($F_{1,16}=23.25$, $P<0.001$), with amethyst sunbirds having longer licks than whitebellied sunbirds ($P<0.001$; Fig. 3). The total time that the birds spent licking in the 3 h period did not differ between sucrose concentrations ($F_{3,48}=0.11$, $P=0.96$). Amethyst sunbirds tended to lick for longer (average of all sucrose concentrations: 3.82 ± 0.58 $\text{min}\bullet 3\ \text{h}^{-1}$) than whitebellied sunbirds (2.07 ± 0.39 $\text{min}\bullet 3\ \text{h}^{-1}$), however, this was not statistically significant ($F_{1,16}=4.25$, $P=0.06$).

The amount of food consumed per lick decreased with increasing sucrose concentration ($F_{3,48}=22.08$, $P<0.001$; Fig. 4). Food intake per lick was lower on the 1.5 M diet than on the three lower sugar concentrations ($P<0.01$). Food intake per lick was also lower on the 1 M diet than on the most dilute diet of 0.25 M ($P<0.01$), while no difference was found between 0.25 and 0.5 M ($P=0.05$, Bonferroni correction: $P=0.06$), and between 0.5 and 1 M ($P=0.53$). Whitebellied and amethyst sunbirds did not differ in the amount of food consumed per lick ($F_{1,16}=0.07$, $P=0.79$).

## Part II: Licking behaviour and a fasting period

Licking frequency did not differ between the control day and the day with a fasting period ($F_{1,16}=0.06$, $P=0.81$; Table 1). Whitebellied and amethyst sunbirds showed similar licking frequencies ($F_{1,16}=0.40$, $P=0.54$). The mean number of licks per feeding event did also not differ between the two days ($F_{1,16}=0.44$, $P=0.52$) and the two species ($F_{1,16}=0.30$, *P*=0.59). Whitebellied and amethyst sunbirds licked on average 10.17 ± 1.76 times per feeding event on the control day, and 9.02 ± 0.94 times per feeding event after the fasting period.

There was also no difference in mean duration of tongue licks ($F_{1,16}$=0.39, *P*=0.54) and the amount of food consumed per lick ($F_{1,16}$=0.38, *P*=0.55; Table 1) between the two treatments. Birds spent on average 3.61 ± 0.67 min•3 $h^{-1}$ licking, with no difference between control and fasting days ($F_{1,16}$=0.01, *P*=0.91) and the two species ($F_{1,16}$=0.08, *P*=0.79). Whitebellied and amethyst sunbirds did also not differ in the mean duration of tongue licks and food consumption per lick ($F_{1,16}$<0.67, *P*>0.43).

## Discussion

### *The effect of sugar concentration on licking behaviour*

The concentration of nectar affects the licking behaviour of whitebellied and amethyst sunbirds. As sugar concentration increases, licking frequency and the amount of food consumed per lick decline, while the duration of individual licks increases. Nectar flows onto the grooved sunbird tongue by capillary action (Cheke et al. 2001; Downs 2004), which is affected by physical properties of nectar. Nectar flow rate increases as surface tension increases, but declines as viscosity of nectar increases (Rowlinson and Widom 1982; Kingsolver and Daniel 1983 and 1995). Surface tension coefficients of simple sugar solutions at an interface with air are large compared to most liquids, and increase slightly with sugar concentration (Kingsolver and Daniel 1995). Viscosity, on the other hand, increases exponentially with concentration at a given temperature (Kingsolver and Daniel 1983; Telis et al. 2007). Thus, sugar solutions of high concentration are much more viscous than dilute solutions. Baker (1975), in offering an explanation for why bird nectars are relatively dilute, suggested that energy intake rates of nectarivorous birds may be constrained by high nectar viscosities. Sunbirds in this study showed lower frequencies and longer durations of tongue licks as nectar sugar concentration increased. This suggests that tongue loading takes longer on very viscous solutions than on less viscous ones. Birds also consumed less food per lick (g) on the most concentrated diet (1.5 M) than on the more dilute diets. In terms of volumetric intake per lick, the difference between diet concentrations would be even greater, since the more concentrated solutions are heavier.

The decline in licking frequency with increasing nectar concentration coincides with findings for a rufous hummingbird, which also licked more slowly on high sucrose concentrations than on more dilute diets (Roberts 1995). However, early studies that investigated the relationship between licking frequency and nectar concentration in avian nectarivores revealed inconsistent trends. Licking frequency of brown honeyeaters did not differ between concentrations (Collins et al. 1980). Blue-throated and black-chinned hummingbirds, on the other hand, licked faster on a concentrated sucrose diet (2 M) than on lower concentrations, although statistical tests were not performed in all cases (Hainsworth 1973).

The effect of sugar concentration on the duration of tongue licks has not been investigated to date. However, the lick duration of a single Anna’s hummingbird feeding on a 0.7 M sucrose diet averaged 105 ms when the distance between the bill and the food was 8.5 mm (Ewald and Williams 1982). The tongue had to be extended further (11 mm) in the present study, and lick duration of amethyst sunbirds on a 0.5 M sucrose diet was slightly higher than that of this hummingbird, while whitebellied sunbirds had much shorter licks. The decline in consumption per lick with increasing sugar concentration observed in my study is in agreement with findings for blue-throated and black-chinned hummingbirds (Hainsworth 1973) and a rufous hummingbird (Roberts 1995). The total time that amethyst and whitebellied sunbirds spent licking did not differ between the sucrose concentrations. In an earlier experiment, the total time that whitebellied sunbirds spent feeding per hour was lower on a 0.6 M sucrose diet than on a 0.3 M diet (Köhler et al. 2006), since birds ingest smaller amounts of a more concentrated diet than of a dilute diet. Despite this decline in nectar intake with increasing concentration, total feeding duration did not decrease beyond 0.6 M sucrose (Köhler et al. 2006), suggesting that it takes longer to ingest concentrated nectar than more dilute nectar. When fed diets ranging from 0.3–1.4 M sucrose in another study, the food intake rates of whitebellied sunbirds decreased with increasing sugar concentration (Köhler et al. 2008). Ingestion rate of suction-feeding hovering hawk moths decreased beyond 0.6 M sucrose, indicating that viscosity impedes ingestion beyond this concentration (Josens and Farina 1997). For future studies, it is recommended that sugar concentration and nectar viscosity be independently manipulated to confirm that nectar viscosity is responsible for the change in licking behaviour, and thus nectar ingestion, observed in the present study.

The separate effects of concentration and viscosity on nectar ingestion have been investigated in earlier studies on insects. Nectarivorous ants (*C. mus*) reduce their intake rate and crop load when encountering high viscosity of a sugar solution, while sugar concentration is kept constant (Medan and Josens 2005). When sugar concentration is increased at a constant viscosity, small and medium sized ants increase their food intake, and thus crop load (Medan and Josens 2005). Hovering hawk moths decrease their nectar intake with increasing sugar concentration, but at constant sugar concentration the intake rate of the moths declines with increasing viscosity (Josens and Farina 2001). Orchid bees demonstrate a similar decline in intake rate as sucrose concentration and viscosity increase (Borrell 2006), decreasing their intake rates when nectar viscosity is increased and sugar concentration is held constant. However, at a constant viscosity, intake rates do not differ between a wide range of sugar concentrations (Borrell 2006). Tezze and Farina (1999) investigated the effect of concentration and viscosity of sucrose solutions on trophallaxis in honeybees (*Apis mellifera*): for a constant sugar concentration, the transfer rate from donor bees to recipient bees decreases with increasing viscosity. At a constant viscosity, the transfer rate increases at high sugar concentrations. When both concentration and viscosity of the solution increase, transfer rate increases up to a maximum concentration of 1 M, and declines beyond this point (Tezze and Farina 1999). Similar to the findings for insects, a recent study indicates that viscosity, and not sugar concentration, determines the nectar intake of whitebellied sunbirds (Leseigneur 2008): when the viscosity of dilute sucrose solutions (0.25–0.7 M) was artificially increased to the viscosity of a 1 M solution, sugar consumption on these diets was lower than on diets of the same sugar concentrations but without altered viscosity. When the viscosity of 1 M sucrose solutions was increased to the viscosities of 1.5–2.5 M solutions, the same birds ingested less sugar than on a pure 1 M solution. The birds further showed reduced energy intake on the highest viscosity (2.5 M sucrose diet), suggesting constraints to ingestion of very viscous solutions (Leseigneur 2008). However, nectar of sunbird-pollinated plants of southern Africa is more dilute: sugar concentrations mainly range from 0.45–1 M, but occasionally up to 2 M (Nicolson and Fleming 2003b).

Preference tests in avian nectarivores also support the hypothesis that high nectar sugar concentrations, resulting in high viscosities, affect nectar ingestion. When given a choice between sugar concentrations, rufous hummingbirds prefer the more concentrated diet, which maximizes their energy intake rate, but they discriminate against very concentrated sugar solutions (Tamm and Gass 1986). Whitebellied sunbirds prefer the more concentrated sucrose solution of a pair up to 1 M, while they are indifferent or tend to ingest more of the lower concentration beyond this point (Leseigneur 2008). Mathematical models demonstrate that the sugar intake rate of avian nectarivores is maximal at intermediate nectar concentrations, since high viscosities of very concentrated solutions affect nectar ingestion (Heyneman 1983; Kingsolver and Daniel 1983). Experimental findings for hummingbirds and honeyeaters, which show highest sugar intake rates at intermediate nectar concentrations, support this theory (e.g. Tamm and Gass 1986; Mitchell and Paton 1990).

## *The effect of experimental devices on licking behaviour*

As discussed earlier, studies investigating the effect of nectar concentration on tongue licking parameters of avian nectarivores revealed different results. Despite physical properties of nectar, differences in feeding devices also influence licking behaviour. Nectarivorous birds show a decreased nectar extraction rate as the corolla length or curvature of a flower increases (Collins 2008), and as corolla diameter decreases at a particular corolla length (Temeles 1996). Consequently, licking behaviour of avian nectarivores can easily be modulated by changing the structure of artificial feeders (Grant and Temeles 1992). In addition to flower structure, nectar volume also affects the licking behaviour of the birds: nectar removal rates of hummingbirds and honeyeaters increase with increasing volume of the food source (Montgomerie 1984; Mitchell and Paton 1990; Collins 2008). If birds can insert the entire tongue into the nectar, the grooves are completely filled and capillarity plays a negligible role (Kingsolver and Daniel 1983). The angle between the tongue and the food source also influences capillary action, as gravity plays a role in vertical upwards flow, but not in horizontal flow (Kingsolver and Daniel 1983). The feeding device was kept constant in the present study, and birds fed from high volume feeders at all times. As confirmed by video recordings, birds inserted their tongues horizontally into the feeding device, with only the tips being inserted into the sugar solution. Floral characteristics and the structure of artificial feeders should be taken into consideration when comparing data on licking behaviour between various studies.

