CHAPTER 3

THE RESPONSE OF TWO AVIAN NECTARIVORES TO INTERRUPTIONS IN FOOD AVAILABILITY

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Abstract

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

Introduction

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

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

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

Materials and methods

Study animals and their maintenance

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

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

Experimental procedure and processing of data

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

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

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

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

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

Statistical analysis

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

Results

Food intake

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

Adjustment of feeding behaviour in sunbirds

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

Body mass

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

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

Discussion

Adjustment of food intake after the fast

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

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

Does the fast lead to an energy deficit?

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

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

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

Physiological constraints to food intake

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

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

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

How do whitebellied sunbirds adjust their food intake?

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

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

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

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Figure legends

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

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

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

Figures

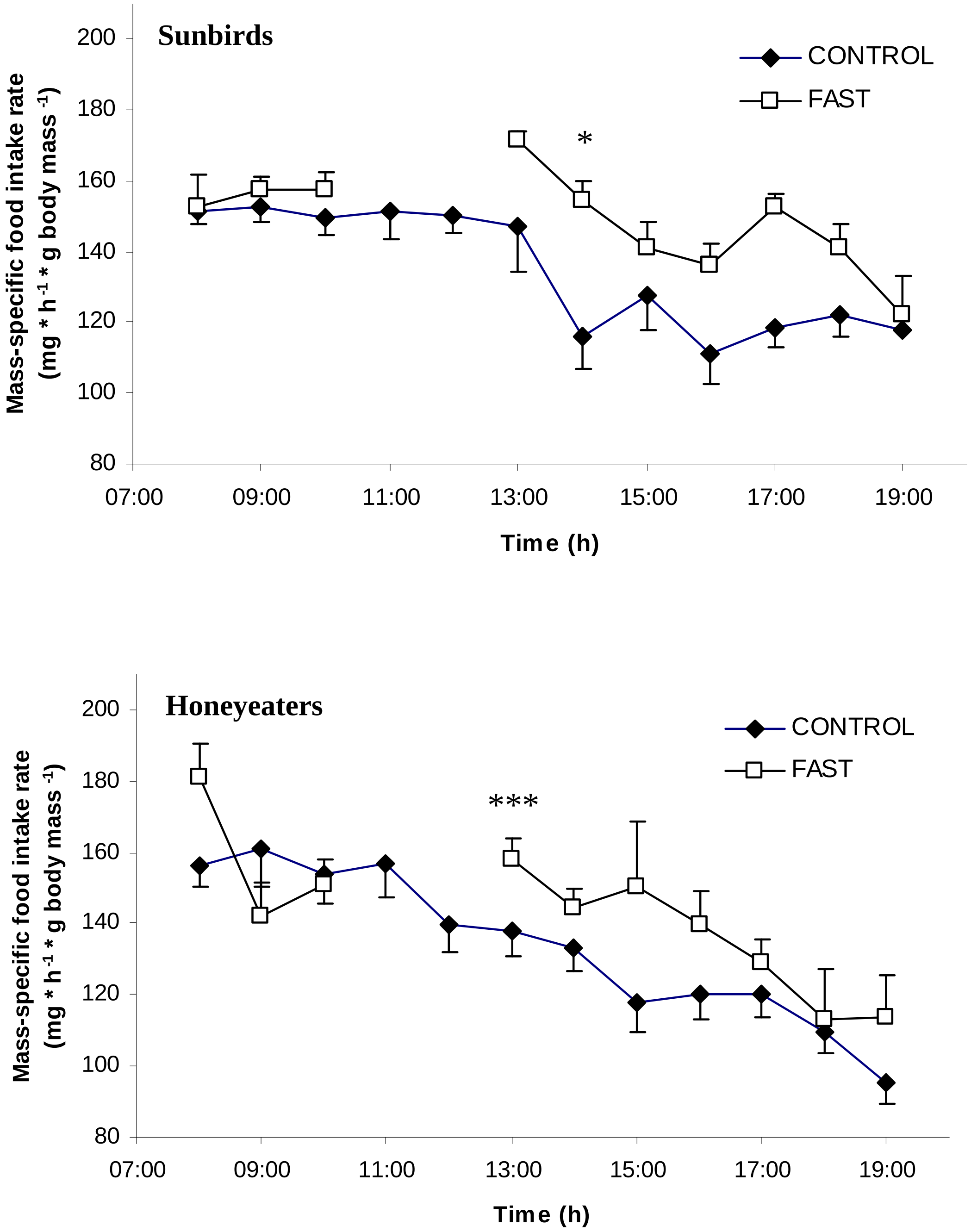

Figure 1.

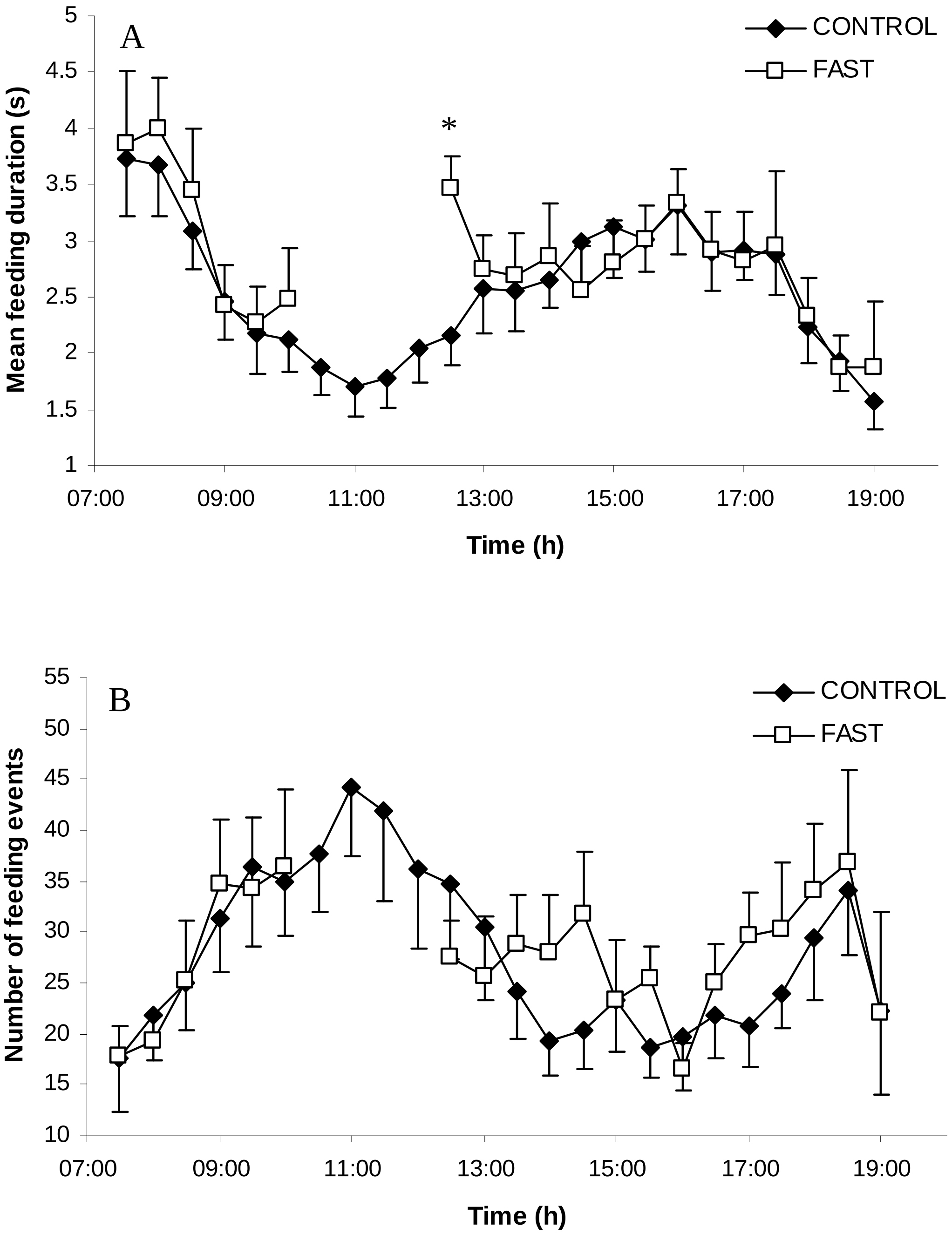
A
CONTROL
FAST
*
Mean feeding duration (s)
5
4.5
4
3.5
3
2.5
2
1.5
1
07:00
09:00
11:00
13:00
15:00
17:00
19:00
Time (h)
B
CONTROL
FAST
Number of feeding events
55
50
45
40
35
30
25
20
15
10
07:00
09:00
11:00
13:00
15:00
17:00
19:00
Time (h)

