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RESEARCH ARTICLE

Effect of diet quality and ambient temperature on the use of torpor by two species of neotropical nectar-feeding bats

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ABSTRACT

Neotropical bats use torpor as a strategy to save energy when they experience a low energy intake and/or low ambient temperature (T_a). Digestive physiology limits the energy intake of several glossophaginid bats, and could play an important role in the onset of torpor in these tropical animals. We measured the effect that diet quality and T_a had on the use of torpor by the nectar-feeding bats *Glossophaga soricina* and *Leptonycteris yerbabuenae*. Captive bats were fed with 5% (low) or 35% (high) sucrose solutions while exposed to two different T_a (17.7 and 23.2°C; low T_a and high T_a) in four different treatments: (1) high sucrose:high T_a, (2) high sucrose:low T_a, (3) low sucrose:high T_a and (4) low sucrose:low T_a. We measured their energy intake, changes in body mass (ΔM_b) and skin temperature (T_{skin}) as response variables. Energy intake (in 10 h) was limited when both species fed on 5% sucrose, but body mass gain was only affected in *G. soricina*. Energy intake and T_a had a negative effect on the minimum T_{skin} of both species, and ΔM_b affected the time that *G. soricina* used torpor. Both species remained normothermic on the high sucrose:high T_a treatment, but used torpor on the other three treatments. Bats used torpor during their resting and activity periods. *Leptonycteris yerbabuenae* spent more time in torpor in the low sucrose:high T_a treatment, while *G. soricina* used this strategy for longer periods of time in the high sucrose:low T_a treatment. We found that diet quality and T_a played an important role in the use of torpor by nectar-feeding bats.

KEY WORDS: T_a, Glossophaginid bats, Nectar, Neotropics, Physiological constraints, Endothermy

INTRODUCTION

The evolution of homeothermic endothermy allowed animals to maintain optimized physiological processes regardless of changes in ambient temperature (T_a) (Crompton et al., 1978). It also improved their ability to invade energetically demanding niches (Ruben, 1995). The emergence of this physiological process gave rise to important challenges associated with maintaining the energetic costs of a high body temperature (Nagy et al., 1999). As a result, a large number of species that show homeothermic endothermy have evolved physiological mechanisms to reduce their energy expenditure. Torpor is one of these strategies, involving a short-term reduction in the animals' metabolic rate from 5% to 30% below its basal values (Geiser and Ruf, 1995; Geiser et al., 2004; Dikic et al., 2008). In this state, animals can reduce their normal body temperature by as much as 27°C, and often become non-responsive to most external stimuli (Wang, 1987). By using torpor, animals are able to save energy, which could help them survive challenging environmental conditions (Lovegrove et al., 1999; Körtner and Geiser, 2000; Warnecke et al., 2008). The use of torpor varies in terms of both the minimal body temperature reached by animals and the time spent in torpor (Wang and Wolowyk, 1988).

Torpor evolved in animals inhabiting high latitudes, mainly as a response to the cold temperatures present during the autumn and winter seasons (Hainsworth and Wolf, 1970; Wang, 1988, 1989). Nevertheless, this mechanism is also present in birds and mammals that are endemic to tropical regions (e.g. Bartels et al., 1998; Körtner and Geiser, 2000, 2009; Geiser and Stawski, 2011), where minimal temperatures tend to be above 20°C (McKnight and Hess, 2000). In these sites, torpor is a physiological response helping animals to cope with other environmental conditions, like low food availability/quality, not just low ambient temperatures (Cruz-Neto and Abe, 1997). Kelm and von Helversen (2007) found that energy limitations triggered the use of torpor in the bat *Glossophaga soricina* in response to limited energy supply, and the depth of torpor was dependent on the body condition of the animals. These results imply that tropical species use torpor to survive changes in food sources like nectar and fruit, which tend to vary widely in their quality and abundance in both space and time (Heithaus et al., 1975; Dinerstein, 1986; Cruz-Neto and Abe, 1997; Pereira et al., 2010).

Neotropical nectar-feeding bats (like *G. soricina*) are small-sized animals that live on the verge of a negative energetic balance (Cruz-Neto and Abe, 1997; von Helversen and Winter, 2003; Ayala-Berdon et al., 2008). Nectar abundance and quality and the T_a they confront in the field limit their energetic budget. Ayala-Berdon et al. (2008) found that the energy intake of three species of bats became limited when they fed on dilute nectar, and that in one species, this reduction in energy intake increased when facing low T_a (Ayala-Berdon et al., 2009). While the effects that energetic constraints related to sugar concentration (Ramírez P et al., 2005; Ayala-Berdon et al., 2008, 2009, 2013; Ayala-Berdon and Schondube, 2011), nectar availability (Kelm and von Helversen, 2007) and foraging behavior (Ayala-Berdon et al., 2011) have on the physiological ecology of bats have been evaluated in the past, the role that the interaction between diet quality, which could limit energy acquisition (Martínez del Rio et al., 2001; Ayala-Berdon et al., 2008, 2009), and T_a has on the use of torpor in tropical nectar-feeding bats remains poorly explored (but see Kelm and von Helversen, 2007).

In this study, we evaluated the effects that diet quality and T_a have on the use of torpor by two species of nectar-feeding bats: *G. soricina* (10.5±0.78 g) and *Leptonycteris yerbabuenae* (22.9±1.17 g). These species show important differences in digestive

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physiology that affect their capacity to obtain energy when feeding on diets of different energy content. While *L. yerbabuenae* reduces its energy intake by about 30% when nectar sugar concentration decreases from 35% to 5%, *G. soricina* decreases its energy intake by 60% in the same range of sugar concentrations (see Ayala-Berdon et al., 2008, 2009; Ayala and Schondube, 2011). In these species, the differences in monosaccharide absorption/transport rates and gut size are the main mechanisms controlling the amount of energy that the animals are able to process per unit of time (Martínez del Rio, 1990; Hernández and Martínez del Rio, 1992; Ayala-Berdon et al., 2008). We hypothesized that both species should use torpor when their physiological constraints limit their energetic intake to close-to or below the energetic costs imposed by T_a. However, because of dissimilarities in digestive physiology and body mass, we expected to find differences in the use of torpor by these two tropical bat species. Because of its lower digestive capacity (Ayala-Berdon et al., 2008; Ayala and Schondube, 2011), *L. yerbabuenae* should be more prone to using torpor than *G. soricina* when feeding on low-quality diets when T_a is not a limiting factor (i.e. high T_a). Furthermore, because of its smaller body mass, and the consequent impact on metabolic costs, we expected *G. soricina* to exhibit torpor more frequently than *L. yerbabuenae* at low T_a, regardless of food quality. We also predicted that energy intake and the capacity to maintain a constant body mass for both species should be more affected at low T_a (Ayala-Berdon et al., 2009).