In addition, recorded licking frequencies depend on the speed of the camera used. Early studies, filming at 18–24 frames•s$^{-1}$ (resolution: 56 up to 42 ms), reported 3–10 licks•s$^{-1}$ for sunbirds, hummingbirds and honeyeaters (Hainsworth 1973; Schlamowitz et al. 1976; Collins et al. 1980). The mean lick durations of whitebellied sunbirds were shorter than 40 ms on low and medium sugar concentrations (Fig. 3), indicating that individual licks are likely to be missed at low resolution. When camera speed was increased to 70 frames•s$^{-1}$ (resolution: 14 ms), maximum rates of licking were above 17 licks•s$^{-1}$ in Anna's hummingbirds (Ewald and Williams 1982). However, the mean lick duration of individual whitebellied sunbirds was as low as 13 ms on comparable diet concentrations. Despite the increased resolution, it is therefore possible that not all tongue licks were recorded in the study by Ewald and Williams (1982). In the present experiment, tongue licks were recorded at a remarkably fine time scale, and licking frequencies ranged from 8–18 licks•s$^{-1}$ in amethyst sunbirds, and 16–31 licks•s$^{-1}$ in whitebellied sunbirds.

## *Licking behaviour and a fasting period*

The licking behaviour of whitebellied and amethyst sunbirds stayed the same in fed and mildly starved birds. This demonstrates that these birds do not adjust their licking frequency and consumption per lick in order to compensate for a loss in foraging time. The lack of adjustment of licking behaviour was predicted, since dietary sugar concentration, which has been demonstrated to affect licking behaviour, was held constant. Note that the licking parameters on comparable sugar concentrations differ between Part I (nectar concentration) and Part II of this study (fasting period), since the experiments were conducted at different ambient temperatures. At a constant sugar concentration the viscosity of a solution decreases with increasing temperature (Nicolson and Thornburg 2007; Telis et al. 2007).

No studies to date have investigated the licking behaviour of nectarivorous birds in relation to food deprivation. Despite differences in tongue morphology, the findings for sunbirds are in agreement with an early study on rats (*Rattus rattus*), which were shown to lick water at a constant rate, irrespective of the level of water deprivation (Stellar and Hill 1952). Fluid licking in rodents is a rhythmic behaviour that has been proposed to be under neural control (Travers et al. 1997). Licking behaviour in rats is very stable and is affected only by the distance between mouth and fluid, changes in the feeding aperture or drug administration (for a review see Weijnen 1998). In contrast to animals that lick fluid, suction-feeding insects are able to adjust their nectar flow rate. Starved nectarivorous ants increased their intake rate of dilute and concentrated sucrose solutions through modulations in pump frequency (Josens and Roces 2000; Falibene and Josens 2008).

## *Differences in licking behaviour between species*

Despite the nectar and flower characteristics discussed earlier, licking also depends on the dimensions of the tongue or proboscis. During suction feeding, flow rate increases with increasing radius and decreasing length of the food canal (Pivnick and McNeil 1985; Daniel et al. 1989; Borrell 2007). Animals using capillary feeding face a trade-off: a small radius of the tongue grooves is required for an effective pressure difference that forces fluid up the tongue, while a large radius results in a lower resistance (Kingsolver and Daniel 1995). Amethyst sunbirds are about twice the size of whitebellied sunbirds, and their tongue is longer and slightly wider than that of the smaller species (C.D.C. Leseigneur and A. Köhler, unpublished data; Downs 2004). Both sunbird species consumed the same amount per lick on all diet concentrations, despite amethyst sunbirds having larger tongues. Avian nectarivores do not fully load their tongues because it is energetically unprofitable for them to do so (Ewald and Williams 1982; Kingsolver and Daniel 1983): as nectar moves up the grooves, the flow rate decreases because of the viscous force opposing the flow, and the mean rate of energy intake consequently decreases as more nectar flows onto the tongue. Amethyst sunbirds licked more slowly and for longer than whitebellied sunbirds over a range of sucrose concentrations, except on the most concentrated diet (1.5 M), where these licking parameters were similar between the species (Figs. 2 and 3). This suggests that the smaller tongue of whitebellied sunbirds is able to draw nectar of low and medium concentrations faster, while very concentrated nectar is loaded more easily onto the larger tongue of amethyst sunbirds.

Few studies have investigated the effect of body size on the licking behaviour of different species within a family. New Holland honeyeaters (*Phylidonyris novaehollandiae*) demonstrated higher licking frequencies than the smaller western spinebills (*Acanthorhynchus superciliosus*), while the volumes per lick ingested by the two species did not differ (Collins 2008). Blue-throated hummingbirds consumed a much higher volume per lick than black-chinned hummingbirds, which are less than half their size, but their licking frequencies were similar (Hainsworth 1973). Interestingly, the differences in licking behaviour found between amethyst and whitebellied sunbirds in the concentration experiment (Part I) were not evident in Part II (fasting period). More data on these and other species are needed to determine whether licking behaviour differs between species within a family of avian nectarivores, and which morphological and physiological factors affect the licking behaviour.

# Conclusion

In summary, nectar concentration affects the licking behaviour of whitebellied and amethyst sunbirds, while a fasting period has no effect on the licking parameters measured in this study. More investigations are needed to identify all factors that determine the licking behaviour of these birds. Future studies could examine whether the length and width of tubular flowers or the nectar volume influence licking behaviour of these sunbird species. If such flower traits affect nectar extraction by bird pollinators, this might influence which flowers are visited. Many studies have highlighted similarities between nectarivore morphology and the structure of flowers on which they feed (e.g. Wolf et al. 1976; Ford and Paton 1977; for a review see Paton and Collins 1989). A recent study by Botes et al. (2008) demonstrated that flower morphology and nectar characteristics of five co-flowering South African *Aloe* species partition bird pollinators: species with long-tubed flowers providing smaller volumes of more concentrated nectar were pollinated by specialist long-billed sunbirds, while species with short corolla tubes and larger amounts of more dilute nectar were associated with short-billed occasional nectarivores. However, striking morphological convergence between avian nectarivores and flowers of their food plants is not evident in other studies (e.g. Brown and Hopkins 1995; Collins 2008) and the sunbird species I investigated also feed on a variety of flower shapes, ranging from open and brush-like (*Callistemon* and *Eucalyptus* spp.) to tubular flowers (*Aloe*, *Erythrina* and *Erica* spp.) (Skead 1967).

According to optimal foraging theory, nectarivorous birds are expected to feed in a manner which maximizes their net rate of energy gain (MacArthur and Pianka 1966; Pyke 1978). In terms of nectar concentration, it can therefore be concluded that sunbirds should favour intermediate sugar concentrations, as dilute nectars may not provide sufficient energy, while very concentrated nectars impede ingestion, thus increasing the feeding time. However, the optimal nectar concentration also depends on the energetic costs of feeding and foraging flights. Heyneman (1983) predicted that pollinators facing high feeding costs, such as hovering hummingbirds, should favour more dilute nectar to minimize ingestion time, while pollinators with high foraging transit costs and low feeding costs, such as sunbirds which perch during meals, should prefer more concentrated nectar. Plant species providing these ideal nectar concentrations are expected to have an evolutionary advantage since they are more likely to be pollinated.

## Acknowledgements

This project was funded by the University of Pretoria and the South African National Research Foundation. Jan Cilliers Park is acknowledged for permission to mist-net sunbirds under permit from the Gauteng Directorate of Nature Conservation. Special thanks to Luke Verburgt for providing the custom-designed software used for data collection and processing, to Wimpie Nortje from Spline Technologies for helpful advice with the electronic equipment, and to Maciej Rossudowski for his assistance with assembling the experimental equipment. Craig Symes and Darren W. Pietersen are thanked for catching sunbirds for this study. The experiments were approved by the Animal Use and Care Committee of the University of Pretoria.

## References

Baker H.G. 1975. Sugar concentrations in nectars from hummingbird flowers. Biotropica 7: 37–41.

Borrell B.J. 2006. Mechanics of nectar feeding in the orchid bee *Euglossa imperialis*: pressure, viscosity and flow. Journal of Experimental Biology 209: 4901–4907.

Borrell B.J. 2007. Scaling of nectar foraging in orchid bees. American Naturalist 169: 569–580.

Botes C., Johnson S.D. and Cowling R.M. 2008. Coexistence of succulent tree aloes: partitioning of bird pollinators by floral traits and flowering phenology. Oikos 117: 875–882.

Brown E.D. and Hopkins M.J.G. 1995. A test of pollinator specifity and morphological convergence between nectarivorous birds and rainforest tree flowers in New Guinea. Oecologia 103: 89–100.

Cheke R.A., Mann C.F. and Allen R. 2001. Sunbirds: A guide to the sunbirds, flowerpeckers, spiderhunters and sugarbirds of the world. Christopher Helm, London, UK.

Collins B.G. 2008. Nectar intake and foraging efficiency: responses of honeyeaters and hummingbirds to variations in floral environments. Auk 125: 574–587.

Collins B.G. and Briffa P. 1983. Seasonal and diurnal variations in the energetics and foraging activities of the brown honeyeater, *Lichmera indistincta*. Australian Journal of Ecology 8: 103–111.

Collins B.G. and Morellini P.C. 1979. The influence of nectar concentration and time of day upon energy intake and expenditure by the singing honeyeater, *Meliphaga virescens*. Physiological Zoology 52: 165–175.

Collins B.G., Cary G. and Packard G. 1980. Energy assimilation, expenditure and storage by the brown honeyeater, *Lichmera indistincta*. Journal of Comparative Physiology B 137: 157–163.

Daniel T.L., Kingsolver J.G. and Meyhöfer E. 1989. Mechanical determinants of nectar-feeding energetics in butterflies: muscle mechanics, feeding geometry, and functional equivalence. Oecologia 79: 66–75.

Downs C.T. 2004. Some preliminary results of studies on the bill and tongue morphology of Gurney's Sugarbird and some southern African sunbirds. Ostrich 75: 169–175.

Ewald P.W. and Williams W.A. 1982. Function of the bill and tongue in nectar uptake by hummingbirds. Auk 99: 573–576.

Falibene A. and Josens R. 2008. Nectar intake rate is modulated by changes in sucking pump activity according to colony starvation in carpenter ants. Journal of Comparative Physiology A 194: 491–500.

Ford H.A. and Paton D.C. 1977. The comparative ecology of ten species of honeyeaters in South Australia. Australian Journal of Ecology 2: 399–407.

Grant V. and Temeles E.J. 1992. Foraging ability of rufous hummingbirds on hummingbird flowers and hawkmoth flowers. Proceedings of the National Academy of Sciences USA 89: 9400–9404.

Hainsworth F.R. 1973. On the tongue of a hummingbird: its role in the rate and energetics of feeding. Comparative Biochemistry and Physiology A 46: 65–78.

Heyneman A.J. 1983. Optimal sugar concentrations of floral nectars – dependence on sugar intake efficiency and foraging costs. Oecologia 60: 198–213.

Howell D.J. and Hodgkin N. 1976. Feeding adaptations in the hairs and tongues of nectar-feeding bats. Journal of Morphology 148: 329–336.

Josens R.B. and Farina W.M. 1997. Selective choice of sucrose solution concentration by the hovering hawk moth *Macroglossum stellatarum*. Journal of Insect Behavior 10: 631–637.

Josens R.B. and Farina W.M. 2001. Nectar feeding by the hovering hawk moth *Macroglossum stellatarum*: intake rate as a function of viscosity and concentration of sucrose solutions. Journal of Comparative Physiology A 187: 661–665.

