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 (%•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•24 h^{-1}•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•24 h^{-1}•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:

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

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.

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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

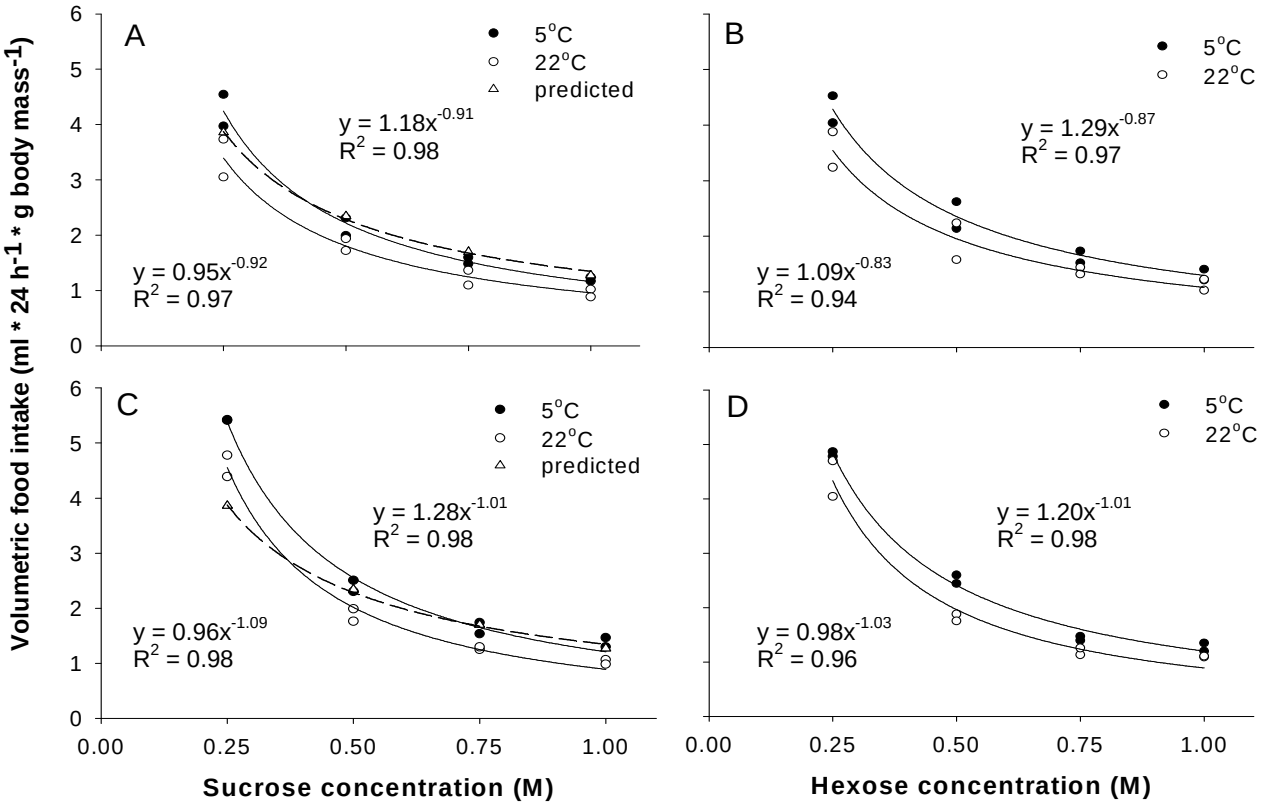

Figure 1.