MATERIALS AND METHODS

Bat care and housing

During April 2012 we captured 10 adult non-reproductive male individuals of each bat species with the use of mist nets in the Chamela-Cuixmala Biosphere Reserve, Mexico (19°22′–19°35′N, 104°56′–105°03′W). Once captured, individuals were kept for 5 days, during which we monitored their body mass and food intake every 12 h to determine their adaptation to captivity. All individuals adapted well to these conditions (they fed by themselves and did not showed changes in body mass). From these individuals, we randomly selected five animals of each species, and transferred them to a laboratory facility at the Institute of Ecosystem Research and Sustainability (IIES) of the National University of Mexico (UNAM), located in the city of Morelia, Michoacán, Mexico (19°38′53.91″N, 101°13′44.31″W). We used only five individuals of each species because of permit restrictions.

Bats were maintained in two colonies of five individuals, separated by species, under controlled conditions (12 h light:12 h dark cycle, 25°C temperature and 30% humidity, 1.5×2×2 m flight cage), and were fed with an aqueous diet of 16.9% ripe banana (mass %), 2.6% mixed grain baby cereal (Gerber, Fremont, MI, USA), 1.9% full cream powdered milk (Nido Clásica, Nestlé, Vevey, Switzerland) and 1.3% sucrose with a 0.3% vitamin supplement (Nekton-S, Guenter Enderle, Tarpon Springs, FL, USA). This diet was designed by Mirón M et al. (2006), and allowed us to maintain colonies of these species for up to 8 months previously (see Ayala-Berdon et al., 2008, 2009; Rodríguez-Peña et al., 2007). Bat activity was monitored using infrared video (DigiOpG2®, USA). During the light part of the daily cycle, bats were maintained in the dark, but the area where we kept them in captivity experienced a small increase in light conditions. Individuals were acclimated for 2 weeks prior to the beginning of our experiments. To asses the health and body condition of the bats, all individuals were marked with a numbered plastic collar and were weighed daily (±0.01 g Ohaus®, Burlington, NC, USA). No bats died during our experiments. All bats maintained their body mass and health condition (activity patterns did not vary, membrane elasticity was maintained and bats did not exhibited hair loss; following Barnard, 2009), and were released at the capture site after 2 months when our experiments concluded.

Use of torpor

Before conducting our torpor experiments, we determined the normothermic zone of our experimental bats. Normothermy is defined as 'the condition of a temperature regulator when its core temperature is within ±1 s.d. of the range associated with the normal post-absorptive resting condition of the species in the thermoneutral zone' (IUPS Thermal Commission, 2001). We determined normothermy for bats individually in small containers that limited their movement to changes position but did not allow movement from one place to another or flying. We measured their temperature for 30 min, 1 h after they fed, at a T_a of 25°C. Additionally, we offered them food, by placing a feeder inside the container, and monitored their temperature during 24 h under the same conditions. The feeder contained a sugar solution of 17% sucrose, as this is the mean sugar concentration found in the chiropterophilic plants they consume in the wild (Rodriguez-Peña et al., 2007, 2016). All bats were video-recorded to assess their behavior. All individuals were awake and feeding during the activity period, and resting during the resting period. We separated body temperature data for the resting (06:00 h to 20:00 h) and the activity period (20:00 h to 6:00 h) and compared the values using a *t*-test. We did not find differences between the post-absorptive body temperature and the body temperature when the bats were offered food ($t_{1,21}$=0.21, P=0.82 and $t_{1,21}$=0.74, P=0.46 for *G. soricina* and *L. yerbabuenae*, respectively). Additionally, we found no differences in body temperature between the resting and the active phases under the above-mentioned conditions ($t_{1,570}$=0.99, P=0.31 and $t_{1,573}$=0.26, P=0.79 for *G. soricina* and *L. yerbabuenae*, respectively).

To determine the role that diet quality and T_a had on the use of torpor, bats were transferred into individual flight cages (1.5×2×2 m), which were monitored by the infrared video system. We defined torpor as a decrease in body temperature to several degrees below normothermic temperature, accompanied by a lack of movement (for details, see 'Data analysis', below). During the experiments, each bat was fed with a sucrose solution of either 5% (w/v, low sucrose) or 35% (high sucrose) sugar concentration (following Ayala-Berdon et al., 2008, 2009), and exposed to one of two T_a: 23.2°C (high T_a) or 17.7°C (low T_a). T_a varied during our experiments (23.2±1.29°C high T_a and 17.7±2.26°C low T_a). This variation occurred during the first hour of the experiment when we modified the temperature of the experimental room. During the rest of the experiment, the variation in temperature was low (0.4–0.7°C around the experimental temperature; Table 1). As a result, bats experienced a difference in temperature among treatments of at least 3.9°C. The sugar concentrations used in the experiments simulated the maximum and minimum nectar concentration found at the capture site (Chamela, Mexico; Rodriguez-Peña et al., 2007, 2016). The temperatures we selected for our experiments represented the minimum T_a registered at night in the warmest and coldest months of the year at the capture site (Rodríguez-Peña et al., 2007; Ayala-Berdon et al., 2009). While these temperatures may not represent extreme temperature conditions in relation to the T_a faced by these species along their geographic distribution, or those used in previous metabolic studies for Glossophaginid bats (Arends et al., 1995; Cruz-Neto and Abe, 1997; Kelm and von Helversen, 2007), they gave us important information on the responses of these two

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Table 1. Experimental design

Bat species	Sucrose concentration (%)	$T_{a,min}$ (°C)	n	$T_{skin,min}$
G. soricina	5	17.73±0.63	5	27.39
		23.16±0.41	5	28.53
	35	17.73±0.56	3	28.81
		23.16±0.59	4	29.94
L. yerbabuenae	5	17.73±0.66	4	22.76
	5	23.16±0.72	4	28.62
	35	17.73±0.70	5	23.05
	35	23.16±0.44	5	33.70

Experiments were designed to evaluate torpor of *Glossophaga soricina* and *Leptonycteris yerbabuena* feeding on nectar sugar concentrations of either 5% or 35% sucrose, and exposed to either high (23.2°C) or low (17.7°C) ambient temperature (T_a). Because of some technical problems with our receiver (data not recorded), the number (n) of bats used in each treatment varied. $T_{a,min}$, minimum T_a; $T_{skin,min}$, minimum skin temperature.

species to the real ecological conditions faced in the field by the individuals from the populations used in this study.