Josens R.B. and Roces F. 2000. Foraging in the ant *Camponotus mus*: nectar-intake rate and crop filling depend on colony starvation. Journal of Insect Physiology 46: 1103–1110.

Kingsolver J.G. and Daniel T.L. 1979. On the mechanics and energetics of nectar feeding in butterflies. Journal of Theoretical Biology 76: 167–179.

Kingsolver J.G. and Daniel T.L. 1983. Mechanical determinants of nectar feeding strategy in hummingbirds: energetics, tongue morphology, and licking behaviour. Oecologia 60: 214–226.

Kingsolver J.G. and Daniel T.L. 1995. Mechanics of food handling by fluid-feeding insects. In: Regulatory mechanisms in insect feeding. R.F. Chapman and G. De Boer (Eds.), Chapman and Hall, New York, USA, pp. 32–73.

Köhler A., Verburgt L. and Nicolson S.W. 2006. Short-term feeding patterns of whitebellied sunbirds (*Nectarinia talatala*): feeding frequency, daily rhythms and individual differences. Journal of Experimental Biology 209: 2880–2887.

Köhler A., Verburgt L. and Nicolson S.W. 2008. Nectar intake of whitebellied sunbirds (*Cinnyris talatala*): Can meal size be inferred from feeding duration? Physiological and Biochemical Zoology 81: 682–687.

Krenn H.W., Plant J.D. and Szucsich N.U. 2005. Mouthparts of flower-visiting insects. Arthropod Structure and Development 34: 1–40.

Leseigneur C.D.C. 2008. The feeding response of whitebellied sunbirds (*Cinnyris talatala*) to sugar concentration and viscosity of artificial nectar. MSc thesis, University of Pretoria, Pretoria, South Africa.

MacArthur R.H. and Pianka E.R. 1966. On optimal use of a patchy environment. American Naturalist 100: 603–609.

Medan V. and Josens R.B. 2005. Nectar foraging behaviour is affected by ant body size in *Camponotus mus*. Journal of Insect Physiology 51: 853–860.

Mitchell R.J. and Paton D.C. 1990. Effects of nectar volume and concentration on sugar intake rates of Australian honeyeaters (Meliphagidae). Oecologia 83: 238–246.

Montgomerie R.D. 1984. Nectar extraction by hummingbirds: response to different floral characters. Oecologia 63: 229–236.

Nicolson S.W. and Fleming P.A. 2003a. Energy balance in the Whitebellied Sunbird *Nectarinia talatala*: constraints on compensatory feeding, and consumption of supplementary water. Functional Ecology 17: 3–9.

Nicolson S.W. and Fleming P.A. 2003b. Nectar as food for birds: the physiological consequences of drinking dilute sugar solutions. Plant Systematics and Evolution 238: 139–153.

Nicolson S.W. and Thornburg R.W. 2007. Nectar chemistry. In: Nectaries and nectar. S.W. Nicolson, M. Nepi and E. Pacini (Eds.), Springer, Dordrecht, Netherlands, pp. 215–264.

Paton D.C. and Collins B.G. 1989. Bills and tongues of nectar-feeding birds: A review of morphology, function and performance, with intercontinental comparisons. Australian Journal of Ecology 14: 473–506.

Pivnick K.A. and McNeil J.N. 1985. Effects of nectar concentration on butterfly feeding: measured feeding rates for *Thymelicus lineola* (Lepidoptera: Hesperiidae) and a general feeding model for adult Lepidoptera. Oecologia 66: 226–237.

Pyke G.H. 1978. Optimal foraging in hummingbirds: testing the marginal value theorem. American Zoologist 18: 739–752.

Roberts W.M. 1995. Hummingbird licking behaviour and the energetics of nectar feeding. Auk 112: 456–463.

Rowlinson J.S. and Widom B. 1982. Molecular theory of capillarity. Oxford University Press, Oxford, UK.

Schlamowitz R., Hainsworth F.R. and Wolf L.L. 1976. On the tongues of sunbirds. Condor 78: 104–107.

Skead C.J. 1967. The sunbirds of southern Africa: also the sugarbirds, the white-eyes and the spotted creeper. The Trustees of the South African Bird Book Fund, Balkema, Cape Town, South Africa.

Stellar E. and Hill J.H. 1952. The rat's rate of drinking as a function of water deprivation. Journal of Comparative Physiology and Psychology 45: 96–102.

Tamm S. and Gass C.L. 1986. Energy intake rates and nectar concentration preferences by hummingbirds. Oecologia 70: 20–23.

Telis V.R.N., Telis-Romero J., Mazzotti H.B. and Gabas A.L. 2007. Viscosity of aqueous carbohydrate solutions at different temperatures and concentrations. International Journal of Food Properties 10: 185–195.

Temeles E.J. 1996. A new dimension to hummingbird-flower relationships. Oecologia 105: 517–523.

Temeles E.J. and Kress W.J. 2003. Adaptation in a plant-hummingbird association. Science 300: 630–633.

Tezze A.A. and Farina W.M. 1999. Trophallaxis in the honeybee, *Apis mellifera*: the interaction between viscosity and sucrose concentration of the transferred solution. Animal Behaviour 57: 1319–1326.

Travers J.B., Dinardo L.A. and Karimnamazi H. 1997. Motor and premotor mechanisms of licking. Neuroscience and Biobehavioral Reviews 21: 631–647.

Van Tets I.G. and Nicolson S.W. 2000. Pollen and the nitrogen requirements of the lesser double-collared sunbird. Auk 117: 826–830.

Weijnen J.A.W.M. 1998. Licking behavior in the rat: measurement and situational control of licking frequency. Neuroscience and Biobehavioral Reviews 22: 751–760.

Winter Y. and von Helversen O. 2003. Operational tongue length in phyllostomid nectar-feeding bats. Journal of Mammalogy 84: 886–896.

Wolf L.L., Stiles F.G. and Hainsworth F.R. 1976. Ecological organization of a tropical, highland hummingbird community. Journal of Animal Ecology 45: 349–379.

# Table

**Table 1.** Frequency of tongue licks (licks•s$^{-1}$), mean duration of licks (ms) and food consumed per lick (mg) of whitebellied (WBSB) and amethyst (ASB) sunbirds recorded over a 3 h period on one control day and after a 2 h fasting period (mean ± SE).

| Species | Treatment | Licking frequency | Mean lick duration | Consumption per lick |
|---|---|---|---|---|
| ASB | Control | 13.81 ± 4.08 | 95.59 ± 15.94 | 2.08 ± 0.30 |
| ASB | Fast | 18.67 ± 3.61 | 71.84 ± 13.00 | 3.10 ± 0.49 |
| WBSB | Control | 19.64 ± 3.90 | 70.53 ± 14.05 | 3.03 ± 0.78 |
| WBSB | Fast | 16.77 ± 2.83 | 75.57 ± 13.10 | 2.38 ± 0.53 |

# Figure legends

**Fig. 1.** The experimental feeding device used to record tongue licks of sunbirds. A black pipe containing photodetectors was mounted between the feeder and a 3 mm thick Perspex plate. The birds extended their tongues through a 1.2 mm hole in the Perspex and the pipe, interrupting the light beam of the infrared phototransmitter. The photodetection system was interfaced to a computer, allowing for continuous recording of tongue licks.

**Fig. 2.** Frequency of tongue licks (licks•$s^{-1}$) of whitebellied and amethyst sunbirds fed different sucrose concentrations over a 3 h period (mean ± SE; error bars partly omitted for clarity).

**Fig. 3.** Mean duration of tongue licks (ms) of whitebellied and amethyst sunbirds fed different sucrose concentrations over a 3 h period (mean ± SE; error bars partly omitted for clarity).

**Fig. 4.** Food intake (mg) per tongue lick of whitebellied and amethyst sunbirds fed different sucrose concentrations over a 3 h period (mean ± SE; error bars partly omitted for clarity).

# Figures

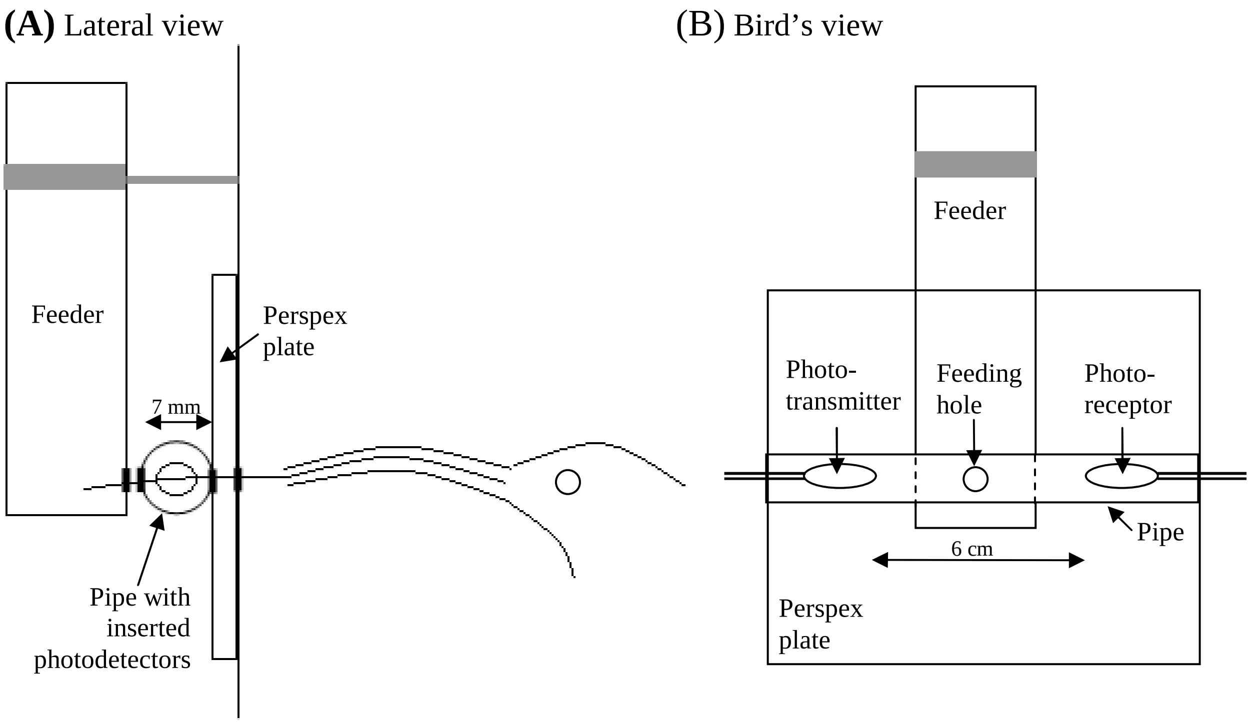
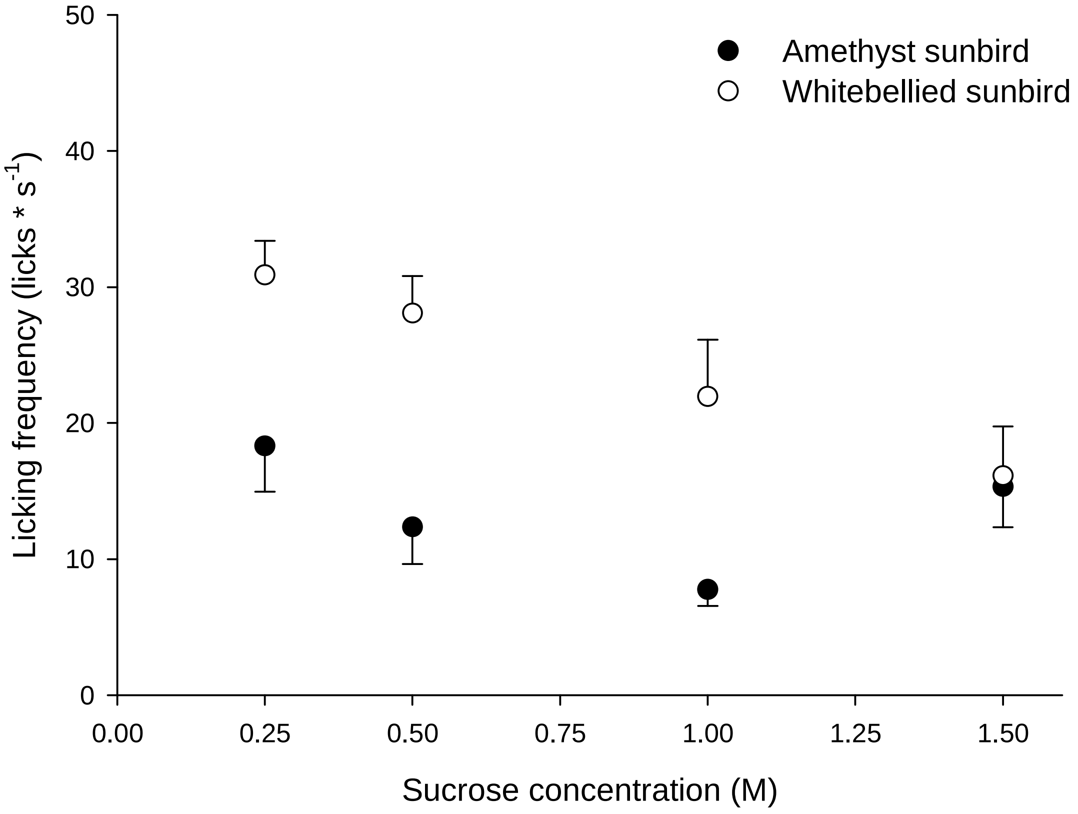