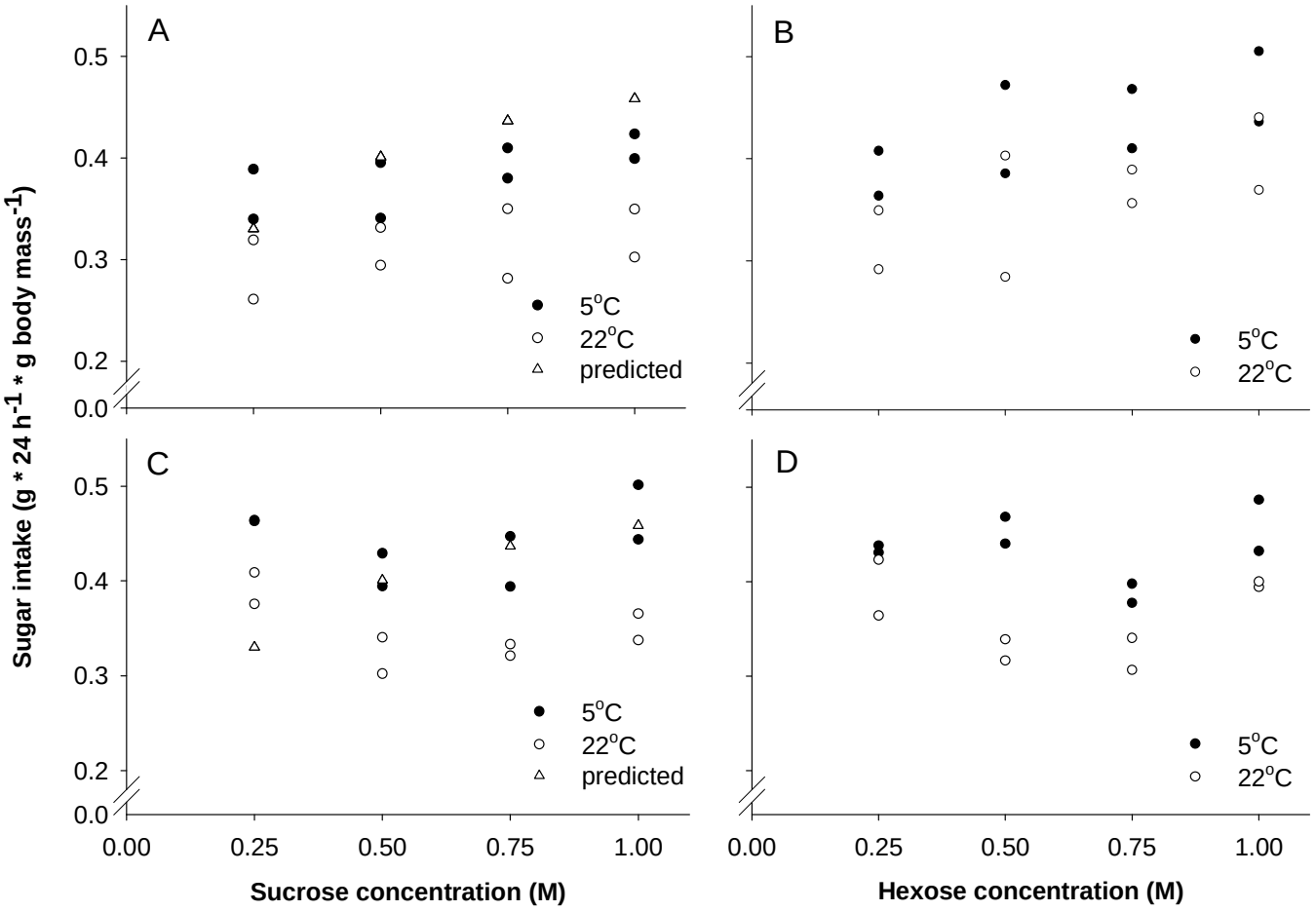
A
B
C
D
Sugar intake (g * 24 h-1 * g body mass-1)
0.0
0.2
0.3
0.4
0.5
0.00
0.25
0.50
0.75
1.00
5°C
22°C
predicted
Sucrose concentration (M)
Hexose concentration (M)

Figure 2.