We decided to use sucrose for our experimental solutions with the purpose of linking the digestive enzymatic activity of the bats with their capacity to obtain energy. Because sucrose cannot be assimilated without first being hydrolyzed by the enzyme sucrase (Sunshine and Kretchmer, 1964; Sestoft, 1983; Martínez del Rio and Stevens, 1988), using sucrose allowed us to directly relate the differences in energy intake shown by the two species when facing different sugar concentrations (Ramírez P et al., 2005; Ayala-Berdon et al., 2008, 2009; Ayala-Berdon and Schondube, 2011), with the existence of a digestive constraint. Both species present a statistically significant digestive constraint (Ayala-Berdon et al., 2008), which can be described using the slope of the log–log relationship between volumetric intake and sugar concentration, and is directly linked, in these species, to the activity of the enzyme sucrase (Martínez del Rio et al., 2001; Ayala-Berdon et al., 2008). Animals exhibiting slope values equal to −1 have a perfect compensation, and an energy intake that is independent of sugar concentration. In contrast, animals with slope values larger than −1 show a positive relationship between sugar ingested and sugar concentration in food (see Martínez del Rio et al., 2001, for details). In previous work, *G. soricina* was shown to have an intake response slope value of −0.76±0.064, while that of *L. yerbabuenae* was −0.61±0.040 (means±s.e.; Ayala-Berdon et al., 2008; Ayala-Berdon and Schondube, 2011).

We used the two sucrose concentrations and the two T_a to generate four treatments that represented different ambient conditions: (1) high sucrose:high T_a, (2) high sucrose:low T_a, (3) low sucrose:high T_a and (4) low sucrose:low T_a. This design allowed us to determine the effects of diet quality and T_a, and the interaction of these two factors in determining the use of torpor by both bat species. Each treatment lasted 3 days, because this is the maximum time that we could keep bats under the low sucrose:low T_a conditions (experiment 4), without negatively affecting their physical condition (Barnard, 2009). We used the same bat individuals in all the treatments. Experimental solutions were offered *ad libitum* in artificial feeders during the activity period of each of the 3 days for each experiment (20:00 h to 06:00 h), and were removed during the rest of the day. Because both bat species are unable to achieve compensatory feeding as a result of digestive constraints (see above), our experimental design with food *ad libitum* constrained their energetic intake when they were supplied with the 5% sucrose solution (see Results). We determined the energy intake (kJ 10 h^{-1}) of each bat during the different experiments by calculating the energetic content of the nectar consumed by bats in each experimental trial (16.6 kJ energy g^{-1} of sugar ingested following Judkin et al., 1971). We measured food intake by weighing the feeders at the beginning and the end of each experimental trial. Feeders were equipped with a leak trap to account for losses caused by the visiting bats. Additionally, each night we placed a feeder full of sugar at each concentration outside the flight cages to control for evaporation and changes in sugar concentration. Control feeders were weighed at the beginning and end of each trial, and the concentration of the sugar solution was measured using a hand-held refractometer (Reichert 10431 0–50 deg compensated Brix temperature, Leica, USA). No changes in volume or concentration were observed in our control feeders. Because experimental sugar solutions lacked nitrogen sources, bats rested for 2 days at the end of each experimental treatment. During this period, animals were maintained at a T_a of 25°C and fed with the maintenance diet described above, which guaranteed adequate nutrition and allowed them to maintain a good physical condition.

In addition to energy intake, we used skin temperature (T_{skin}) and changes in bat body mass between the beginning and end of each 3 day experiment (ΔM_b) as response variables to our experiments. T_{skin} allowed us to determine the use of torpor, and was obtained using temperature transmitters and a data-logger receiver (Pip Ag376 w/thermistor transmitter, SRX800-D receiver, Lotek®, Newmarket, Ontario, Canada). Transmitters were glued to a shaved area of the bat's back between the shoulder blades using surgical glue (Skin-Bond, Smith & Nephew, Inc., Largo, FL, USA). These transmitters vary the number of pulses they emit in response to changes in temperature. We related the number of pulses that each transmitter emitted at different temperatures by placing the transmitter on a heat plate with a known temperature (measured using a laboratory mercury thermometer: 0–50°C; PC-420D stirring hot plate, Corning®, NY, USA). To determine transmitter accuracy, each transmitter was tested three times, with a temperature gradient from 20 to 40°C. There was a very small variation in the measurements of the transmitters used in our experiments (r^2 values of regression lines between number of pulses and temperature were equal to or higher than 0.98). Transmitters were programmed to emit continuous pulses that allowed us to register T_{skin} every 5 min during the 3 day duration of each experiment. However, the data-loggers captured noise and sometimes recorded inaccurate data (~5–10% of the total measurements). We excluded these data from our analyses. For this reason, we report different degrees of freedom for the different treatments in the Results. Additionally, because of some technical problems with our receiver (i.e. data not recorded), the number of individual bats we included in our analyses varied for the different treatments (Table 1).

Bats were captured and used in experiments with permission from the Dirección General de Vida Silvestre, Mexico to J.E.S. (FAUT-0193). Procedures and animal management were conducted following the official Mexican guidelines for the care and use of laboratory animals (NOM-062-ZOO-1999), and approved by the Dirección General de Vida Silvestre, Mexico, and the IIES-UNAM Ethics Committee.

Data analysis

For both bat species, we evaluated the role that sugar concentration and T_a had on energy intake, and the capacity of bat individuals to gain body mass (ΔM_b) and use torpor (minimum skin temperature during each experiment, $T_{skin,min}$). We used the mean T_a registered during each experiment to conduct our analyses (Table 1). First, we determined the intake response to changes in sugar concentration (following Ayala-Berdon et al., 2008). Briefly, we estimated the

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slopes and intercepts of the relationship between food intake and sugar concentration with least squares regression analysis on the log-transformed data for each individual bat. We used an ANCOVA to account for differences in the intake responses among individuals, and between species. We then compared the value of the intake response slope with the −1 value expected from compensatory feeding, using a one-sample *t*-test (following Martínez del Rio et al., 2001). Additionally, we determined the effect of diet quality on energy intake using a mixed-effect model. Because energy intake was strongly related to sugar concentration in the diet (see Results), we used sugar intake as a measure of the bats' responses to the two experimental diets in the rest of our analyses. Second, we used mixed-effect models to analyze: (1) the effect of T_a on energy intake and (2) the effect of energy intake on the ΔM_b experienced by the bats. Third, we investigated the effect of energy intake, ΔM_b and T_a on $T_{skin,min}$ reached by the bats. Because energy intake and ΔM_b were correlated (see Results), we investigated the effect of each variable on $T_{skin,min}$ in separated models to prevent collinearity between explanatory variables. Finally, we used another mixed-effect model to determine the role that energy intake, ΔM_b and T_a had on the duration (in min) of torpor in individual bats. In the analyses in which we looked for an effect of energy intake and T_a on $T_{skin,min}$, we searched for an interaction between the effects of energy intake and T_a; however, we did not find any significant interactions and removed the interaction factor from our models. Mixed-effect models were designed based on the results of Ayala-Berdon et al. (2008, 2009), who found that the physiological constraints of both bat species were properly determined by analyzing the individual intake responses of the bats (*sensu* Martínez del Rio et al., 2001) to changes in sugar concentration and T_a. We included bat identity as a random factor to account for our repeated measurement design. In all models, we nested the factor day within the random effect (individual). Each model was conducted separately for each bat species.

