Energy management on a nectar diet: can sunbirds meet the challenges of low temperature and dilute food?

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Running headline: Energy challenges in sunbirds

Summary

1. Animals commonly experience variation in both food quality and metabolic requirements, and must regulate their food intake to maintain energy balance.
2. We exposed captive whitebellied (*Cinnyris talatala*) and amethyst (*Chalcomitra amethystina*) sunbirds (Nectariniidae) to different 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.
3. Both species compensated for decreased dietary sugar concentration by increasing food intake. While whitebellied sunbirds showed a constant sugar intake over the range of diet concentrations, amethyst sunbirds ingested less sugar on 0.25 M than on 0.5 M sucrose. During acute short-term exposure to 5˚C, birds increased food and thus sugar intake on all diet concentrations by almost 27%, compared to the similar intake at 15 and 25˚C. Despite increased food consumption, all birds lost more body mass at 5 and 15˚C than at 25˚C.
4. Adjustment of food intake in both species took place via changes in feeding frequency, with the duration of feeding events unchanged.
5. Apparent sugar assimilation in whitebellied sunbirds was >99%, irrespective of diet concentration and temperature. A chemical reactor model of digestive capacity based on measurements of intestinal hydrolytic capacity mostly underestimated the maximal food intake of whitebellied sunbirds in the cold, but predicted higher maximal intake for amethyst sunbirds than observed on most diet concentrations.

6. We conclude that physiological constraints impose upper limits on compensatory feeding in sunbirds. These constraints are mainly digestive at low temperature and osmoregulatory on dilute diets, while the combination of both stresses leads to additional metabolic costs.
7. The moderate mass loss of both species when exposed to these energy challenges suggests that behavioural changes and/or hypothermia may be used to reduce energy expenditure.

Key-words: ambient temperature, compensatory feeding, digestive capacity modelling, feeding frequency, nectar concentration, physiological constraint, sugar assimilation

Introduction

The balance between energy intake and expenditure in animals is of major importance for survival and fitness. When the energy content of their food decreases, animals commonly compensate by ingesting larger amounts. Similarly, when energy demands increase, as occurs during reproduction, exercise and cold exposure, food consumption also increases (for a review see Karasov & Martínez del Rio 2007). Nectarivorous birds are particularly interesting subjects for investigating these two kinds of compensation - for food quality (*i.e.* varying nectar sugar concentration) and increased energy demands (low ambient temperatures, Ta). 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 (Hiebert 1991). In addition, avian nectarivores often ingest large volumes of dilute nectar to meet their high metabolic requirements (Martínez del Rio *et al*. 2001). When birds face the dual challenges of low environmental temperature and low food concentration, warming food to body temperature is an additional problem (Lotz, Martínez del Rio & Nicolson 2003).

When avian nectarivores are acutely exposed to low *T*a and different nectar sugar concentrations, they show varying degrees of compensation in response to these energetic challenges. Southern double-collared sunbirds *Cinnyris chalybeus*, when 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 unable to compensate on very dilute diets of 0.1 M (Fleming *et al*. 2004). In the same study, Fleming *et al*. (2004) found that broadtailed hummingbirds *Selasphorus platycercus* coped better with the combination of dilute diet and low temperature than the sunbirds, although this was not apparent in a previous study of the same species (McWhorter & Martínez del Rio 2000). The effects of acute cold exposure on hummingbirds have also been investigated on a shorter time frame: rufous hummingbirds *Selasphorus rufus* kept at 5˚C for 4 h periods were able to maintain body mass on a 30% w/v (0.88 M) sucrose diet, but not on lower concentrations (Gass, Romich & Suarez 1999). In most laboratory studies of nectarivore feeding behaviour, food intake has been recorded only hourly or daily. In studies where feeding patterns were monitored continuously via photocell detectors, birds visit feeders more frequently when the food sugar concentration (*i.e.* quality) is reduced

(Gass *et al*. 1999; Köhler *et al*. 2008). Nothing is known, however, of the effect of low temperatures on short-term feeding patterns.

When energy assimilation cannot be increased sufficiently to maintain body mass whilst animals are subjected to acute increases in energy demands or decreases in food quality (*i.e.* they are forced to feed maximally), various physiological constraints to food intake should be considered. Food intake in birds and mammals can be limited by the volumetric capacity of the intestine and/or the rate at which food is digested (Starck 1999; Karasov & McWilliams 2005). Indeed, mathematical modelling of digestive function clearly illustrates the fact that digestive capacity is a function of both enzyme and transporter activity levels and gut size (Martínez del Rio & Karasov 1990; McWhorter 2005). Sucrose hydrolysis rates and hexose absorption may be limiting in nectarivorous birds ingesting large amounts of nectar (Martínez del Rio & Karasov 1990; McWhorter & Martínez del Rio 2000; McWhorter *et al*. 2006; Napier *et al*. 2008). Birds may be further constrained by the elimination of excess water (McWhorter & Martínez del Rio 1999; Hartman Bakken & Sabat 2006) and by retention of electrolytes when consuming large diet volumes (Fleming & Nicolson 2003). Consumption of dilute nectar in the cold adds a further requirement: the need to warm the food to body temperature (Lotz *et al*. 2003). If intake cannot be increased sufficiently in response to increased energy requirements or low food quality because of these various constraints, birds may save energy by reducing their activity, as shown in green-backed firecrowns *Sephanoides sephanoides* (Fernández, López-Calleja & Bozinovic 2002) or reducing body temperature, such as food-deprived rufous hummingbirds utilising torpor (Tooze & Gass 1985). If body mass is still lost in spite of such energy-saving changes in behaviour, this provides robust evidence of physiological constraint(s) to energy assimilation (McWhorter & Martínez del Rio 2000).

In this study we investigated the interplay between compensatory feeding and physiological constraints in two species of Nectariniidae, whitebellied sunbirds *Cinnyris (Nectarinia) talatala* (A Smith) and amethyst sunbirds *Chalcomitra (Nectarinia) amethystina* (Shaw) (Fig. 1). We varied both food quality and *T*a and examined the effect on food intake, feeding patterns and body mass. We predicted that both species would compensate for decreased nectar sugar concentration by increasing their food intake, irrespective of *T*a. We further hypothesized that, owing to thermogenic costs, birds would increase their food intake at low *T*a on all sugar concentrations. 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 & Hainsworth 1977; Köhler, Verburgt & Nicolson 2006). Despite the expected increase in food intake, sunbirds were expected to lose body mass in the cold due to physiological limitations to the processing of nectar meals. Food intake in the cold was compared with the maximal intake predicted by a mathematical model, using measurements of intestinal hydrolytic capacity (McWhorter & 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 simultaneously encountering food of low energy content.