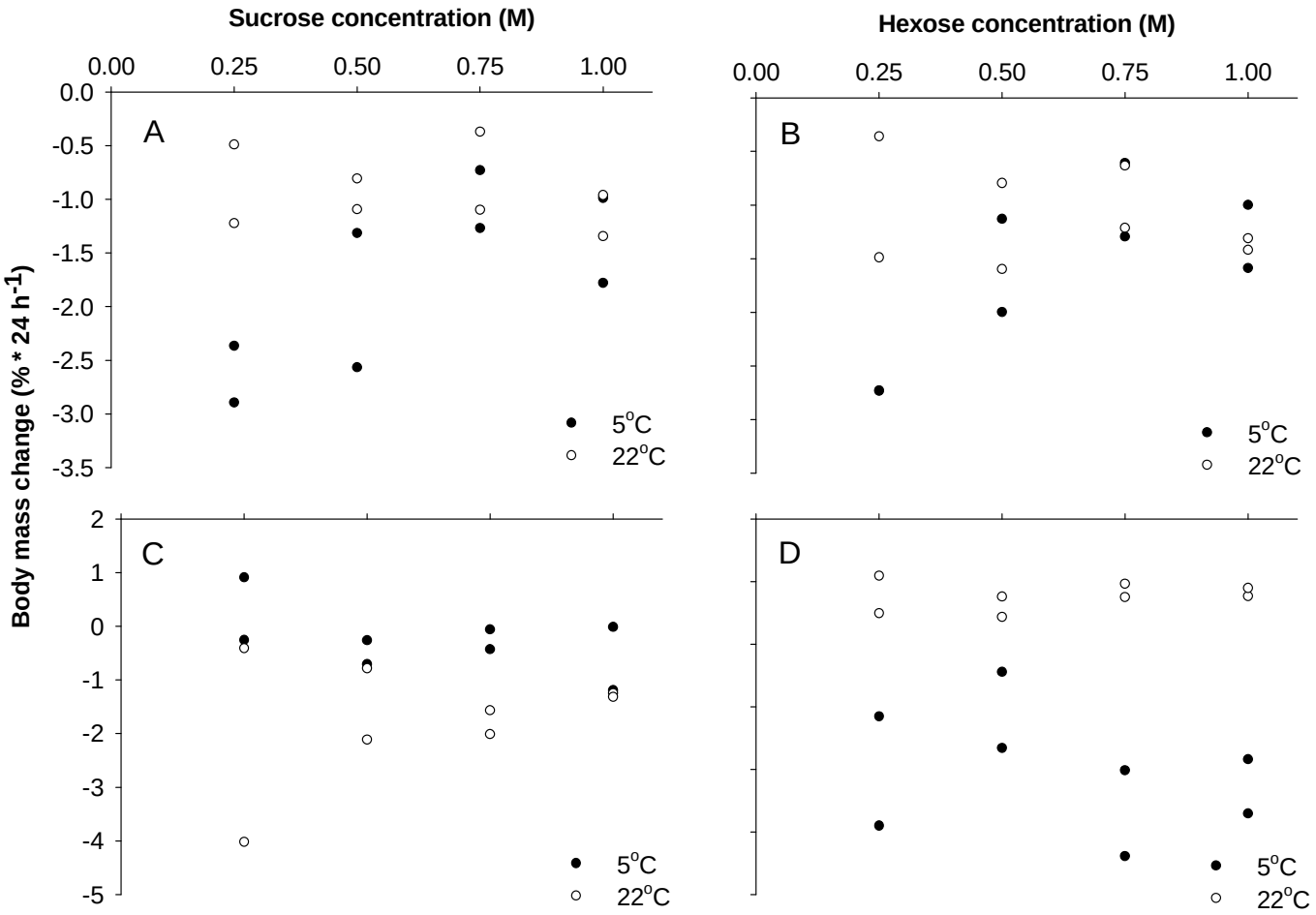
Sucrose concentration (M)
Hexose concentration (M)
Body mass change (% * 24 h-1)
0.00
0.25
0.50
0.75
1.00
0.0
-0.5
-1.0
-1.5
-2.0
-2.5
-3.0
-3.5
2
1
0
-1
-2
-3
-4
-5
A
B
C
D
5°C
22°C

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 $\mu l \cdot s^{-1}$ of 0.5–1.0 M sucrose solutions, and three honeyeater species, weighing 10–25 g, ingested 20–74 $\mu l \cdot s^{-1}$ 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 $\mu l \cdot s^{-1}$ 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 $licks \cdot s^{-1}$ (Hainsworth 1973; Schlamowitz et al. 1976). Brown (*Lichmera indistincta*) and singing (*Meliphaga virescens*) honeyeaters licked at a speed of 8–10 $licks \cdot s^{-1}$ (Collins and Morellini 1979; Collins et al. 1980). When licking frequencies were filmed at higher resolutions, maximum licking rates above 17 $licks \cdot s^{-1}$ 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 ($g \bullet 3\ h^{-1}$). 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 min•3 h^{-1}) than whitebellied sunbirds (2.07 ± 0.39 min•3 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.