Because of our small sample size, in addition to the previously described mixed-effect models, we used randomized resampling tests. We created 1000 data sets for each of our models, and extracted the alpha values obtained from the analysis of each data set. We calculated the percentage of alpha values that were lower than 0.05. We considered the result of our initial analysis to be significant if ≥70% of the estimated alpha values obtained during the randomized resampling tests were lower than 0.05 (following Wetzels et al., 2011).

Because the use of torpor by tropical animals is a controversial topic (McNab, 1969; Audet and Thomas, 1997; Cruz-Neto and Abe, 1997), we considered animals to be using torpor when their T_{skin} dropped below 34°C. When we determined the normothermic zone of our experimental bats, we found that they showed a small variation in their T_{skin} (37.55±1.41 and 37.66±1.15 for *G. soricina* and *L. yerbabuenae*, respectively). For our analyses, we divided each trial into periods of 12 h (i.e. the resting phase during the day and the activity phase during the night), and compared whether the mean T_{skin} of each period was below the temperature of 34°C using a one-sample *t*-test. Comparisons were conducted individually for each bat on each day of treatment.

To estimate the frequency and duration of torpor, we counted the number of minutes each bat was under the temperature limit of 34°C for each day in each of the treatments where we detected torpor. Additionally, we modeled the thermic responses of bats exposed to the different treatments to determine the time elapsed between the resting or activity periods and the onset of torpor. For this analysis, we conducted generalized additive models of T_{skin} as a function of time (first, second or third experimental day) and treatment. This modeling procedure uses a smooth function to predict the response variable when the exact parametric form of the response is complex or unknown. We modeled T_{skin} as a smooth function of the interaction between treatment and time. We used the smooth factor (f_s) as a smoothing basis to allow modeling of the interaction between factor (treatment) and the numeric variable (time). We set the knot value of 20 to model the maximum number of inflections in the thermic response experienced by bats during each treatment. The two species were analyzed using separate models. All analyses were carried out in R 3.0.2 (mixed models using function *lme*; additive models using function *gam* library *mgcv*; http://www.R-project.org/). Values in our results are given as means±s.e.m., unless noted otherwise.

RESULTS

Effect of diet quality and T_a on energy intake

The two species of bats increased their volumetric intake when the sugar concentration in the experimental solutions decreased (5%; regression formulas: log food intake=−0.78 log concentration+2.08 and log food intake=−0.57log concentration+2.1 for *G. soricina* and *L. yerbabuenae*, respectively); however, they were unable to achieve compensatory feeding. The slope of the relationship between sugar concentration and food intake differed significantly from the compensatory feeding value of −1 in both bat species ($t_{1,4}$=−5.18, P=0.006 and $t_{1,3}$=−7.17, P=0.005 for *G. soricina* and *L. yerbabuenae*, respectively). The intake responses did not differ among individual bats from each species ($F_{1,1}$=0.05, P=0.81 and $F_{1,1}$=0.008, P=0.92 for *G. soricina* and *L. yerbabuenae*, respectively); however, the intake responses differed among the two bat species ($F_{1,1}$=9.42, P=0.002).

Our mix-effect model analysis also showed that the energy intake of both bat species was affected by the sucrose concentration of the two experimental diets (*G. soricina*: $t_{1,36}$=5.65, P<0.001; and *L. yerbabuenae*: $t_{1,32}$=10.6, P<0.001; the randomized resampling test showed that 99% of the alpha values were <0.05 for both species; Fig. 1). *Glossophaga soricina* reduced its energy intake by 35.4% and 31.9% when facing a change in sugar concentration from 35% to 5% in the low and the high experimental T_a, respectively, while *L. yerbabuenae* exhibited a 64.5% and 69.1% reduction

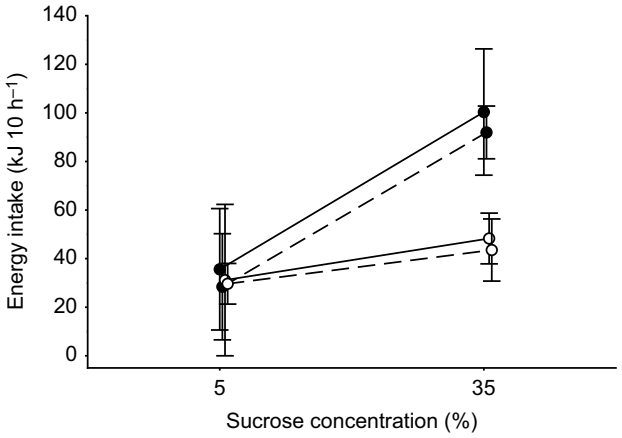

Fig. 1. Energy intake versus sucrose concentration for nectar-feeding bats at two ambient temperatures (T_a). Energy intake for *Glossophaga soricina* (open circles, n=5) and *Leptonycteris yerbabuenae* (filled circles, n=5) was positively related to sugar concentration (35% and 5%) at T_a 23.2±1.2°C (dashed line) and 17.7±2.2°C (solid line; P<0.01 for both). Data are presented as means±s.d.

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(Fig. 1). Neither of the bat species showed an effect of T_a on their energy intake ($t_{1,36}$=−1.46, P=0.15 and $t_{1,32}$=−1.05, P=0.3 for *G. soricina* and *L. yerbabuenae*, respectively).

Effect of energy intake, ΔM_b and T_a on $T_{skin,min}$ and time spent in torpor

We found an effect of energy intake on ΔM_b in *G. soricina* ($t_{1,46}$=3.78, P<0.01; the randomized resampling test showed that 85.5% of the alpha values were <0.05) but not in *L. yerbabuenae* ($t_{1,42}$=1.73, P=0.09). $T_{skin,min}$ was not affected by ΔM_b in either species ($t_{1,36}$=2.28, P=0.13 and $t_{1,32}$=1.98, P=0.16 for *G. soricina* and *L. yerbabuenae*, respectively). However, we found an effect of energy intake and T_a on $T_{skin,min}$ reached by both bat species (*G. soricina*: $t_{1,35}$=5.04 and 3.61 and *L. yerbabuenae*: $t_{1,31}$=4.99 and 3.97 for energy intake and T_a, respectively; all P<0.01; the randomized resampling tests showed that >87% of the alpha values were <0.05 for both species; Fig. 2). Finally, we found an effect of ΔM_b on the duration of torpor in *G. soricina* ($t_{1,7}$=3.42, P=0.01; 89.1% of the alpha values were <0.05 in the randomized resampling test; P=0.01). We did not find this effect in *L. yerbabuenae* ($t_{1,3}$=0.006, P=0.93).