Figure 1.



50
40
30
20
10
0
Licking frequency (licks * s-1)
0.00
0.25
0.50
0.75
1.00
1.25
1.50
Sucrose concentration (M)
● Amethyst sunbird
○ Whitebellied sunbird

Figure 2.

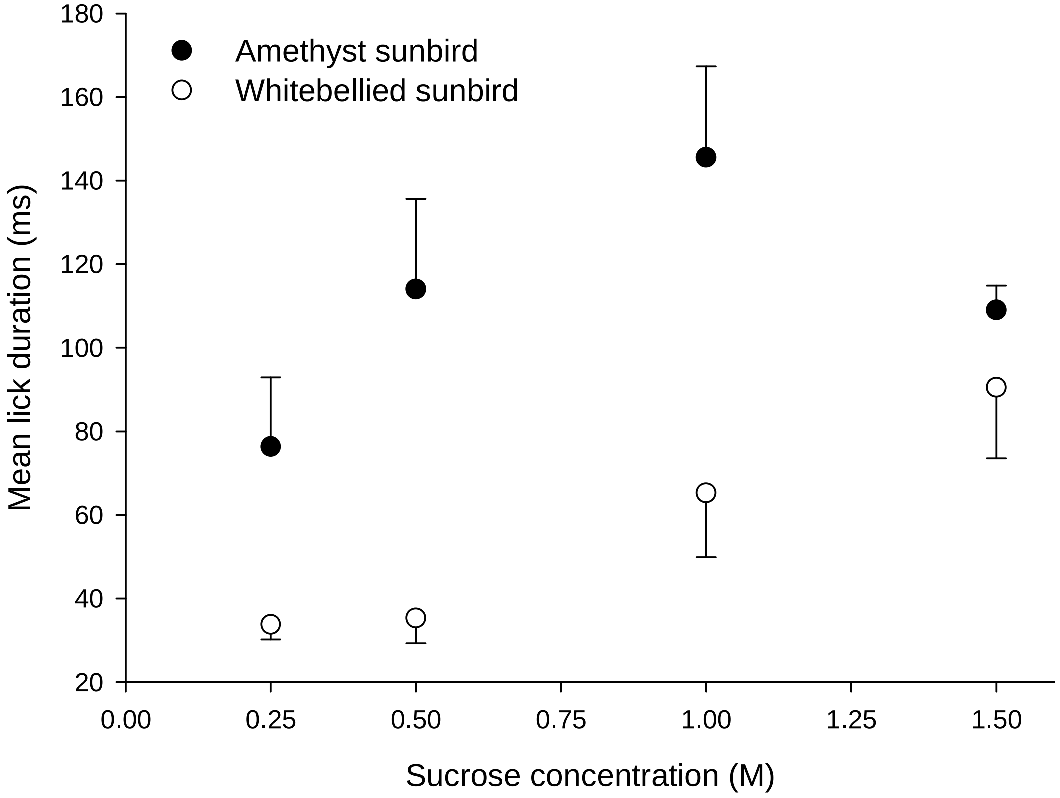

180
160
140
120
100
80
60
40
20
Mean lick duration (ms)
● Amethyst sunbird
○ Whitebellied sunbird
0.00
0.25
0.50
0.75
1.00
1.25
1.50
Sucrose concentration (M)

Figure 3.

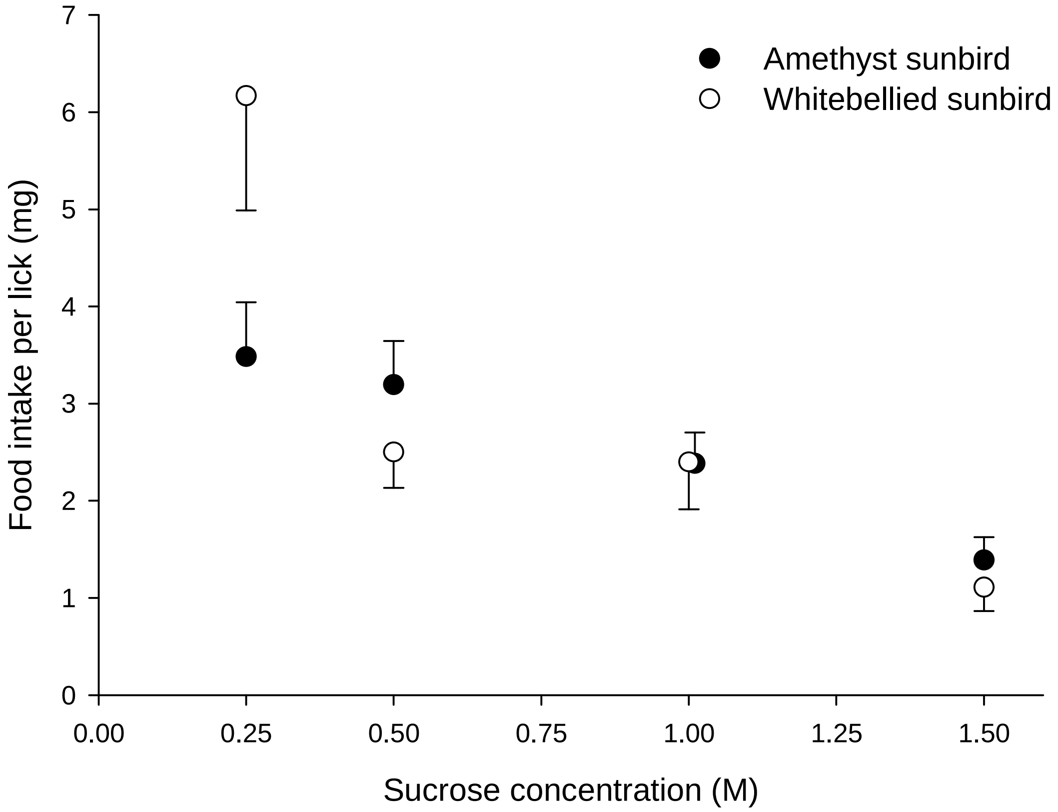

Amethyst sunbird
Whitebellied sunbird
Food intake per lick (mg)
0
1
2
3
4
5
6
7
Sucrose concentration (M)
0.00
0.25
0.50
0.75
1.00
1.25
1.50

Figure 4.

# CONCLUSION

## *The importance of studying animal responses to energy challenges*

The regulation of food intake allows an animal to balance its energy intake and expenditure, which is crucial for survival and fitness. When the energy content of the food source is low or energy requirements are high, animals must increase their food intake. Exposure to low food quality, fasting periods and low temperature under laboratory conditions provides an understanding of how animals cope in terms of foraging energetics with adverse environmental conditions. All three energy challenges in my study are ecologically relevant. Firstly, nectarivorous birds encounter food plants providing low nectar concentrations, or nectar may be diluted by rain. Secondly, the time available for foraging may be restricted by storms and heavy rain, or chases by predators and competitors may prevent feeding. Thirdly, the birds also experience sudden drops in environmental temperature during a storm, or are exposed to longer-term periods of cold during winter.

Studies in the field of ecological physiology provide insight into animal physiology and behaviour, including energy availability and utilization, thermoregulation, body size effects on physiological traits and behavioural adjustments (Bennett 1987a; Karasov and Martínez del Rio 2007). Data deriving from these studies can be used to predict the physiological responses of animals exposed to changing environmental conditions. Since the physiology of an animal determines the range of environmental conditions under which it can persist, this knowledge may serve conservation purposes. It can help biologists to anticipate future problems, find explanations for population declines, develop countermeasures and monitor the success of management strategies (Wikelski and Cooke 2006).

The response of hummingbirds to energetically challenging conditions, such as low ambient temperature and fasting periods, has been extensively investigated (e.g.

Beuchat et al. 1979; Tooze and Gass 1985; Carpenter and Hixon 1988; Gass et al. 1999; McWhorter and Martínez del Rio 2000; Fernández et al. 2002). Similar challenges in sunbirds (Lotz 1999; Nicolson et al. 2005) and honeyeaters (Collins and Briffa 1984) have received less attention, but it is just as important to investigate the effect of energy stress on these two families of avian nectarivores. My study therefore provides an important contribution, as it focuses on the behavioural and physiological responses of the main families of passerine nectarivores to variations in food quality and availability and low ambient temperature.

## *Suitability of avian nectarivores for my study*

Avian nectarivores are diurnal and can easily be observed while foraging. For this reason, the feeding behaviour and inter-individual interactions of wild avian nectarivores have been investigated in various field studies (e.g. Gill and Wolf 1975 and 1977; Vaughton 1990; Lott 1991; Mendonça and Dos Anjos 2006). Nectarivorous birds are also ideal subjects for investigating the compensatory feeding response and physiological constraints to this response under energetically challenging conditions. Firstly, their diet consists mainly of nectar, which is a relatively simple food and easily assimilated. The energy intake rates of nectarivorous birds can be readily quantified, since nectar consists mainly of sugar and water. Secondly, the energy balance of the birds is linked to their water balance. Birds compensate for low nectar concentrations and increased energy demands by ingesting larger amounts of the watery diet, which may pose osmoregulatory challenges (Lotz and Nicolson 1999; Fleming and Nicolson 2003). Thirdly, avian nectarivores are characterized by high mass-specific metabolic rates and energetic lifestyles. The birds have to feed frequently to meet their high energy requirements. As shown for hummingbirds, the metabolism is fuelled by recently ingested sugars, rather than stored fat (Carleton et al. 2006; Welch and Suarez 2007). These features make the birds susceptible to low temperatures and periods of food scarcity.