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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

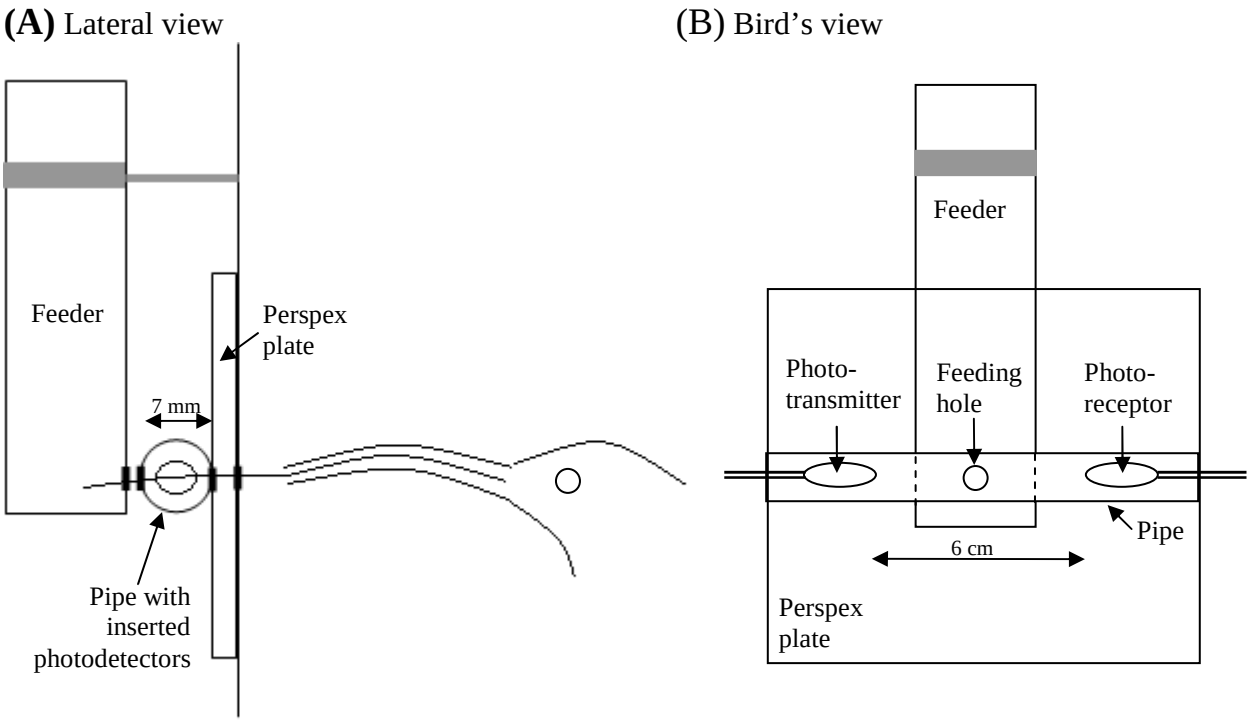

Figure 1.

