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Digestive capacities allow the Mexican long-nosed bat (*Leptonycteris nivalis*) to live in cold environments

Jorge Ayala-Berdon [a], Rubén Galicia [b], Cesar Flores-Ortíz [c], Rodrigo A. Medellín [b], Jorge E. Schondube [a,*]

[a] Centro de Investigaciones en Ecosistemas, Universidad Nacional Autónoma de México, Apartado Postal 27–3 (Xangari), 58089, Morelia, Michoacán, México
[b] Instituto de Ecología, Universidad Nacional Autónoma de México, Ap. Postal 70–275, 04510, Ciudad Universitaria, Distrito Federal, México
[c] Laboratorio de Fisiología Vegetal, UBIPRO, Facultad de Estudios Superiores Iztacala, Universidad Nacional Autónoma de México, Avenida de los Barrios # 1, Los Reyes Iztacala, Tlanepantla, Estado de México, 54090, México

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ABSTRACT

Digestive capabilities of nectar-feeding vertebrates to assimilate sugars affect their ability to acquire and store energy and could determine the minimal temperatures at which these animals can survive. Here, we described the sugar digestive capability of *Leptonycteris nivalis* and related it with its capacity to live in cold environments. We measured the enzymatic activity, food intake rate and changes in body mass of bats feeding at four different sucrose concentrations (from 5 to 35% wt./vol.). Additionally, we used a mathematical model to predict food intake and compared it with the food intake of bats. *L. nivalis* was able to obtain ~111.3 kJ of energy regardless of the sugar concentration of their food. Also, bats gained ~2.57 g of mass during the experimental trials and this gain was independent of sugar concentration. The affinity ($1/K_m$) of sucrase (EC 3.2.1.48) was one order of magnitude higher relative to that reported for its sister species *Leptonycteris yerbabuenae* (0.250 and 0.0189 $mmol^{-1}$ L, respectively), allowing this species to have a higher energy intake rate. We propose that the high ability to acquire energy conferred *L. nivalis* the faculty to invade cold environments, avoiding in this way the ecological competition with its sympatric species *L. yerbabuenae*.

1. Introduction

In recent decades, many studies have explored the relationship between the physiological capabilities of animals and the environmental conditions in which they live (Karasov and Diamond, 1988; Martínez del Rio et al., 1992; Ayala-Berdon et al., 2008, 2009; Ayala-Berdon and Schondube, 2011, among others). Some of these studies have demonstrated that the digestive capabilities of nectar-feeding vertebrates to assimilate sugars and their ability to process large volumes of water, affect their ability to acquire and store energy (Karasov and Diamond, 1988; Karasov, 1990; Diamond, 1991; Martínez del Rio et al., 1992; Ayala-Berdon et al., 2008, 2009; Ayala-Berdon and Schondube, 2011). Following these ideas, Ayala-Berdon et al. (2009), hypothesized that the capability to acquire energy should limit the ecological role and geographic distribution of nectar-feeding bats.

Nectar-feeding bats respond immediately to changes in the quality of their food resources by regulating food intake in relation to the concentration of sugar in floral nectar (Ayala-Berdon et al., 2008, 2009). While the Mexican long-tongued bat *Choeronycteris mexicana* is able to obtain a constant energy intake while feeding on a wide range of sugar concentrations (i.e. achieving compensatory feeding), other species of phyllostomid bats are unable to do so (Ayala-Berdon and Schondube, 2011). Digestive limitations make *Glossophaga soricina* and *Leptonycteris yerbabuenae* obtain 40 and 60% less energy when they feed on dilute nectars (<15% wt./vol.), than when feeding on concentrated nectars (≥25% wt./vol.; Ayala-Berdon et al., 2008). These differences in energy assimilation affect both how bats partition food resources inside their ecological communities (Ayala-Berdon and Schondube, 2011), and how they are able to cope with cold weather (Ayala-Berdon et al., 2009).

Ayala-Berdon et al. (2009) proposed that the bats' capability to acquire and store energy sets an upper limit to the energy that these animals can use to fuel their metabolism. The interaction between gut capacity to acquire energy and the metabolic costs will determine the minimal temperatures at which bats can survive, affecting their geographical and altitudinal distribution (Ayala-Berdon et al., 2009). These authors proposed that animals able to gain body mass independently of food energy density (i.e. exhibiting compensatory feeding) could inhabit colder sites than those presenting physiological constraints (Schondube and Martínez del Rio, 2003; Ayala-Berdon et al., 2009). For example, it appears that *G. soricina* cannot tolerate sites with minimum temperatures below 10 °C. This could partially explain why this species is usually found at lower elevations in tropical environments (Álvarez et al., 1991), while other species of nectar-feeding bats, like *Leptonycteris nivalis*, are able to survive in sites with minimum temperatures close to 0 °C (Arita, 1991; Brown, 2008; J.E. Schondube, pers. observations).

