UNIVERSIDAD NACIONAL AUTÓNOMA DE MÉXICO

POSGRADO EN CIENCIAS BIOLÓGICAS

CENTRO DE INVESTIGACIONES EN ECOSISTEMAS

Efecto de la Calidad del Néctar
Sobre las tasas de Consumo y Patrones
de Forrajeo de los Murciélagos
Nectarívoros

T E S I S

QUE PARA OBTENER EL GRADO ACADÉMICO DE

**MAESTRO EN CIENCIAS BIOLÓGICAS
(BIOLOGÍA AMBIENTAL**)

P R E S E N T A

JORGE ISRAEL AYALA BERDÓN

DIRECTOR DE TESIS: DR. JORGE E. SCHONDUBE FRIEDEWOLD

MÉXICO, D.F.   **NOVIEMBRE, 2006**

AGRADECIMIENTOS

- Muy especialmente al Chon que además de ser un exelente asesor es un exelente amigo.

- Con mucho afecto a la gente de mi laboratorio: Lore, Moni, El tio, Ian, Carlita.

- Al personal de la Estación de Biología de Chamela: al Dr. Ricardo por las facilidades que otorgó para que fuese posible la realización de la tesis. A la señora Eva y la señora Elena, Nachita, Normita, Vero, Daniel, Tania murciélago, Tania perico, Kathy Renton, Aletz, Carlitos, Lucy e Irma, por hacer de mi estancia en la Estación de Biología una experiencia inolvidable.

- Muy especialmente al Dr. Carlos Martínes del Rio, por su ayuda en el escrito de los artículos, por ser una exelente persona además de un exelente investigador. A su esposa Martha , sus hijos Kormack y Aurora.

- Con mucho cariño a mi comité tutoral: Jorge Scondube, Kathy Stoner y Alberto Burquez, que ademas de ser exelentes personas me hicieron crecer en mi formación como maestro.

- A mis revisores por las obsevaciones y el apoyo en la elaboración de la tesis: Victor Sánchez y Gerardo Herrera.

- Al Centro de Investigaciones en Ecosistemas, especialmente a la gente con la que tuve el gusto de convivir.

- A mi maravillosa familia que amo, la cual me ha dado una nueva lección de vida: Mama Carmen, Papa Jorge, hermanos Cesar y Sandra, Cuñados Carlitos e Idalia.

- A mis maravillosos sobrinos: Beto, Dany y Kathy.

- Muy cariñosamente a Nelly por su apoyo en el campo y como amiga.

- Con mucho cariño a Dianita y Cristian por su apoyo en el campo y su amistad.

- A la señora Olga y a toda la familia Peña por formar parte de mi familia.

- Al Consejo Nacional de Ciencia y Tecnología por el apoyo de beca.

- A PAPITT por el apoyo económico al proyecto.

ÍNDICE.

Discussion

CAPITULO 2: Cambios en la respuesta de consumo asociados a diferencias estacionales de temperatura

Introducción

El papel ecológico que juegan los murciélagos nectarívoros en los ecosistemas es de fundamental importancia, ya que polinizan una gran cantidad de especies de plantas (Fleming y Sosa 1994, Kearns y Inouye 1997, Allen-Wardell 1998, Kearns *et al*. 1998). En el neotropico se ha calculado que alrededor de 750 especies de plantas dependen total o parcialmente de ellos para su reproducción (Heithaus *et al*. 1975, Dobat 1985). El néctar producido por las flores es un alimento alto en energía y fácilmente asimilable. Sin embargo el alimentarse de néctar representa un reto importante para los murciélagos, ya que este puede presentar marcadas diferencias de calidad, al variar la concentración entre especies e individuos de una misma especie (Pyke y Waser 1981). El contenido de azúcar en el néctar de flores polinizadas por murciélagos en el neotropico puede variar entre 5 y 29% (peso/volumen en equivalentes de sacarosa; Helversen 1993).

Esta variación en la concentración de nutrientes en el alimento tiene efectos importantes sobre los patrones de conducta de los organismos, afectando la cantidad de alimento que estos requieren consumir para satisfacer sus necesidades energéticas (McWhorter y Martínez del Rio 2000, Martínez del Rio *et al*. 2001, Schondube y Martínez del Rio 2003). De esta manera, una disminución en la concentración en el néctar tiene como efecto un incremento en el consumo del mismo (Collins 1981, Downs 1997, López-Calleja *et a*l. 1997, McWhorter y Martínez del Rio 2000, Schondube y Martínez del Rio 2003). Este fenómeno ha sido denominado respuesta de consumo (Figura I;

Castle y Wunder 1995), y tiende a ser resultado del esfuerzo de los organismos para lograr una alimentación compensatoria (Simpson *et al.* 1989).

En este tipo de comportamiento de alimentación, los organismos regulan la cantidad de alimento que consumen para mantener un flujo constante de energía o nutrientes asimilados (Montgomery y Bumgardt 1965, Slansky y Wheeler 1992). En aves nectarívoras se ha encontrado que la relación negativa entre la concentración y el consumo de néctar tiende a generar tasas relativamente constantes de ingestión de azúcares (López-Calleja *et al*. 1997, Levey y Martínez del Rio 1999). Sin embargo el proceso de alimentación compensatoria puede estar limitado por la habilidad de los organismos para procesar los nutrientes contenidos en el alimento (Levey y Martínez del Rio 1999, Martínez del Rio *et al.* 2001), y/o su capacidad para excretar materiales indigeribles que ocupen espació dentro de su tracto digestivo. En animales nectarívoros, debido a los grandes volúmenes de agua que pueden ingerir cuando se alimentan de néctar diluido, el consumo de alimento puede verse limitado por su capacidad para procesar agua (Martínez del Rio *et al* 2001).

Los factores que pueden afectar las respuestas de consumo de los murciélagos nectarívoros pueden separarse en dos categorías: 1) factores pre-ingesta y 2) factores post-ingesta. Los factores pre-ingesta consisten en la interacción entre las características morfológicas de los murciélagos (forma del rostro, boca y lengua), la morfología floral y el volumen y la concentración del néctar presente en las flores que visitan. Los factores pre-ingesta determinan las tasas de extracción e ingestión de néctar de estos animales (Hainsworth

1973, Montgomerie 1984, Tamm y Gass 1986, Paton y Collins 1989, Mitchell y Paton 1990). Los factores post-ingesta incluyen todos aquellos que determinan las tasas de asimilación de nutrientes y la capacidad para eliminar los grandes volúmenes de agua contenidos en el néctar sin perder electrolitos (Diamond *et al.* 1986, Karasov y Diamond 1988, Martínez del Rio y Stevens 1989, Karasov 1990, Martínez del Rio 1990, Diamond 1991, Martínez del Rio y Restrepo 1993, Karasov y Hume 1997).
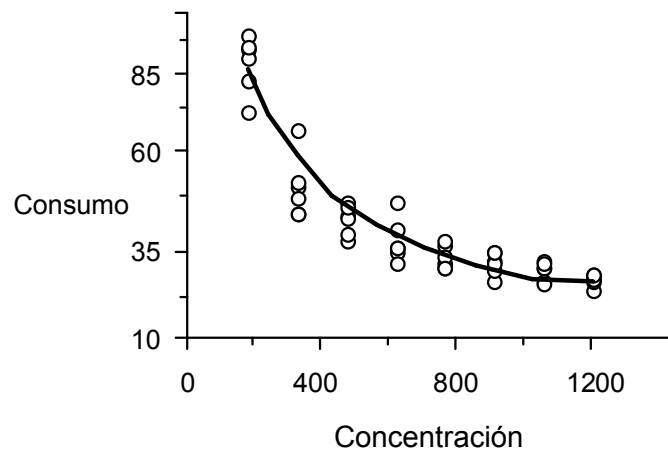
Figura I. Respuesta de consumo típica en donde el volumen ingerido decrece como función exponencial de la concentración de nutrientes presentes en el alimento (Castle y Wunder 1995).

Determinación de limitantes a las respuestas de consumo de organismos que se alimentan de néctar

McWorther y Martínez del Rio (2000) y Martínez del Rio *et al* (2001) desarrollaron un método analítico para determinar si exísten limitantes a la respuesta de consumo. Debido a que el volumen consumido de alimento (**V**) decrece como una función exponencial de la concentración (**C**), la cantidad de nutrientes ingeridos (**N**) es también una función exponencial de su concentración (Figura I):

$$N = aC^{-b}C = aC^{1-b}$$

Por lo tanto, animales que presentan valores de exponente *b* iguales a 1, presentan una alimentación y un consumo de nutrientes compensatorio que es independiente de la concentración (McWhorter y Martínez del Rio 2000, Martínez del Rio *et al.* 2001). En contraste, animales con valores del exponente *b* menores a 1, presentan una relación positiva entre nutrientes ingeridos y la concentración de estos en su alimento, lo que sugiere la existencia de una restricción fisiológica que impide que los animales mantengan un consumo energético constante. La mayoría de las respuestas de consumo reportadas a la fecha presentan exponentes que van de 0.65 a 1 (ver McWhorter y López-Calleja 2000 para una revisión más profunda sobre este tema en aves).

Dos tipos de limitaciones fisiológicas pueden restringir la cantidad de energía que los animales nectarívoros pueden ingerir ante un cambio de la concentración de azúcares en el néctar del que se alimentan: 1) limitantes

digestivas y 2) limitantes renales. Cuando al alimentarse a diversas concentraciones un animal mantiene tasas de ingestión de nutrientes constantes, pero pierde masa corporal, una limitante digestiva esta restringiendo la cantidad total de energía que el organismo puede asimilar (McWhorter y Martínez del Rio 2000). Por otro lado, debido a la baja densidad de energía contenida en néctares diluidos, los animales nectarívoros que se alimenten de ellos deberán consumir grandes volúmenes de agua para tratar de obtener la energía necesaria para cubrir sus necesidades metabólicas. Ante estas circunstancias, la habilidad renal del organismo para eliminar agua, y vaciar el tracto digestivo, puede limitar la obtención de energía (Martínez del Rio *et al* 2001).

Determinar si el consumo de alimento esta limitado por procesos fisiológicos nos permite estimar la cantidad máxima de energía que un organismo puede procesar, y como esta se relacionan con la composición y la disponibilidad de néctar en su ambiente natural. En la primera parte de esta tesis (Capítulo 1) se describe la respuesta de consumo de 3 especies de murciélagos filostómidos y se discuten los posibles factores que las limitan. Finalmente con el objetivo de relacionar las características del tracto digestivo con el consumo de alimento, en este capítulo se utilizó un modelo matemático que permite predecir las respuestas de consumo en organismos que se alimentan de néctar (ver McWhorter y Martínez del Rio 2000).

Cambios en respuesta de consumo asociados a cambios estacionales

Estudiar la respuesta de consumo puede proveer información sobre los patrones de alimentación y forrajeo de los animales nectarívoros en campo

(Martínez del Rio *et al*. 2001). Sin embargo las limitantes fisiológicas de estos pueden ser modeladas por cambios en las características físicas del ambiente. Por ejemplo Owen (1960), West (1968) y Case y Robel (1974) encontraron que una disminución en temperatura causa un incremento en el consumo de alimento en aves. McWhorter y Martínez del Rio y McWhorter y López-Calleja discuten que los presupuestos energéticos de los organismos están limitados por factores centrales (limitantes fisiológicas) y periféricos (características físicas del ambiente). Estas características pueden no solo moldear las respuestas de consumo, sino que en conjunto pueden actuar como limitantes en la distribución de los organismos. Por este motivo, la segunda parte de la tesis esta dirigida a entender las respuestas de consumo asociadas a cambios estacionales en donde las temperaturas mínimas en un ambiente estacional tienden a ser muy diferentes en época de lluvias y secas. Esperamos que aunque los murciélagos estén limitados fisiológicamente, incrementarán el consumo de alimento en respuesta al un incremento en gasto metabólico cuando la temperatura disminuye en época de secas (Figura II)

Log Consumo

Limitante fisiológica
Secas
Lluvias

Log Concentración
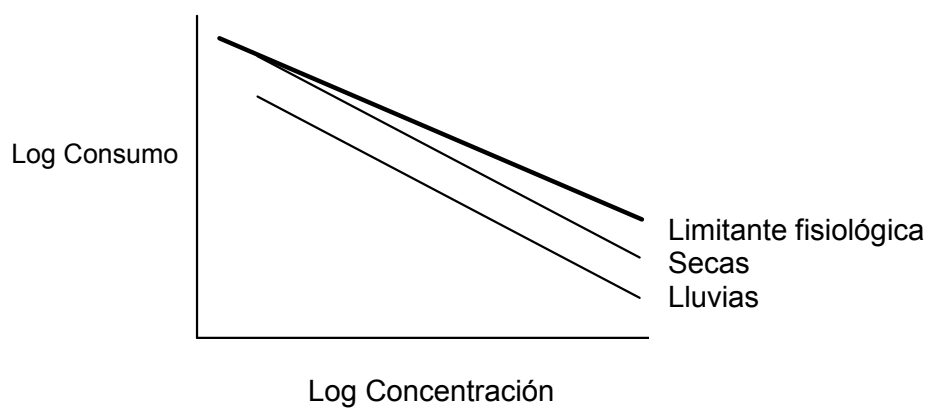
Figura II. Predicciones de respuestas de consumo en dos estaciones del año: secas y lluvias. Asumimos que la cantidad de alimento consumido se incrementará en respuesta a un incremento en el gasto metabólicocuando la temperatura disminuye (época de secas).