Use of torpor

Bats of the two species remained normothermic when they were subjected to the high sucrose:high T_a treatment, and started to use torpor when they were exposed to the other three treatments (Fig. 3). Surprisingly, both species of bats used torpor not only during the resting period (day) but also at night between feeding bouts. For the resting period, two individuals of *G. soricina* used torpor in the high sucrose:low T_a and the low sucrose:high T_a treatments, and five individuals used torpor in the low sucrose:low T_a treatment. Similarly, one individual of *L. yerbabuenae* used torpor in the high sucrose:low T_a and the low sucrose:high T_a treatments, while three individuals used torpor in the low sucrose:low T_a treatment (Fig. 3, Table 2).

Some bats of both species also used torpor during the activity period, particularly when feeding at low T_a. For *G. soricina*, one individual used torpor between activity bouts in the high sucrose: low T_a treatment and two individuals in the low sucrose:low T_a treatment, while only one individual of *L. yerbabuenae* entered torpor during the activity period in the high sucrose:low T_a treatment (Fig. 3, all t-values are presented in Table 2; all P<0.001).

The generalized additive models adequately explained the thermal responses of both bat species during the four treatments (explaining 49.7% and 32% of the variance for *G. soricina* and *L. yerbabuenae*, respectively; Fig. 4). In both cases, we observed a pattern where the thermal curve of bats in the high sucrose:high T_a treatment was close to the normothermic zone and dropped to lower T_{skin} in the high sucrose:low T_a, the low sucrose:high T_a and the low sucrose:low T_a treatments. This indicates that bats reduced their T_{skin} when confronted with experimental conditions that reduced energy intake and/or temperature. The lowest T_{skin} reached by both bat species occurred with the low sucrose:low T_a treatment: *G. soricina* reached a $T_{skin,min}$ of 27.3°C (mean±s.d. 29.96±0.8°C) while *L. yerbabuenae* dropped its $T_{skin,min}$ to 22.76°C (mean±s.d. 25.69± 4.1°C; see Table 1). Time spent in torpor varied between species depending on the experimental treatment (Table 3). Both species reached their $T_{skin,min}$ near midday (12:00 h) and then slowly increased T_{skin} to their normothermic zones (Fig. 4).

DISCUSSION

We found that both species of glossophaginid bats reduced their energy intake when feeding on the low-quality diet. Additionally, bats used torpor when they faced the low sucrose concentration

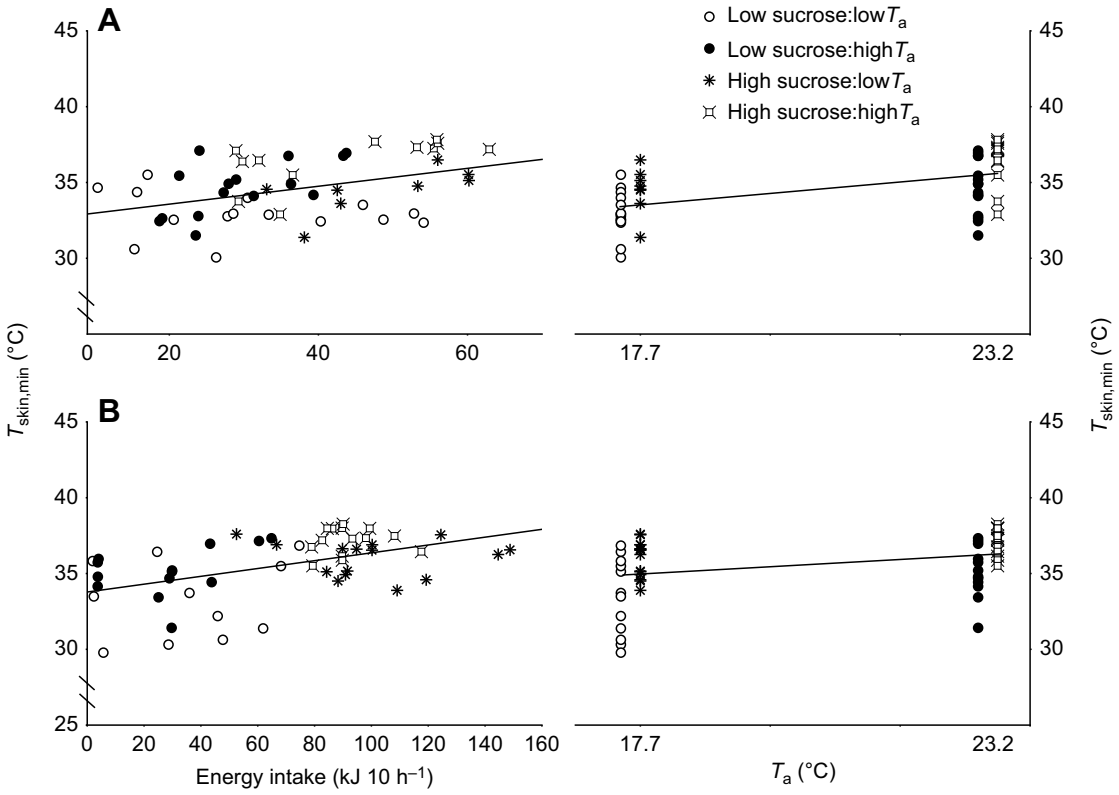

Fig. 2. Effect of energy intake and T_a on minimum skin temperature ($T_{skin,min}$). $T_{skin,min}$ of *G. soricina* (A; n=5) and *L. yerbabuenae* (B; n=5) versus energy intake (sucrose concentration; left) and T_a (right). Symbols in the right panel are displaced to show the effect of each treatment on $T_{skin,min}$.

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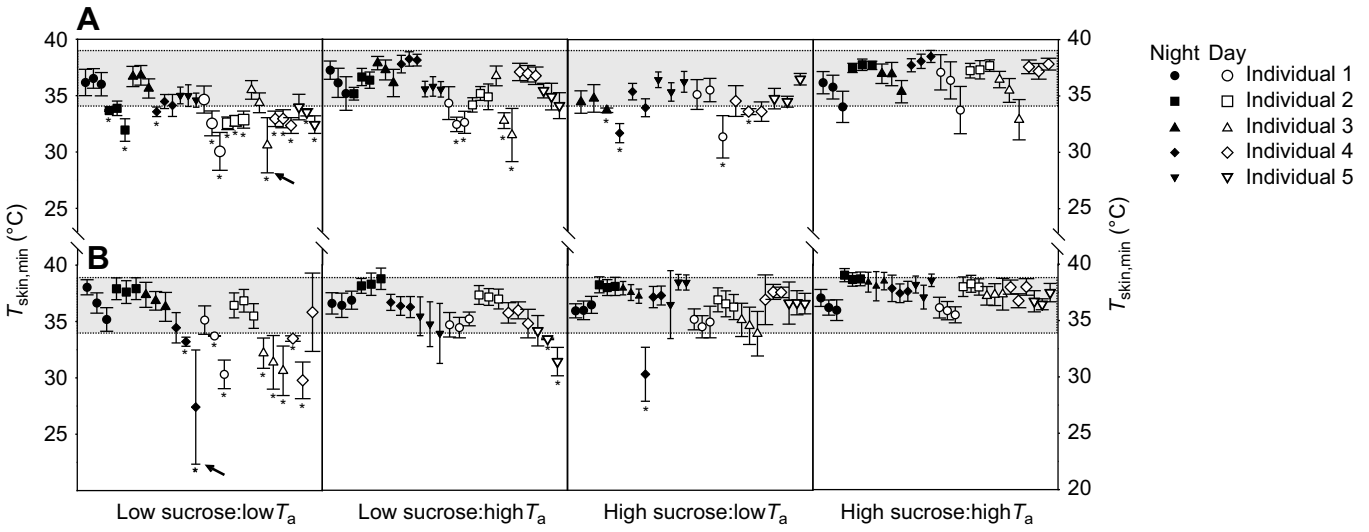