Materials and methods

STUDY ANIMALS AND THEIR MAINTENANCE

Nine whitebellied sunbirds and nine amethyst sunbirds were mist-netted at Jan Cilliers Park in Pretoria. 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 of 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 & 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 experiments. They were released at the capture site on completion of the study.

EXPERIMENTAL PROCEDURE

Whitebellied and amethyst sunbirds were exposed to T_a 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

(Nicolson & Fleming 2003a). Each bird received all three dietary sugar concentrations at all three *T*a, *i.e.* the nine birds of each species were exposed to each temperature three times. The sequence of *T*a and sugar concentration was randomised, with three birds receiving the same sugar concentration at a time. 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 became accustomed to the experimental cages, *T*a and diet, followed by one test day. Between the different parts of the experiment, birds were given five days on the maintenance diet at 20°C to regain energy balance as they generally lose body mass on the experimental sugar-only diets (Nicolson & Fleming 2003b). Repeated exposures to 5˚C were at least two weeks apart as we were interested in the response to acute energy challenges and wanted to avoid physiological adjustments to high feeding rates, such as increased gut size and thus increased amounts of nutrient transporters and digestive enzymes (Starck 1999).

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 enable a comparison between the different sugar types found in nectar of sunbird-pollinated plants (Nicolson & 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 (0.01 g, Denver Instrument PK-352, 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. Evaporative mass loss from the syringes containing the test diets was determined using additional feeders, with all diet concentrations and both sugar types, on cages without birds. These feeders were weighed before and after one full day at each *T*a. The mass of food consumed was corrected for losses from both dripping and evaporation. On all test days, the time spent feeding and the number of feeding events were automatically recorded 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 the nine whitebellied sunbirds produced over 24 h were collected in plastic trays placed underneath the cages on one test day at each *T*a on sucrose diets, and on both test days on hexose diets (5 and 25˚C). Excreta samples were allowed to evaporate and were 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) 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 killed by a halothane overdose. The small intestine was immediately removed from each bird and flushed clean with ice-cold saline before being cut into sections and dissected lengthwise. The length and nominal surface area of each section were measured and used to calculate the luminal volume of each section, and later the total luminal 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 & Karasov (2006). In short, 30 µl tissue homogenates diluted with 300 mM mannitol in 1 mM Hepes/KOH were 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).

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 *T*a.

The amount of each sugar (sucrose, glucose and fructose) 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. Apparent sucrose, glucose and fructose assimilation efficiencies (AE*) were calculated for each bird on each test day as the proportion of ingested sugar that was not excreted:

$$AE^* = \frac{\text{sugar in} - \text{sugar out}}{\text{sugar in}} \bullet 100$$

where sugar in ($\text{mg} \bullet 24\text{h}^{-1}$) is the concentration ($\text{mg} \bullet \text{ml}^{-1}$) of a particular sugar in the ingested diet multiplied by the volume of food ingested (ml), and sugar out ($\text{mg} \bullet 24\text{h}^{-1}$) is the amount of the particular sugar excreted.

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 & Martínez del Rio (2000) and Martínez del Rio *et al*. (2001). 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 data on gut morphology and intestinal sucrase activity for each sunbird species. Predicted daily maximal volumetric food and sucrose intake rates were 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 those on 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 on 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, 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}, arcsine transformed) were separately subjected to repeated measures ANOVA (RM-ANOVA), with *T*a 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. Sugar intake (mg•24 h^{-1}•g body $mass^{-1}$) showed a different pattern in whitebellied than in amethyst sunbirds, therefore separate RM-ANOVA were performed for each species. To test whether the birds show compensatory feeding, we plotted the mass-specific food intake of each bird on each diet concentration (for each temperature separately), calculated a power regression and compared the exponent of this power function to -1 using a *t*-test (single sample). Exponents that are not significantly different from -1 indicate perfect compensatory feeding (Martínez del Rio *et al*. 2001). A *t*-test (single sample) was used to compare the observed food (and sugar) intake of each bird on each sucrose concentration at 5˚C to the maximal intake predicted by the digestive model for the particular concentration.

The amounts of sucrose, glucose and fructose excreted and the total amounts of sugar excreted ($mg \cdot 24\ h^{-1}$) were subjected to Spearman rank correlations to test for correlations with diet sugar concentration (for each *T*a and sucrose and hexose diets separately). RM-ANOVA was used to determine differences in total sugar excreted between 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 *T*a 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). The level of significance was $\alpha \leq 0.05$ for all tests. 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 *T*a is shown in Fig. 2. 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 per g body mass than amethyst sunbirds ($P<0.01$).

Sugar intake (mg•24 h^{-1}•g body $mass^{-1}$) of both whitebellied and amethyst sunbirds was found to differ between temperatures ($F_{2,16}>5.56$, $P<0.01$; Fig. 3). Sugar intake of whitebellied sunbirds was higher at 5˚C than at 15 and 25˚C ($P<0.001$). Amethyst sunbirds also ingested more sugar at 5˚C than at 15˚C ($P=0.01$), but not more than at 25˚C ($P=0.09$). Sugar intake of both species was similar on 15 and 25˚C ($P>0.61$). Whitebellied sunbirds showed a similar sugar intake on all dietary sugar concentrations ($F_{2,16}$=1.07, $P=0.37$; Fig. 3) and the exponents of the food intake responses did not differ from -1 at any of the three temperatures ($t_8<1.98$, $P>0.08$), indicating perfect compensatory feeding over the range of dietary sugar concentrations. The exponents of the food intake responses of amethyst sunbirds, on the other hand, were significantly different from -1 at all three temperatures ($t_8>2.90$, $P<0.02$), and sugar intake differed significantly between sugar concentrations ($F_{2,16}=8.83$, $P<0.01$). *Post-hoc* analysis revealed that amethyst sunbirds ingested less sugar on the dilute diet (0.25 M) than on the 0.5 M diet ($P<0.01$). Sugar intake on this dilute diet also tended to be lower than on the 1 M diet but this was not significant ($P=0.05$). Sugar intake of amethyst sunbirds did not differ between the 0.5 and 1 M diets ($P=0.27$).