REFERENCIAS

Allen-Wardell, G., P. Bernhardt, R. Bitner, A. Burquez, S. Buchmann, J. Cane, P. A. Cox, V. Dalton, P. Feinsinger, M. Ingram, D. Intuye, C. E. Jones, K. Kennedy, P. Kevan, H. Koopowitz, R. Medellín, R., S. Morales, G. P. Nabhan, B. Pavlik, V. Tepedino, P. Torchio, y S. Walter. 1998. The potential consequences of pollinator declines on the conservation of biodiversity and stability of food crop yields. *Conservation Biology* 12: 8-17.

Carpenter, F. L., M. A. Hixon, A. Hunt y R. W. Russell. 1991. Why hummingbirds have such large crops. *Evolutionary Ecology* 5: 405-414.

Carter, R. R. y W. L. Grovum. 1990. Factors affecting the voluntary intake of food by sheep. 5. The inhibitory effect of hypertonicity in the rumen. *British Journal of Nutrition* 64: 285-299.

Case R. M. y Robel, R. J. 1974. Bionergetics of the bobwithe. *Journal of wildlife management* 38: 638-652.

Castle, K. T. y B. A. Wunder. 1995. Limits to food intake and fiber utilization in the prairie vole, *Microtus orchrogaster*: effects of food quality and energy need. *Journal of Comparative Physiology B* 164: 609-617.

Collins, B. G. 1981. Nectar intake and water balance for two species of Australian honeyeater, *Lichmera indistincta* and *Acanthorynchus superciliosis*. *Physiological Zoology* 54: 1-13.

Denbow, D. M. 2000. Gastrointestinal anatomy and physiology. En: G. C. Whittow (ed.) Sturkie's. *Avian physiology*. New York, Academic Press. Paginas 299-325.

Diamond, J. 1991. Evolutionary design of intestinal nutrient absorption: enough but not too much. *News in Physiological Sciences* 6: 92-96.

Diamond, J. M., W. H. Karasov, D. Phan y F. L. Carpenter. 1986. Digestive physiology is a determinant of foraging bout frequency in hummingbirds. *Nature* 320: 62-63.

Dobat, K. y T. Peikert-Holle. 1985. Blüten und fledermäuse. Verlag Waldemar Kramer, Frankfurt, Alemania.

Downs, C. T. 1997. Sugar digestion efficiencies of Gurney's Sugarbirds, Malachite Sunbirds, and Black Sunbirds. *Physiological Zoology* 70: 93-99.

Fleming, T. y V. J. Sosa. 1994. Effects of nectarivorous and frugivorous mammals on reproductive success of plants. *Journal of Mammalogy* 75: 845-851.

Hainsworth, F. R. 1973. On the tongue of a hummingbird: its role in the rate and energetics of feeding. *Comparative Biochemistry and Physiology A* 46: 65-78.

Heithaus, E. R., T. H. Fleming y P. A. Opler. 1975. Foraging patterns and resource utilization in seven species of bats in a seasonal tropical forest. *Ecology* 56: 841-854.

Helversen, O. v. 1993. Adaptations of flowers to the pollination by Glossophaginae bats. En: W. Barthlott (ed.). *Animal-plant interactions in tropical environments.* Museum Alexander Koenig, Bonn, Alemania. Paginas 41-59.

Karasov, W. H. 1990. Digestion in birds: chemical and physiological determinants and ecological implications. *Studies in Avian Biology* 13: 391-415.

Karasov, W. H. y J. M. Diamond. 1988. Interplay between physiology and ecology in digestion. *Bio Science* 38: 602-611.

Karasov, W. H. y I. D. Hume. 1997. Vertebrate gastrointestinal system. En: W. Dantzler (ed.). *Handbook of comparative physiology*. Bethesda, MD, American Physiological Society. Paginas 409-480.

Kearns, C. y D. Inouye. 1997. Pollinators, flowering plants, and conservation biology. *Bioscience* 47: 297-307.

Kearns, C., D. Inouye, y N. Waser. 1998. Endangered mutualisms: the conservation of plant-pollinator interactions. *Annual Review of Ecology and Systematics* 29: 83-112.

Levey, D. J. y C. Martínez del Rio. 1999. Test, rejection, and reformulation of a chemical reactor-based model of gut function in a fruit-eating bird. *Physiological and Biochemical Zoology* 72: 369-383.

López-Calleja, M. V., F. Bozinovic y C. Martínez del Rio. 1997. Effects of sugar concentration on hummingbird feeding and energy use. *Comparative Biochemistry and Physiology* 118A: 1291-1299.

Martínez del Rio, C. y W. H. Karasov. 1990. Digestion strategies in nectar- and fruit-eating birds and the sugar composition of plant rewards. *The American Naturalist* 136: 618-637.

Martínez del Rio, C. y C. Restrepo. 1993. Ecological and behavioral consequences of digestion in frugivorous animals. *Vegetatio* 107/108: 205-216.

Martínez del Rio, C., J. E. Schondube, T. J. McWhorter y L. G. Herrera. 2001. Intake responses in nectar feeding birds: digestive and metabolic causes, osmoregulatory consequences, and coevolutionary effects. *American Zoologist* 41: 902-915.

Martínez del Rio, C. y B. R. Stevens. 1989. Physiological constraint on feeding behavior: intestinal membrane disaccharides of the starling. *Science* 243: 794-796.

McWhorter, T. J. y M. V. López-Calleja. 2000. The integration of diet, physiology, and ecology of nectar-feeding birds. *Revista Chilena de Historia Natural* 73: 415-460.

McWhorter, T. J., y C. Martínez de Rio. 1999. Food ingestion and water turnover in hummingbirds: how much dietary water is absorbed? *Journal of Experimental Biology* 202: 2851-2858.

Mitchell, R. J. y D. C. Paton. 1990. Effects of nectar volume and concentration on sugar intake rates of Australian honeyeaters (Meliphagidae). *Oecologia* 83: 238-246.

Montgomery, M. J. y B. R. Baumgardt. 1965. Regulation of food intake in ruminants. 2. Pelleted rations varying in energy concentration. *Journal of Dairy Science* 48: 569-577.

Montgomerie, R. D. 1984. Nectar extraction by hummingbirds: response to different floral characters. *Oecologia* 63: 229-236.

Owen, R.B. 1970. The bioenergetics of captive Blue-winged Teal under controled and outdoor conditions. *Condor* 72: 156-173.

Paton, D. C. y B. G. Collins. 1989. Bills and tongues of nectar-feeding birds: a review of morphology, function and performance, with intercontinental comparisons. *Australian Journal of Ecology* 14: 473-506.

Pyke, G. H. and N. M. Waser. 1981. The production of dilute nectars by hummingbird and honeyeater flowers. *Biotropica* 13: 260-270.

Schondube, J. E. y C. Martínez del Rio. 2003. Concentration-dependent sugar preferences in nectar-feeding birds: mechanisms and consequences. *Functional Ecology* 17: 445-453.

Simpson, S. J., L. Barton-Browne y A. C. M. van Gerwen. 1989. The patterning of compensatory feeding in the Australian sheep blowfly. *Physiological Entomology* 14: 91-105.

Slansky, F. y G. S. Wheeler. 1992. Caterpillars compensatory feeding response to diluted nutrients leads to toxic allelochemical dose*. Entomiologia Experimentalis et Aplicatta* 65: 171-186.

Tamm, S. y C. L. Gass. 1986. Energy intake rates and nectar concentration preferences by hummingbirds. *Oecologia* 70: 20-23.

CAPITULO 1. FACTORES QUE AFECTAN LA RESPUESTA DE CONSUMO EN MURCIÉLAGOS NECTARÍVOROS

The intake responses of three species of leaf-nosed Neotropical bats

JORGE AYALA-BERDON[1], JORGE E. SCHONDUBE[1], KATHRYN E.
STONER[1], NELLY RODRIGUEZ-PEÑA[1], AND CARLOS MARTÍNEZ DEL RIO[2]

[1]*Centro de Investigaciónes en Ecosistemas, Universidad Nacional Autónoma*

de México, Apartado Postal 27-3 (Xangari), 58089, Morelia, Michoacán, México

[2]*Department of Zoology and Physiology, University of Wyoming, 82071-*

3166, Laramie, Wyoming, USA.

Abstract

We investigated intake response to sugar concentration in food of three Neotropical bat species: *Leptonycteris curasoae*, *Glossophaga soricina* and *Artibeus jamaicensis*. Our experiments were conducted in the forest in outdoor enclosures (2.0 x 4.0 x 1.6m) where bats could fly freely while foraging. We measured food intake at various concentrations of sucrose or 1:1 mixtures of glucose-fructose (292, 438, 584, 730, 876 and 1022 sucrose equivalents). Physiological mechanisms limited food intake in the three species of bats. Curiously, bats did not show differences in the intake between sucrose and the 1:1 glucose-fructose solutions. This indicates that digestion and absorption in bat intestines are coupled. Our results suggest that, on the basis of energy intake, bats should not prefer hexoses over sucrose. We used a mathematical model of the rate of sucrose hydrolysis and the small intestinal volume of bats to predict intake responses. The model described the intake response of the nectarivores *Leptonycteris curasoae* and *Glossophaga soricina* adequately but was a very poor descriptor of the intake response of the frugivorous *Artibeus jamaicensis*. The intake responses of bats to sugar solutions seem to be shaped by post-ingestional physiological factors in the case of *Leptonycteris curasoae* and *Glossophaga soricina*, and by pre and post-ingestional factors in *Artibeus jamaicensis*.

Introduction

The behavioural response of animals to foods that vary in energy concentration has been described in a variety of animals, ranging from insects (Slansky and Wheeler 1992; Josens et al. 1998) to large mammals (Spiegel 1973; Hansen et al. 1981). Typically, animals decrease intake as the concentration of assimilable energy in food increases (Montgomery and Bumgardt 1965; Slansky and Wheeler 1992). Castle and Wunder (1995) named the reciprocal relationship between intake and energy content "intake response".

The intake response has been well studied in nectar-feeding birds (Collins 1981; López-Calleja et al. 1997; McWhorter and Martínez del Rio 2000; Schondube and Martínez del Rio 2003). In these animals, intake decreases as a power function of sugar concentration (intake = a(concentration)^b; where b < 0 (McWhorter and Martínez del Rio 2000). Although less well known in bats, three recent studies, one on an old-world bat (Rousettus aegyptiacus; Korine et al. 2004) and two on New Word bat Glossophaga soricina (Helverson and Winter 2003; Ramírez et al. 2005) suggest that the responses of nectar-feeding bats to sugar concentration in food are similar to those of nectar-feeding birds.

The power function relating intake with sugar concentration has two non-exclusive explanations (Martínez del Rio et al. 2001). One is that nectar-feeding animals simply vary ingestion to maintain a constant sugar intake. In this compensatory feeding scenario, the exponent of the power function equals -1 (Montgomery and Bumgardt 1965; Slansky and Wheeler 1992), implying that sugar intake remains constant as concentration varies. Helverson and Winter

(2003) reported that Glossophaga soricina was able to maintain daily energy intakes of 48 kJ d^{-1} when faced with concentrations ranging from 292 to 876 mmol L^{-1} sucrose equivalents under laboratory conditions. The alternative explanation to compensatory feeding is that a physiological process constrains food-intake (Levey and Martínez del Rio 1999; Martínez del Rio et al. 2001).

McWhorter and Martínez del Rio (2000) constructed a mathematical model that predicts nectar intake from data on the kinetics of sucrose hydrolysis and the morphological characteristics of the small intestine. This model accurately predicts the relationship between intake and concentration in broad-tailed hummingbirds. In contrast with the compensatory feeding explanation, the power function resulting from McWorther and Martínez del Rio's model has an exponent of -0.7. An exponent with such a value leads to a positive relationship between total sucrose intake and sugar concentration in food. Ramírez et al. (2005) applied the model to the nectar-feeding bat Glossophaga soricina under laboratory conditions and found that it describes the observed intake response of this species adequately. The exponent of the intake response power function in these bats was -0.52, indicating that the bats were physiologically limited to ingest food while feeding on sucrose solutions. Ramírez et al. (2005) hypothesized that sucrose digestion may be the limiting step shaping the intake response of this species.

In this paper we explored how bats modulate their nightly food intake in response to food of various concentrations and sugar compositions. Specifically, we investigated the role that sucrose digestion has over the intake

responses of three Neotropical bat species in the family Phyllostomidae: *Leptonycteris curasoae*, *Glossophaga soricina* and *Artibeus jamaicensis*. We measured the intake responses of bats to food containing various concentrations of sucrose or 1:1 mixtures of glucose-fructose. We expected bats to be like some passerine birds in which sucrose hydrolysis is more limiting than hexose uptake (Nicolson 2001; Nicolson and Fleming 2003; Schondube and Martínez del Rio 2003), and hence that these animals would ingest more hexoses than sucrose if food contains the same caloric concentration. We use published data on the rate of sucrose hydrolysis and the small intestinal volume of bats (Hernández and Martínez del Rio 1992; Schondube et al. 2001) to examine whether the model predicts the bats´ intake response accurately.