The study species, namely amethyst (*Chalcomitra amethystina*) and whitebellied (*Cinnyris talatala*) sunbirds and brown honeyeaters (*Lichmera indistincta*), were chosen for this study as they occur in similar habitats, but belong to two different families of avian nectarivores. The body size of the brown honeyeater is comparable to that of the two sunbird species. The brown honeyeater is one of the smallest honeyeater species and mainly nectarivorous, while larger honeyeaters also ingest fruits and insects as major portions of their diet (Pyke 1980). Since the species are ecologically alike, they are expected to experience similar energy challenges in their natural environment. If sunbirds and honeyeaters differ in their response to energy challenges, phylogenetic effects are likely, since other ecological and physiological aspects are comparable (Liknes and Swanson 1996).

Brown honeyeaters and whitebellied sunbirds accustom easily to captivity, and have consequently been useful research subjects (e.g. Collins et al. 1980; Collins and Briffa 1984; Nicolson et al. 2005; Leseigneur et al. 2007). Whitebellied sunbirds, in particular, learn to feed from artificial feeders within a few hours of capture and settle down quickly in individual cages. They can also be kept together in an aviary, without major competitive and aggressive behaviour (pers. observation). Amethyst sunbirds do not habituate to captivity so easily: individual birds take several days to learn to feed from artificial feeders, and the birds are generally more alert even after long periods in captivity. When both species were kept in a group in an aviary, I observed adult male amethyst sunbirds chasing each other and whitebellied sunbirds of both sexes, with the subordinate birds being excluded from feeding, even though plenty of feeding stations were provided.

## *The response of sunbirds and honeyeaters to energy challenges*

In response to a decrease in nectar sugar concentration, whitebellied sunbirds increased their food intake to maintain a constant energy intake. This supports the compensatory feeding hypothesis, which states that nectarivorous birds adjust their food intake and defend a constant sugar intake over a wide range of nectar concentrations (Martínez del Rio et al. 2001). On a very dilute diet (2.5% w/w), however, sunbirds were not able to increase their food intake sufficiently and had a lower daily sugar intake than on more concentrated diets, indicating the influence of physiological constraints. When switched from a very dilute to a more concentrated diet, the birds increased their food intake instantly in an attempt to compensate for the previous low energy intake. In contrast, the response was much slower following a decrease in nectar sugar concentration: birds increased their food intake within 10 min after the diet was changed. The ecological significance of this may be that when wild birds encounter low nectar sugar concentrations, they may first search for flowers that provide nectar with higher energy content before increasing their intake of dilute nectar. How fast nectarivorous birds respond to a change in dietary sugar concentration has not been determined before, except for one preliminary study in which a single rufous hummingbird (*Selasphorus rufus*) responded to an increase in concentration faster than to a decrease (Gass 1978).

When whitebellied sunbirds and brown honeyeaters were exposed to a fasting period, food intake and energy accumulation were accelerated following the fast. However, compensation for the loss in foraging time was incomplete, as the birds had a lower daily sugar intake and weighed less in the evening than on a day with uninterrupted feeding. During acute exposure to low ambient temperature, which increases energy requirements for thermoregulation, all three nectarivore species increased their food and thus energy intake. Despite this increased food consumption, however, the birds lost more body mass in the cold than at the highest temperature.

It is therefore concluded that the intake response of whitebellied and amethyst sunbirds and brown honeyeaters is shaped by both compensatory feeding and physiological constraint. As discussed in the individual chapters, limitations to nectar ingestion (Bednekoff and Houston 1994; Mbatha et al. 2002), digestion (McWhorter and Martínez del Rio 2000; McWhorter et al. 2006) and osmoregulatory processes (Fleming and Nicolson 2003; Hartman Bakken and Sabat 2006) may result in energy deficits. To overcome digestive constraints, avian nectarivores may show physiological adjustments that lead to a higher rate of digestion. Brown honeyeaters in my study were able to increase their food intake after only a few days of exposure to high feeding rates, which suggests that physiological adjustments had taken place. These may consist of upregulation of digestive processes or an increase in intestine size, and thus increased amounts of nutrient transporters and digestive enzymes (McWilliams and Karasov 2001; Karasov and McWilliams 2005). These physiological changes occur in a wide variety of animals and may occur rapidly (Starck 1999).

In terms of food ingestion, my study showed that the rate at which nectar can be licked may constrain ingestion at high nectar concentrations. As diet concentration increased, lick durations of sunbirds increased, and licking frequency and food consumption per lick decreased. High nectar concentrations impede the ingestion of the solution, thus reducing energy intake rates. Consequently, birds and other nectarivorous animals face a trade-off between feeding on concentrated nectar, which provides more energy per unit volume than dilute nectar, and feeding on nectar that is dilute enough for optimal ingestion (Nicolson 2007). The effect of nectar concentration on the licking behaviour of avian nectarivores was investigated in early studies (e.g. Hainsworth 1973; Collins et al. 1980) but improved techniques allow for more accurate results in the present study. Future studies should include independent manipulation of the concentration and viscosity of sugar solutions to investigate which fluid characteristic affects the licking behaviour of the birds.

When looking at the feeding patterns of sunbirds, it was apparent throughout this study that nectar intake rate is mainly adjusted by increasing feeding frequency and not meal size. However, sunbirds do not seem to be constrained by the amount of food that they can ingest and process at once, as starved individuals that were previously exposed to a very dilute diet or deprived of food were able to ingest larger meals. So why do sunbirds not adjust their food intake rate by changing meal size? A large meal results in an increase in body mass, which is associated with higher energetic costs for flight (DeBenedictis et al. 1978). The adjustment of nectar intake via feeding frequency rather than meal size may therefore be interpreted as optimal feeding behaviour based on energetic costs and benefits. The hovering capacity of ruby-throated hummingbirds (*Archilochus colubris*) was reduced when body mass was increased (Chai and Dudley 1999; Chai et al. 1999). A reduction in body mass, on the other hand, saves energy as shown in European starlings (*Sturnus vulgaris*) (Bautista et al. 1998). It must be noted however, that the metabolic costs of short flights of sunbirds are relatively inexpensive compared to those of starlings (Hambly et al. 2004), and the hovering flight of hummingbirds. Alternatively, the increase in feeding frequency may also represent the natural foraging behaviour of sunbirds. The birds visit many different flowers to meet their energy requirements, as the volume of nectar per flower is limited. Consequently, sunbirds ingest frequent small meals instead of few large meals.

Comparing the responses of sunbirds and honeyeaters to energy challenges, it is concluded that they showed convergence in their behavioural and physiological responses. Both sunbirds and honeyeaters compensated for a period of food deprivation, low food quality and increased energy requirements by increasing their food intake. However, species of both lineages of nectarivorous birds appear to face constraints to food intake and processing, since their compensatory feeding response was incomplete. Although sunbirds and honeyeaters are unrelated and geographically isolated (except for the far northeast of Australia, New Guinea and nearby islands), the adaptation to a similar nectar-feeding lifestyle and comparable environmental conditions probably has resulted in similar behaviour and physiology. In my study, major phylogenetic effects on the responses to energy challenges were not apparent.

## *Individual variation*

The feeding patterns of sunbirds were recorded on a novel short-term scale using a photodetection system. The detailed measurements highlighted unexpectedly large variation in feeding behaviour between individual birds. Meal sizes, feeding frequencies and licking behaviour differed greatly between birds, but the behaviour of individual birds was very consistent. For instance, if one bird took many short meals, while a second bird took few long meals, they would demonstrate the same characteristic feeding pattern on different days and under various experimental conditions. However, the data are insufficient to statistically demonstrate this repeatability since birds were not exposed to the same experimental conditions several times to avoid training effects and physiological adjustments.

Between-individual differences have already been observed on a coarse time scale, when the energy intake of whitebellied sunbirds was measured in an earlier study (Nicolson and Fleming 2003). Jackson et al. (1998) found variations in sugar type preferences between individual southern double-collared sunbirds (*Cinnyris chalybeus*) and Cape sugarbirds (*Promerops cafer*). The responses of rufous hummingbirds (*Selasphorus rufus*) and European starlings exposed to energetically challenging conditions differed between individuals (Tooze and Gass 1985; Bautista et al. 1998). Individual differences may conceal trends in the behavioural response of nectarivorous birds to experimental conditions. Earlier studies on avian nectarivores sometimes tested only one individual (Carpenter and Hixon 1988; Roberts 1995) or very few (e.g. Wolf and Hainsworth 1977; Hainsworth et al. 1981; Tamm and Gass 1986; Roberts 1996). Researchers should increase their sample size in order to be able to draw conclusions that are representative of the entire species.

However, the investigation of between-individual differences has the potential to contribute to physiological and ecological studies. In earlier ecophysiological studies, the uniqueness of an individual often received limited attention, with researchers rather attempting to describe the physiological and behavioural response of the average animal of an experimental group (Bennett 1987b). If researchers find repeatable individual traits of animals that are in good physical condition, and experimental errors have been ruled out, the causes and consequences of individual variation can be analyzed (Bennett 1987b). What are the physiological and morphological factors that underlie individual differences? Is the performance of an animal correlated with differential survivorship under natural conditions and what are the evolutionary consequences?

## *The integration of physiology and behaviour*

The exposure of avian nectarivores to energetically challenging conditions was short-term in my study. Since the results indicate physiological limitations, it remains a question how sunbirds and honeyeaters cope with longer-term unfavourable environmental conditions. The birds are likely to adjust their behaviour, such as reducing their flight activity to save energy. Behavioural adjustments probably occurred, especially when birds maintained their body mass despite an insufficient energy intake. During cold exposure, for instance, sunbirds and honeyeaters were observed to be less active and ptiloerected their feathers to conserve body heat. Birds may also have reduced their body temperature to reduce the amount of energy needed to maintain body heat (McKechnie and Lovegrove 2002).

This emphasizes that physiological and behavioural responses are linked and can not be investigated in isolation. In addition to an increased food intake, animals may conserve energy by reducing their activity and metabolic rate when they need to maintain energy balance under energetically stressful conditions. Behavioural adjustments have been observed in earlier studies in which birds were exposed to energetically challenging conditions (e.g. Dall and Witter 1998; Fernández et al. 2002). If the perches are connected to electronic balances interfaced to a computer, and the bird can not rest anywhere else, activity can be calculated from missing and unstable body mass recordings. In Chapter 2, the flights between the two perches in the cage were used as a measure of flight activity. The daily number of flights did not differ when birds were fed different nectar concentrations. On the dilute diet treatment however, sunbirds were found to reduce the number of flights in the afternoon, which indicates activity adjustments to conserve energy.

## *Directions for future research*

Several new questions and directions for future experiments have arisen during the course of my research. Firstly, a more detailed study on the activity and time budget of the birds may enhance our understanding of behavioural adjustments during energy challenges.