FEEDING PATTERNS

The number of feeding events for whitebellied and amethyst sunbirds during the 12 h light period of the test days differed significantly between temperatures ($F_{2,32}=13.20$, $P<0.001$) and sugar concentrations ($F_{2,32}=28.51$, $P<0.001$; Fig. 4). *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 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, no significant difference between individual temperatures was found ($P>0.05$). 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 the 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, $P<0.001$). 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

All birds lost body mass during test days (Fig. 5) because the experimental diets were not supplemented with protein. The change in body mass (%•24 h^{-1}) differed significantly among 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

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, when the data were combined for all sugar concentrations and all *T*a. Total amounts of sugar excreted was not correlated with diet sugar concentration at all *T*a and on both sucrose and hexose diets ($R_s>-0.53$, $P>0.14$). Data for all concentrations were therefore pooled for each sugar type to determine differences between *T*a. Total amounts of sugar excreted were independent of *T*a 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 at all three temperatures ($R_s>-0.74$, $P>0.02$; sequential Bonferroni: $P>0.006$, n.s.). Data for all concentrations at a given *T*a were pooled to determine differences between sugar types. The amounts of sucrose, glucose and fructose excreted were found to differ significantly at all temperatures ($F_{2,16}>4.94$, $P<0.02$). 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 efficiencies of whitebellied sunbirds were exceptionally high, irrespective of sugar concentration, dietary sugar type and *T*a (sucrose AE*=99.81 ± 0.01%, n=27; glucose AE*=99.66 ± 0.05%, n=18; fructose AE*=99.74 ± 0.02%, n=18).

GUT MORPHOLOGY, SUCRASE ACTIVITY AND PREDICTED MAXIMAL INTAKE

Data on intestinal 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 $\mu mol \cdot min^{-1}$ in whitebellied and 13.30 ± 0.94 $\mu mol \cdot min^{-1}$ in amethyst sunbirds. Apparent Michaelis constant (K_m) and pH optima for intestinal sucrase activity were 15.40 ± 2.24 mM and 5.5 for whitebellied sunbirds, and 14.08 ± 2.46 mM and 5.5 for amethyst sunbirds.

Since amethyst sunbirds have larger intestines and thus higher sucrase activities than whitebellied sunbirds, they are able to digest more nectar of a particular concentration per unit time. The maximal daily volumetric food intake predicted by the chemical reactor model of digestive capacity was higher in amethyst than in whitebellied sunbirds, as was daily food intake measured on all sugar concentrations at 5˚C (Table 1). Comparing the predicted maximal and observed food intake in the cold, whitebellied sunbirds fed more than predicted on the 0.25 M (t_8=7.38, P<0.001) and 0.5 M sucrose diets (t_8=3.31, P=0.01; Table 1). On the 1 M diet, whitebellied sunbirds appeared to ingest more food than predicted, but this was not significant (t_8=1.96, P=0.09; Table 1). Consequently, the predicted maximal daily sugar intake of whitebellied sunbirds at 5˚C was lower than the observed sugar intake on the 0.25 and 0.5 M sucrose diets (t_8>3.31, P≤0.01), but not on the 1 M diet (t_8=1.96, P=0.09; Table 1). For amethyst sunbirds, the predicted maximal daily food intake and sugar intake were higher than the observed intakes at 5˚C (t_8<-3.35, P≤0.01), except on the 0.5 M diet where the predicted and observed intakes were similar (t_8=0.15, P=0.88; Table 1).

Discussion

When we manipulated both food quality (*i.e.* energy density) and energy requirements (*i.e.* ambient temperature), both sunbird species showed compensatory feeding as expected. However, the extent of the compensation was apparently limited by physiological constraints because birds assimilated less sucrose and lost more body mass at low ambient temperatures, and lost the most body mass when feeding on energy-dilute diets in the cold. We will first discuss the possible constraints in the case of low temperatures, dilute food, and combinations of the two and then discuss reasons for the discrepancy between observed maximal food intake and that predicted by a chemical reactor model of digestive capacity. Finally, we consider possible adjustments in body temperature and behaviour which may be necessary when nectarivorous birds are subjected to energy challenges and physiological constraints prevent them from meeting these challenges. We were particularly interested in the acute response to energetically stressful conditions.

COMPENSATORY FEEDING IN RESPONSE TO TEMPERATURE AND FOOD CONCENTRATION

When nectar sugar concentration is decreased, birds typically compensate by increasing their volumetric food intake. Whitebellied sunbirds have previously been shown to defend a constant energy intake over a 10-fold range in diet concentration from 0.25 to 2.5 M sucrose (Nicolson & Fleming 2003b), and this compensatory feeding response is characteristic of avian nectarivores in general (for a review see Martínez del Rio *et al*. 2001). When energy demands increase, birds also have to ingest more food. Our sunbirds compensated for a decrease in nectar concentration or *T*a by increasing the number of feeding events, confirming our earlier finding that food intake is mainly adjusted via feeding frequency (Köhler *et al*. 2006; Köhler *et al*. 2008). When whitebellied sunbirds were fed a 0.25 M sucrose diet in an earlier study, they ingested on average 1000 meals per day at 20˚C (Köhler *et al*. 2008), which is comparable to their feeding frequency at the two higher temperatures in the present study; and was increased to 1200 feeding events in the cold. Meal duration did not increase, as volumetric intake at each feeding event may be limited by the absence of a crop (Mbatha, Downs & Penning 2002), although under some circumstances sunbirds can ingest relatively large meals (Köhler *et al*. 2008). More frequent feeding at low *T*a has also been shown for hummingbirds (Beuchat, Chaplin & Morton 1979; Gass *et al*. 1999). However, sunbirds in our study ingested only 27% more of the sugar solution at 5˚C than at 25˚C. In previous studies involving both acute exposure and acclimation to cold, the metabolic rate of sunbirds was twice as high at 5ºC as at 25ºC (Leon & Nicolson 1997; Lindsay, Downs & Brown 2009), suggesting that food intake should double in order to meet energy requirements in the cold. Comparing the different diet concentrations, whitebellied sunbirds showed perfect compensatory feeding by defending a constant sugar intake, while amethyst sunbirds ingested less sugar on the most dilute diet than on the other two diets, despite the increase in nectar intake with decreasing sugar concentration. In summary, sunbirds attempted to compensate for a decrease in nectar sugar concentration and *T*a by increasing their food intake. However, sugar intake of amethyst sunbirds was still lower on the most dilute diet and both species lost more body mass at the two lower temperatures, suggesting that the birds face physiological constraints that inhibit compensation at environmental extremes.