Materials and methods

BAT CARE AND HOUSING

Adult non-reproductive individuals of the three species were captured using mist nets and transferred to a room temperature laboratory were they were maintained in colonies in cages (0.6x0.6x0.6m). Bats were fed on the maintenance diet described by Mirón (2005) that offered 22.2% sucrose and 4.4% of protein. The diet was supplemented with NEKTON-Plus®. (Guenter Enderle, Tarpon Springs, FL, USA) Individuals were marked on the forearm with a numbered band and weighed daily at the beginning and end of each feeding trial using an electronic balance (0.01g precision). Captive bats maintained mass for the duration of the experiments and were released at their capture site when experiments were finished.

INTAKE RESPONSES

We measured nightly food intake of individual bats in outdoor enclosures (2.0 x 4.0 X 1.6m) placed within the forest where bats could forage freely. During each feeding trial we offered a solution of sucrose or a 1:1 mixture of glucose-fructose from 20:00 to 06:00. Six individuals were used for each of the three bat species. Each bat was offered 7 solutions of sucrose and 7 solutions of glucose-fructose. The sugar concentrations of these solutions were 146, 292, 438, 584, 730, 876, and 1022 mmol L^{-1} sucrose equivalents. We offered individual bats each of the concentrations in a random order. We weighed solutions at the beginning and end of each feeding trial to quantify food intake (electronic balance 0.01-g precision). Because experimental solutions lacked nitrogen sources, our experiments consisted in three nights of experiments per one day of resting. During the "resting day" bats received maintenance diet. Each night we placed a feeder full of each concentration outside of the flying cages to control for evaporation and changes in concentration. These feeders were covered with a mosquito mesh, to avoid insects and other nocturnal animals from drinking from them. 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.

MODEL AND DATA ANALYSIS

We estimated the slopes and intercepts of the relationships between food intake and concentration with least squares regression analysis on the log-transformed data of each individual bat. We compared the slopes and intercepts of the intake responses of the sucrose, and the glucose-fructose diets with a two-sample t-test. We compared the value of the intake responses' exponents to the -1 value expected from the compensatory feeding expectation using a one-sample t-test. We expected the change in mass (Δ**M**) experienced by bats to correlate positively with their sugar intake (**SI**). To test this conjecture we correlated Δ**M** against the **SI** of each bat with Spearman's rank correlation (r_s) and tested whether the average r_s was significantly greater than 0. This procedure is appropriate because it avoids the pseudoreplication that one would incur in 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 Odd 1994). Additionally, we compared our results with intake predictions from McWother and Martínez del Rio's (2000) model. This model assumes that the intestine is analogous to a chemical reactor, in which sucrose hydrolysis (-r_s) follows Michaelis-Menten kinetics:

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

Where $S_{\max}$ is the rate of hydrolysis along the intestine (µmol min⁻¹µL⁻¹), K_m is sucrase's Michaelis-Menten constant (µmol µL⁻¹), and C_s is the concentration of sucrose (µmol µL⁻¹) along the intestine. The time (τ) required to reduce the initial concentration of sucrose (C_{50}) to a given final value (Cs_f) can then be integrated from equation (1) to:

$$\tau = \frac{K_m \ln(C_{50} / C_{sf}) + (C_{50} - C_{sf})}{S_{max}}, \ (2)$$

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

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

The parameters that we used to fit the model and the sources from which we obtained them 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-Spechel 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).

Results

DOES THE INTAKE RESPONSE TO SUCROSE SOLUTIONS DIFFER FROM THAT OF HEXOSE MIXTURES?

The three bat species decreased intake as sugar concentration increased and the relationship between food intake and sugar concentration was well described by power functions (Table 2, Fig. 1). The response of bats to sucrose solutions did not differ from their response to hexose mixtures. We found that the intercepts and slopes of these relationships did not differ (Table 2). The exponent of the functions relating intake with concentration was in all cases significantly different from -1 (Table 2). Sugar intake increased significantly with sugar concentration in food for the nectarivores *Leptonycteris curasoae* and *Glossophaga soricina* (Fig 2; r_s = 0.83 ± 0.04 SE and 0.67 ± 0.04 respectively, t > 17.40, P < 0.001), however, in the frugivore *Artibeus jamaicensis* sugar intake and sugar concentration in food were not significantly correlated (average r_s = 0.386 ± 0.16 SE, t = 2.41, P= 0.061). The average Spearman-rank coefficient of correlation between **SI** and **ΔM** was significantly different from 0 for *Leptonycteris curasoae* (average r_s = 0.350 ± 0.08 SE, t = 4.03, P= 0.020), *Glossophaga soricina* (average r_s = 0.210 ± 0.06 SE, t = 3.35, P= 0.015) and *Artibeus. jamaicensis* (average r_s = 0.580 ± 0.12 SE, t = 4.68, P= 0.005). However the positive relationship between **SI** and **ΔM** in the three species were variable and weak (Fig 3).

DOES THE MODEL ACCURATELY PREDICT THE BAT'S INTAKE RESPONSE?

The model described the shape of the relationship between intake and sucrose concentration in *Leptonycteris curasoae* (r^2 = 0.539) and *Glossophaga soricina* (r^2 = 0.673) well. However, the model underestimated intake in *Leptonycteris curasoae* and overestimated it slightly in *Glossophaga soricina* (Fig 4a and b). The power functions estimated by non-linear regression provided better fits to the data than the model (r^2 = 0.861 and r^2 = 0.837 for *Leptonycteris curasoae* and *Glossophaga soricina* respectively). In the case of *Artibeus jamaicensis* the model greatly overestimated intake (Fig 4c). The residual sum of squares was much larger than the total sum of squares, yielding a negative value for r^2. This result implies a very poor fit of the model to the data. Indeed, it implies that the mean intake at all concentrations is a better descriptor of the relationship between intake and concentration than the model (Anderson-Sprechel 1994). A power function fitted using non-linear regression fitted the data well (R^2 = 0.69, fig 4c).

Discussion

The three species of bats we studied had intake responses similar to those that have been observed in bats (Korine *et al.* 2004; Ramírez *et al.* 2005) and birds (Martínez del Rio *et al.* 2001; McWhorter and Lopez-Calleja 2000 among others). Furthermore there were no differences in the intake between sucrose and the 1:1 glucose-fructose solutions. The exponents of the intake responses for the three species of bats were different from -1, showing that sugar intake is positively correlated with sugar concentration (Levey and Martínez del Rio 1999; Martínez del Rio *et al.* 2001). Here we first discuss the potential prevalence of intake responses among nectar-feeding vertebrates and the

consequences of this prevalence for osmoregulation in nectar and fruit-eating bats. Next we consider the implications of no differences in the intake response of bats to sucrose and hexose solutions for the interaction between bats and plants and the plants that they pollinate. Finally we evaluate the role that digestive processes, like sucrose hydrolysis and hexose transport, have over the food intake of bats.

The osmoregulatory consequences of the prevalence of intake responses among nectar-feeding vertebrates

Our results demonstrate that three Neotropical bat species, with different levels of dietary specialization for nectar, show intake responses similar to those being found in other nectar and fruit-eating animals. The intake responses of *Leptonycteris curasoe*, *Glossophaga soricina* and *Artibeus jamaisensis* were similar to those reported for nectar and fruit-eating bats in the past (Thomas 1984; Helverson and Winter 2003; Korine *et al.* 2004; Ramirez *et al.* 2005). As in these studies we found that bats respond to sugar concentration in food in the same fashion as birds do. We suggest that their response probably leads to the same consequences observed in birds. For example, Bakken *et al.* (2004) and McWhorter *et al.* (2004) found that intake responses in birds lead to very high water ingestion rates at low sugar concentrations. Similarly at low concentrations *Leptonycteris curasoae* and *Glossophaga soricina* ingested from 1.0 to 3.5 and 1 to 5 times respectively of their body mass in water (Fig 1).

Bakken *et al.* (2004) identified the osmoregulatory quandary faced by hummingbirds. When feeding, these birds must dispose of ingested water

rapidly. However, while they are fasting, hummingbirds must conserve water.

This predicament is aggravated by the apparent inability of hummingbirds to

concentrate urine (Lotz and Martínez del Rio 2004). Our intake response

results suggest that nectar-feeding bats probably share this osmoregulatory

dilemma with hummingbirds. They ingest, and must dispose of large amounts

of water when they are feeding. However, when they are fasting, they must

conserve water. Like hummingbirds, the kidneys of nectar and fruit-eating bats

seem better suited to dispose of water and to recover electrolytes than to

concentrate urine (Studier and Wilson 1983; Herrera *et al.* 2001; Schondube *et*

al. 2001). The threat of dehydration in fasting bats may be exacerbated by

evaporative water losses through their thin wing membranes (Baudinette *et al.*

2000). The mechanisms used by bats to resolve the osmoregulatory

predicament posed by intake responses are unknown and need to be explored.

The intake responses of bats did not differ between sugar types

Bats´ intake responses to sucrose and hexoses were indistinguishable. This

implies that for equicaloric concentrations bats ingested the same amount of

energy of glucose and fructose than of sucrose. This result is surprising on two

accounts. First, in the New World nectar-pollinated flowers secrete primarily

hexose-dominated flowers (Pyke and Waser 1981; Baker and Baker 1983;

Baker *et al.* 1998). Our results suggest that, on the basis of energy intake, bats

should *not* prefer hexoses over sucrose. Second, Herrera (1999) found that

nectar-feeding bats preferred sucrose to equicaloric hexose solutions. Our

results suggest that they should be indifferent. One possible explanation is that

bats are using cues other than energy intake, such as taste, when they make

choices between different sugar solutions (Herrera 1999; Herrera *et al.* 2000).
The preferences of bats for different sugars and the reasons that explain the
prevalence of hexose-dominated nectars among New-world bat-pollinated
plants remain unexplained.

Is food intake constrained in bats?

In all cases the exponents of the intake responses were higher than -1 (Table
2). This exponent implies a positive correlation between sugar/energy intake
and the sugar concentration of ingested food, and for this reason it has been
interpreted as evidence of feeding constrained by one or many physiological
processes (McWorter and Martínez del Rio 2000). The existence of a
constraint is further supported by the positive correlation between sugar intake
and sugar concentration. In compensatory feeders, energy intake remains
relatively constant across concentrations (Lopez-Calleja *et al.* 1997; Martínez
del Rio *et al.* 2001; Helverson and Winter 2003). Ramirez *et al.* (2005)
suggested that sucrose hydrolysis might be the process that constrains intake in
Glossophaga soricina. However, we found that there were no differences in the
bats´ intake response to glucose and fructose. This result is similar to that
reported by Schondube and Martínez del Rio (2003) in magnificent
hummingbirds (*Eugenes fulgens*). The absence of a difference in the response
of bats to sucrose and hexose solutions suggests that neither sucrose
hydrolysis nor the uptake of hexoses is limiting. Sugar digestion and absorption
in bat intestines seem to be designed according to the principle of
symmorphosis, which postulates that physiological processes in series are
designed so that no step is more limiting than others (Weibel 2000).

Although the intake responses of the three species were qualitatively similar, there were some interesting differences. These differences are best highlighted by considering how well McWhorter and Martínez del Rio's (2000) model described the intake responses of bats. The model performed very poorly for the frugivore *Artibeus jamaicencis*. These bats appear to ingest much less food than their intestines are capable of processing. Indeed, when all trials are considered, *Artibeus jamaicensis* was the only species to maintain neutral mass ($\Delta W = 0.48 \pm 0.75$ SD, $t = 1.80$, $p > 0.05$). Both of the nectarivores, *Glossophaga soricina* ($\Delta W = 0.59 \pm 0.75$, $t = 6.7$, $p < 0.001$) and *Leptonycteris curasoae* (mean $\Delta W = 1.7 \pm 0.62$ SE, $t = 22.8$, $p < 0.001$) gained mass in these trials. Intake response studies in nectar-feeding birds emphasize potential post-ingestional constraints on feeding. Since *Artibeus jamaicensis* is a frugivore that only ingests nectar occasionally, its oral morphology is better suited to chew fruit than to lap nectar (Ortega and Castro-Arellano 2001). We hypothesize that the intake response of *Artibeus jamaicensis* was constrained more by pre-ingestional than by post-ingestional factors (Paton and Collins 1989; Mitchell and Paton 1990).

Both Helverson and Winter (2003) and Ramirez *et al.* (2005) measured the intake response of *Glossophaga soricina*. While Helverson and Winter found that bats were able to maintain a constant daily energy intake under laboratory conditions, Ramirez and collaborators (2005) reported that *Glossophaga soricina* was unable to perform compensatory feeding. Our field experiments support the last result. Ramirez *et al.* (2005) found that *Glossophaga soricina* had an exponent of -0.52, higher than the one we found

(-0.76), and that bats ingested significantly less food per hour than in our experiments. One possible explanation for differences among the three studies is the use of different experimental designs. Helverson and Winter (2003) conducted their experiments in large flying cages, controlling temperature, relative humidity and availability of food per visit. Ramirez *et al.* (2005) also conducted their experiments under controlled temperature and humidity, but used small cages (0.2 x 0.18 x 0.18m) that did not allow flying, and offered the bats an unlimited amount of food. Our experiments were conducted under natural conditions (in the forest) within medium size cages (2 x 4 x 1.6m) that allowed flying and *ad libitum* food. We suspect that the difference in experimental conditions led to the differences found among studies. These differences emphasize that experimental conditions, especially those that lead to differences in energy expenditures and water balance, can have a significant influence on the intake response of nectar-feeding animals.