Figure 2.

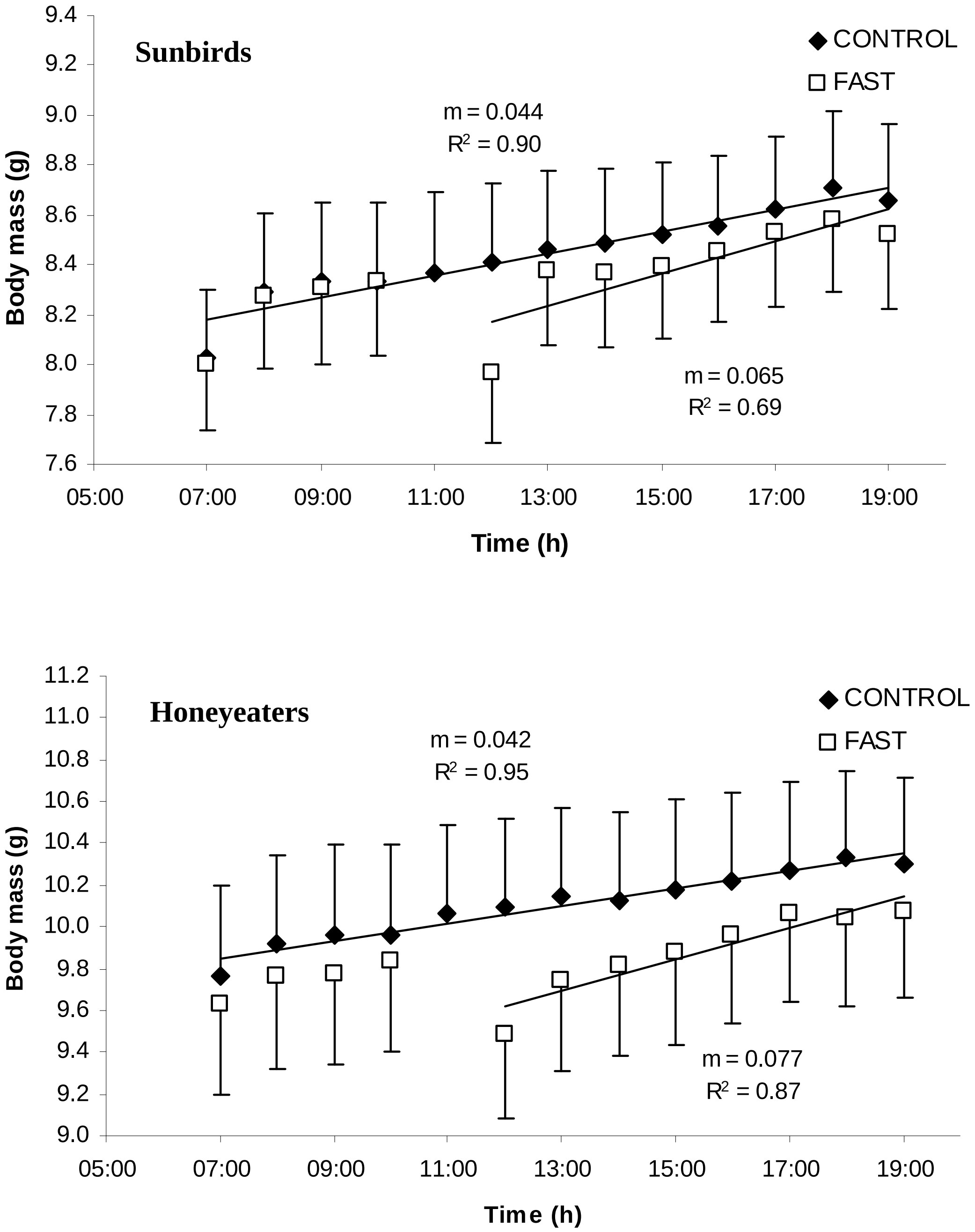
Sunbirds
CONTROL
FAST
m = 0.044
R² = 0.90
m = 0.065
R² = 0.69
Body mass (g)
9.4
9.2
9.0
8.8
8.6
8.4
8.2
8.0
7.8
7.6
05:00
07:00
09:00
11:00
13:00
15:00
17:00
19:00
Time (h)
Honeyeaters
CONTROL
FAST
m = 0.042
R² = 0.95
m = 0.077
R² = 0.87
Body mass (g)
11.2
11.0
10.8
10.6
10.4
10.2
10.0
9.8
9.6
9.4
9.2
9.0
05:00
07:00
09:00
11:00
13:00
15:00
17:00
19:00
Time (h)