LOW TEMPERATURES: DIGESTIVE CONSTRAINTS

At low temperatures, the additional food intake required to compensate for higher metabolic costs may be limited by constraints on nectar digestion. Sucrose hydrolysis rates have been proposed as a limiting factor when large amounts of nectar are ingested (McWhorter & Martínez del Rio 2000). Birds may also be constrained by the passive and carrier-mediated absorption of glucose and fructose (McWhorter *et al*. 2006; Napier *et al*. 2008). When nectar sugar concentrations are high, it is more likely for birds with a substantial passive component to hexose absorption to be limited by sucrose hydrolysis (Martínez del Rio & Karasov 1990). However, hexose diets do not improve the ability of whitebellied sunbirds to maintain energy balance (Fleming *et al*. 2004). Whitebellied sunbirds in our experiment assimilated more than 99% of the ingested sugar, irrespective of diet sugar concentration, sugar type and *T*a. This confirms previous studies in specialist nectar-feeding birds where sugar assimilation efficiency has been found to be 95% or higher (Collins, Cary & Packard 1980; Downs 1997; Jackson, Nicolson & van Wyk 1998; McWhorter & Martínez del Rio 2000). Despite this very high assimilation efficiency, the rate of sugar assimilation may still have constrained the food intake rate of sunbirds (see Ayala-Berdon *et al*. 2008).

In mammals and birds acclimated to high feeding rates, gastrointestinal tracts show an increase in size, with a concomitant increase in amounts of digestive enzymes and nutrient transporters (Starck 1999). This plasticity of the digestive system ensures a long-term spare capacity of 100–125% above routine rates (reviewed by Karasov & McWilliams 2005). However, 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; this is termed immediate spare capacity and may not be sufficient to maintain energy balance (Karasov & McWilliams 2005). The 27% increase that we measured (in the middle of this range) was for sunbirds exposed to low *T*a under acute, short-term conditions, with sufficient maintenance days between cold exposures. There was no evidence of digestive adjustments in our experiment, since food intake did not increase with repeated cold exposure. Such rapid-adjustment experiments, yielding data on immediate spare capacity, are less common than those involving gradual adjustment to high energy demand (Karasov & McWilliams 2005).

DILUTE NECTAR: OSMOREGULATORY CONSTRAINTS

As a result of compensatory feeding, avian nectarivores may ingest extraordinary volumes of food. For example, food intake of *C. talatala* varies from 4 ml/day on 2.5 M sucrose solutions to 32 ml/day (~4 times body mass) on 0.25 M sucrose (Nicolson & Fleming 2003b). On very dilute diets energy intake is likely to be limited by the processing of the excess water (McWhorter &

Martínez del Rio 1999; Fleming & Nicolson 2003; Hartman Bakken & Sabat 2006). Sunbirds, however, may be able to modulate intestinal water absorption and thus reduce the load on the kidneys, as shown for Palestine sunbirds *Cinnyris oseus* by McWhorter *et al.* (2004). Regulation of ion levels also represents a challenge for birds when water fluxes are high: recovery of ions is impressive, but the total ion output in whitebellied sunbirds is still higher on extremely dilute sucrose-only diets on which they cannot maintain energy balance (Fleming & Nicolson 2003). There may also be digestive constraints associated with very dilute diets: fast passage rates and low substrate concentrations may limit sucrose hydrolysis and subsequent absorption of hexoses in nectarivorous and frugivorous birds (Martínez del Rio & Karasov 1990; Witmer 1999).

DILUTE NECTAR IN THE COLD: ADDITIONAL METABOLIC CONSTRAINTS

The dual challenges of low environmental temperature and low food concentration impose additional thermoregulatory costs, because ingested nectar must be warmed to body temperature. The cost of this warming increases substantially with decreasing nectar temperature (*i.e.* the difference between body and nectar temperatures) and with decreasing nectar concentration (*i.e.* the volume consumed), as shown by a mathematical model and empirical measurements in rufous hummingbirds (Lotz *et al.* 2003). The cost of warming nectar, measured as a change in metabolic rate of feeding hummingbirds, changed exponentially with nectar concentration and linearly with nectar temperature, and whether the birds hovered or perched to feed made little difference. Similarly, 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 & Nicolson 2002). Other than the study by Lotz *et al.* (2003), investigations of acute or chronic cold exposure in nectar-feeding birds have always used food provided at ambient temperature, so this additional constraint has usually been overlooked. Nectar warming costs will be important for migrating rufous hummingbirds that refuel in subalpine meadows in early mornings (Gass *et al*. 1999), or for sunbirds feeding on dilute nectar of *Aloe* species during South African winters (Botes, Johnson & Cowling 2008).

OBSERVED AND PREDICTED MAXIMAL FOOD INTAKE

Assuming that sucrose hydrolysis rates limited the food intake of sunbirds in our study, we predicted their maximal food and sugar intake using a chemical reactor model of digestive capacity that takes into account the decline in sucrose concentration along the length of the gut (McWhorter & Martínez del Rio 2000). Use of this model in studies of nectar-feeding birds has provided strong evidence of physiological constraints to food intake (McWhorter 2005). In whitebellied sunbirds, observed intake values in birds challenged by low *T*a generally exceeded the predicted values; while for amethyst sunbirds the predicted values were higher, except on the intermediate sugar concentration (Table 1).

In earlier studies on nectarivorous birds, the model accurately predicted maximal food intake rates of broadtailed hummingbirds challenged by low *T*a, providing an explanation for the inability of acutely cold-exposed birds to compensate for their increased energy demands (McWhorter & Martínez del Rio 2000). The model also accurately predicted maximal food intake rates 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 under caged conditions, the predicted maximal food intake exceeded the observed intake by 40–70%, indicating that the bats did not feed at their maximal rates (Ramírez, Herrera & Mirón 2005). However, better agreement between observed and expected intake was found for this species when bats had to fly to feed (Ayala-Berdon *et al*. 2008). Flight not only increases energy requirements but also evaporative water loss, which may contribute to the elimination of excess water on dilute diets. When *G. longirostris* were forced to maximize their food intake by limiting the time available for foraging (Winter 1998), their intake rates were remarkably similar to the maximal intake rates predicted by the sucrose hydrolysis model of Ramírez *et al*. (2005).