We found that McWhorter and Martínez del Rio's model (2000) explained 53% and 67% of the variation in intake response for *Leptonycteris curasoae* and *Glossophaga soricina* respectively. Although, power functions described the data better than this model (Fig 4a and b), the adequate fit of the model is remarkable. It is more remarkable given that the enzyme and morphology data that we used to fit the model was obtained from the literature (Hernandez and Martínez del Rio 1992; Schondube *et al.* 2001), and represents a different set of individuals. In addition, we used Winter's assimilation efficiency (1998) data to test the model sensitivity to variation in this parameter. A relatively small decrease in sugar assimilation efficiency from 0.998 (Winter 1998) to 0.991

(data obtained from bats´ excreta), leads to a large change (17%) in intake at the lowest concentration (fig 4). We suggest that the model could describe accurately the intake responses of bats, but ideally its parameters must be drawn from the same population of bats in which the intake response is measured.

Both our experimental results and the predictions from the model indicate that both sucrose digestion and the uptake of hexoses can limit the food intake of nectar-feeding bats. These bats have high sucrose digestion rates that seem to be coupled with high transport rates of glucose and fructose. We speculate that food intake is not limited by a single factor but potentially by many steps along the digestion to catabolism pathway. The high water intakes of bats at the lowest sugar concentrations also lead us to speculate that getting rid of excess absorbed water may represent a problem to these animals. Limitations to food intake, while feeding on nectar, in fruit- and nectar-feeding bats seem to be caused by both pre- and post-ingestional factors, such as mouth and tongue morphology in the fruit-eating bat *Artibeus jamaicensis*, and digestive, osmoregulatory and metabolic processes in *Leptonycteris curasoae* and *Glossophaga soricina*.

References

Anderson-Sprecher R (1994) Model comparisons and R^2. Am Stat 48:113–117.

Bakken BH, McWorther TJ, Tsahar E, Martínez del Rio C (2004). Hummingbirds arrest their kidneys at night: diel variation in glomerular filtration rate in *Selasphorus platycercus*. J Exp Biol 207:4383–4391.

Baker HG, Baker IS (1983) Floral nectar constituents in relation to pollinator type. In: C. E. Jones and R. J. Little (eds.) Handbook of Pollinators. New York Scientific and Academic, pp. 117-141.

Baker HG, Baker IS, Hodges A (1998) Sugar composition of nectars and fruits consumed by birds and bats in the tropics and subtropics. Biotropica 30: 559–586.

Baudinette RV, Churchill SK, Christian KA, Nelson JE, Hudson PJ (2000). Energy, water balance and the roost microenvironment in three australian cave-dweling bats (Microchiroptera). J Comp Physiol [B] 170: 439–446.

Bullock S.H., 1995. Plant reproduction in neotropical dry forest. In: S. H. Bullock, H. A. Mooney and E. Medina (eds) Seasonally dry tropical forest. Cambridge University Press, Cambridge, pp. 277–203.

Castle KT, Wunder BA (1995) Limits to food intake and fiber utilization in the prairie vole *Microtus orchrogaster*: effects of food quality and energy need. J Comp Physiol [A] 164: 609–617.

Collins BG (1981) Nectar intake and water balance for two species of Australian honeyeater, *Lichmera indistincta* and *Acanthorynchus superciliosis*. Physiol Zool 54: 1–13.

Hansen CB, Jen KC, Kribs P (1981) Regulation of food Intake in monkeys.

    Physiol Behav 26: 479–486.

Hernandez A, Martínez del Rio C (1992) Intestinal disaccharidases in five

    species of phyllostomid bats. J Comp Physiol [B] 103: 105–11.

Herrera LG (1999) Preferences for different sugars in Neotropical nectarivorous

    and frugivorous bats. J Mammal 80: 683–688.

Herrera LG, Leblanc D, Nassar J (2000) Sugar discrimination and gustatory

    thresholds in captive-born frugivorous Old World bats. Mammalia 64: 135–

    143.

Herrera LG, Martínez del Rio C, Braun E, Hobson K (2001) Renal structure in

    phyllostomid bats: using stable isotopes to explore relationships between

    diet and morphology. Isot Environ Healt S 37: 1–11.

Hiebert SM, Calder WA, III (1983) Sodium, potassium, and chloride in floral

    nectars: energy-free contributions to refractive index and salt balance.

    Ecology 64: 399-402.

Inouye DW, Favre ND, Lanum JA, Levine DM, Meyers JB, Roberts MS, Tsao

    FC, Wang Y-Y (1980) The effects of non-sugar nectar constituents on

    estimates of nectar energy content. Ecology 61: 992-996.

JMP 5.1® (2003) SAS institute, Cary, NC, USA.

Josens RB, Farina WM, Roces F (1998) Nectar feeding by the ant *Camponotus*

    mus: intake rate and crop filling as a function of sucrose concentration. J

    Insect Physiol 44: 579–585.

Korine C, Speakman J, Arad Z (2004). Reproductive energetics of captive free-

    ranging egyptian fruit bats *Rossetus aegymtiacus*. Ecology 85: 220–230.

Levey DJ, Martínez del Rio C (1999). Test, rejection, and reformulation of a chemical reactor-based model of gut function in a fruit-eating bird. Physiol Biochem Zool 72: 369–383.

López-Calleja MV, Bozinovic F.,Martínez del Rio C (1997) Effects of sugar concentration on hummingbird feeding and energy use. Comp Biochem Physiol A 118: 1291–1299.

Lott EJ (1993) Annotated checklist of the vascular flora of the Chamela bay region Jalisco, Mexico. Occasional papers of the California Academy Science 148: 1-60.

Lott EJ, Bullock SH, Solis-Magallanes JA (1987) Floristic diversity and structure of upland and arroyo forest in coastal Jalisco. Biotropica 19: 228-235.

Lotz C, Martínez del Rio C (2004) The ability of rufous hummingbirds (*Celasphorus rufus*) to dilute and concentrate urine. J Avian Biol 35: 54–62.

Martínez del Rio C, Schondube JE, McWhorter TJ, Herrera LG (2001) Intake responses in nectar feeding birds: digestive and metabolic causes, osmoregulatory consequences, and coevolutionary effects. Am Zool 41: 902–915.

McWhorter TJ, López-Calleja MV (2000) The integration of diet, physiology and ecology of nectar-feeding birds. Rev Chil Hist Nat 73: 415–460.

McWorther TJ, Martínez del Rio C, Pinshow B, Roxburg L (2004) Renal function in Palestine Sunbirds: elimination of excess water does not constrain energy intake. J Exp Biol 23: 3391–3398.

Mirón, M.L., 2005. Efecto de la dieta en el fraccionamiento isotópico de 13C y 15N en el murciélago Glossophaga soricina (Chiroptera: Phillostomidae).

Tesis de maestría (Maestría en ciencias biológicas). Facultad de Ciencias UNAM.

Mitchell RJ, Paton DC (1990) Effects of nectar volume and concentration on sugar intake rates of Australian honeyeaters (Meliphagidae). Oecologia 83: 238–246.

Montgomery MJ, Baumgardt BR (1965) Regulation of food intake in ruminants. 2. Pelleted rations varying in energy concentration. J Dairy Sci 48: 569–577.

Nicolson SW (2001) Pollination by passerine birds: why are nectars so dilute?. Comp Biochem Physiol 4: 1–8.

Nicolson SW, Fleming PA (2003) Nectar as food for birds: the physiological consequences of drinking dilute sugar solutions. Plant Syst Evol 238: 139–153.

Ortega J, Castro-Arellano I (2001) *Artibeus jamaicensis*. Mamma Spec 662: 1–9.

Paton DC, Collins BG (1989) Bills and tongues of nectar-feeding birds: a review of morphology, function and performance, with intercontinental comparisons. Aust J Ecol 14: 473–506.

Pyke GH, Waser NM (1981) The production of dilute nectars by hummingbird and honeyeater flowers. Biotropica 13: 260-270.

Ramírez NP, Herrera GM, Mirón L (2005) Physiological constraint to food ingestion in a New World nectarivorous bat. Physiol Biochem Zool 78: 1032–1038.

Schondube JE, Herrera LG, Martínez del Rio C (2001) Diet and the evolution of digestion and renal function in phyllostomid bats. Zoology 104: 59–73.

Schondube JE, Martínez del Rio C (2003) Concentration-dependent sugar preferences in nectar-feeding birds: mechanisms and consequences. Funct Ecol 17: 445–453.

Slansky F, Wheeler GS (1992) Caterpillars compensatory feeding response to diluted nutrients leads to toxic allelochemical dose. Entomol Exp Appl 65: 171–186.

Spiegel TA (1973) Caloric regulation of food intake in man. J Comp Physiol Psychol 84: 24–37.

Stuart A, Ord JK (1994) Kendall's advanced theory of statistics. Volume 1. Edward Arnold, New York.

Studier EH, Wilson DE (1983) Natural urine concentrations and composition in Neotropical bats. Comp Biochem Physiol 75: 509–515.

Thomas DW (1984) Fruit intake and energy budgets of frugivorous bats. Physiol Zool 57: 457–467.

Weibel ER (2000) Symmorphosis: on form and function in shaping life. Harvard University Press, Cambridge.

Winter Y (1998) In vivo measurement of near maximal rates of nutrient absorption in a mammal. Comp Biochem Physiol 119: 853–859.

Table 1. Parameters used to fit the McWorter and Martínez del Rio's (2000) model. S_{max} is the rate of hydrolysis along the intestine, K_m is the sucrase Michaelis-Menten constant, C_{50} is the digestion efficiency of sucrose, and *G* is the volume of the intestine.

	Leptonycteris curasoae	*Glossophaga soricina*	*Artibeus jamaicensis*
[a] S_{max} (µmol min^{-1}µL^{-1})	*0.049*	*0.115*	*0.055*
[a] K_m (µmol µL^{-1})	*0.052*	*0.044*	*0.059*
[b] C_{50}	*0.009*	*0.009*	*0.009*
[a]G (µL)	*0.750*	*0.304*	*2.320*

[a] From Schondube *et al.* (2001).
[b] We measured the digestion 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 Martinez del Rio 2003). Because solutes other than sugars bias refractometer readings (Hiebert and Calder 1983; Inouye *et al..* 1980), our measurements of sugar concentration in excreta were used only to generate a relative measurement of digestion efficiency.

Table 2. Intake responses to sucrose and hexose mixtures with their respective exponents, intercepts, and coefficients of determination.

	Exponent ± SE	[a] Intercept ± SE	r^2
Glossophaga soricina			
S	-0.76* ± 0.064	3.40 ± 0.180	0.94 ± 0.020
G:F	-0.75* ± 0.076	3.38 ± 0.090	0.92 ± 0.020
t-test	*0.18*	*0.08*	*0.55*
P	*0.86*	*0.93*	*0.60*
Leptonycteris curasoae			
S	-0.61* ± 0.040	3.24 ± 0.110	0.9 ± 0.030
G:F	-0.62* ± 0.026	3.22 ± 0.090	0.96 ± 0.020
t-test	*0.19*	*0.28*	*1.76*
P	*0.86*	*0.80*	*0.15*
Artibeus jamaicensis			
S	-0.70* ± 0.079	3.46 ± 0.234	0.85 ± 0.050
G:F	-0.77* ± 0.078	3.57 ± 0.217	0.85 ± 0.039
t-test	*0.65*	*0.38*	*0.05*
P	*0.54*	*0.71*	*0.96*

S= Sucrose G:F= Glucose-fructose
*Mean exponent was significantly higher than –1 (t > 2.95, P < 0.005)
[a]=This intercept equals Log(a) in the equation log(intake)= Log(a) + bLog(concentration).

Figure legends

Figure 1. Intake response of the three species of phyllostomid bats. The axes are logarithmic, and a different symbol and a different regression line represent each individual. The heavy line is the common regression line obtained from the average of all intercepts and slopes. Closed symbols represent sucrose whereas open symbols represent hexoses.

Figure 2. Sugar intake increased significantly with sugar concentration in food for *Leptonycteris curasoae* and *Glossophaga soricina* but not in *Artibeus jamaicensis* . Closed symbols represent sucrose whereas open symbols represent hexoses.

Figure 3. Mass change (Δ**M**) increased as a function of sugar intake (**SI**). In the three species the average Spearman-rank correlation coefficient was significantly different from 0. Individuals are represented by different symbols. Closed symbols represent sucrose whereas open symbols represent hexoses.

Figure 4. Predictions generated by McWorther and Martínez del Rio's model (2000) are shown as a dashed line. A power function fitted with a non-linear square routine is represented with the solid line. The grey area represents the range of values predicted by the model when sugar assimilation is varied from from 0.998 (from Winter 1998) to 0.991 (data obtained from bats´ excreta). Data are only for the intake response of bats to sucrose solutions.