Figure 3.

CHAPTER 4

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

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Abstract

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

Introduction

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

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

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

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

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

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

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

Materials and methods

Study animals and their maintenance

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

Experimental procedure

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

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

Data collection

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

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

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

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

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

Data processing

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

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

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

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

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

Statistical analysis

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

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

Results

Food and sugar intake

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

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

Feeding patterns

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

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

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

Body mass

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

Sugar assimilation in whitebellied sunbirds

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

Gut morphology, sucrase activity and predicted maximal intake

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

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

Discussion

Compensatory feeding

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

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

Physiological constraints to food intake

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

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

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

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

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

Maximal food intake in sunbirds

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

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

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

Energy-saving mechanisms

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

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

Feeding patterns

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

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

Conclusion

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

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Table

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

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

Figure legends

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

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

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

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

Figures

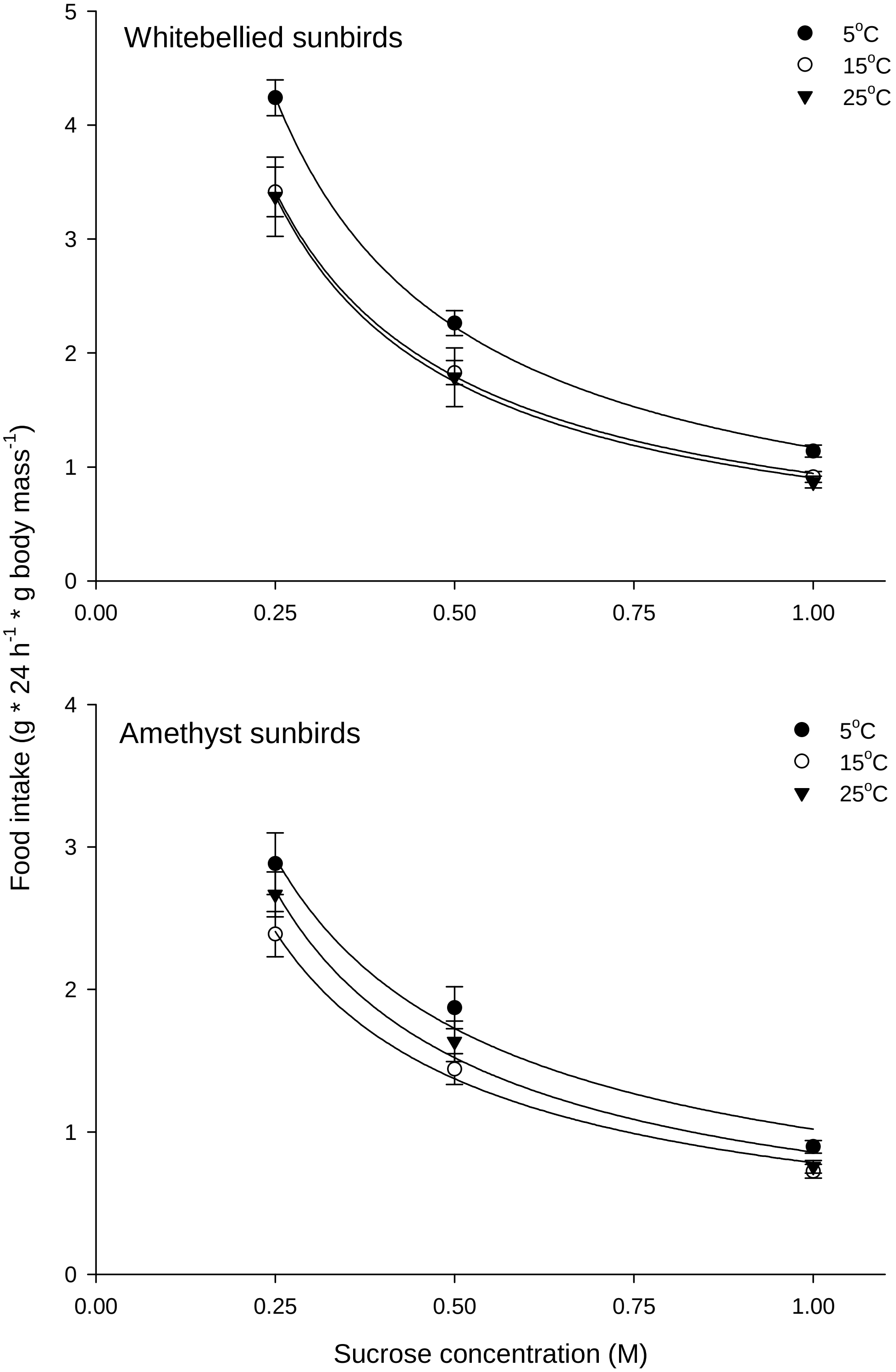

Figure 1.