* Corresponding author.
E-mail addresses: jorgeayalaberdon@gmail.com (J. Ayala-Berdon), rgp5b@ciencias.unam.mx (R. Galicia), cmflores@servidor.unam.mx (C. Flores-Ortíz), medellin@ecologia.unam.mx (R.A. Medellín), chon@cieco.unam.mx (J.E. Schondube).

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In this study we describe the gut capacity of *L. nivalis*, a bat species that is able to live in colder and higher sites than other nectar feeding bats. We explore the hypothesis that this bat has higher capacity to assimilate energy than other nectar-feeding bats that live in sites at lower elevations or in warmer climates. We measured the activity of the enzyme sucrase–isomaltase in its gut (EC for sucrase and maltase: 3.2.1.48 and 3.2.1.20, respectively), modeled its capacity to assimilate energy, and quantified the food intake and changes in body mass when feeding at different sugar concentrations. We expected this species to have higher activity of the enzyme than other nectar-feeding phyllostomids, and to be able to achieve compensatory feeding. Because nectar-feeding bats exhibiting compensatory feeding have shown increments in body mass independent of sugar intake in the past, we expected the same ability in our study bat species (Ayala-Berdon and Schondube, 2011).

2. Materials and methods

2.1. Study species

L. nivalis was first collected in 1860 near the snowline of Mt. Orizaba, in the Veracruz state, Mexico. The habitat in the type locality was the reason of the specific epithet "*nivalis*" which means snowy (Hensley and Wilkins, 1988). *L. nivalis* migrates from central Mexico to northern of Mexico and southern United States during the summer, returning to Mexico during the winter season (Barbour and Davis, 1969; Schmidly, 1977; Kunz, 1982). In some areas of central and northern Mexico, this species is sympatric with *L. yerbabuenae* (Arita and Humphrey, 1988), but it prefers cooler places and inhabits habitats located at higher altitudes (Koestner, 1941; Barbour and Davis, 1969). This has been interpreted as a strategy to avoid competition with its sister species (Arita, 1991). Brown (2008) showed that *L. nivalis* preferred cool roosts at high elevations, even when they have to fly longer distances to forage at lower elevations. This species feeds on nectar and pollen of plants distributed mainly in five families: Cactaceae, Bombacaceae, Convolvulaceae, Fabaceae and Agavaceae (Sánchez and Medellín, 2007; López-Segurajáuregui, 2010).

2.2. Capture site

Adult non-reproductive bats were captured using mist nets in "La Cueva del Diablo" (18°59′43″ N, 99°03′40″ W, 1960 masl), a cave located in the municipality of Tepoztlán, in the state of Morelos, México. The site is located in a transition zone with vegetation being composed by tropical deciduous forest and temperate perennial forest (*Pinus–Quercus*). Average minimum temperature is ~13 °C. However, night temperature often drops near or below 0 °C during the winter months when the species is present at this site (Mexican National Weather Service, http: smn.cna.gob.mx/climatologia/).

2.3. Bat care and housing

After capture, 12 adult bats were transported to the animal facility of the Laboratorio de Ecología Funcional, Universidad Nacional Autónoma de México, in the city of Morelia (19° 38′ 53.91″N, 101° 13′ 44.31″W, 1900 masl; www.oikos.unam.mx), located in the state of Michoacán, Mexico. Bats were kept in a room with controlled ambient temperature (21.3–25.7 °C), inside individual cages (0.6 × 0.6 × 0.6 m). This allowed us to assess any changes in body condition experienced by the bats. To avoid nutritional problems, all bats were fed with a maintenance diet composed by weight 20% of sucrose and 4.4% protein (following Mirón et al., 2006). After a period of acclimation of two weeks, bats were trained to feed in experimental feeders and used in the intake response experiments. Due to difficulties of the species to adapt to captive conditions, we only used four bats for the intake response experiments (mean body mass ± SD = 24.46 g ± 1.53 g). At the end of the trials, these bats were euthanized in an ether chamber, and their tissues were used for morphological and biochemical measurements. Bats not well adapted to captivity and not used in the experiments were released at their capture site. All the experiments were done following the UNAM's Animal Care and Use Committee.