The explanation for why our model may have over- or underestimated the maximal food intake of the sunbirds probably lies in the fact that individuals used for measurements of gut morphology and sucrase activity differed from those used in the experiment. Firstly, birds that were killed were captured early in April (South African autumn), while birds used in the experiment were caught one month later in the following year. Secondly, the maintenance diet in captivity may have affected digestive capacities. Sunbirds used for morphological and sucrase analysis were killed a few days after capture, while experimental birds were acclimated to a sucrose maintenance diet for several weeks. Either exposure to lower *T*a or an exclusive sucrose diet could potentially increase digestive performance through an increase in intestinal size; the tissue-specific amount of enzymes and transporters are less likely to change (McWilliams, Caviedes-Vidal & Karasov 1999). Future studies could investigate the effect of season, acclimation to maintenance diets and constant laboratory conditions on gut morphology and digestive enzyme activity of nectarivorous birds. These factors might explain conflicting findings in a single species, such as broadtailed hummingbirds, which increased their nectar intake at low *T*a in one study (Fleming *et al*. 2004), but not in another (McWhorter & Martínez del Rio 2000).

POTENTIAL ENERGY-SAVING MECHANISMS

In spite of digestive and osmoregulatory constraints on food intake, and the additional metabolic costs of warming cold nectar, birds showed relatively low energy deficits under cold conditions. Reductions in energy expenditure through hypothermia, and thus reduced metabolism, may have helped sunbirds to minimise mass losses. Many species of birds have been shown to save energy under unfavourable environmental conditions through facultative hypothermia or torpor (for review see McKechnie & Lovegrove 2002). Contrasting strategies are evident in hummingbirds exposed to the combined energy stresses of low *T*a and restricted or dilute food: *S. rufus* regulates energy loss through the use of torpor (Beuchat *et al.* 1979; Hiebert 1991; Gass *et al.* 1999), while *Calypte anna* regulates energy gain mainly through increased food intake (Beuchat *et al.* 1979). Torpor was not apparent in sunbirds in the present study, as they were active when caught for weighing in the mornings, irrespective of *T*a and diet concentration. Reductions in rest-phase body temperature, however, cannot be ruled out. Small birds, which need to generate more heat (per g body mass) to maintain a constant body temperature, generally reduce their temperature more when inactive than do larger birds: this increases the amplitude of day-night differences in body temperature (McKechnie & Lovegrove 2002). Facultative hypothermic responses are not always easy to distinguish from the normothermic circadian rhythm of body temperature (McKechnie & Lovegrove 2003). They occur in singing honeyeaters (*Meliphaga* *virescens*) deprived of food for 1–2 h at the end of the day (Collins & Briffa 1984), and in southern double-collared sunbirds subjected to low *T*a (Leon & Nicolson 1997). Sunbirds do appear to have labile body temperatures, with torpor having been reported in the malachite sunbird *Nectarinia famosa* (Downs & Brown 2002). Temporal heterothermy may represent an adaptive strategy in endotherms: when they experience low food availability, or increasing costs of homeothermy at low *T*a, animals should favour less precise thermoregulation (Angilletta *et al*. 2010). It is therefore important that future studies include body temperature measurements of sunbirds during similar temperature challenges.

Besides adjustments in body temperature, birds can reduce their activity to conserve energy. Sunbird activity was not quantified in our study, but at 5˚C the birds sat 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 (A. Köhler, pers. observation). Green-backed firecrowns also became less active when thermoregulatory and feeding costs were high (Fernández *et al*. 2002). Tiebout (1991) manipulated energy demands of two hummingbird species by varying food delivery (equivalent to low nectar secretion rate) and foraging distance in a flight cage (equivalent to low flower density). Birds showed considerable flexibility in daily energy management, reducing their activity when food availability was restricted, while engaging in more non-foraging flights when food was freely available. This resulted in lower total energy expenditure during food restriction, compared to *ad libitum* feeding. Birds additionally showed a short-term reduction in perching metabolic rates when food was limited or foraging distance increased (Tiebout 1991). Hummingbirds have also been shown to reduce their energy expenditure during flight by actively regulating their body mass (Altshuler & Dudley 2002). A lower body mass could decrease energetic costs for flight in sunbirds, although this is probably negligible as birds in our study could only make short flights. The importance of reductions in energy expenditure, as opposed to increases in food intake, needs further investigation.

ENERGY CHALLENGES IN THE WILD

Free-living whitebellied and amethyst sunbirds probably face similar limitations to energy intake to the individuals in our study. Sugar concentrations vary widely in their nectar plants, such as *Greyia*, *Erythrina*, *Strelitzia*, *Leonotis* and *Protea* (Nicolson 2002; Hockey, Dean & Ryan 2005). In winter, sunbirds forage widely on dilute *Aloe* nectar, which may pose an energetic challenge in addition to low temperature. *Aloe* species producing the most dilute nectar tend to be pollinated by generalised bird pollinators rather than specialised sunbirds (Botes *et al.* 2008), and this association between nectar properties of plants and bird pollination systems extends to other plant genera (Johnson & Nicolson 2008), but sunbirds commonly visit the extremely dilute nectar (average 0.3 M) of flowers adapted to generalised bird pollinators. In southern African winters, temperatures may exceed 20˚C during the day, but can drop to 5˚C or less at night, thus exposing sunbirds to large daily temperature fluctuations. Amethyst sunbirds occur along a steep altitudinal gradient in South Africa, with habitats in mountain ranges where temperatures can drop far below 0˚C in winter months (Lindsay *et al.* 2009). Some avian nectarivores of southern Africa breed in winter, and must meet the increased energy requirements of incubation in the cold (Jackson 1999). Future studies should investigate how sunbirds cope with the dual challenge of low ambient temperature and dilute nectar under natural or semi-natural conditions.