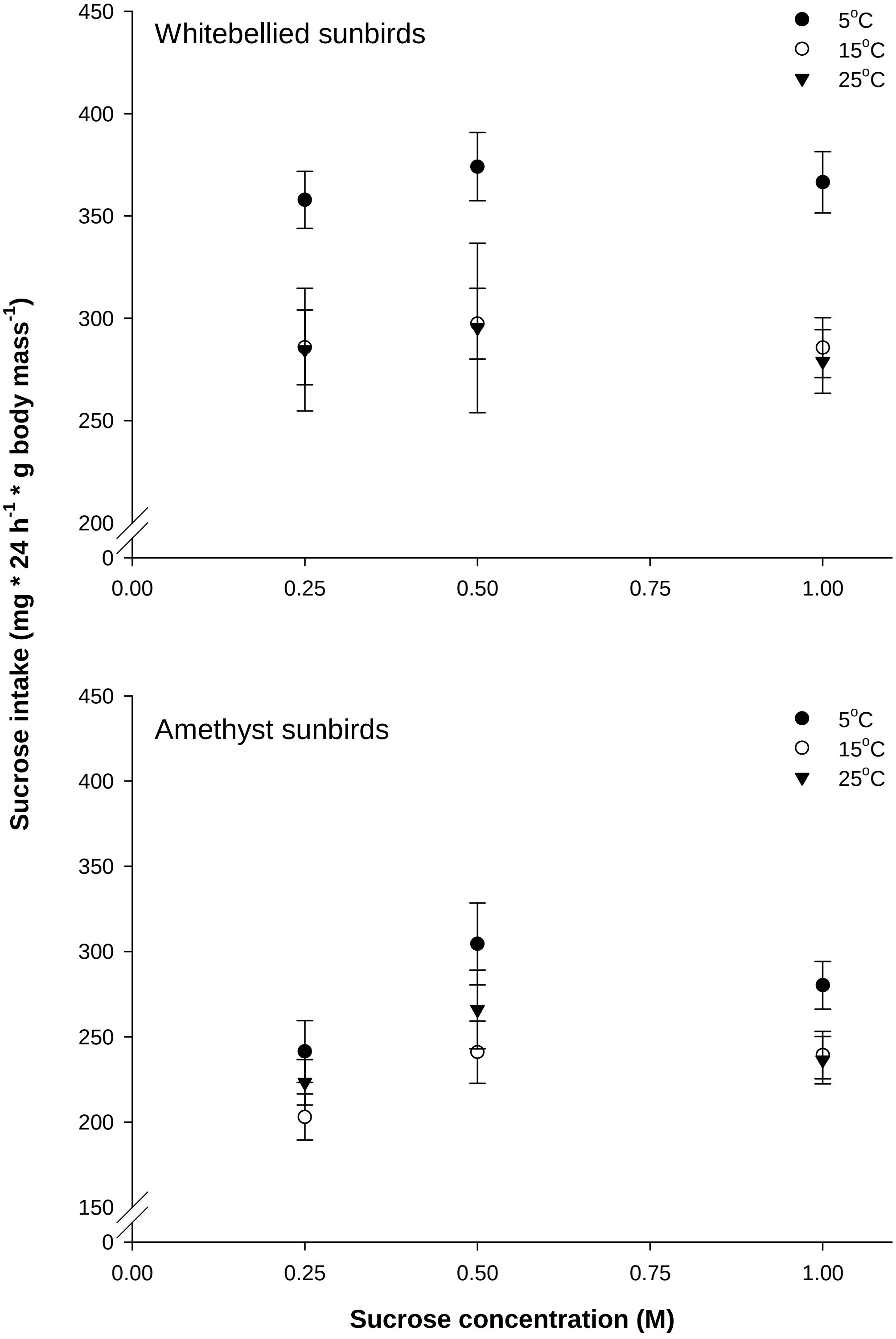
Whitebellied sunbirds
Amethyst sunbirds
● 5°C
○ 15°C
▼ 25°C
450
400
350
300
250
200
150
0
0.00
0.25
0.50
0.75
1.00
Sucrose intake (mg * 24 h-1 * g body mass-1)
Sucrose concentration (M)

Figure 2.