Fig. 3. Mean T_{skin} under the four experimental conditions. Nectar-feeding bats (A, *G. soricina*, *n*=5; B, *L. yerbabuenae*, *n*=5) were exposed to low or high T_a in combination with low or high energy (sucrose) intake. The relationships to the bats' resting periods are shown to highlight the effect of energy intake on the use of torpor in these species. We considered that bats used torpor when their mean T_{skin} was statistically different (asterisks) from the normothermic value of 34°C in each period. The gray shaded area represents normothermia. Means±s.e.m. are plotted consecutively for each individual bat for the first, second and third day of the experiment. Arrows indicate the minimum T_{skin} registered for each species. Statistical values are presented in Table 2.

(5%) and/or the low T_a (17.7°C) treatment. We found no interaction between the effects of diet quality and T_a in the use of torpor by both species. The physical condition of the bats (ΔM_b) negatively affected the duration of torpor in *G. soricina*, but this was not the case in *L. yerbabuenae*. As we predicted, the capacity to obtain energy, limited by the digestive physiology of the bats (sucrase

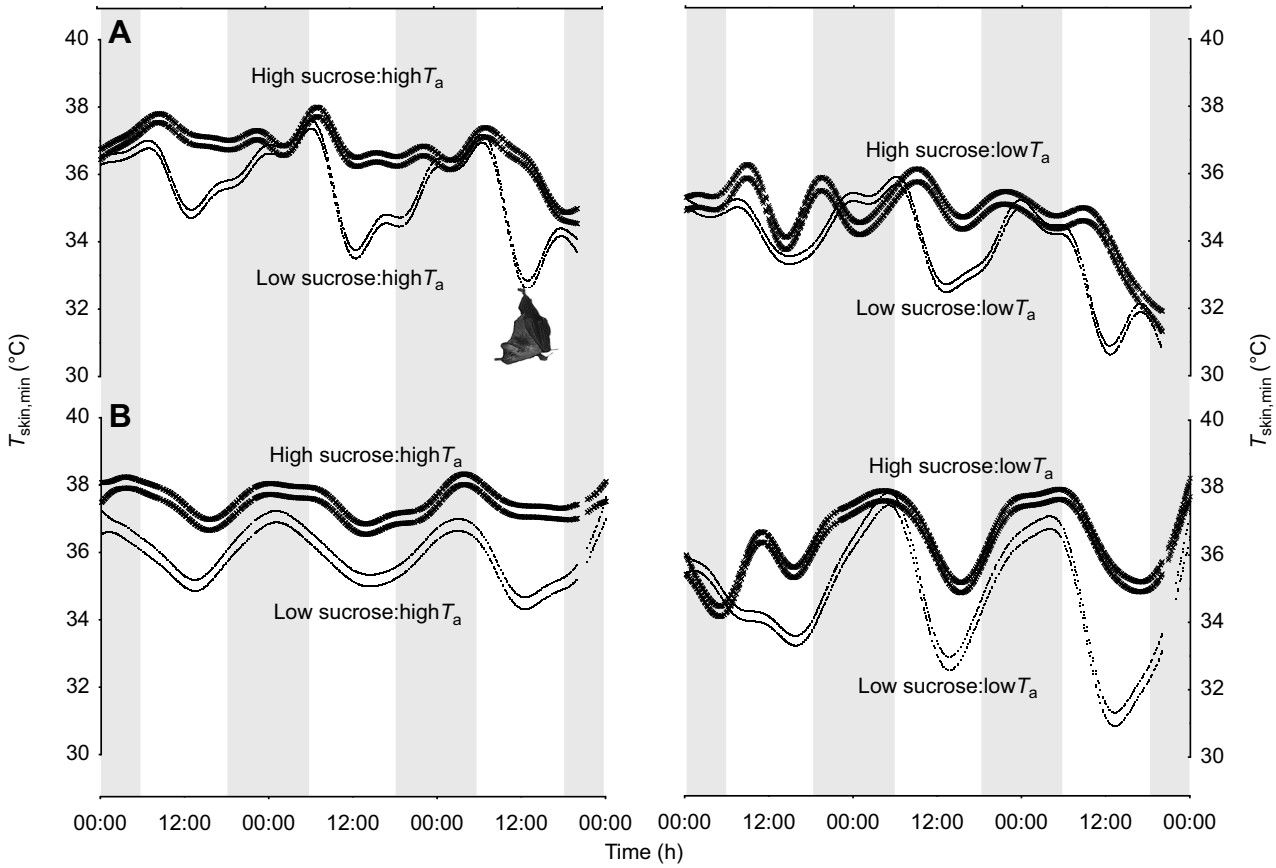

Fig. 4. Modeling the thermic responses to the four experimental treatments. Nectar-feeding bats (A, *G. soricina*, *n*=5; B, *L. yerbabuenae*, *n*=5) were exposed to low or high T_a in combination with low or high energy (sucrose) intake. T_{skin} was modeled using generalized additive models. Each model includes the bats' responses to the four treatments; however, we separated the graph into two panels to show the data clearly. The two lines represent the 95% confidence intervals of the mean for each thermic response.