Figure 1.
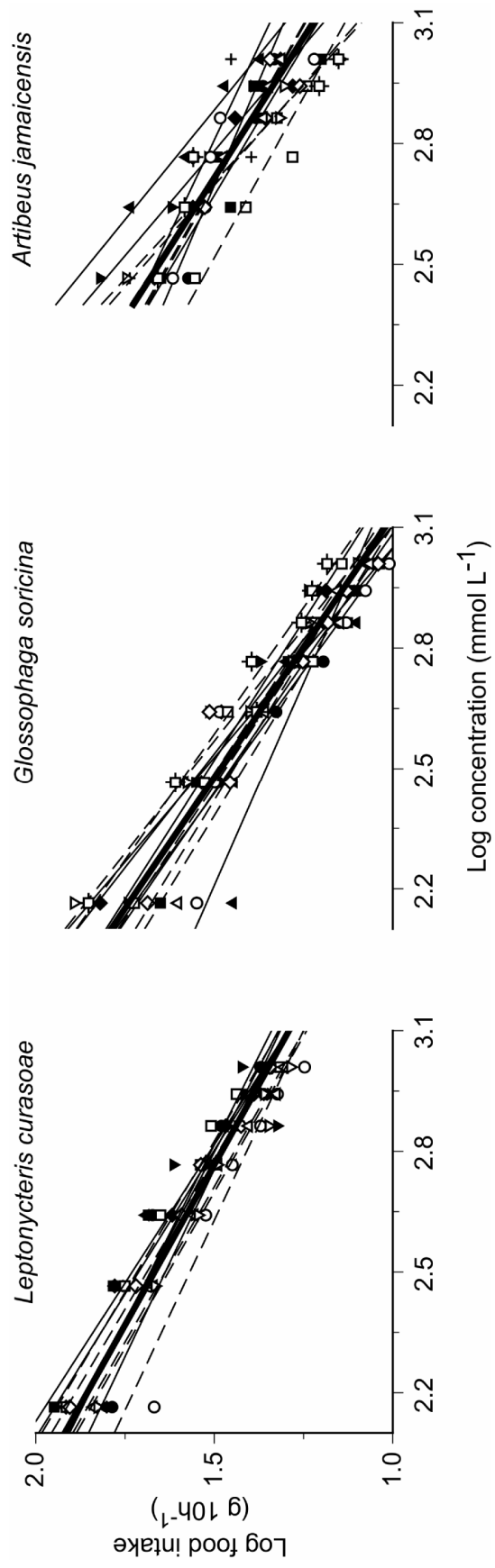
Figure 2.
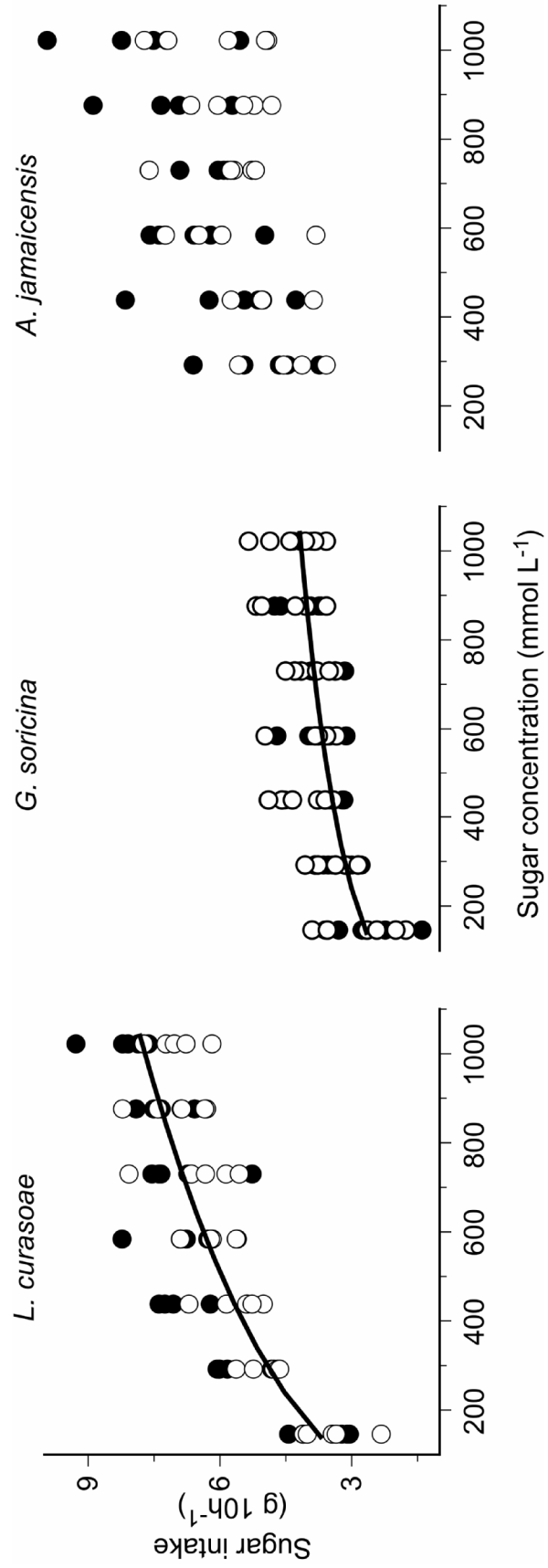
Figure 3.
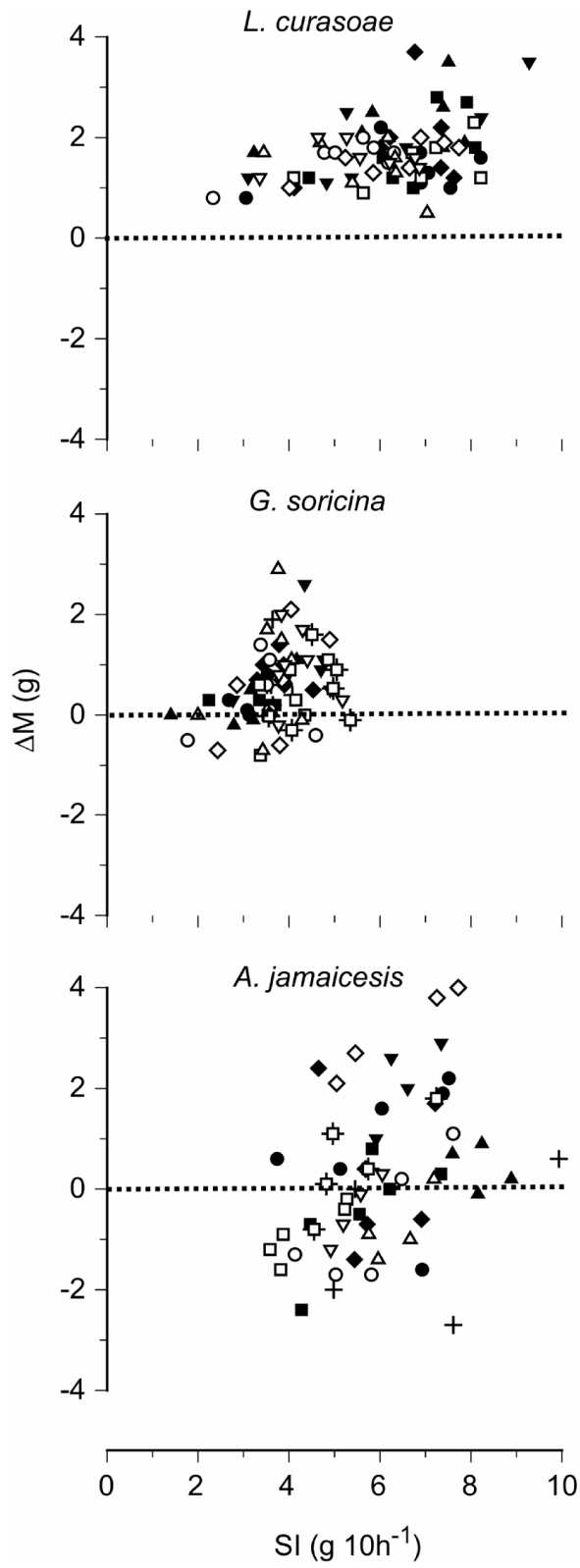
Figure 4
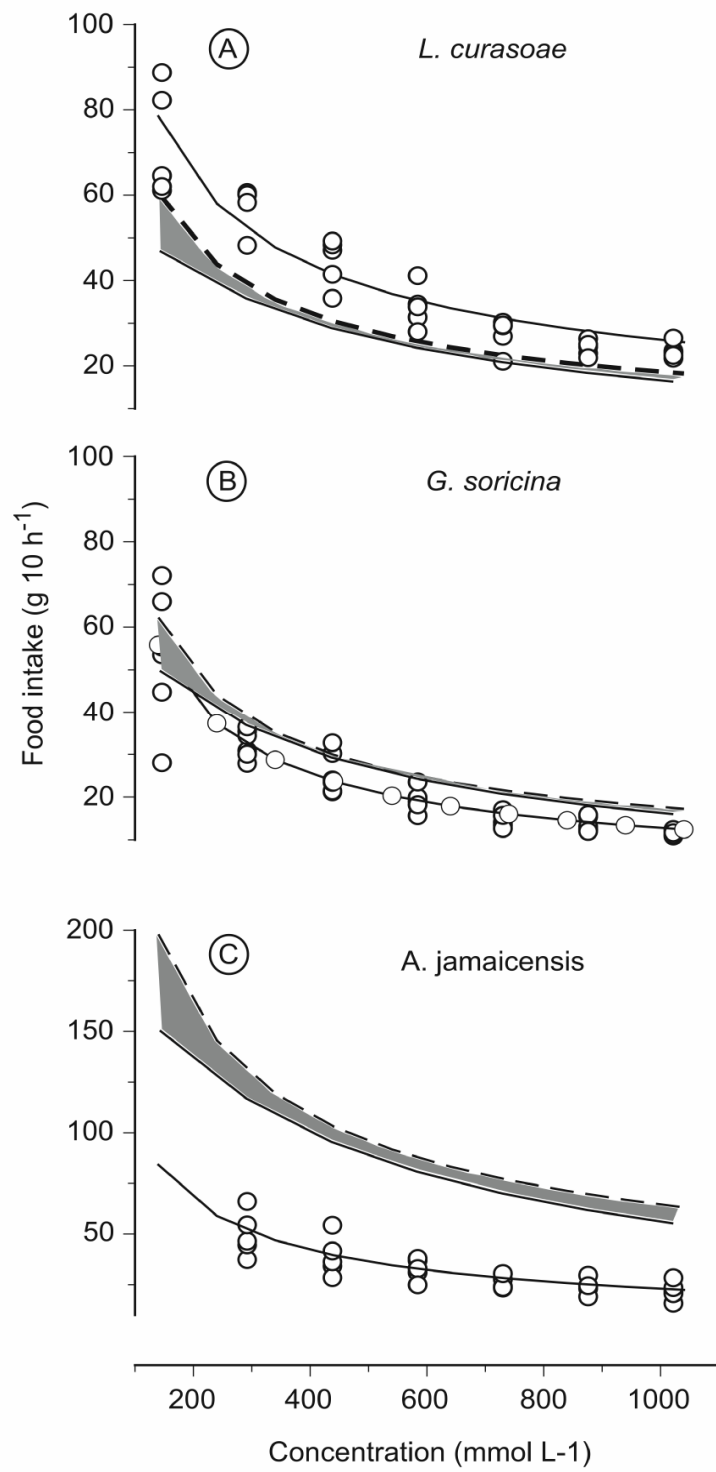
CAPITULO 2. CAMBIOS EN RESPUESTA DE CONSUMO ASOCIADOS A DIFERENCIAS ESTACIOALES DE TEMPERATURA

Seasonal intake responses in the nectar-feeding bat *Glossophaga soricina.*

JORGE AYALA-BERDÓN, JORGE E. SCHONDUBE[*] AND KATHRYN E. STONER

Centro de Investigaciónes en Ecosistemas, Universidad Nacional Autónoma de México, Apartado Postal 27-3 (Xangari), 58089, Morelia, Michoacán, México.

Abstract

We evaluated how both a seasonal change in minimum temperature and physiological constraints affected intake responses in the nectar-feeding bat *Glossophaga soricina*. We measured nightly food intake rate during both the wet/warm and dry/cool seasons at sucrose concentrations ranging between 146 and 1022 mmol L^{-1}. Our experiments were conducted in outdoor enclosures placed within the forest (2.0 x 4.0 x 1.6m) where bats could forage freely while flying. Season affected both the intake response and food intake rate of bats. Differences in energy expenditure between the wet/warm and dry/cool seasons may account for the differences we observed. Regardless of season, bats were unable to achieve compensatory feeding. This finding suggests that the rate of food intake is physiologically constrained in *G. soricina*. Using the rate of sucrose hydrolysis and the small intestine volume of bats, we modelled their intake responses. During both seasons, the predicted maximum intake rates from our model were generally higher than the intake rates we observed. Additionally, our model predicts "broken" intake responses when the metabolic expenditures of bats are increased above 50 kJ d^{-1}. A physiological constraint on the rate of food intake may explain the geographic distribution of *G. soricina*.

Key Words: digestive constraints, energy intake, metabolic demands, nectar-feeding bats, temperature.