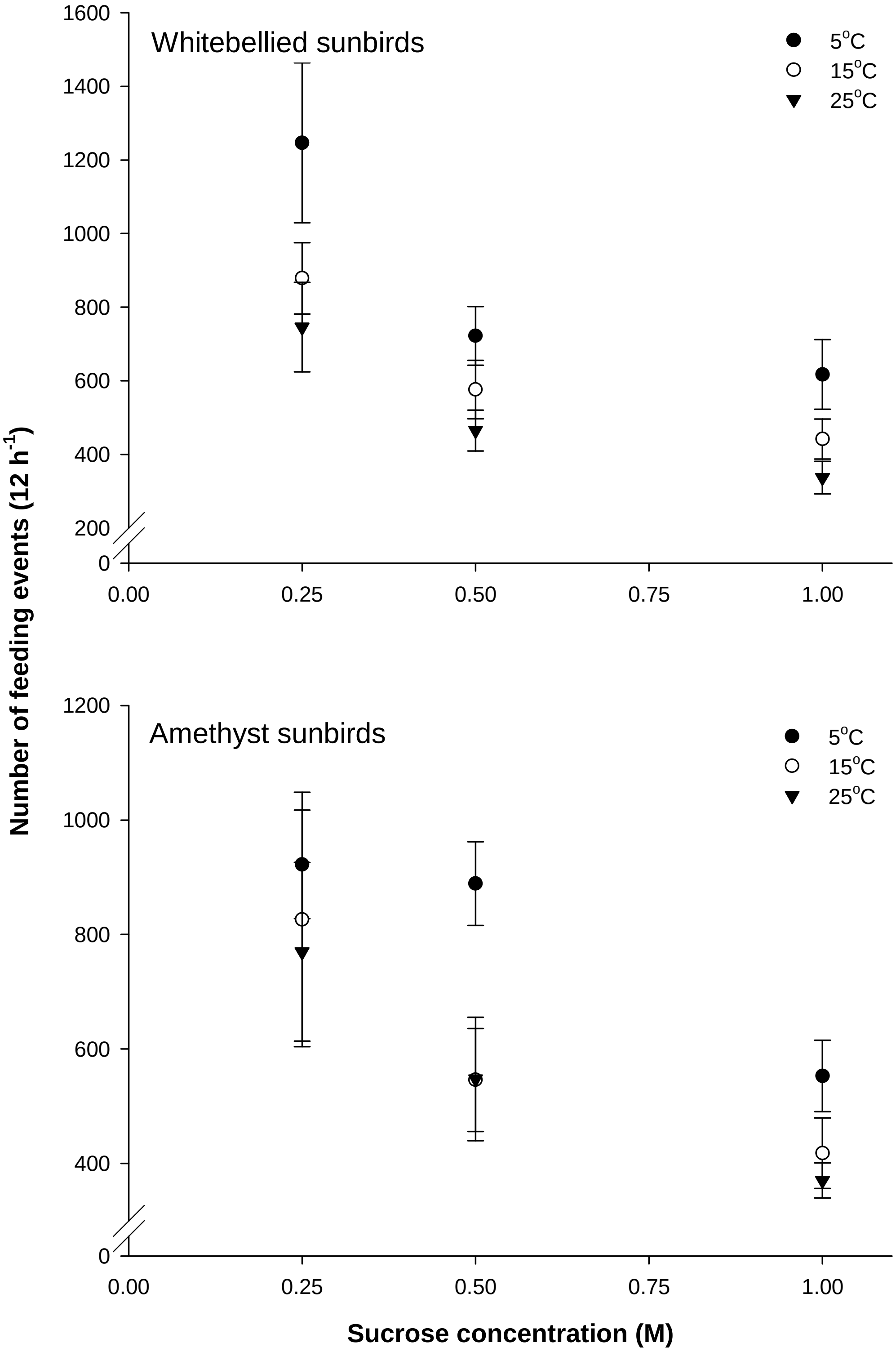
Whitebellied sunbirds
Amethyst sunbirds
● 5°C
○ 15°C
▼ 25°C
1600
1400
1200
1000
800
600
400
200
0
0.00
0.25
0.50
0.75
1.00
Number of feeding events (12 h⁻¹)
Sucrose concentration (M)

Figure 3.