2.4. Intake responses

To establish the digestive capabilities of bats, we measured the food intake responses and the capacity of each individual bat to gain body mass when they fed at four sucrose solutions with different concentrations (5, 15, 25 and 35% of sugar-wt./vol.). We know from previous studies that the digestive capabilities of nectar-feeding bats are well represented by sucrose digestion rates, gut transit time, and gut volume (Ayala-Berdon et al., 2008). Also, these capabilities are closely matched by volumetric food intake when animals face changes in sugar concentration (Ayala-Berdon et al., 2008, 2009). We only used sucrose solutions because phyllostomid bats' food intake is not affected by sugar composition (Rodríguez-Peña et al., 2007; Ayala-Berdon et al., 2008). Individuals were transferred from maintenance colonies to flying cages (3 × 2 × 1.6 m), which had a feeder in their center. Flying cages were located in the periurban forest close to the university campus, a place that presented similar climate conditions to the capture site. This allowed us simulate semi-natural conditions of ambient temperature and humidity. Bats received one sugar solution per night. We used a Latin-square design (4 × 4) to ensure that we had dilute (5%), intermediate (15 and 25%) and concentrated sugar solutions (35%) being offered to different individuals during the same night. Because experimental sugar solutions lacked nitrogen sources, our experiments consisted of one night of experiment followed by one day of resting. During the resting day bats received the maintenance diet and were kept under controlled conditions (see above). Solutions were weighed at the beginning (W_i) and the end (W_f) of each feeding trial from 20:00 to 04:00 h. This period of time corresponds to the normal foraging period of the bats at their capture site (R. Galicia, pers. commun.). Food consumed was estimated by subtracting W_i of W_f. Each night we placed a feeder of each sugar concentration outside the flight cages to control for changes in concentration and volume due to evaporation. These feeders were covered with a mosquito mesh to prevent drinking by insects and other nocturnal animals. Control feeders were weighed at the beginning and end of each trial, and the concentration of the solution was measured using a hand-held refractometer (Reichert 10431; 0–50° compensated Brix temperature, Leica, Buffalo NY, USA), to account for changes in concentration. No changes in volume or concentration were observed in our control feeders.

2.5. Capability to store energy

To evaluate the capability of bats to store energy, we calculated the changes in body mass (Δ_{bm} in g 8 h^{-1}) exhibited by the bats, by weighing each animal at the beginning and the end of each trial. To assess if this capacity was ecologically realistic, we captured and weighted bats at the entrance of "La Cueva del Diablo" when they exited to feed at sunset (20:00 h) and when they returned to the roost after foraging (04:00 h).

2.6. Gut morphology and enzymatic activity

To assess gut morphology and enzymatic activity, our experimental bats were euthanized. Duodenum, jejunum and ileum sections were dissected lengthwise to measure nominal surface area (i.e. length × width), and placed in 1.5 mL cryovials. Then guts were frozen immediately at − 70 °C and stored. Prior to conducting disaccharidase activity assays, guts were thawed at 5 °C and homogenized (30 s, OMNI 5000 homogenizer at setting 6) in nine volumes of 350 mmol L^{-1} mannitol

in 1 mmol L^{-1} Hepes/KOH, pH 7.5. Disaccharidase activities were measured following Schondube et al. (2001) with modifications of Martínez del Rio et al. (1995) methodology. In brief, tissue homogenates (100 μL) diluted with 350 mmol L^{-1} mannitol in 1 mmol L^{-1} Hepes/KOH were incubated at 37 °C with 100 μL of 56 mmol L^{-1} sugar (sucrose or maltose) solutions in 0.1 mol L^{-1} maleate/NaOH buffer, pH 6.5. After 10–20 min of incubation, reactions were stopped by adding 3 mL of a glucose assay kit (GAGO20, Sigma-Aldrich, St. Louis, MO, USA) in 250 mL, 1.0 mol L^{-1} Tris/HCl, pH 7.0, plus 250 mL of 0.5 mol L^{-1} NaH_2PO_4/Na_2HPO_4, pH 7.0. Instead of reading each sample individually (Schondube et al., 2001), after 15 min at 20 °C, the absorbances of the resulting solutions were measured simultaneously at 550 nm with a Spectra Elisa reader for 96-well microplates (Oxford, USA).

To determine pH optima, we used a 0.1 mol L maleate/NaOH buffer system (for sucrose and maltose), with pH ranging from 5.0 to 7.5. Disaccharide (56 mmol L^{-1}) concentration was held constant. Measurements reported in results were conducted at optimal pH (to the nearest 0.5). Kinetics parameters were measured at concentrations ranging from 0.5 to 200 mmol L^{-1} for sucrose and maltose.