Introduction

Animals change their food intake in response to both intrinsic and extrinsic factors. Intrinsic factors include the physiological and morphological characteristics that affect assimilation efficiency and gut transit time (Diamond *et al.* 1986; Karasov & Diamond 1988; Martínez del Rio & Stevens 1989; Karasov 1990; Martínez del Rio 1990; Diamond 1991; Martínez del Rio & Restrepo 1993; Karasov & Hume 1997). Extrinsic factors affecting food intake include food abundance, nutritional quality (Pyke & Waser 1981; Baker, Baker & Hodges 1998), and environmental conditions. The latter by affecting both the production of food resources and the nutritional/energetic demands of animals (McWhorter & Martínez del Rio 2000; Martínez del Rio *et al.* 2001; Schondube & Martínez del Rio 2003). One approach to understanding how intrinsic and extrinsic factors affect the feeding strategies of animals is to measure the relationship between food intake and food quality (Castle & Wunder 1995). These relationships, referred to by Castle and Wunder (1995) as the intake response, have informed our understanding of nutritional physiology in a diversity of organisms, ranging from insects to mammals (Spiegel 1973; Hansen, Jen & Kribs 1981; Slansky & Wheeler 1992; Josens, Farina & Roces 1998).

In nectar-feeding animals, food ingestion increases in response to decreases in the sugar concentration of nectar (Martínez del Rio *et al.* 2001; Helversen & Winter 2003; Schondube & Martínez del Rio 2003; Ramirez *et al.* 2005; Ayala *et al.* unpublished data). This response is often attributed to compensatory feeding (Simpson, Barten-Browne & Van Gerwen 1989;

McWhorter & Martínez del Rio 1999; McWhorter & López-Calleja 2000; Martínez del Rio *et al.* 2001). According to this explanation, animals regulate food intake to maintain a constant flux of nutrients or energy (Montgomery & Baumgardt 1965; Slansky & Wheeler 1992). For example, Helversen and Winter (2003) found that the nectar-feeding bat *Glossophaga soricina* compensated perfectly when feeding on different concentrations of sucrose. However, physiological processes have been shown to limit food intake rates and, therefore, prevent animals from maintaining constant rates of energy intake. In nectar-feeding animals, food intake could be constrained by their ability to assimilate the sugars contained in nectar (Carter & Grovum 1990; Savory 1999; Denbow 2000; Schondube & Martínez del Rio 2003) or by their capacity to process large amounts of water when feeding on concentrated and dilute nectars, respectively (Studier & Wilson 1983; Hernández & Martínez del Rio 1992; Herrera *et al.* 2001; Martínez del Rio *et al.* 2001; Schondube, Herrera & Martínez del Rio 2001). Ramirez *et al.* (2005) and Ayala-Berdón *et al.* (unpublished data) found evidence that physiological traits may constrain the intake response in *G. soricina*. The differences between the studies above may be attributed to differences in experimental conditions. That is, under mild conditions, most animals can achieve compensatory feeding; however, physiological constraints tend to appear when animals confront environmental conditions that increase energetic demands (McWhorter & Martínez del Rio 1999; Lotz *et al.* 1999). For example, McWhorter and Martínez del Rio (1999) and McWhorter and López-Calleja (2000) found that hummingbirds exposed to an acute drop in temperature could no longer maintain constant rates of energy intake when feeding on dilute nectars. These studies illustrate the influence

that both ambient temperature and quality of food have on an organism's ability to maintain energy balance. However, our understanding of how metabolic demands shape an organism's intake response was not fully elucidated by these studies. When compared to nectar-feeding birds, nectarivorous mammals are a poorly studied group. Consequently, studies on the energetics of mammalian nectarivores are of interest to determine if their physiology shows patterns similar to those observed in nectarivorous birds.

In this study, we evaluated the effects of both a seasonal changes in minimum temperature and physiological constraints on the intake response of *G. soricina* under natural conditions. We compared the nightly food intake rate of bats during the dry/cool season to those during the wet/warm season. We expected that food intake and metabolic demands would be greater during the dry/cool season (Cruz-Neto & Abe 1997; McWhorter & Martínez del Rio 1999; McWhorter & López-Calleja 2000; Helversen & Winter 2003). To determine the limitations imposed by both physiological and ecological factors on the intake response of *G. soricina*, we compared energy intake rates at different temperatures to the maximum energy intake rates predicted by a gut function model (Martínez del Rio *et al.* 2000).

Materials and methods

STUDY SITE

Our study site was located in the Chamela-Cuixmala Biosphere Reserve (19º22'-19º35'N, 104º56'-105º03´W), which is situated on the western coast of Jalisco, México. The vegetation is dominated by lowland deciduous forest with small patches of riparian forest. The climate is tropical subhumid with a marked

dry season. The warmest part of the year is from June to September, and the coldest months are January through March (García-Oliva, Camou & Mass 2002). Average monthly temperature is 25°C with little variation in monthly mean maximum temperature (29-32ºC); however large seasonal differences are found in monthly mean minimum temperature (15-24.5ºC; García-Oliva *et al* 2002). Largely because of the influence of the Pacific Ocean, nightly values of relative humidity are above 75% during the entire year (Maass *et al.* 2002).

BAT CARE AND HOUSING

Adult non-reproductive individuals of *G. soricina* were captured using mist-nets and transferred to a laboratory where they were maintained in colonies in cages (0.6x0.6x0.6m). Bats were fed on the maintenance diet described by Mirón (2005), that we supplemented with a mixture of vitamins and minerals (Nekton-S$^{®}$; Guenter Enderle, Tarpon Springs, FL, USA). Individuals were marked on the forearm with a numbered ring and weighed immediately before and after each feeding trial (± 0.01 g). Bats maintained mass in captivity and were released at their site of capture when the experiments were finished.

INTAKE RESPONSES

We measured the nightly food intake rate of individual bats in outdoor enclosures (2.0 x 4.0 x 1.6m) placed within the forest where bats could forage freely while flying. To determine differences in food intake associated with differences in temperature, trials were conducted during the wet/warm season of 2005 (July; minimum temperature = 24.2 ± 3.6ºC) and the dry/cool season of 2006 (February; minimum temperature = 16.3 ± 2.4ºC; Fig. 2). During each feeding trial we offered bats a sucrose solution from 20:00 to 06:00 hrs. We

used six individuals per season, offering each bat seven different solutions of sucrose. The concentrations of these solutions were 146, 292, 438, 584, 730, 876, and 1022 mmol L^{-1} [~5, 10, 15, 20, 25, 30 and 35% sucrose (mass percent), respectively]. We offered individual bats each of the concentrations in a random order. We weighed solutions at the beginning and end of each feeding trial to quantify food intake (± 0.01 g). During each trial, we placed a feeder of each sucrose concentration outside of the flying cages to control for evaporation and changes in concentration. These feeders were covered with a mosquito mesh to prevent consumption. 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. During the dry/cool season trials in February 2006, we confronted each bat with the seven sucrose solutions. However, due to Tropical Storms Dora and Eugene we were unable to confront each bat with all seven concentrations during the wet/warm season trials in July 2005. For the 146, 292, 438, 584, 730, 876, and 1022 mmol L^{-1} sucrose solutions we tested 3, 4, 4, 3, 3, 4, and 4 bats, respectively.

DATA ANALYSIS

We estimated the slope and intercept of the relationship between food intake and concentration with least squares regression analysis on the log-transformed data of each individual bat for the dry/cool and wet/warm seasons. A value of -1 is expected if bats are exhibiting compensatory feeding (McWhorter &

Martínez del Rio 1999; Martínez del Rio *et al.* 2001). For each season, we compared the value of the intake responses' exponents to a -1 value using a one-sample *t*-test. To test for differences in intake responses associated with seasonal changes in temperature, we compared the individual log-log regression lines between seasons using analysis of covariance (ANCOVA). To determine the effect of temperature on food ingestion, we conducted least squares regression analysis using the data for all individuals at each concentration against the minimum temperature of the day in which the experiment was conducted. Finally, we compared the slopes and intercepts of these regression lines using ANCOVA.

To determine if digestive physiology could limit the food intake at low temperatures, we used McWorther and Martínez del Rio's (2000) model of sucrose digestion. This model assumes that sucrose hydrolysis ($-r_s$) follows Michaelis-Menten kinetics, such that:

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

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}) along the intestine. The time (τ) required to reduce the initial concentration of sucrose (C_{50}) to a given final value (Cs_f) can then be integrated from equation 1 to:

$$\tau = \frac{K_m \ln(C_{50} / C_{sf}) + (C_{50} - C_{sf})}{S_{max}}. \quad (2)$$

Intake rate (V_0 in µL min^{-1}) can then be estimated using the volume of the small

intestine G (in μL) as:

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

Parameters used to fit the model were collected during the dry/cool season (Hernandez & Martinez del Rio 1992; Table 1). We compared the observed intake of sucrose solutions in dry/cool and wet/warm seasons with those predicted from the model using ANCOVA. If the experimentally obtained intake response curve intersected the line predicted from the model, we interpreted this as evidence of a physiological constraint "breaking" the intake response and limiting food intake (Martínez del Rio et al. 2001).

To describe the role that both physiological and environmental factors play shaping the intake responses of G. soricina; we constructed a graphical relationship between food intake, sucrose concentration in food, and metabolic demands. In the graphical model we plot: 1) the maximum food intake rate at different sucrose concentrations as predicted by McWorther and Martínez del Rio's (2000) model and 2) food intake rates needed to achieve compensatory feeding at different levels of energetic demands. Because bats maintained body mass regardless of sucrose concentration in food or ambient temperature, we assumed that all the energy ingested was used to cover metabolic demands.

Results

SEASONAL DIFFERENCES IN INTAKE RESPONSES

Glossophaga soricina decreased food intake as sugar concentration increased during both the dry/cool and wet/warm seasons, and these relationships were well described by power functions (Fig. 1). In both the dry/cool and wet/warm

seasons, the value of the exponents relating intake with concentration were statistically different from the expected compensatory value of –1 (dry/cool: -0.83±0.04, t_5=8.71, p<0.0003; wet/warm: -0.59±0.04, t_5=24.07, p<0.0003), suggesting that food intake is physiologically constrained during both seasons. The slopes of the intake responses were significantly different between seasons ($F_{(slope)1,62}$=10.07, p=0.002; Fig. 1).

TEMPERATURE EFFECTS ON FOOD INTAKE

Maximum temperatures were comparable between the dry/cool and wet/warm seasons (32.55±1.98 and 33.83±1.50ºC, respectively); however, minimum temperature was significantly lower during the dry/cool season (t_{64}=34.32 p<0.0001, Fig. 2,). Minimum temperatures during the dry/cool and wet/warm seasons were 16.3 ± 2.4 and 24.2 ± 3.6ºC, respectively (Fig. 2).

For all sucrose concentrations, food intake rate decreased in response to increasing temperatures (Fig. 2). Food intake was higher during the dry/cool season compared to that during the wet/warm season (Table 2). For all sucrose concentrations, slopes between food intake and temperature differed from 0 (all values of t≥8.03, all values of p< 0.002; Table 3) and varied among different sucrose concentrations (Fig. 2; Table 3). At low to medium concentrations (146 to 584 mmol L^{-1}), slopes were steeper compared to slopes observed at higher concentrations (876 to 1022 mmol L^{-1}; Fig. 2, Table 3).

PREDICTED MAXIMUM FOOD INTAKE

Maximum intake rates predicted from the McWhorter and Martínez del Rio's (2000) model were higher than the intake rates we measured during both the dry/cool and wet/warm seasons (Fig.1). An exception was the food intake rate at 146 mmol L^{-1} in the dry/cool season, which was accurately predicted by the model. The intake response obtained during the wet/warm season had a different intercept and similar slope to that predicted by the model ($F_{(intercept)1,33}$=98.37, p<0.001 and $F_{(slope)1,33}$=0.45, p=0.50). Both the intercept and slope of the intake response from the dry/cool season differed from those predicted by the model ($F_{(intercept)1,51}$=16.35, p=0.001 and $F_{(slope)1,51}$=5.04, p<0.03).

The graphical model we constructed relating food intake, sucrose concentration, and metabolic demands suggests that bats should be capable of compensatory feeding when metabolic demands are ≤ 50 kJ d^{-1} (Fig. 3). However, if energy expenditures are increased above 50 kJ d^{-1}, the model predicts a break in the intake response at low sucrose concentrations and no compensatory feeding (Fig 3). As metabolic demands increase, the point were the food intake rate of bats is constrained physiologically moves to the right, suggesting that bats will need to feed on more concentrated nectars to maintain energy balance.

Discussion

The individuals of *G. soricina* we studied exhibited classical intake responses, where food intake rate increased when the sucrose concentration of food decreased. We found seasonal differences in the slopes of their intake

responses. During both the dry/cool and wet/warm seasons, slopes were different from the expected compensatory value of –1. The differences in food intake rate we observed between seasons were related to seasonal differences in minimum temperature. In our discussion, we first consider effect ambient temperature had on food intake rates. Next, we examine potential physiological causes for our observation of seasonal intake responses. We conclude our discussion by proposing that the interactions between physiological constraints and environmental conditions explain the geographic distribution of *G. soricina*.