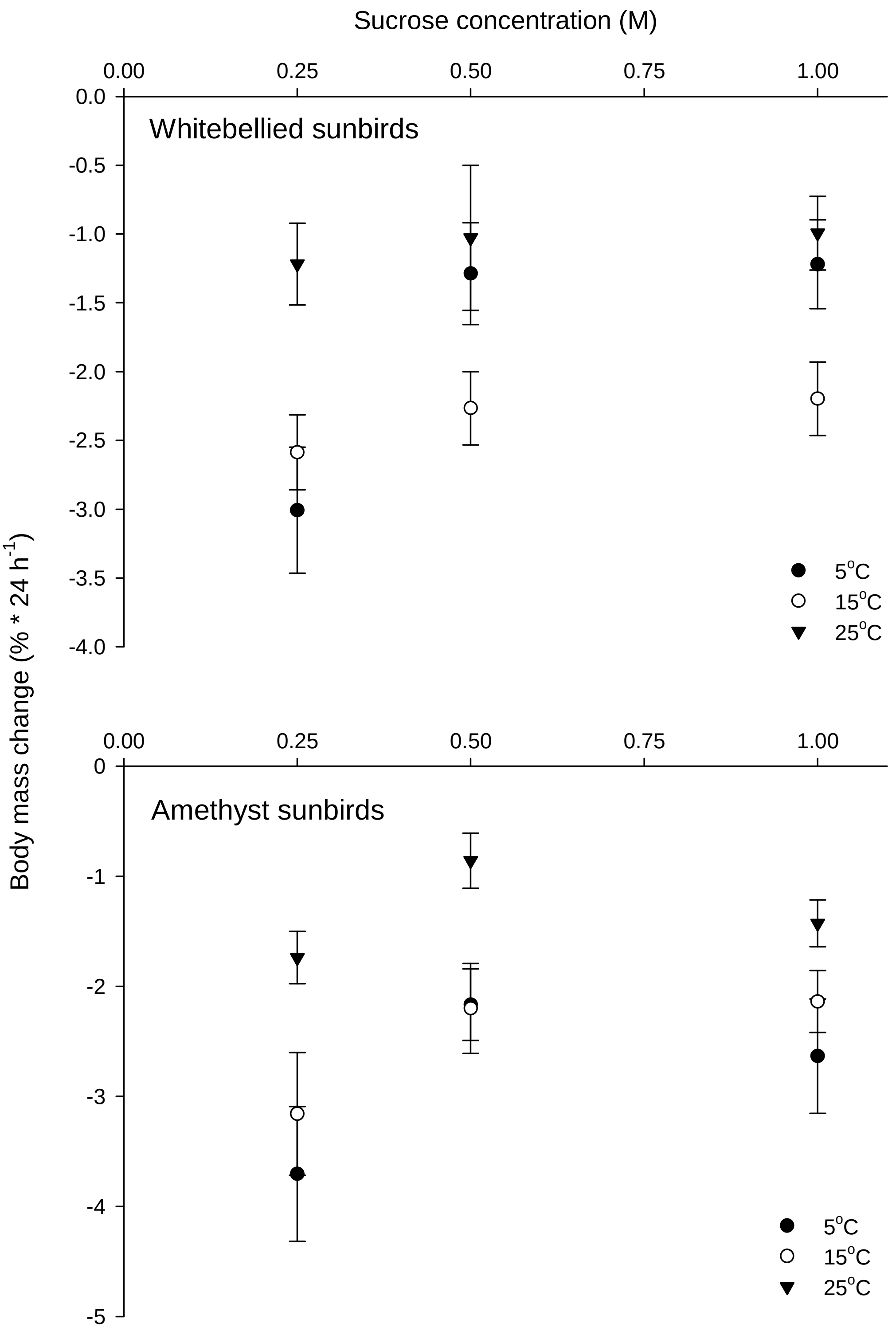
Sucrose concentration (M)
0.00
0.25
0.50
0.75
1.00
0.0
-0.5
-1.0
-1.5
-2.0
-2.5
-3.0
-3.5
-4.0
Whitebellied sunbirds
● 5°C
○ 15°C
▼ 25°C
Body mass change (% * 24 h-1)
0
-1
-2
-3
-4
-5
Amethyst sunbirds
● 5°C
○ 15°C
▼ 25°C

Figure 4.