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Table 2. Results of one-sample *t*-tests performed on the mean T_{skin} of *G. soricina* and *L. yerbabuenae* in the different experimental treatments

Bat species	Treatment	Individual	Day (period)	*t*
G. soricina	High sucrose:low T_a	1	3 (d)	$t_{1,121}$=15.42
		3	3 (n)	$t_{1,107}$=3.10
		4	2 (d)	$t_{1,109}$=4.72
	Low sucrose:high T_a	1	2 (d)	$t_{1,74}$=8.18
			3 (d)	$t_{1,101}$=6.97
		3	2 (d)	$t_{1,99}$=7.15
			3 (d)	$t_{1,114}$=11.33
	Low sucrose:low T_a	1	2 (d)	$t_{1,99}$=13.24
			3 (d)	$t_{1,96}$=23.12
		2	1 (d)	$t_{1,104}$=26.59
			1 (n)	$t_{1,79}$=3.82
			2 (d)	$t_{1,101}$=26.47
			3 (d)	$t_{1,108}$=14.94
			3 (n)	$t_{1,92}$=13.29
		3	3 (d)	$t_{1,99}$=13.90
		4	1 (d)	$t_{1,96}$=10.59
			1 (n)	$t_{1,96}$=2.89
			2 (d)	$t_{1,111}$=10.11
			3 (d)	$t_{1,98}$=14.53
		5	2 (d)	$t_{1,102}$=3.66
			3 (d)	$t_{1,87}$=8.26
L. yerbabuenae	High sucrose:low T_a	4	1 (d)	$t_{1,89}$=12.08
			2 (n)	$t_{1,97}$=4.02
			3 (n)	$t_{1,107}$=10.84
	Low sucrose:high T_a	5	2 (d)	$t_{1,73}$=2.50
			3 (d)	$t_{1,57}$=16.91
	Low sucrose:low T_a	1	2 (d)	$t_{1,111}$=2.91
			3 (d)	$t_{1,84}$=26.80
		3	1 (d)	$t_{1,92}$=7.44
			2 (d)	$t_{1,87}$=10.34
			3 (d)	$t_{1,96}$=14.65
		4	2 (d)	$t_{1,105}$=4.24
			3 (d)	$t_{1,96}$=24.76

Bats were fed on nectar sugar concentrations of either 5% (low) or 35% (high) sucrose at a T_a of 23.2°C (low) and 17.7°C (high). We divided each experiment into periods of 12 h [day (d)/night (n)], and interpreted bats as using torpor when the $T_{skin,min}$ of each period was below the normothermic temperature of 34°C. No bats entered torpor in the high sucrose:high T_a treatment. Only significant values are presented: P<0.01 for all.

Table 3. Time spent in torpor by *G. soricina* and *L. yerbabuena* in the different experimental treatments

Bat species	Treatment	No. of bats using torpor	Time in torpor (min)
G. soricina	High sucrose:low T_a	2	169±173.9
	Low sucrose:high T_a	2	150±110.3
	Low sucrose:low T_a	5	287±317.4
L. yerbabuenae	High sucrose:low T_a	1	80.5±94
	Low sucrose:high T_a	1	174.5±140.7
	Low sucrose:low T_a	3	127±176

Bats were fed a sucrose solution of either 5% (low) or 35% (high) sucrose and exposed to one of two T_a: 23.2°C (high) or 17.7°C (low). Time in torpor is given as means±s.d. Bats remained normothermic in the high sucrose:high T_a treatment.

activity), played an important role in the use of torpor in both species when they faced high T_a, with the species that had the highest digestive limitation (*L. yerbabuenae*) using torpor for longer periods of time. Additionally, *G. soricina* was more sensitive to T_a, using torpor for more time than *L. yerbabuenae* in the low T_a treatment, regardless of diet quality.

Effect of sugar concentration and T_a on the energy intake of nectar-feeding bats

Our results show that *G. soricina* and *L. yerbabuenae* decreased their energy intake by 31.7% and 54.2%, respectively, when they fed on the 5% sugar concentration compared with when they fed on the 35% sugar concentration (w/v; Fig. 1). This finding is similar to those reported by Ramírez P et al. (2005), Ayala-Berdon et al. (2008, 2009), Herrera M and Mancina G (2008) and Ayala-Berdon and Schondube (2011) for the same bat species, and supports the existence of a physiological limitation controlling energy intake in these species. Several species of nectar-feeding animals differ in their capacity to obtain energy when the sugar concentration of their food varies (Martínez del Rio et al., 2001; McWhorter and López-Calleja, 2000; Ramírez P et al., 2005; Ayala-Berdon et al., 2008). While some species are able to maintain a constant energy intake (Simpson et al., 1989; López-Calleja et al., 1997), other species present physiological constraints that limit their energy intake, especially when they feed on sugar concentrations below 15% (Levey and Martínez del Rio, 1999; Martínez del Rio et al., 2001; Ayala-Berdon et al., 2008). Studies performed with glossophaginid bats have found large differences in their capacity to acquire energy when the sugar concentration in their diets varies (Ramírez P et al., 2005; Ayala-Berdon et al., 2008; Ayala-Berdon and Schondube, 2011). While the nectar-feeding bats *Leptonycteris nivalis* and *Choeronycteris mexicana* are capable to achieve compensatory feeding, and have an energy intake independent of sugar concentration (Ayala-Berdon and Schondube, 2011; Ayala-Berdon et al., 2013), other species like *G. soricina* and *L. yerbabuenae* exhibit limited energy intake when they face a reduction in the energetic content of their food (Ramírez P et al., 2005; Ayala-Berdon et al., 2008). Previous studies have shown that disaccharidase activity, monosaccharide absorption/transport rates and gut size are the main mechanisms controlling the total energy intake in nectar-feeding birds and glossophaginid bats (Martínez del Rio, 1990; Hernández and Martínez del Rio, 1992; Ayala-Berdon et al., 2008, 2013). In the case of our study species, the activity of the disaccharidase sucrase and monosaccharide absorption/transport rates are paired (Ayala-Berdon et al., 2008; Herrera M and Mancina G, 2008); as a consequence, when these bats feed on sucrose, the activity of the enzyme is the mechanism that determines the upper limit of their energy intake (Martínez del Rio and Stevens, 1989; Ayala-Berdon et al., 2008, 2009).

Contrary to our expectations, we did not find an effect of T_a on the energy intake of the bats at any of the sugar concentrations tested. Ayala-Berdon et al. (2009) showed that *G. soricina* was able to increase its energy intake during the winter in semi-natural conditions in a tropical dry forest when animals faced a decrease in T_a in two different seasons. The authors found that the effect of T_a on energy intake when bats fed at medium to high sugar concentrations (i.e. >15%) was minimal, and energy intake increased more than 75% when sugar concentration was below 15%. Why were our bats unable to increase their energy intake when they confronted a reduction in T_a? McWhorter and Martínez del Rio (2000) generated a mathematical model to predict the maximum amount of food that nectar-feeding animals would be able to process per unit of time when faced with changes in sugar concentration, given their digestive capacities. By using this model, Ayala-Berdon et al. (2008) determined the maximum energy intake of our study species when feeding on sucrose solutions with different concentrations. We compared our data on the energy intake of bats feeding on 5% sucrose concentration with those published by Ayala-Berdon et al. (2008) using a one sample *t*-test, and found that both species were ingesting the maximum amount of energy their

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gut could process (*G. soricina*: predicted 49 kJ 10 h^{-1}, observed 35±24.9 kJ 10 h^{-1}, $t_{1,4}$=1.25, P=0.27; *L. yerbabuenae*: predicted 46 kJ 10 h^{-1}, observed 31.1±31 kJ 10 h^{-1}, $t_{1,4}$=1.07, P=0.34). Because both bat species were feeding at the limit of their digestive constraints, they were unable to increase their energy intake when we decreased T_a in our experiments.