Effect of ambient temperature on food intake rates

In our study, *G. soricina* had dramatically greater rates of food and energy consumption during the dry/cool season compared to those rates during the wet/warm season. This finding is similar to the increased nutrient consumption rates reported for hummingbirds and sunbirds when they were acutely confronted with reduced ambient temperature (Lotz *et al* 1999; McWhorter & Martínez del Rio 1999; McWhorter & López-Calleja 2000). Additionally, we found that the effect of temperature on food intake was affected by the sucrose concentration of nectar (Fig. 2). Bats feeding on medium to concentrated nectars (584-1022 mmol l^{-1}) were affected by changes in temperature in a similar fashion, increasing their nightly food intake by approximately 1.8 g 10 h^{-1} of food per each drop in 1°C. However when nectar concentrations were of 438 mmol l^{-1} or less, the increase in food intake per each drop in 1°C was higher (2.05, 2.16, and 3.01 g 10 h^{-1} at 438, 292, and 146 mmol L^{-1}, respectively; Fig. 2, Table 3). Our findings suggest that bats must increase foraging when

feeding on dilute nectars in cold environments. This behavior would increase

energy expenditure and reduce the net gain of nutrients.

Minimum night time temperatures at our study site are ~15 and 25ºC

during the dry/cool and the wet/warm seasons, respectively (García-Oliva *et al.*

2002). These values are below the thermoneutral zone of *G. soricina* (30-35ºC;

Cruz-Neto & Abe 1997). A 10ºC reduction in ambient temperature, which is

similar seasonal temperature changes at our study site, doubles the oxygen

consumption of *G. soricina* (Cruz-Neto & Abe 1997). This increase in

metabolism is close to the 86% increase in food intake rate that we found

between seasons when bats were feeding on the 146 mmol L^{-1} sucrose

solution. The similarity between these values suggests that seasonal changes

in food intake rate that we observed may be caused by seasonal differences in

metabolic rates.

Differences in intake responses between seasons
Glossophaga soricina showed intake responses similar to those found in nectar-

feeding birds (Collins 1981; López-Calleja, Bozinovic & Martínez del Rio 1997;

McWhorter & Martínez del Rio 2000; Schondube & Martínez del Rio 2003) as

well as other fruit and nectar-feeding bats (Thomas 1984; Helversen & Winter

2003; Korine *et al* 2004; Ramirez *et al* 2005; Ayala-Berdón *et al.* unpublished

data). We found that *G. soricina* increased their food intake by 4.9 fold during

the dry/cool season, and by 3.5 fold during the wet/warm season when they

faced a change in nectar concentration from 1022 to 146 mmol L^{-1}. This

increase in food intake involved ingesting up to seven times their body mass in

water per night during the dry/cool season when feeding on dilute nectar (146 mmol L^{-1}).

Our results also show that in both seasons bats were incapable of compensatory feeding. The presence of a physiological mechanism limiting food intake has been reported before for this species (Ramirez *et al* 2005). Surprisingly we found that the slopes of intake responses differed between the dry/cool and wet/warm seasons (Fig. 1). This observed difference could be attributed to increased metabolic demands during the dry/cool season.

In the dry/cool season, *G. soricina*'s food intake, when feeding on dilute nectar, is similar to the maximum food intake predicted by McWhorter and Martínez del Rio's (2000) model. This match between our field data and the model supports the hypothesis of a digestive constraint limiting food intake in this species (Ramirez *et al.* 2005). However, during the wet/warm season, *G. soricina* consumed only half of what they ingested in the dry/cool season. This poses a perplexing question. Why is *G. soricina* limiting its food intake during the wet/warm season? Our results offer two complementary explanations. First, *G. soricina* may exhibit phenotypic digestive plasticity. This would allow for greater nutrient assimilation by increasing sucrase activity and/or gut size when energetic demands increase during the colder months of the year. Karasov and Hume (1997) reported that the activity of intestinal hydrolases could vary up to three fold as a result of dietary modulation in response to a change in diet. The seasonal changes in food intake we describe are within this range; however, sucrase activity per area of the gut is not plastic in birds (Sabat *et al.* 1998).

The plasticity of sucrase activity has not been investigated in bats. A second explanation for the observation of a significantly reduced food intake during the wet/warm season is that energy demands were lower compared to the dry/cool season. In this scenario, the seasonal difference in food intake may be a physiological "safety margin", allowing bats to survive when environmental conditions are harsh. Support for this hypothesis comes from the observation that bats maintained body mass during our experiments (Fig. 1, upper panel). Lotz and collaborators (1999), McWhorter and Martínez del Rio (1999), and Schondube (2003) showed that different species of nectar-feeding birds have spare digestive capacity that allows them to increase energy intake when confronted with higher energetic demands. If nectar-feeding animals pair their food intake with their energetic demands, intake responses could be used to obtain estimates of metabolic rate both under controlled conditions and in the field.

Do seasonal intake responses account for the geographic distribution of Glossophaga soricina?

The interaction between physiological and environmental factors is well described by our graphical model (Fig. 3). The model predicts that bats should be compensatory feeders when their metabolic expenditures are ≤ 50 kJ day^{-1}. This prediction is supported by Helverson and Winter (2003), who reported that *G. soricina*, under mild laboratory conditions, was able to achieve a constant energy intake of 48 kJ d^{-1}. However, bats should be unable to achieve compensatory feeding when confronted with higher energetic demands, as found by Ramirez and collaborators (2005).

Based on the predictions of our model, *G.soricina* have two means of maintaining energy balance when energy demands are increased: 1) select for concentrated nectars, which increases energy intake while minimizing foraging costs; or 2) use behavioral energy conserving strategies, such as reducing activity, migrate to warmer regions, or use torpor (Coburn & Geiser 1998; Soriano, Ruiz & Arends 2002). Our model predicts that in colder sites, feeding on dilute nectar will result in negative energy balances. This prediction suggests that both ambient temperature and nectar quality limit the geographic distribution of bats. Specifically, it appears that *G. soricina* cannot tolerate sites with minimum temperatures below 10ºC. This could partially explain why this species is usually found at lower elevations in tropical environments (Alvarez 1999). To test this hypothesis, it would be interesting to compare the digestive and energetic physiology of *G. soricina* with that of other nectar-feeding bats found in colder sites.

with permission from the Oficina de Fauna Silvestre, Mexico, for JES (FAUT-0193).

References

Alvarez, J.M., Willig, M.R., Jones, J. & Webster, D. (1999) *Glossophaga soricina. Mammalian Species* **379**, 1–7.

Baker, H.G., Baker, I.S. & Hodges, A. (1998) Sugar composition of nectars and fruits consumed by birds and bats in the tropics and subtropics. *Biotropica*, **30**, 559–586.

Carter, R. R. & Grovum. W. L. (1990) Factors affecting the voluntary intake of food by sheep. 5. The inhibitory effect of hypertonicity in the rumen. *British Journal of Nutrition*, **64**, 285-299.

Castle, K.T. & Wunder, B.A. (1995) Limits to food intake and fiber utilization in the prairie vole *Microtus orchrogaster*: effects of food quality and energy need. *Journal of Comparative Physiology*, **164**, 609–617.

Coburn, D.K. & Geiser, F. (1998) Seasonal changes in energetics and torpor patterns in the subtropical blossom-bat *Synconycteris australis* (Megachiroptera). *Oecologia, 113, 467-473.*

Collins, B.G. (1981) Nectar intake and water balance for two species of Australian honeyeater, *Lichmera indistincta* and *Acanthorynchus superciliosis*. *Physiological Zoology*, **54**, 1–13.

Cruz-Neto, A.C.& Abe, A.S. (1997) Metabolic rate and thermoregulation in the nectarivorous bat *Glossophaga soricina* (Chiroptera, Phillostomidae). *Revista Basileira de Biologia, 57,* 203-209.

Denbow, D. M. (2000) Gastrointestinal anatomy and physiology. *Sturkie's Avian physiology* (eds G. C. Whittow). pp. 299-325. New York, Academic Press. New York.

Diamond, J. (1991) Evolutionary design of intestinal nutrient absorption: enough but not too much. *News in Physiological Sciences*, **6**, 92-96.

Diamond, J. M., Karasov, W. H., Phan D. & Carpenter F. L. (1986) Digestive physiology is a determinant of foraging bout frequency in hummingbirds. *Nature*, **320**, 62-63.

Downs, C. T. (1997) Sugar digestion efficiencies of Gurney's Sugarbirds, Malachite Sunbirds, and Black Sunbirds. *Physiological Zoology*, **70**, 93-99.

García-Oliva, F., Camou, A. & Mass, J.M. (2002) El clima de la región central de la costa del pacífico mexicano. *Historia Natural de Chamela* (eds F. Noguera, S.H. Vega, A.N. García-Aldrete and M. Quesada) pp. 3-10. Universidad Nacional Autónoma de México. México.

Hansen, C.B., Jen, K.C. & Kribs, P. (1981) Regulation of food Intake in monkeys. *Physiology and Behaviour*, **26**, 479–486.

Helversen, O. & Winter, Y. (2003) Glossophagine bats and their flowers: Costs and benefits for plants and pollinators. *Bat Ecology* (eds T.H. Kunz & M.B. Fenton) pp.346-397. University of Chicago press. Chicago.

Hernandez, A. & Martínez del Rio, C. (1992) Intestinal disaccharidases in five species of phyllostomoid bats. *Comparative Biochemical and Physiology B*, **103**, 105–11.

Herrera, L.G., Martínez del Rio, C., Braun, E. & Hobson, K. (2001) Renal structure in phyllostomid bats: using stable isotopes to explore relationships between diet and morphology. *Isotopes in Environment and Health Sciences*, **37**, 1–11.

Josens, R.B., Farina, W.M. & Roces, F. (1998) Nectar feeding by the ant
Camponotus mus: intake rate and crop filling as a function of sucrose
concentration. *Journal of Insect Physiology*, **44**, 579–585.

Karasov, W. H. (1990) Digestion in birds: chemical and physiological
determinants and ecological implications. *Studies in Avian Biology*, **13**,
391-415.

Karasov, W. H. & Diamond. J. M. (1988) Interplay between physiology and
ecology in digestion. *BioScience*, **38,** 602-611.

Karasov, W. H. & Hume I. D. (1997) Vertebrate gastrointestinal system.
Handbook of comparative physiology (eds W. Dantzler), pp 409-480.
Bethesda, MD, American Physiological Society.

Korine, C., Speakman, J. & Arad, Z. (2004) Reproductive energetics of captive
free-ranging egyptian fruit bats *Rossetus aegymtiacus*, *Ecology* **85**, 220–
230.

Levey, D.J. & Martínez del Rio, C. (1999) Test, rejection, and reformulation of a
chemical reactor-based model of gut function in a fruit-eating bird.
Physiological and Biochemical Zoology, **72**, 369–383.

López-Calleja, M.V., Bozinovic, F. & Martínez del Rio, C. (1997) Effects of
sugar concentration on hummingbird feeding and energy use.
Comparative Biochemistry and Physiology, **118A**, 1291–1299.

Martínez del Rio, C. (1990) Sugar preferences in hummingbirds: the influence
of subtle chemical differences on food choice. *The Condor*, **92**, 1022-
1030.

Martínez del Rio, C. & Restrepo C. (1993) Ecological and behavioral

   consequences of digestion in frugivorous animals. *Vegetatio*, **107/108**,

   205-216.

Martínez del Rio, C., Schondube J.E., McWhorter T.J. & Herrera, L.G. (2001)

   Intake responses in nectar feeding birds: digestive and metabolic causes,

   osmoregulatory consequences, and coevolutionary effects. *American*

   Zoologist, **41**, 902–915.

Martínez del Rio, C. & Stevens, B. R. (1989) Physiological constraint on feeding

   behavior: intestinal membrane disaccharides of the starling. *Science*, **243**,

   794-796.

McWhorter, T.J. & López-Calleja, M.V. (2000) The integration of diet,

   physiology and ecology of nectar-feeding birds. *Revista Chilena de*

   Historia Natural, **73**, 415–460.

McWhorter, T. J. & Martínez de Rio C. (1999) Food ingestion and water

   turnover in hummingbirds: how much dietary water is absorbed?. *Journal*

   of Experimental Biology, **202**, 2851-2858.

Mirón, M.L. (2005) Efecto de la dieta en el fraccionamiento isotópico de 13C y

   15N en el murciélago *Glossophaga soricina (Chiroptera: Phillostomidae).*

   Tesis de maestría (Maestría en ciencias biológicas). Facultad de

   Ciencias, Univesidad Nacional Autónoma de México. México.

Montgomery, M.J. & Baumgardt, B.R. (1965) Regulation of food intake in

   ruminants. 2. Pelleted rations varying in energy concentration. *Journal of*

   Dairy Science, **48**, 569–577.

Mass, J.M., Jaramillo, V., Martínez-Yrízar, A., García-Oliva, F., Pérez-Jiménez,

   A. & Sarukhán, J. (2002) Aspectos funcionales del ecosistema de la selva

baja caducifolia en Chamela, Jalisco. *Historia Natural de Chamela* (eds F. Noguera, S.H. Vega, A.N. García-Aldrete and M. Quesada) pp. 525-542. Universidad Nacional Autónoma de México. México.

Ortega, J. & Castro-Arellano, I. (2001) *Artibeus jamaicensis*. *Mammalian Species*, **662**, 1–9.

Pyke, G.H. & Waser, N.M. (1981). The production of dilute nectars by hummingbird and honeyeater flowers. *Biotropica*, **13**, 260-270.