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Table 1. Observed daily food and sugar intake (mean ± SE) of nine whitebellied (WBSB) and nine amethyst (ASB) sunbirds on three sucrose concentrations at 5°C and maximal daily food and sugar intake predicted by a chemical reactor model of digestive capacity (McWhorter & Martínez del Rio 2000). Statistical results derive from *t*-tests (single sample) that were used to compare predicted and observed food (and sugar) intakes (n.s. $P>0.05$, ** $P\leq0.01$, *** $P\leq0.001$; see text for details).

Species	Sucrose concentration (M)	Volumetric food intake ($ml \bullet 12\ h^{-1}$)		Sugar intake ($g \bullet 12\ h^{-1}$)		
		Observed (mean ± SE)	Predicted	Observed (mean ± SE)	Predicted	Observed vs. Predicted
WBSB	0.25	36.13 ± 0.81	30.15	3.09 ± 0.07	2.58	***
WBSB	0.5	19.25 ± 0.69	16.97	3.30 ± 0.12	2.90	**
WBSB	1	9.58 ± 0.27	9.05	3.28 ± 0.09	3.10	n.s.
ASB	0.25	41.25 ± 1.56	46.67	3.53 ± 0.13	3.99	**
ASB	0.5	26.27 ± 1.55	26.04	4.50 ± 0.27	4.46	n.s.
ASB	1	12.29 ± 0.46	13.82	4.21 ± 0.16	4.73	**

Figure legends

Fig. 1. (a) Male whitebellied sunbird (*Cinnyris talatala*) and (b) male amethyst sunbird (*Chalcomitra amethystina*).

Fig. 2. 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 at three ambient temperatures (mean ± SE). The equations and R^2-values of the power functions are: whitebellied sunbirds: 5˚C: $y=1.15x^{-0.95}$, $R^2=0.99$; 15˚C: $y=0.92x^{-0.95}$, $R^2=0.99$; 25˚C: $y=0.88x^{-0.98}$, $R^2=0.99$; amethyst sunbirds: 5˚C: $y=0.94x^{-0.84}$, $R^2=0.98$; 15˚C: $y=0.75x^{-0.86}$, $R^2=0.99$; 25˚C: $y=0.79x^{-0.91}$, $R^2=0.98$.

Fig. 3. 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. 4. 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. 5. 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

(a)

Tim Jackson

(b)

Warwick Tarboton

Fig. 1.

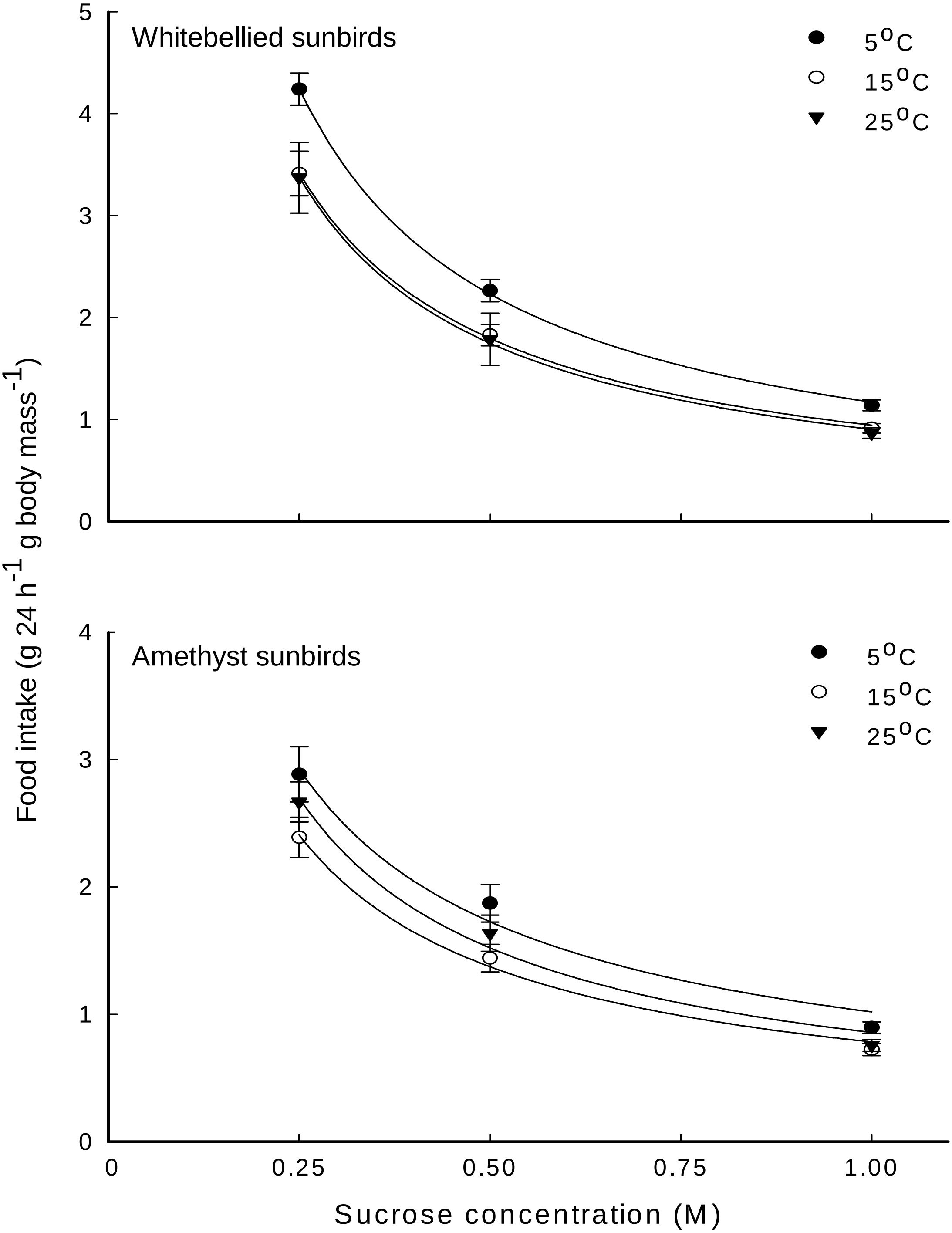
Food intake (g 24 h^{-1} g body mass^{-1})
Whitebellied sunbirds
● 5°C
○ 15°C
▼ 25°C
5
4
3
2
1
0
Amethyst sunbirds
● 5°C
○ 15°C
▼ 25°C
4
3
2
1
0
0
0.25
0.50
0.75
1.00
Sucrose concentration (M)

Fig. 2.