2.7. Data analysis

We estimated the slopes and intercepts of the relationships between food intake and sugar concentration using regression analysis on the log-transformed data of each individual bat. The relationship between volumetric intake and sugar concentration is well described by power functions of the form $V = aC^{-b}$. Where V equals volumetric intake, C equals sugar concentration, and the intercept (a) and the exponent (b) are empirically derived constants (McWhorter and Martínez de Río, 1999; McWhorter and Martínez del Rio, 2000; Martínez del Rio et al., 2001). Because volumetric intake (V) decreases as a power function of concentration (C), the amount of sugar ingested (A) is also a power function of sugar concentration ($A = aC^{-b}C = aC^{1-b}$; Martínez del Rio et al., 2001). Animals exhibiting exponents equal to 1 show perfect compensation with sugar intake independent of concentration ($1 - b = 0$). In contrast, animals with values of exponents smaller than 1 will show a positive relationship between sugar ingested and sugar concentration in food (i.e. energy density). We compare the intake responses of the four bats using a generalized linear model with food intake as the dependent variable, and sugar concentration and individual as independent variables. We tested the values of the intake response exponents to the expected value for compensatory feeding (Eq. (1)) using a one-sample t test.

To obtain the maximal hydrolysis rates for each of the different substrates (V_{max}) and their apparent binding constants $\left(K_m$, the concentration at which the rate of hydrolysis equals $\frac{V_{max}}{2}\right)$, we used a nonlinear Gauss–Newton routine. On the basis of absorbance standards constructed for glucose, we calculated intestinal activities standardized per unit of nominal area (cm^2). Martínez del Rio et al. (1995) provide a justification for our choice of standardization.

Additionally, we compared our intake response results with intake predictions from a mathematical model (McWhorter and Martínez del Rio, 2000). This model assumes that the intestine is analogous to a tubular chemical reactor, in which sucrose hydrolysis ($-r_s$) follows Michaelis–Menten kinetics:

$$-r_s = \frac{S_{max} C_s}{K_m + C_s}, \quad (1)$$

where S_{max} is the rate of hydrolysis along the intestine (μmol min^{-1} μL^{-1}), K_m is sucrase's Michaelis–Menten constant (μmol μL^{-1}), and $C_s$is the concentration of sucrose (μmol μL^{-1}) down the intestine. The time (τ) required to reduce the initial concentration of sucrose (C_{S0}) to a given final value (Cs_f) can then be integrated from Eq. (1) to:

$$\tau = \frac{K_m \ln\left(C_{S0}/C_{sf}\right) + \left(C_{S0} - C_{sf}\right)}{S_{max}}. \quad (2)$$

Maximum intake rate (V_0 in μL min^{-1}), can then be estimated using the volume of the small intestine G (in μL) as:

$$V_0 = \frac{G}{\tau}. \quad (3)$$

We used gut morphology and enzymatic data from the collected individuals to fit the model. We calculated sucrose affinity as the inverse of the K_m. Gut parameters are presented in Table 1. To compare observed intake of sucrose solutions with those predicted from the model, we used the coefficient of determination as a descriptor of goodness of fit (Anderson-Sprecher, 1994). We compared this coefficient of determination with that of a power function fitted to the same data set using a non-linear regression routine (JMP 5.1®, 2003).

In addition, we calculated Δ_{bm} (in g 8 h^{-1}) experienced by the bats, by weighing each individual bat at the beginning and the end of each trial. We used simple linear regressions to see the effect of concentration on Δ_{bm} against nectar concentration and sugar intake (SI). We correlated Δ_{bm} against SI of each bat using Spearman's rank correlations (r_s) and tested whether the average r_s was significantly greater than 0 using a t-test. This procedure avoids the pseudo-replication that one would incur when estimating r_s for pooled data. The average r_s values for a sample of bats satisfy the central limit theorem and hence, one can make inferences about whether they are positive or negative (Stuart and Ord, 1994). Also we calculated Δ_{bm} for wild animals in the field using the data collected at "La Cueva del Diablo". We weighted bats when they were leaving the cave, and when they came back to roost after foraging. We captured a total of 75 bats (17 males and 58 females), when animals were exiting (2000 h; 36 individuals) and arriving from foraging (0400 h; 39 individuals) to "La Cueva del Diablo". Since we weighed different individuals, the data we obtained represented a population mean. We compared this value with the mean Δ_{bm} for the experimental bats feeding at 15% of sugar (value close to the mean of the concentration of quiropterophilic plants; Rodríguez-Peña et al., 2007) using a t test. Finally, we assigned an alpha value of 0.05 to all tests performed to determine the existence of statistical differences. Due to our small sample size, we conducted power tests on all of our parametric analyses. In all cases the power values (beta) were higher than 0.83.

Table 1
Sucrase, and maltase activity parameters for the nectar-feeding bat *Leptonycteris nivalis*. Enzyme activities are standardized by intestinal nominal area (cm^2) and wet mass of tissue (g) for both enzymes. We used sucrase parameters to fit McWhorter and Martínez del Rio's model (2000) to our experimental data.