Under laboratory conditions, the activity of birds can easily be quantified by connecting all available perches to electronic balances and recording body mass. Alternatively, the activity of a caged bird can be analyzed using video recordings or motion detectors. Future studies could quantify flight activity, along with other activities such as hopping, jumping from perch to perch and grooming. From these records, researchers can calculate what proportions of the day the bird is active or resting. The recording of the time spent feeding, using a photodectection system or motion detectors next to the feeder, completes the time budget data.

These time budget data can then be used to calculate the energy requirements of the birds. The basal metabolic rate can be determined by measuring the oxygen consumption of resting birds in a metabolic chamber. The energetic costs for flights can be calculated from the body mass, time spent flying and the unit flight cost for the particular species (Wolf et al. 1975; Collins and Morellini 1979). This would result in a complete daily energy budget, which could then be compared between control and energetically challenging conditions. Such calculations of energy budgets have been done for hummingbirds exposed to different ambient temperatures and diet qualities (López-Calleja and Bozinovic 2003). In a future experiment, one could also show whether energetic costs for flight increase with increasing body mass in sunbirds, and whether the size of a large meal affects flight costs. If so, this would explain why sunbirds in the present study adjusted their food intake via feeding frequency rather than meal size.

In addition to activity measurements and energy budgets, the body temperature of avian nectarivores during energy challenges should be recorded. This was not incorporated in my study, as regular measurements with a thermocouple involve catching of the birds and this would interrupt their normal feeding patterns. Alternatively, iButtons could be implanted and body temperatures monitored using telemetry. Various bird species reduce their body temperature (leading to either shallow hypothermia or torpor), which in turn leads to a reduction in metabolic rate (McKechnie and Lovegrove 2002). For example, southern double-collared sunbirds and malachite sunbirds (*Nectarinia famosa*) reduce their body temperature with decreasing ambient temperature (Leon and

Nicolson 1997; Downs and Brown 2002). Body temperature measurements in future experiments involving energy challenges would enable us to detect possible facultative hypothermic responses, and would complete the investigations of physiological and behavioural responses of avian nectarivores to energy challenges.

A different aspect, that could not be resolved in the present study, is the physiological constraint to food intake. Body mass losses during individual energy challenges indicate that the birds were not able to increase their food intake sufficiently to maintain body mass. Which factors are limiting? Sucrose hydrolysis rates may constrain food intake in avian nectarivores (McWhorter and Martínez del Rio 2000). If all experiments were to be repeated on hexose diets and the birds maintained their body mass, then sucrose hydrolysis is likely to be the constraining factor. However, repetition of parts of the experiment on hexose diets also revealed limitations to food intake (Chapters 4 and 5). This limitation on hexose diets is in agreement with other studies, and suggests constraints to hexose absorption rates (Levey and Martínez del Rio 1999; Fleming et al. 2004). Furthermore, birds may be challenged by the handling of large amounts of ingested fluid (Fleming and Nicolson 2003) and by electrolyte and metabolite losses in the excreta (Lotz and Nicolson 1999; Lotz and Martínez del Rio 2004). In future experiments, the nectar diets could be supplemented with electrolytes to investigate whether this allows the birds to maintain energy balance during energetically stressful conditions.

Lastly, the knowledge on feeding behaviour and energy balance of avian nectarivores gained from experiments on captive birds should be confirmed by studies on free-living individuals. Captivity is associated with reduced activity levels compared to wild individuals and affects the basal metabolic rate of birds (Warkentin and West 1990; Nudds and Bryant 2001). The present study showed that housing conditions affect the activity of avian nectarivores and their energy requirements. When kept in isolation in a Perspex cage, individual whitebellied sunbirds were less active than when they were able to see or hear the other birds (A. Köhler, pers. observation). Consequently, their sugar intake was lower in the Perspex cage (Chapter 1: 220–240 mg•24 $h^{-1}$•g body $mass^{-1}$ at

20˚C on 0.32–1.35 M sucrose diets) than when birds were kept in separate cages standing next to each other (Chapter 4: 280–300 mg•24 $h^{-1}$•g body $mass^{-1}$ at 15 and 25˚C on 0.25–1 M sucrose diets). Furthermore, it is possible that the pure sucrose diet (with added Ensure®), which was available *ad libitum* during captivity, leads to upregulation of intestinal sucrase activity, increased food intake and body mass gain. Since the sunbirds were kept together in a large outdoor aviary between experiments, with exposure to natural vegetation and weather conditions, they were in good physical condition and did not gain mass during captivity. They were kept in captivity for up to one year, after which they were released and new individuals were captured. Honeyeaters were captured and kept in the laboratory for six weeks prior to experiments, and were kept for a total of three months. Their diet consisted of honeyeater and lorikeet nectar (Wombaroo®) and additional sucrose, and body mass did not change after capture. I am therefore confident that the responses of the sunbirds and honeyeaters used in my study can be extrapolated from captive to wild individuals. However, captivity and the presence of a researcher induce stress in the birds and may affect physiological and behavioural results.

Wild birds can be caught in mist-nets and colour coded rings attached to their legs for recognition during later observations. Alternatively, radio-transmitters can be implanted, so that released birds can be tracked. Many sunbird and honeyeater species are resident, at least seasonally, and can easily be observed foraging on flowers, especially when territorial (Gill and Wolf 1975 and 1977; Frost and Frost 1981). The feeding behaviour, activity and roosting of a single rufous hummingbird was recorded by observing the wild bird (Carpenter and Hixon 1988). In addition, body temperature can be recorded with implanted iButtons. Field metabolic rate and water turnover can be quantified using the doubly labelled water method, as has been done in free-living female orange-breasted sunbirds (*Anthobaphes violacea*) during the incubation period (Williams 1993). Field studies are time-consuming and often difficult to implement, but they are necessary to confirm the responses of avian nectarivores to energy challenges under natural conditions.

# References

Bautista L.M., Tinbergen J., Wiersma P. and Kacelnik A. 1998. Optimal foraging and beyond: how starlings cope with changes in food availability. American Naturalist 152: 543–561.

Bednekoff P.A. and Houston A.I. 1994. Avian daily foraging patterns: effects of digestive constraints and variability. Evolutionary Ecology 8: 36–52.

Bennett A.F. 1987a. The accomplishments of ecological physiology. In: New directions in ecological physiology. M.E. Feder, A.F. Bennett, W.W. Burggren and R.B. Huey (Eds.), Cambridge University Press, Cambridge, UK, pp. 1–8.

Bennett A.F. 1987b. Interindividual variability: an underutilized resource. In: New directions in ecological physiology. M.E. Feder, A.F. Bennett, W.W. Burggren and R.B. Huey (Eds.), Cambridge University Press, Cambridge, UK, pp. 147–166.

Beuchat C.A., Chaplin S.B. and Morton M.L. 1979. Ambient temperature and the daily energetics of two species of hummingbirds, *Calypte anna* and *Selasphorus rufus*. Physiological Zoology 53: 280–295.

Carleton S.A., Hartman Bakken B. and Martínez del Rio C. 2006. Metabolic substrate use and the turnover of endogenous energy reserves in broad-tailed hummingbirds (*Selasphorus platycercus*). Journal of Experimental Biology 209: 2622–2627.

Carpenter F.L. and Hixon M.A. 1988. A new function for torpor: fat conservation in a wild migrant hummingbird. Condor 90: 373–378.

Chai P. and Dudley R. 1999. Maximum flight performance of hummingbirds: capacities, constraints, and trade-offs. American Naturalist 153: 398–411.

Chai P., Altshuler D.L., Stephens D.B. and Dillon M.E. 1999. Maximal horizontal flight performance of hummingbirds: effects of body mass and molt. Physiological and Biochemical Zoology 72: 145–155.

Collins B.G. and Briffa P. 1984. Nocturnal energy expenditure by honeyeaters experiencing food shortage and low environmental temperatures. Comparative Biochemistry and Physiology A 78: 77–81.

Collins B.G. and Morellini P.C. 1979. The influence of nectar concentration and time of day upon energy intake and expenditure by the singing honeyeater, *Meliphaga virescens*. Physiological Zoology 52: 165–175.

Collins B.G., Cary G. and Packard G. 1980. Energy assimilation, expenditure and storage by the brown honeyeater, *Lichmera indistincta*. Journal of Comparative Physiology B 137: 157–163.

Dall S.R.X. and Witter M.S. 1998. Feeding interruptions, diurnal mass changes and daily routines of behaviour in the zebra finch. Animal Behaviour 55: 715–725.

DeBenedictis P.A., Gill F.B., Hainsworth F.R., Pyke G.H. and Wolf L.L. 1978. Optimal meal size in hummingbirds. American Naturalist 112: 301–316.

Downs C.T. and Brown M. 2002. Nocturnal heterothermy and torpor in the Malachite sunbird (*Nectarinia famosa*). Auk 119: 251–260.

Fernández M.J., López-Calleja M.V. and Bozinovic F. 2002. Interplay between the energetics of foraging and thermoregulatory costs in the green-backed firecrown hummingbird *Sephanoides sephanoides*. Journal of Zoology 258: 319–326.

Fleming P.A. and Nicolson S.W. 2003. Osmoregulation in an avian nectarivore, the whitebellied sunbird *Nectarinia talatala*: response to extremes of diet concentration. Journal of Experimental Biology 206: 1845–1854.

Fleming P.A., Hartman-Bakken B., Lotz C.N. and Nicolson S.W. 2004. Concentration and temperature effects on sugar intake and preferences in a sunbird and a hummingbird. Functional Ecology 18: 223–232.

Frost S.K. and Frost P.G.H. 1981. Sunbird pollination of *Strelitzia nicolai*. Oecologia 49: 379–384.

Gass C.L. 1978. Experimental studies of foraging in complex laboratory environments. American Zoologist 18: 729–738.

Gass C.L., Romich M.T. and Suarez R.K. 1999. Energetics of hummingbird foraging at low ambient temperature. Canadian Journal of Zoology 77: 314–320.

Gill F.B. and Wolf L.L. 1975. Economics of feeding territoriality in the golden-winged sunbird. Ecology 56: 333–345.

Gill F.B. and Wolf L.L. 1977. Nonrandom foraging by sunbirds in a patchy environment. Ecology 58: 1284–1296.

Hainsworth F.R. 1973. On the tongue of a hummingbird: its role in the rate and energetics of feeding. Comparative Biochemistry and Physiology A 46: 65–78.

Hainsworth F.R., Tardiff M.F. and Wolf L.L. 1981. Proportional control for daily energy regulation in hummingbirds. Physiological Zoology 54: 452–462.

Hambly C., Pinshow B., Wiersma P., Verhulst S., Piertney S.B., Harper E.J. and Speakman J.R. 2004. Comparison of the cost of short flights in a nectarivorous and a non-nectarivorous bird. Journal of Experimental Biology 207: 3959–3968.

Hartman Bakken B. and Sabat P. 2006. Gastrointestinal and renal responses to water intake in the green-backed firecrown (*Sephanoides sephanoides*), a South American hummingbird. American Journal of Physiology (Regulatory, Integrative and Comparative Physiology) 291: R830–R836.