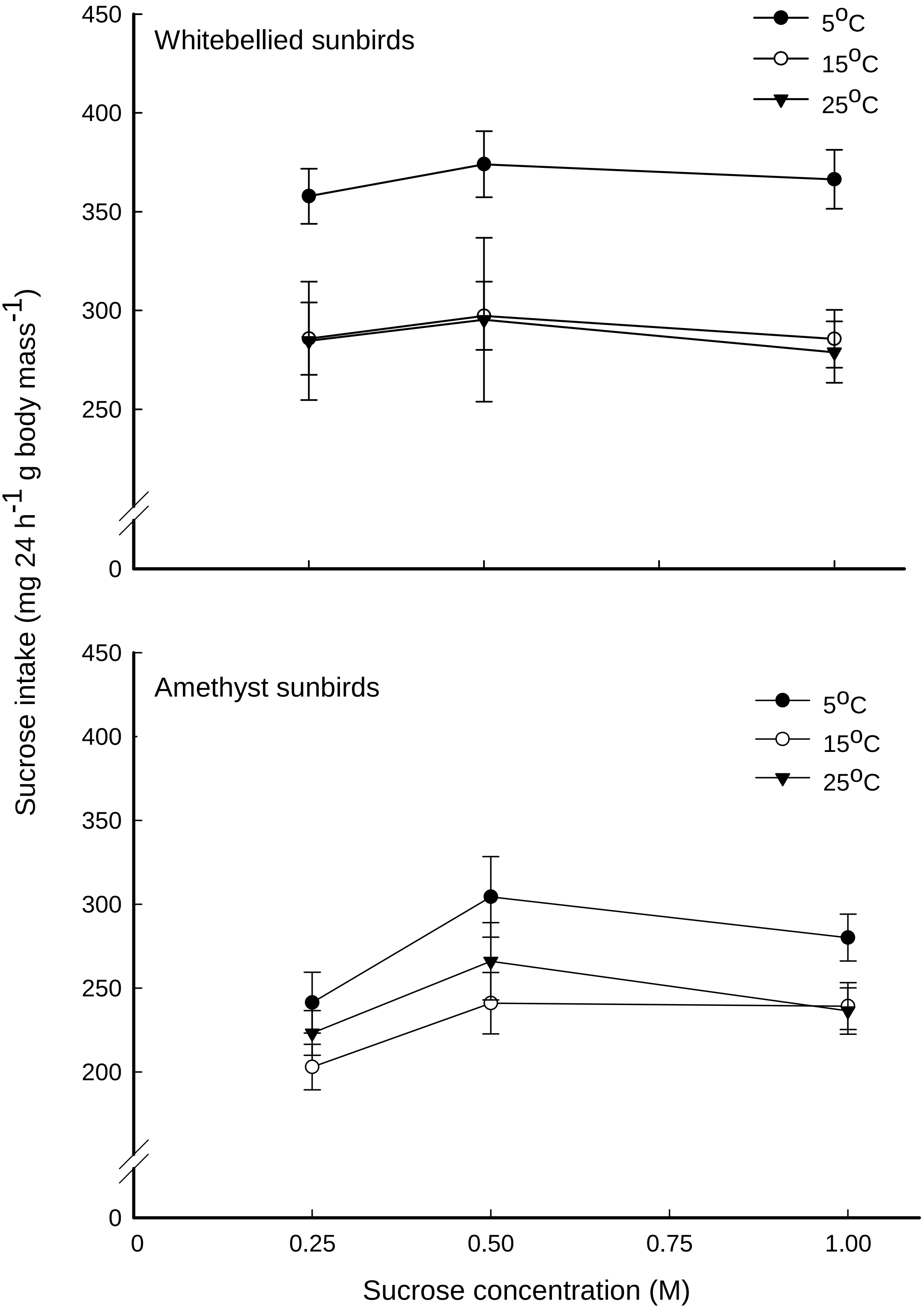
Whitebellied sunbirds
Amethyst sunbirds
5oC
15oC
25oC
Sucrose intake (mg 24 h-1 g body mass-1)
450
400
350
300
250
200
0
0
0.25
0.50
0.75
1.00
Sucrose concentration (M)

Fig. 3.

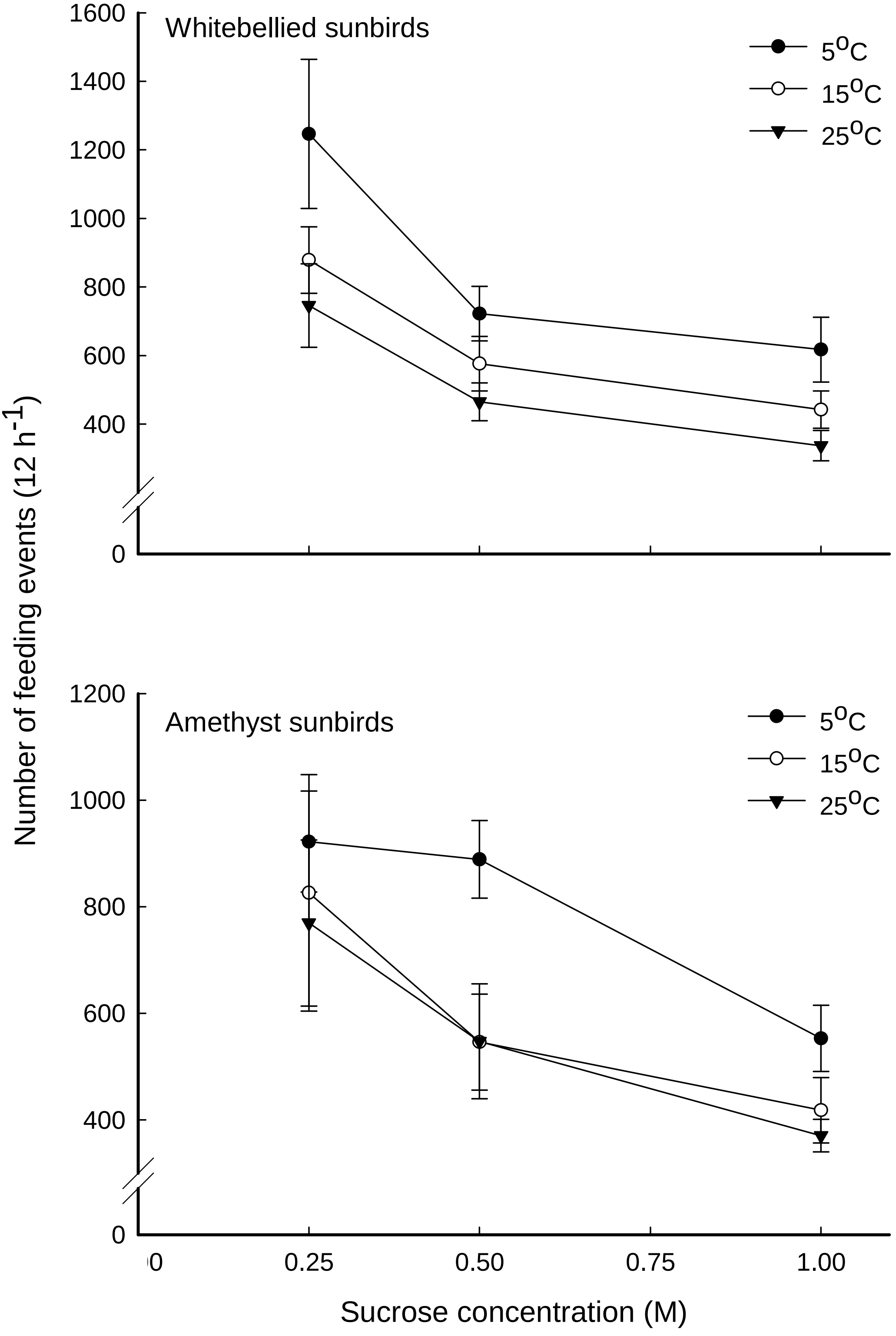
Number of feeding events (12 h^{-1})
Whitebellied sunbirds
1600
1400
1200
1000
800
600
400
0
5°C
15°C
25°C
Amethyst sunbirds
1200
1000
800
600
400
0
5°C
15°C
25°C
0
0.25
0.50
0.75
1.00
Sucrose concentration (M)