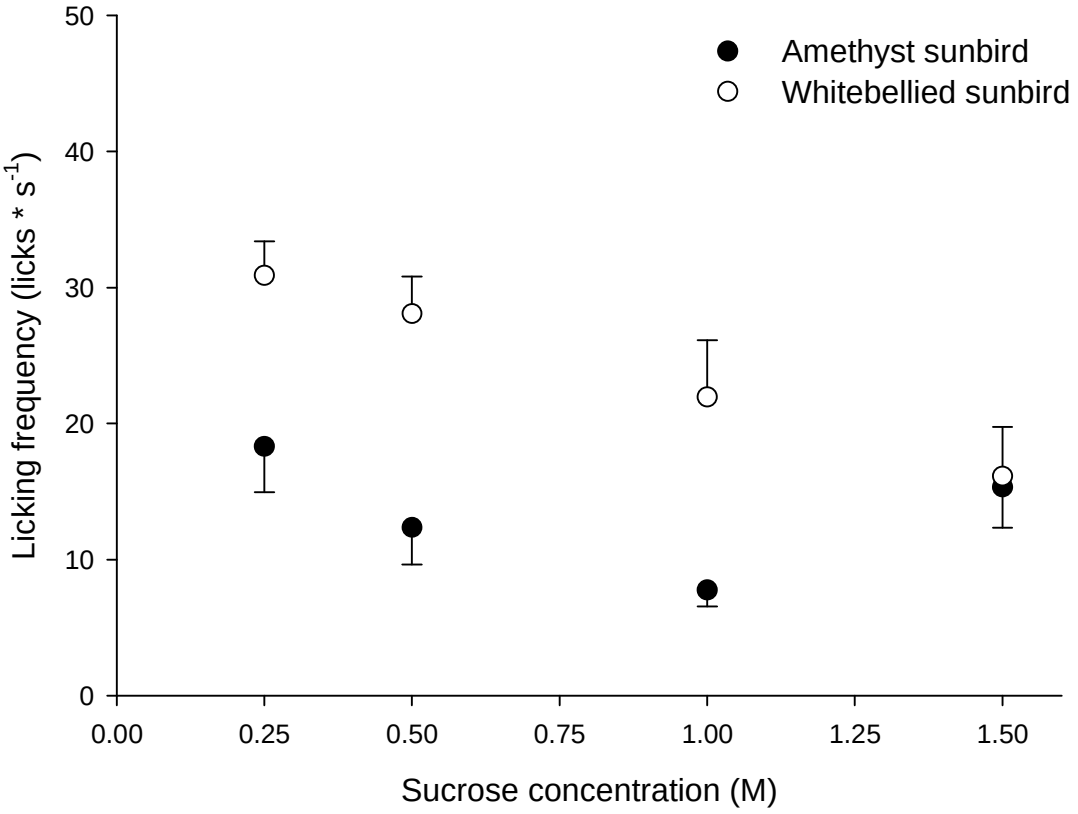
Amethyst sunbird
Whitebellied sunbird
Licking frequency (licks * s-1)
Sucrose concentration (M)
0
10
20
30
40
50
0.00
0.25
0.50
0.75
1.00
1.25
1.50

Figure 2.

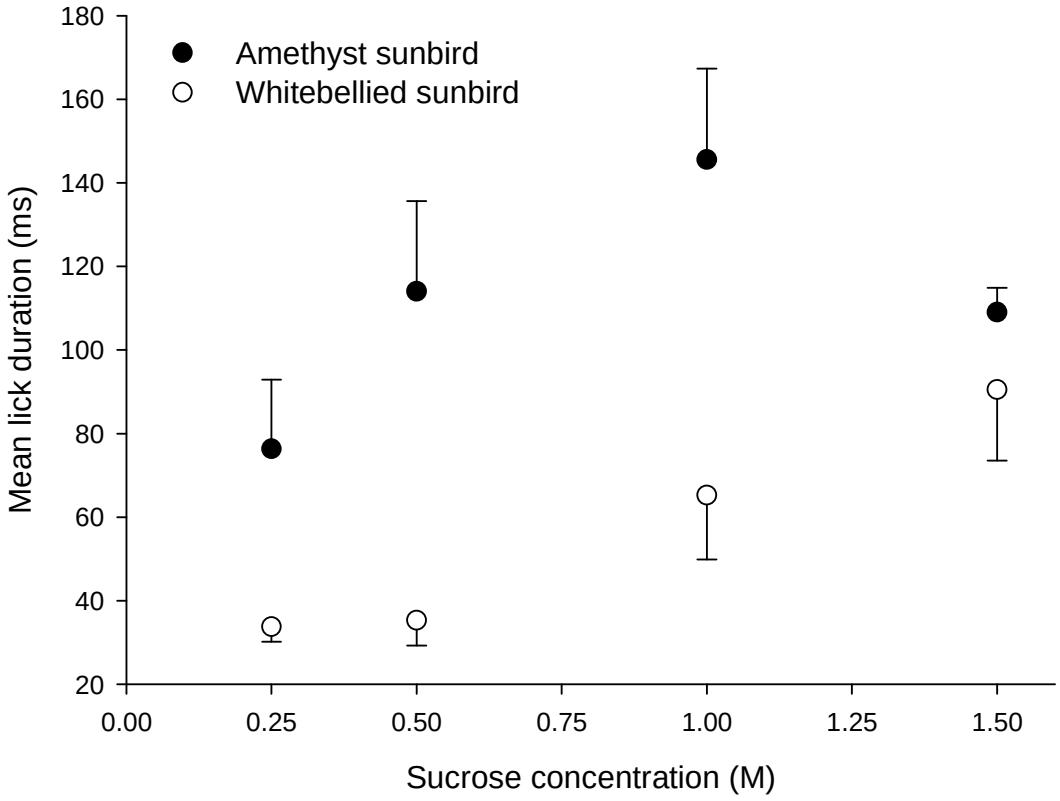
Amethyst sunbird
Whitebellied sunbird
Mean lick duration (ms)
180
160
140
120
100
80
60
40
20
0.00
0.25
0.50
0.75
1.00
1.25
1.50
Sucrose concentration (M)