Jackson S., Nicolson S.W. and Lotz C.N. 1998. Sugar preferences and “side bias” in Cape sugarbirds and lesser double-collared sunbirds. Auk 115: 156–165.

Karasov W.H. and Martínez del Rio C. 2007. Physiological ecology: how animals process energy, nutrients, and toxins. Princeton University Press, New Jersey, USA.

Karasov W.H. and McWilliams S.R. 2005. Digestive constraints in mammalian and avian ecology. In: Physiological and ecological adaptations to feeding in vertebrates. J.M. Starck and T. Wang (Eds.), Science Publishers, Enfield, New Hampshire, USA, pp. 87–112.

Leon B. and Nicolson S.W. 1997. Metabolic rate and body temperature of an African sunbird, *Nectarinia chalybea*: daily rhythm and the effect of ambient temperature. South African Journal of Zoology 32: 31–36.

Leseigneur C.D.C., Verburgt L. and Nicolson S.W. 2007. Whitebellied sunbirds (*Nectarinia talatala*, Nectariniidae) do not prefer artificial nectar containing amino acids. Journal of Comparative Physiology B 177: 679–685.

Levey D.J. and Martínez del Rio C. 1999. Test, rejection, and reformulation of a chemical reactor-based model of gut function in a fruit-eating bird. Physiological and Biochemical Zoology 72: 369–383.

Liknes E.T. and Swanson D.L. 1996. Seasonal variation in cold tolerance, basal metabolic rate, and maximal capacity for thermogenesis in White-breasted Nuthatches *Sitta carolinensis* and Downy Woodpeckers *Picoides pubescens*, two unrelated arboreal temperate residents. Journal of Avian Biology 27: 279–288.

López-Calleja M.V. and Bozinovic F. 2003. Dynamic energy and time budgets in hummingbirds: a study in *Sephanoides sephanoides*. Comparative Biochemistry and Physiology A 134: 283–295.

Lott D.F. 1991. Bronzy sunbirds tolerate intrusion on foraging territories by female golden-winged sunbirds that perform “begging” display. Journal of Field Ornithology 62: 492–496.

Lotz C.N. 1999. Energy and water balance in the lesser double-collared sunbird, *Nectarinia chalybea*. PhD thesis, University of Cape Town, South Africa.

Lotz C.N. and Martínez del Rio C. 2004. The ability of rufous hummingbirds *Selasphorus rufus* to dilute and concentrate urine. Journal of Avian Biology 35: 54–62.

Lotz C.N. and Nicolson S.W. 1999. Energy and water balance in the lesser double-collared sunbird (*Nectarinia chalybea*) feeding on different nectar concentrations. Journal of Comparative Physiology B 169: 200–206.

Martínez del Rio C., Schondube J.E., McWhorter T.J. and Herrera L.G. 2001. Intake responses in nectar feeding birds: digestive and metabolic causes, osmoregulatory consequences, and coevolutionary effects. American Zoologist 41: 902–915.

Mbatha K., Downs C.T. and Penning M. 2002. Nectar passage and gut morphology in the Malachite Sunbird and the Black-capped Lory: implications for feeding in nectarivores. Ostrich 73: 138–142.

McKechnie A.E. and Lovegrove B.G. 2002. Avian facultative hypothermic responses: a review. Condor 104: 705–724.

McWhorter T.J. and Martínez del Rio C. 2000. Does gut function limit hummingbird food intake? Physiological and Biochemical Zoology 73: 313–324.

McWhorter T.J., Hartman Bakken B., Karasov W.H. and Martínez del Rio C. 2006. Hummingbirds rely on both paracellular and carrier-mediated intestinal glucose absorption to fuel high metabolism. Biology Letters 2: 131–134.

McWilliams S.R. and Karasov W.H. 2001. Phenotypic flexibility in digestive system structure and function in migratory birds and its ecological significance. Comparative Biochemistry and Physiology A 128: 579–593.

Mendonça L.B. and Dos Anjos L. 2006. Feeding behavior of hummingbirds and perching birds on *Erythrina speciosa* Andrews (Fabaceae) flowers in an urban area, Londrina, Paraná, Brazil. Revista Brasileira de Zoologia 23: 42–49.

Nicolson S.W. 2007. Nectar consumers. In: Nectaries and nectar. S.W. Nicolson, M. Nepi and E. Pacini (Eds.), Springer, Dordrecht, Netherlands, pp. 289–342.

Nicolson S.W. and Fleming P.A. 2003. Energy balance in the Whitebellied Sunbird *Nectarinia talatala*: constraints on compensatory feeding, and consumption of supplementary water. Functional Ecology 17: 3–9.

Nicolson S.W., Hoffmann D. and Fleming P.A. 2005. Short-term energy regulation in nectar-feeding birds: the response of Whitebellied Sunbirds (*Nectarinia talatala*) to a midday fast. Functional Ecology 19: 988–994.

Nudds R.L. and Bryant D.M. 2001. Exercise training lowers the resting metabolic rate of Zebra Finches, *Taeniopygia guttata*. Functional Ecology 15: 458–464.

Pyke G.H. 1980. The foraging behaviour of Australian honeyeaters: a review and some comparisons with hummingbirds. Australian Journal of Zoology 5: 343–369.

Roberts W.M. 1995. Hummingbird licking behaviour and the energetics of nectar feeding. Auk 112: 456–463.

Roberts W.M. 1996. Hummingbirds' nectar concentration preferences at low volume: the importance of time scale. Animal Behaviour 52: 361–370.

Starck J.M. 1999. Structural flexibility of the gastro-intestinal tract of vertebrates – implications for evolutionary morphology. Zoologischer Anzeiger 238: 87–101.

Tamm S. and Gass C.L. 1986. Energy intake rates and nectar concentration preferences by hummingbirds. Oecologia 70: 20–23.

Tooze Z.J. and Gass C.L. 1985. Responses of rufous hummingbirds to midday fasts. Canadian Journal of Zoology 63: 2249–2253.

Vaughton G. 1990. Seasonal variation in honeyeater foraging behaviour, inflorescence abundance and fruit set in *Banksia spinulosa* (Proteaceae). Australian Ecology 15: 109–116.

Warkentin I.G. and West N.H. 1990. Impact of long-term captivity on basal metabolism in birds. Comparative Biochemistry and Physiology A 96: 379–381.

Welch K.C., Jr. and Suarez R.K. 2007. Oxidation rate and turnover of ingested sugar in hovering Anna's (*Calypte anna*) and rufous (*Selasphorus rufus*) hummingbirds. Journal of Experimental Biology 210: 2154–2162.

Wikelski M. and Cooke S.J. 2006. Conservation physiology. Trends in Ecology and Evolution 21: 38–46.

Williams J.B. 1993. Energetics of incubation in free-living orange-breasted sunbirds in South Africa. Condor 95: 115–126.

Wolf L.L. and Hainsworth F.R. 1977. Temporal patterning of feeding by hummingbirds. Animal Behaviour 25: 976–989.

Wolf L.L., Hainsworth F.R. and Gill F.B. 1975. Foraging efficiencies and time budgets of nectar-feeding birds. Ecology 56: 117–128.

# APPENDIX

## *The model of sucrose hydrolysis*

The chemical reactor model of digestive capacity was developed by McWhorter and Martínez del Rio (2000) and was extended by Martínez del Rio et al. (2001). In this approach, it is assumed that the intestine of avian nectarivores functions as a plug-flow chemical reactor (Penry and Jumars 1987).

<u>The assumptions of the model</u>

1) Digesta flows unidirectionally (Jumars and Martínez del Rio 1999)
2) Rate of sucrose hydrolysis in the gut ($-r_s$) follows simple Michaelis-Menten kinetics

Therefore

$$-r_s = S_{max} \bullet C_s \bullet (K_m + C_s)^{-1} \qquad (1)$$

| | |
|---|---|
| $S_{max}$ | Rate of hydrolysis along the gut [$\mu$mol•min$^{-1}$•$\mu$l$^{-1}$] |
| $K_m$ | Michaelis constant of sucrase [$\mu$mol•$\mu$l$^{-1}$] |
| $C_s$ | Sucrose concentration [$\mu$mol•$\mu$l$^{-1}$] down the intestine or over time (Jumars and Martínez del Rio 1999) |

The integration of Equation (1) yields the throughput time ($\tau$) that is required to reduce the initial sucrose concentration ($C_{s0}$) to a given final sucrose concentration ($C_{sf}$) which is based on the assimilation efficiency:

$$\tau = S_{max}^{-1} \bullet [K_m \bullet \ln(C_{s0} \bullet C_{sf}^{-1}) + (C_{s0} - C_{sf})] \qquad (2)$$

If the volume of gut contents (G) and the throughput time ($\tau$) are known, intake rate ($\upsilon_0$) can be estimated for plug-flow reactors:

$$\upsilon_0 = G \bullet \tau^{-1} \qquad (3)$$

| | |
|---|---|
| $\upsilon_0$ | Intake rate [$\mu$l•min$^{-1}$] |
| G | Volume of gut contents [$\mu$l] |
| $\tau$ | Throughput time [min] |

## References

Jumars P.A. and Martínez del Rio C. 1999. The tau of continuous feeding on simple foods. Physiological and Biochemical Zoology 72: 633–641.

Martínez del Rio C., Schondube J.E., McWhorter T.J. and Herrera L.G. 2001. Intake responses in nectar feeding birds: digestive and metabolic causes, osmoregulatory consequences, and coevolutionary effects. American Zoologist 41: 902–915.

McWhorter T.J. and Martínez del Rio C. 2000. Does gut function limit hummingbird food intake? Physiological and Biochemical Zoology 73: 313–324.

Penry D.L. and Jumars P.A. 1987. Modeling animal guts as chemical reactors. American Naturalist 129: 69–96.

## *Photographs of the experimental equipment*

**Fig. 1.** The experimental equipment used in Chapters 1–3. The cage was constructed from Perspex with one-way mirrors on two sides to minimize disturbance of the bird. An infrared photodetection system was mounted inside the cage on either side of the feeding aperture to record feeding events. The feeder was mounted on an electronic balance and the two perches were also connected to electronic balances. A tray with liquid paraffin was placed on the balance beneath the feeder to collect dripping fluid. The photodetectors and all balances were interfaced to a computer and feeding events, food intake and body mass of the bird were recorded continuously, using custom-designed software (L. Verburgt).

**Fig. 2.** The Perspex cages used for the fasting experiment in brown honeyeaters (Chapter 3). One side of each cage consisted of a one-way mirror allowing for observation of the bird with minimal disturbance. The only perch available in the cage was suspended from a balance which was placed on top of the cage. Body mass was recorded manually when the bird was resting on the perch. The feeder was attached outside the cage, allowing for weighing of the feeder without disturbance of the bird.