Fig. 4.

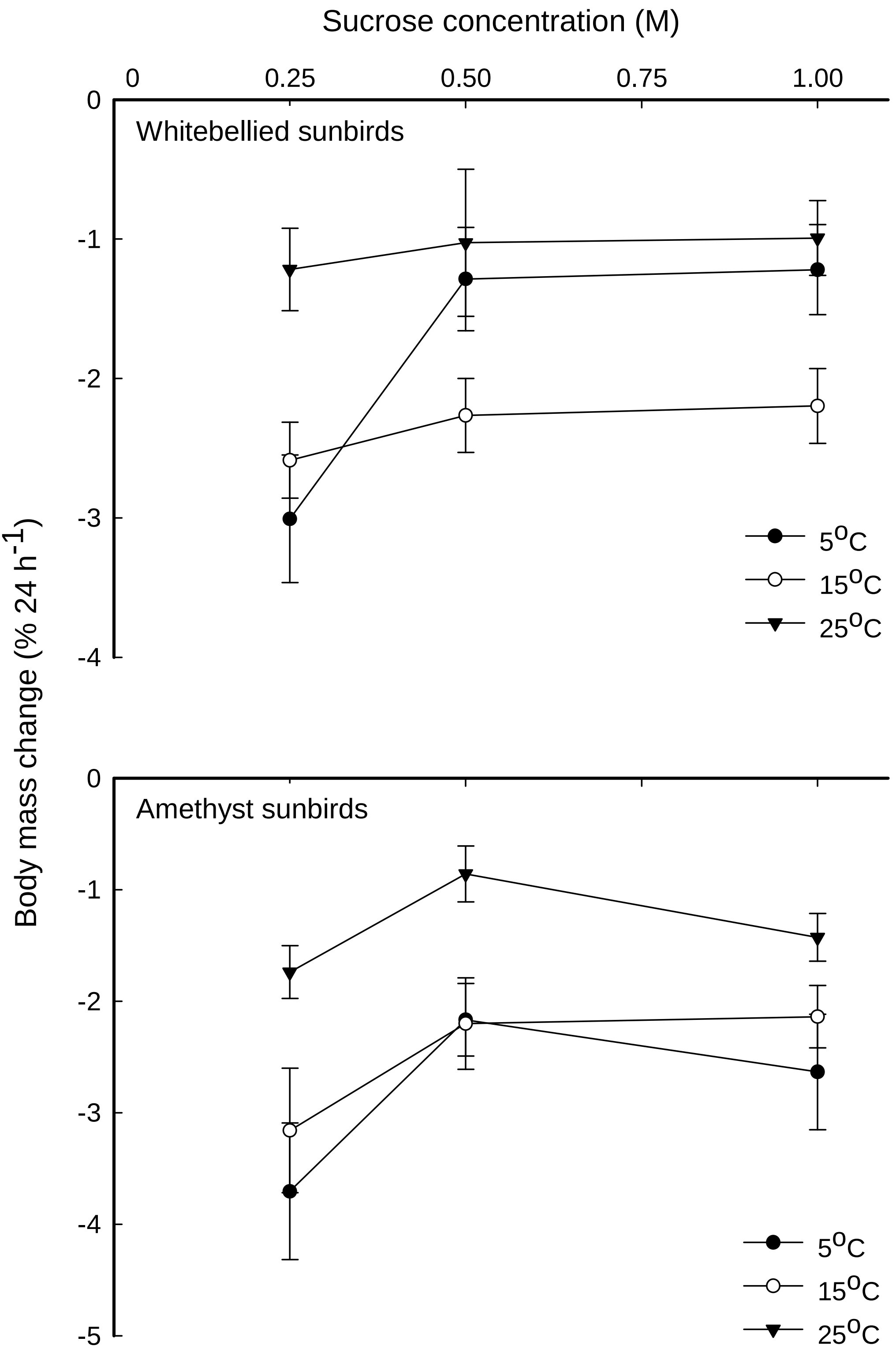
Sucrose concentration (M)
0
0.25
0.50
0.75
1.00
Body mass change (% 24 h^{-1})
Whitebellied sunbirds
0
-1
-2
-3
-4
5°C
15°C
25°C
Amethyst sunbirds
0
-1
-2
-3
-4
-5
5°C
15°C
25°C

Fig. 5.