	Sucrase	Maltase
Total activity (μmol min^{-1})	14.10	67.3
pH optimum	6.0	6.5
S_{max} (mmol min^{-1} L^{-1})	0.048	6.85
K_m (mmol L^{-1})	4.0	10.17
Cs_f *	0.009	0.009
G (μL^{-1})	0.724	
Gut mass (g)	0.51 ± 0.02	

S_{max}: rate of hydrolysis along the intestine, K_m: sucrase Michaelis–Menten constant, Cs_f: final concentration of sucrose after digestion, G volume of the intestine.

* We measured the digestive efficiency by quantifying the sugar content in the excreta of bats feeding exclusively on sugar solutions with a hand-held refractometer (Accuracy; Reichert 10431 0–50°Brix temperature compensated, Leica, Buffalo NY, USA; Schondube and Martínez del Rio, 2003). Because solutes other than sugars bias refractometer readings (Inouye et al., 1980; Hiebert and Calder, 1983) our measurements of sugar concentration in excreta were used only to generate a relative measurement of digestion efficiency.

3. Results

3.1. Intake responses and capacity to store energy

L. nivalis increased food intake when sugar concentration in nectar decreased. The relationship between food intake and concentration was described by a power function (regression formula: log food intake = 2.7445–0.936 log concentration, $r^2 = 0.94$, $\beta = 0.99$). We did not find an effect of individual on the food intake of the four bats ($x^2_2 = 0.0355$, $p = 0.85$ from a GLM). Bats ingested 119.3 ± 20.8 g of food when feeding at low concentrations (145 mmol L^{-1}), and reduced their intake to 18.5 ± 2.2 when feeding on the most concentrated diet (1022 mmol L^{-1}). The maximum volume of nectar ingested represented up to 5 times their body mass in one night. The exponents of the individual intake responses did not differ statistically from the compensatory value of 1 (mean exponent = 0.93, $t_3 = -1.45$, $P = 0.24$, $\beta = 0.82$). This implies that the changes in volumetric intake allowed bats to always obtain the same amount of sugar regardless of the sucrose concentrations tested ($F_{14} = 0.28$, $P = 0.60$). Finally, their Δ_{bm} was independent of sugar concentration ($F_{14} = 0.21$, $P = 0.64$) and sugar intake ($F_{14} = 0.38$, $P = 0.54$).

We did not find any statistical differences in Δ_{bm} of bats captured in the field at "La Cueva del Diablo" respective to that exhibited by our experimental species feeding at 15% of sucrose (Δ_{bm} field 3.57 g ± 1.46 vs. 2.57 g ± 1.21 for the experiments; $t_1 = 3.0$, $P = 0.17$, $\beta = 0.96$).

3.2. Enzymatic activity

Sucrase and maltase activity in *L. nivalis* followed classical Michaelis–Menten kinetics (Table 1). Enzyme activities standardized by intestinal nominal surface area (cm^2) and wet mass of tissue (g) were linearly and tightly correlated ($0.94 < r < 0.99$). Subsequently we standardize enzymatic activity by nominal area only. Sucrase and maltase activities standardized by nominal area were positively correlated (Fig. 1). Optimal pH was 6.0 and 6.5 for sucrase and maltase respectively. Both sucrase and maltase activity decreased along the intestine (Fig. 1).

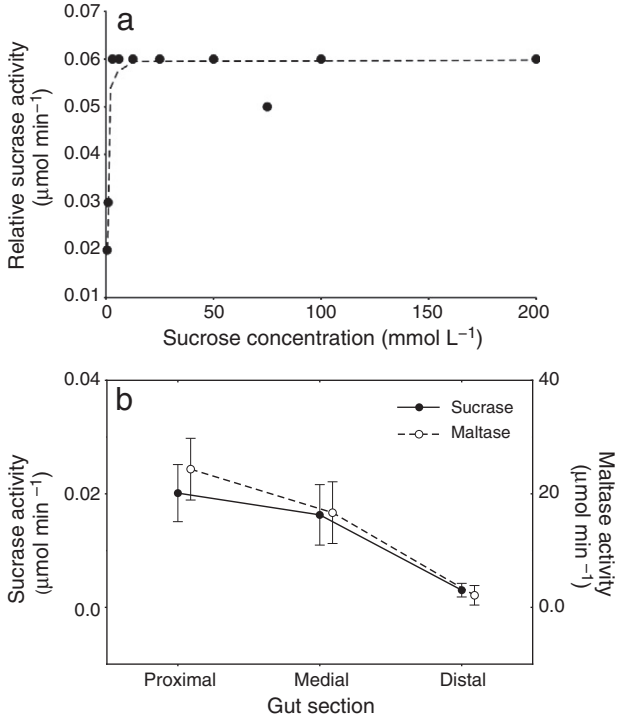

Fig. 1. a) Sucrase kinetics for the nectar-feeding bat *Leptonycteris nivalis*. b) sucrase and maltase activity in the three portions of the bat's small intestine. Data are presented as averages with their respective SD.