Figure 3.

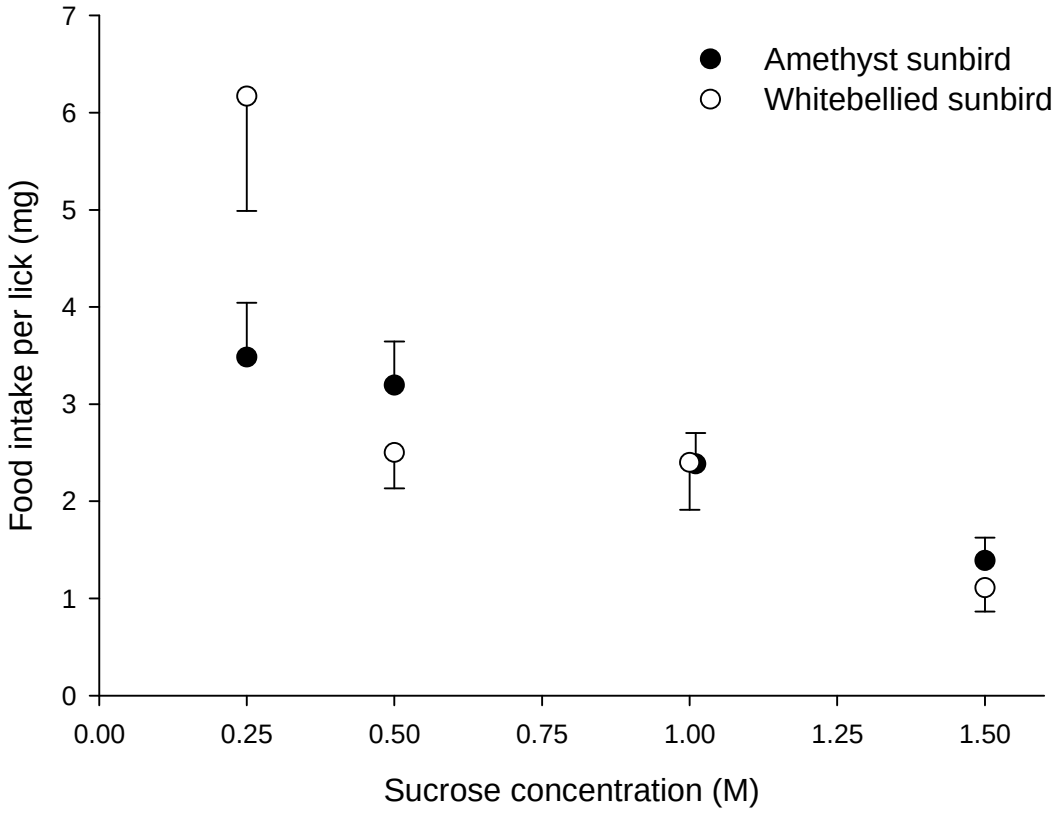
Amethyst sunbird
Whitebellied sunbird
Food intake per lick (mg)
Sucrose concentration (M)
0
1
2
3
4
5
6
7
0.00
0.25
0.50
0.75
1.00
1.25
1.50

Figure 4.