Role of energy intake and T_a in ΔM_b and the use of torpor in nectar-feeding bats

We found that while energy intake positively affected ΔM_b in *G. soricina*, this did not happen in *L. yerbabuenae*. This result is similar to that obtained by Ayala-Berdon and Schondube (2011) for the same bat species. Those authors found that while energy intake positively affected ΔM_b in *G. soricina*, *L. yerbabuenae* presented an erratic pattern of body mass gain when they fed on nectar ranging from 5% to 35% sugar concentration. This result may be associated with the low physiological capacity of *L. yerbabuenae* to process the energy content of nectar. Ayala-Berdon et al. (2013) found that differences in the affinity of the enzyme sucrase for its substrate in the nectar-feeding bats *L. yerbabuenae* and *L. nivalis* generate an important difference in the energy intake of these two species. According to these authors, the low sucrase affinity of *L. yerbabuenae* (0.018 mmol l^{-1}; Hernández and Martínez del Rio, 1992) could be the reason why this species is only common at low elevations (<600 m above sea level; Cole and Wilson, 2006). This could also explain why *L. yerbabuenae* does not show a significant increment in body mass when feeding on more concentrated sugar solutions, while *G. soricina*, which has a higher sucrase affinity (0.022 mmol l^{-1}), does (see Schondube et al., 2001).

Although physiological limitations controlling the energy intake of nectar-feeding vertebrates have been well studied, the relationship between digestive capacity and use of torpor had not previously been explored. In this study, we found a positive effect of energy intake and T_a on the $T_{skin,min}$ presented by the bats. This result shows that digestive limitations (sucrase activity and kinetics) reducing energy intake, and the energetic costs imposed by T_a play important roles in the onset of torpor in neotropical nectar-feeding bats. In tropical environments, where T_a is usually high (i.e. higher than 20°C; McKnight and Hess, 2000), our study species use torpor mainly in response to variation in nectar quality and/or availability (Coburn and Geiser, 1998; Kelm and von Helversen, 2007). In subtropical and high elevation regions, the use of this energy-saving strategy may help neotropical bats to survive when low T_a increases their energetic demands (Cruz-Neto and Abe, 1997; Bartels et al., 1998).

The use of torpor by *G. soricina* has been demonstrated previously by Cruz-Neto and Abe (1997) and by Kelm and von Helversen (2007). Cruz-Neto and Abe (1997) suggested that the combination of unsuccessful foraging and low T_a might be the underlying cause of the use of torpor in this species. While Kelm and von Helversen (2007) demonstrated that an energy limitation caused by nectar availability was responsible for the use of torpor by *G. soricina*, our study indicates that not only nectar availability but also nectar quality and low temperatures could lead to the use of torpor in this species. Additionally, we found that a second species of glossophagine bat (*L. yerbabuenae*) also uses this energy-saving strategy in a similar fashion to *G. soricina*. Our results suggest that different species of tropical nectar-eating animals could be using torpor in response to similar stimuli (nectar quality and temperature), and that the onset of torpor could be caused by similar physiological mechanisms in these species (digestive capacity); however, more research on this topic is needed to assess this hypothesis.

While our results on the use of torpor by *G. soricina* are similar to those found by Kelm and von Helversen (2007), there is an important difference between the two studies. Kelm and von Helversen (2007) found that bats from this species remained normothermic when they were provided with an unrestricted food supply, independently of T_a, while in our study, *G. soricina* individuals facing the high sucrose:low T_a treatment used torpor. This difference is perplexing, because in our experiments bats could ingest a large volume of nectar on each visit to the feeder (300–850 µl per visit), while in Kelm and von Helversen's (2007) experiments, they obtained only a small volume per visit (15 µl), expending more energy while foraging as a result of the larger time they spent flying. Unfortunately, important methodological differences between the two studies (for example, the geographic origin of the bats, differences in temperature regimes used in the experiments and length of the experiments, use of different energy limitations) limit our capacity to compare these results and explain their differences. However, our results indicate that, under certain conditions, T_a could also trigger the use of torpor in this species.

In this study, we found that in accordance with our hypothesis, our bat species responded to changes in food quality and T_a in different ways. While *L. yerbabuenae* spent more time using torpor when confronted with the low sucrose:high T_a diet, *G. soricina* became torpid for longer periods of time when bats were exposed to the high sucrose:low T_a and low sucrose:low T_a treatments. These results suggest that animals with a low digestive capacity to process the energy content of nectar, such as *L. yerbabuenae*, are more prone to using torpor when fed on dilute sugar concentrations even when the T_a is high (Ayala-Berdon et al., 2008, 2009). In contrast, bats with lower digestive constraints, like *G. soricina*, would became torpid when faced with the energetic limitations related to their small body mass, despite the fact that the food they ingest is of a high sugar concentration (Geiser and Stawski, 2011; Table 3).

Ecological and ambient factors affecting the use of torpor in neotropical nectar-feeding bats

What are the ecological and ambient factors that could determine the use of torpor in nectar-feeding bats? While several authors have contributed to the notion of little environmental variation in the tropics (Mac Arthur, 1972; Warman and Moles, 2009), nectar availability, its sugar concentration and nightly T_a could be highly variable in tropical ecosystems. Many authors have reported that nectar availability is dramatically affected by season in different tropical ecosystems (Heithaus et al., 1975; Lemke, 1984; Tschapka, 2004, among others), and because food restrictions can activate the use of torpor in different species of bats (*Syconycteris australis* and *G. soricina*; Coburn and Geiser, 1998; Kelm and von Helversen, 2007), this variation in food availability could have played a crucial role in the evolution of torpor in neotropical bats (Geiser and Stawski, 2011). With respect to the sugar concentration of nectar and the T_a present in some tropical areas, Rodriguez-Peña et al. (2007, 2016) and Ayala-Berdon et al. (2009) found that in a tropical dry forest of central Mexico inhabited by our study species, the nectar of chiropterophilic plants varies from 3% to 33% in its sugar content, and while the mean nightly temperature in the wet/warm season (i.e. from June to September) is 26°C, it drops to 16°C in the dry/cool season (i.e. from January to May). So, neotropical areas may represent a changing scenario for nectar-feeding bats, and the use of torpor may allow individuals to achieve a positive

energy balance when they confront changes in the availability and energetic content of the nectar they consume, and night temperatures in the different seasons of the year.

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Competing interests
The authors declare no competing or financial interests.

Author contributions
J.A.-B. and J.E.S. jointly developed the experimental design of the project. J.E.S. obtained financial support for the experimental work. J.A.-B. and R.V.-F. carried out the experiments and collected the data. R.B.-B. executed the statistical analysis. All authors jointly wrote the manuscript.

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