3.3. McWhorter and Martínez del Rio's (2000) digestive model

Food intake predictions from the model were almost identical to the food intake exhibited by our study species (Fig. 2). Predictions from the model were tightly and positively correlated with the experimental data ($r^2 = 0.994$, $t_2 = 23.98$, $P = 0.001$, $\beta = 0.99$). This tight relation between food intake predicted and observed suggest that the digestive capacity to process sucrose is responsible of the shape of the intake response in *L. nivalis*.

4. Discussion

Our results demonstrated that the nectar-feeding bat *L. nivalis* can conduct compensatory feeding when animals fed in the range of sugar concentrations tested. Also, we reported new data on disaccharidase activity for this species. The McWhorter and Martínez del Rio's (2000) model fitted our experimental data very well. Finally, Δ_{bm} was independent of sugar concentration. In this section we first discuss the relative role that digestion and renal function play in shaping the intake responses of nectar-feeding bats. Second, we relate the enzymatic information with physiological capacities of bats and evaluate the efficiency of the gut function model to predict food intake in nectar-feeding bats. Finally, we discuss the relationship between the capacities of *L. nivalis* to acquire energy and increase body mass with its aptitude to live in cold environments.

4.1. Digestive and renal limitations for energy acquisition

Studies conducted with birds have demonstrated that nectar-feeding vertebrates have different capacities to acquire the energy present in the nectar they consume (López-Calleja et al., 1997; Levey and Martínez del Rio, 1999; McWhorter and López-Calleja, 2000; Martínez del Rio et al., 2001). These studies have shown that while some animals are able to achieve compensatory feeding (López-Calleja et al., 1997; Levey and Martínez del Rio, 1999), others exhibit physiological constraints to energy acquisition, especially when animals feed on dilute concentrations (Levey and Martínez del Rio, 1999; Martínez del Rio et al., 2001). Nectar-feeding bats respond to changes in the sugar concentration of nectar in a similar fashion to birds (Ramírez et al., 2005; Ayala-Berdon et al., 2008, 2009). While the nectar-feeding bats *C. mexicana* (Ayala-Berdon and Schondube, 2011) and *L. nivalis* (this study) are able to achieve compensatory feeding and obtain the same amount of energy when feeding on concentrations ranging from 5 to 35% (wt./vol.),

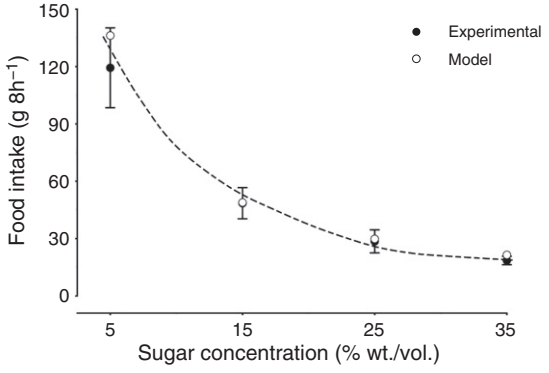

Fig. 2. Intake response obtained from experimental data compared with the McWhorter and Martínez del Rio's model (2000). We used enzymatic parameters obtained from our study species to obtain the gut function model. Experimental data are presented as means with their respective standard deviations.

G. soricina and *L. yerbabuenae* exhibit physiological constraints that limit their energy intake when animals feed on concentrations ≤15% (wt./vol.; Ramírez et al., 2005; Ayala-Berdon et al., 2008, 2009). In this context, nectar feeding bats differ in their digestive capacities to obtain energy with some species being able to satisfy their energetic budget by feeding at any nectar sugar concentration, while others exhibit energetic deficits when they feed on dilute nectars (Ayala-Berdon and Schondube, 2011). This must have implications for their spatial ecology, including daily and migratory movements, probably restricting or otherwise shaping the way these bats use food resources in the field.