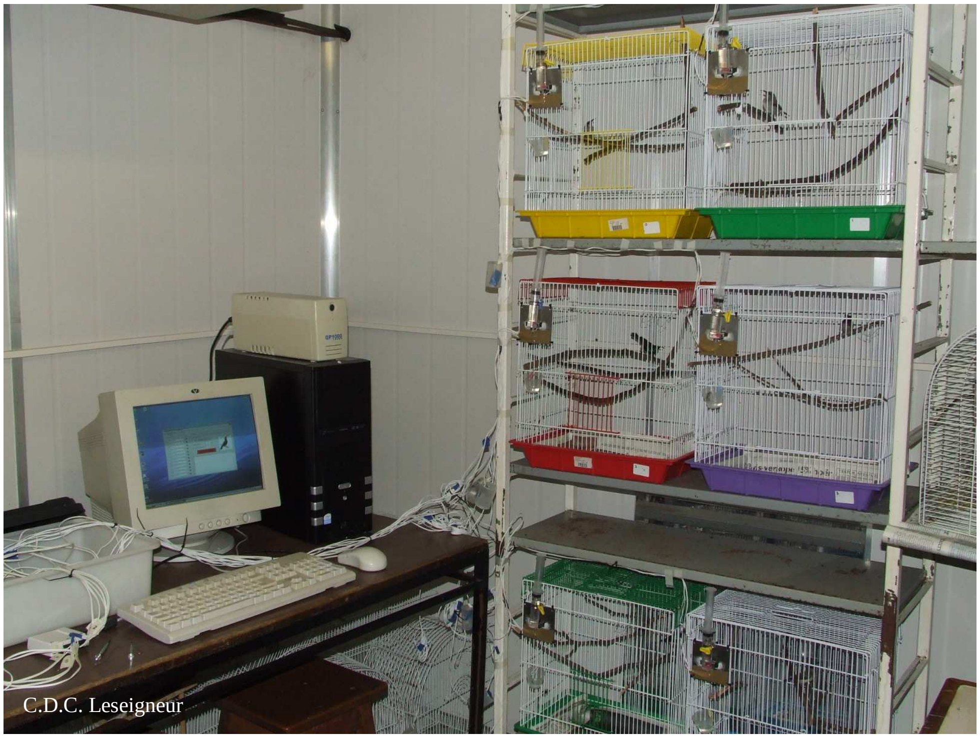

C.D.C. Leseigneur

**Fig. 3.** The experimental equipment used in Chapter 4. Feeding behaviour of nine sunbirds was recorded simultaneously by photodetection systems interfaced to a computer. Custom-designed software (L. Verburgt) was used.

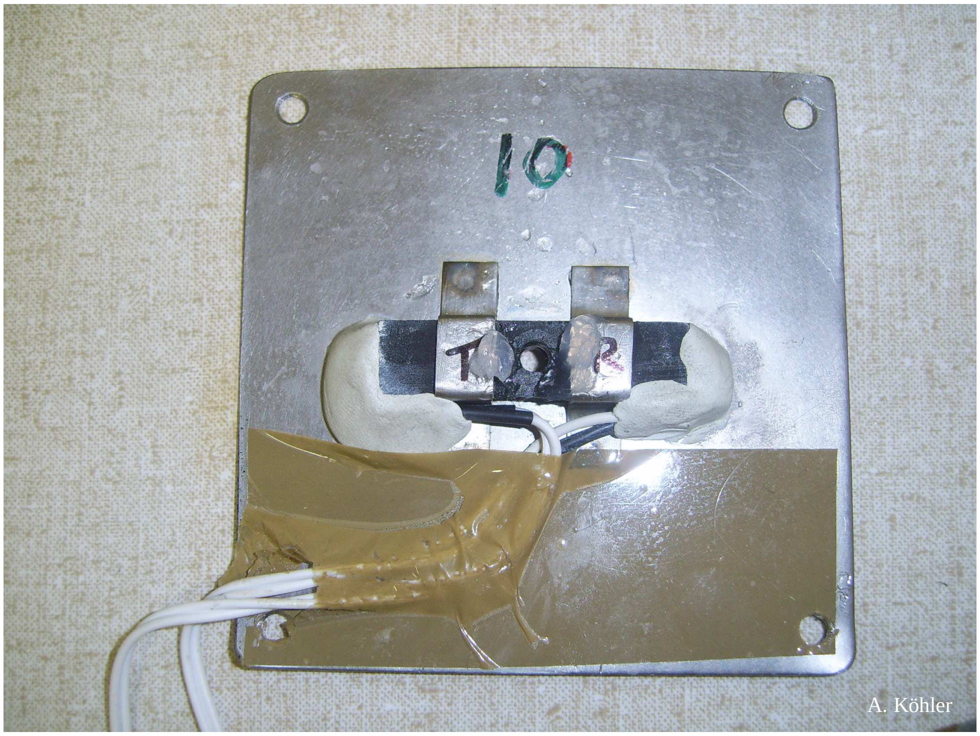


**Fig. 4.** The photodetection system used in Chapter 4. Transmitter (T) and receiver (R) were mounted on either side of the 3 mm feeding aperture, between a metal plate and the feeder (not shown). Photodetectors were surrounded by a plastic cover to shield them from light and protect them from contamination. The light beam was interrupted by the insertion of the bill into the hole for feeding, recorded as a feeding event.

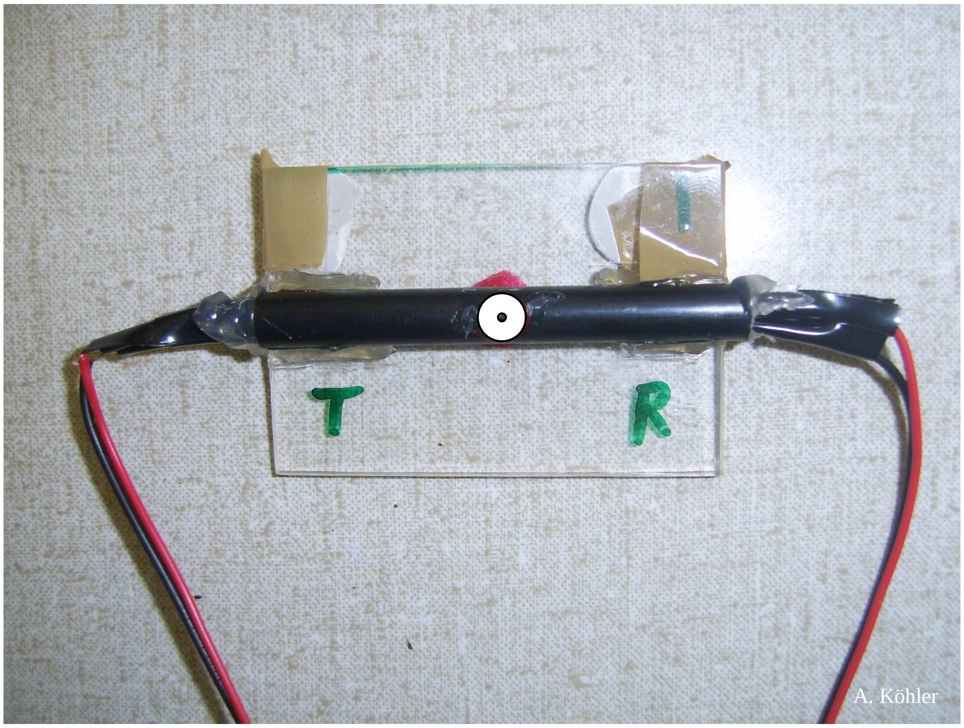
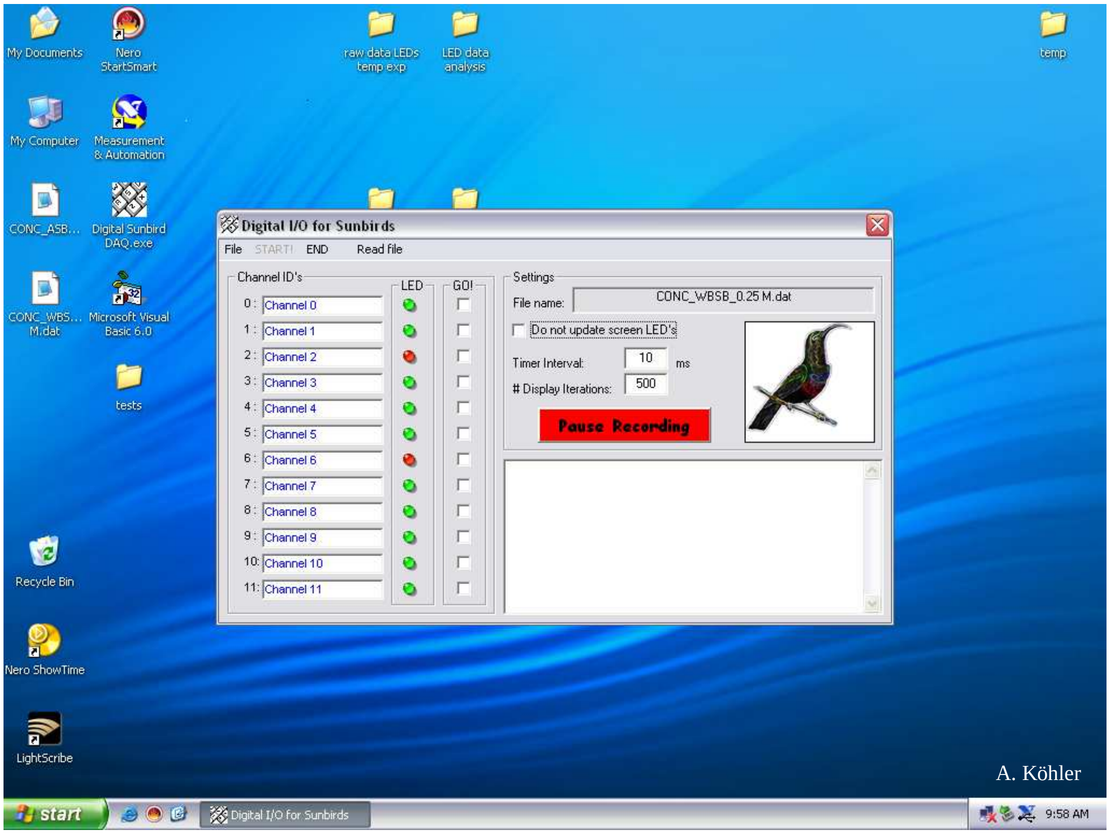


**Fig. 5.** The feeding aperture used to record licking behaviour of sunbirds (Chapter 6). A black pipe containing photodetectors was mounted between the feeder (not shown here) and a 3 mm thick Perspex plate. The birds extended their tongues through a 1.2 mm hole in the Perspex and the pipe, and this movement interrupted the light beam of the infrared light source. The photodetection system was interfaced to a computer, and tongue licks were recorded continuously.



Channel ID's
0 : Channel 0 ☐
1 : Channel 1 ☐
2 : Channel 2 ☐
3 : Channel 3 ☐
4 : Channel 4 ☐
5 : Channel 5 ☐
6 : Channel 6 ☐
7 : Channel 7 ☐
8 : Channel 8 ☐
9 : Channel 9 ☐
10: Channel 10 ☐
11: Channel 11 ☐

File name: CONC_WBSB_0.25 M.dat
☐ Do not update screen LED's
Timer Interval: 10 ms
# Display Iterations: 500

A. Köhler

**Fig. 6.** Image of the custom-designed software developed by Luke Verburgt, which was used to record licking behaviour of up to 12 birds simultaneously (Chapter 6). Licking events of individual birds were indicated by colour changes next to the channel. The recording could be started separately for each bird. The time interval for recordings could be set manually; data were recorded every 1 ms. Recorded data could be displayed on the screen.