What are the mechanisms that limit the bats' energy intake? Several studies performed with birds and bats have proposed that the size of the gut, the rate of sugar assimilation (Martínez del Rio, 1990; Hernandez and Martínez del Rio, 1992; Ayala-Berdon et al., 2008, 2009, 2011), the rate at which the water is absorbed in the intestine, and the capacity of animals to filter in the kidney the large volumes of water ingested when they feed on dilute concentrations could limit energy intake in nectar-feeding animals (Karasov, 1990; Martínez del Rio, 1990; Karasov and Hume, 1997). Although the kidneys set an upper limit to water management that could affect food intake when bats are feeding on dilute nectars, the gut function model used by us in this study, and by Ayala-Berdon et al. (2008), accurately predicted the intake responses of nectar-feeding phyllostomid bats using only the digestive characteristics of these animals. These results suggest that digestion, and not renal function, is the main factor shaping the intake responses of phyllostomid bats. Hartman Bakken et al. (2008) provided additional evidence for the lack of a renal constraint limiting food intake in nectarivorous bats. These authors analyzed water management in the nectar-feeding bat *G. soricina* and identified that this species is able to handle water fluxes even greater than those experienced by marine fishes of the same body-mass. Our findings and those reported by Ayala-Berdon et al. (2008) and Hartman Bakken et al. (2008) strongly suggest that digestive characteristics play a major role in food intake regulation in nectar-feeding bats.

4.2. Digestive characteristics of *L. nivalis*

Several digestive parameters of *L. nivalis* do not differ with those of other nectar-feeding bats. Both gut size and sucrase S_{max} in *L. nivalis* are very similar to those exhibited by its sister species *L. yerbabuenae* (gut size: 0.72 and 0.75 μL^{-1}; S_{max}: 0.048 and 0.049 μmol min^{-1} L^{-1} for *L. nivalis* and *L. yerbabuenae* respectively; Hernandez and Martínez del Rio, 1992; Schondube et al., 2001). However, *L. nivalis* is able to ingest ~59% more energy when feeding at dilute nectars compared to *L. yerbabuenae* (Ayala-Berdon et al., 2008; Ayala-Berdon and Schondube, 2011). If both species have similar digestive traits, why does *L. nivalis* have a higher capacity to process food in its gut than its sister species?

Ayala-Berdon et al. (2008) proposed that the affinity of sucrase for its substrate and rates of hexose assimilation in the small intestine may strongly affect the amount of food that an animal is able to ingest. According to this prediction, we found that the affinity of sucrase of *L. nivalis* is one order of magnitude higher than the affinity shown by *L. yerbabuenae* (0.25 $mmol^{-1}$ L — the inverse of $K_m = 4$ mmol L^{-1} obtained in this study- and 0.0189 $mmol^{-1}$ L — the inverse of $K_m =$ 52.77 mmol L^{-1}, obtained from Schondube et al., 2001). In this context, the high affinity of sucrase for its substrate may confer to *L. nivalis* the ability to achieve compensatory feeding when they encounter different sugar concentrations. Furthermore, it has been shown that rates of sugar digestion and absorption are closely matched in phyllostomid bats (Ayala-Berdon et al., 2008; Herrera and Mancina, 2008). If this is true for *L. nivalis*, this species should exhibit closely matched sugar digestion and absorption capacity and may have the ability to feed from any source of nectar regardless of sugar composition or concentration. However this hypothesis remains to be explored.

4.3. McWhorter and Martínez del Rio's model (2000) as predictor of food intake in nectar-feeding bats

McWhorter and Martínez del Rio (2000) proposed that a plug flow chemical reactor (Penry and Jumars, 1986) could be used as a method for modeling the food intake of nectar-feeding animals. The authors showed that this model predicted food intake accurately in broad-tailed hummingbirds. Ayala-Berdon et al. (2008) applied this model on two species of nectar-feeding bats. Their results showed that the model explained the experimental data accurately (53 and 67% of the variation of the intake responses for *L. yerbabuenae* and *G. soricina* respectively). However, the power functions fit to their experimental data described the intake responses better (86 and 83% for *L. yerbabuenae* and *G. soricina* respectively). These authors assumed that the differences between the predictions of the model and their food intake observation were caused by the fact that the enzymatic data they used was from different individuals than their food intake data. In this study, we demonstrated that the gut function model explained food intake data remarkable well when enzyme activity and gut morphology data used were from the same individuals as the food intake data (99.4% of the variation of the intake response explained by the model; Fig. 2). Our results clearly show that the model proposed by McWhorter and Martínez del Rio (2000) accurately predicts food intake in nectar-feeding bats.

The fact that the McWhorter and Martínez del Rio's model accurately predicted food intake in our study species, indicates that bats are living at the edge of their digestive capacities. This result is similar of that observed for hummingbirds in which gut volume and digestive traits direct the amount of food that animals are able to ingest (Diamond et al., 1986). By maximizing energy assimilation rates, bats should enhance their energy intake when they are feeding at different sugar concentrations.

4.4. Do the physiological capabilities of *L. nivalis* allow it to live in cold environments?

The presence of physiological constraints limiting the capacity to achieve compensatory feeding has effect on the energy balance of nectar-feeding bats (Ayala-Berdon et al., 2008, 2009). These constraints affect the way that bats acquire and store energy and influence the behavior and ecology of these animals (Ayala-Berdon and Schondube, 2011; Ayala-Berdon et al., 2011). *L. yerbabuenae* and *G. soricina* exhibit physiological constraints in digestion, and show a positive relationship between Δ_{bm} and sugar concentration (Ayala-Berdon and Schondube, 2011), while *L. nivalis* (this study) and *C. mexicana* (Ayala-Berdon and Schondube, 2011), present compensatory feeding, and their $\Delta_{bm}$was independent of sugar concentration.

Physiological capabilities limiting the maximum amount of energy that an animal can acquire should have important effects in the way they interact with their environment (Ayala-Berdon et al., 2009; Gaston, 2009; Kuo and Sanford, 2009; Rodríguez-Serrano and Bozinovic, 2009; Szathmary et al., 2009). Several studies have suggested that gut capabilities to acquire energy have effects on the behavior, ecology and geographical and altitudinal distribution of bats (Ayala-Berdon et al., 2009; Ayala-Berdon and Schondube, 2011; Ayala-Berdon et al., 2011). *G. soricina* and *L. yerbabuenae* compensated for their inability to achieve compensatory feeding by reducing flight time and increasing feeding time when their energy intake was lower due the presence of a physiological constraint (Ayala-Berdon et al., 2011). Additionally, differences in the selection and use of food resources by an assemblage of a nectar-feeding bat community appear to be driven by the physiological capabilities of these animals. Bats able to achieve compensatory feeding have the capacity to feed on any nectar resource present in their environment, having the potential to act as ecological generalists and being able to live in a wider range of environmental conditions (both in terms of temperature and quality of food resources; Ayala-Berdon and Schondube, 2011). On the other extreme, bats exhibiting physiological constraints would benefit

from feeding on more concentrated nectars, becoming ecologically more specialized and having a narrower capacity to cope with environmental variability (Ayala-Berdon and Schondube, 2011).

In this study we showed that the nectar-feeding bat *L. nivalis* is able to obtain the same amount of energy when animals fed at sucrose concentrations ranging from 5 to 35% (wt./vol.). Also, this species was able to increase its body mass independent of sugar concentration, even when the night temperatures dropped to 0 °C. Here, we propose that the ability to acquire and store energy allowed this species the capacity to invade cold environments and/or high altitudes (Arlettaz et al., 2000; McKechnie, 2008). The use of these new habitats could have reduced the ecological competition with its sympatric species, *L. yerbabuenae* as proposed by Arita (1991). Our results suggest that a change in the affinity of a disaccharidase could have dramatic effects on the ecology of one species, by changing its capacity to assimilate sugars and the total amount of energy they can obtain in a day. The relationship between gut capacities and geographical and altitudinal distribution in *L. nivalis* suggests that the capacity of this species to live in cold environments is the result of a change in its digestive capacities. Our results suggest that a small biochemical change that affects the capacity to obtain energy can affect the ecological niche of a species by modifying its capacities to withstand colder weather, and/or use a wider diversity of food resources with different qualities.

Although *L. nivalis* is highly capable of achieving a constant rate of energy assimilation when feeding on nectars of different concentrations, and it potentially can exploit more resources to survive, the Mexican Long-nosed bat is actually endangered, even more critically than *L. yerbabuenae*, which is physiologically constrained to survive feeding on dilute nectars. Our results indicate that while digestive capacity could explain altitudinal segregation between this two species, is important to consider the role of competition for food resources to understand their ecology and distribution. Our results indicate that *L. nivalis* could exploit more nectar resources than other nectarivorous bats; however it is feeding on similar sources to *L. yerbabuenae* (Stoner et al., 2003; Sánchez and Medellín, 2007). If both species prefer the same food resources, *L. nivalis* could have used its ability to inhabit colder places to avoid competition for food resources with it sister species. While the digestive capacities of *L. nivalis* could have granted it the ability to differentiate its ecological niche from that of *L. yerbabuenae*, it is important to understand how other factors are affecting these species to understand why *L. nivalis* is more critically endangered than *L. yerbabuenae*. Moreover, both species of *Leptonycteris* are able to conduct long-distance migrations. The role, balance, and dynamics of energy in the context of the day-to-day movements and annual migrations is crucial to fully appreciate and model the migratory behavior of these bats and their capacity to respond to changes in temperature and food resource abundance and quality.

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