Ramírez, N.P., Herrera G.M. & Mirón, L. (2005). Physiological constraint to food ingestion in a New World nectarivorous bat. *Physiological and Biochemical Zoology*, **78,** 1032–1038.

Sabat, P., Novoa F., Bozinovic, F., & & Martínez del Rio, C. (1998) Dietary flexibility and intestinal plasticity in birds: a field and laboratory study. *Physiological Zoology*, **71**, 226-236.

Savory, C.J. (1999) Temporal control of feeding behavior and its association with gastrointestinal function. *Journal of Experimental Zoology*, **283**, 339-347.

Schondube, J. E. (2003) *Flowerpiercers and Hummingbirds: a compartive study of nectar-feeding strategies in birds*. Ph.D. Dissertation. Department of Ecology and Evolutionary Biology, University of Arizona, Tucson, Arizona.

Schondube, J.E., Herrera, L.G. & Martínez del Rio, C. (2001) Diet and the evolution of digestion and renal function in phyllostomid bats. *Zoology*, **104**, 59–73.

Schondube, J. E. & Martínez del Rio, C. (2003) Concentration-dependent sugar preferences in nectar-feeding birds: mechanisms and consequences. *Functional Ecology*, **17**, 445–453.

Simpson, S. J., Barton-Browne L. & van Gerwen A. C. M. (1989) The patterning of compensatory feeding in the Australian sheep blowfly. *Physiological Entomology*, **14**, 91-105.

Slansky, F. & Wheeler, G.S. (1992) Caterpillars compensatory feeding response to diluted nutrients leads to toxic allelochemical dose. *Entomiologia Experimentalis et Aplicatta*, **65**, 171–186.

Soriano, P., Ruiz, A. & Arends, A. (2002) Physiological responses to ambient temperature manipulation by three species of bats from Andean cloud forests. *Journal of Mammalogy*, **83**, 445-457.

Spiegel, T.A. (1973) Caloric regulation of food intake in man. *Journal of Comparative and Physiological Psychology*, **84**, 24–37.

Studier, E.H. & Wilson, D.E. (1983) Natural urine concentrations and composition in neotropical bats. *Comparative Biochemistry and Physiology*, **75**, 509–515.

Thomas, D.W. (1984) Fruit intake and energy budgets of frugivorous bats. *Physiological Zoology*, **57**, 457–467.

Tables

Table 1. Parameters we used to fit McWhorter and Martínez del Rio's (2000) model to *Glossophaga soricina*. S_{max} is the rate of sucrose hydrolysis along the intestine, K_m is the sucrase Michaelis-Menten constant, C_{50} is the digestion efficiency of sucrose, and *G* is the volume of the intestine. Digestive activity data collected during the month of January.

	G. soricina
S_{max} (µmol min^{-1}µL^{-1})	0.115[1]
K_m (µmol µL^{-1})	0.044[1]
C_{50}	0.009[2]
G (µL)	0.304[1]

[1]From Hernandez & Martínez del Rio (1992).
[2]We measured the digestion efficiency by quantifying the sugar content in the feces with a hand-held refractometer (Reichert 10431 0-50°compensated Brix temperature, Leica, Buffalo NY, USA, from Schondube & Martínez del Rio 2003).

Table 2. Food intake rates between seasons for each sucrose concentration.

	Dry/cool season		Wet/warm season	
Concentration (mmol L^{-1})	Mean food intake (g 10 h^{-1})	SD	Mean food intake (g 10 h^{-1})	SD
146	68.53	9.41	38.45	1.48
292	36.80	6.40	23.85	4.27
438	27.78	3.50	20.12	3.84
584	21.88	2.38	17.86	3.01
730	18.65	1.87	16.50	3.73
876	16.51	1.92	12.87	1.39
1022	14.19	1.66	10.97	1.48

Table 3. Effect of minimum temperature on the energy intake rates of bats feeding on different sucrose concentrations.

Concentration (mmol L^{-1})	Concentration (weight/volume)	[1]Slope	[1]Intercept	r^2
146	5%	3.002	106.100	0.90
292	10%	2.165	94.240	0.70
438	15%	2.051	101.006	0.73
584	20%	1.751	100.088	0.70
730	25%	0.882	90.050	0.31
876	30%	1.850	110.090	0.66
1022	35%	1.720	108.450	0.66

[1]Slopes and intercepts obtained from regression analyses were all statistically different from 0.

Figure legends

Figure 1. Seasonal changes in the intake responses of *Glossophaga soricina* compared to the maximum food intake rate predicted by McWhorter and Martínez del Rio's model (dark line). *Glossophaga soricina* did not show significant changes in body mass (ΔM; upper panel). Bats decreased food intake as sugar concentration increased (lower panel). In both the dry/cool (open circles; log sucrose intake = - 0.790 log concentration + 3.5298) and the wet/warm seasons (closed circles; log intake Sucrose = -0.600 log concentration + 2.878), food intake rates were physiologically constrained. The slopes of the intake responses differed between seasons. During the dry/cool season, *G. soricina* exhibited a "broken" intake response. This finding suggests that food intake rates are constrained at low sucrose concentrations.

Figure 2. Effect of seasonal changes in ambient temperature on food intake rate in *Glossophaga soricina*. We found significant differences in minimum temperatures between seasons (top panel). For all sucrose concentrations, food intake rate decreased in response to increasing temperatures (lower panel). Food intake rate was higher during the dry/cool season compared to the wet/warm season. Slopes of the relationships between food intake rate (g $10 \ h^{-1}$) and temperature (°C) were different among sucrose concentrations (mmol L^{-1}). At low to medium concentrations (146 to 584 mmol L^{-1}) the values of the slopes were steeper than at higher concentrations (876 to 1022 mmol L^{-1}; Fig. 2, Table 3).

Figure 3. Graphical model relating food intake rate, sucrose concentration in food, and metabolic demands in *Glossophaga soricina*. The model predicts that

bats should be compensatory feeders when their metabolic demands ≤ 50 kJ day^{-1}. When bats have higher energy demands, they will not be able to maintain constant rates of energy intake. This explains the "broken" appearance of the predicted intake response. As metabolic demands increase, the point where the food intake rate of bats is constrained moves to the right. Consequently, this model predicts that bats must feed on concentrated nectars when metabolic expenditures are high.
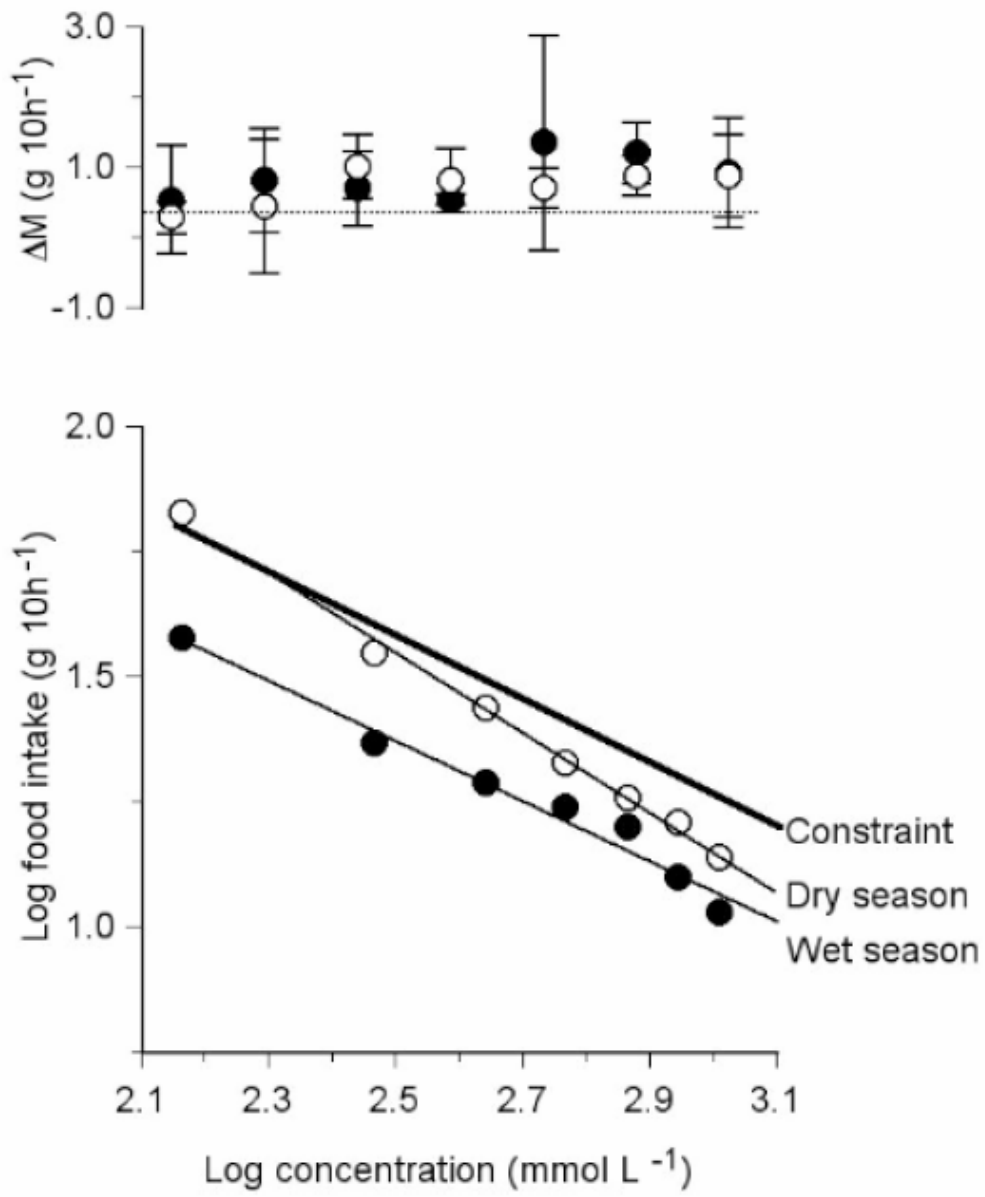
Figure 1.

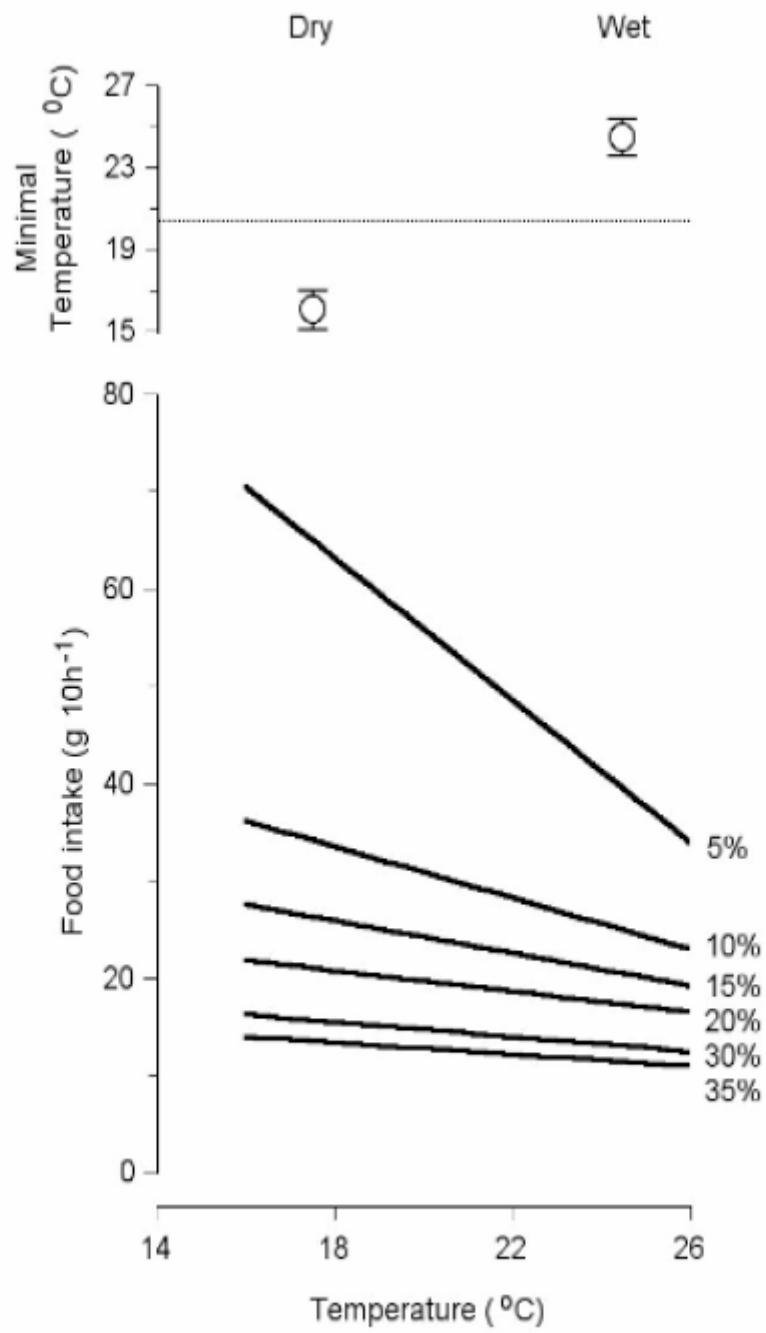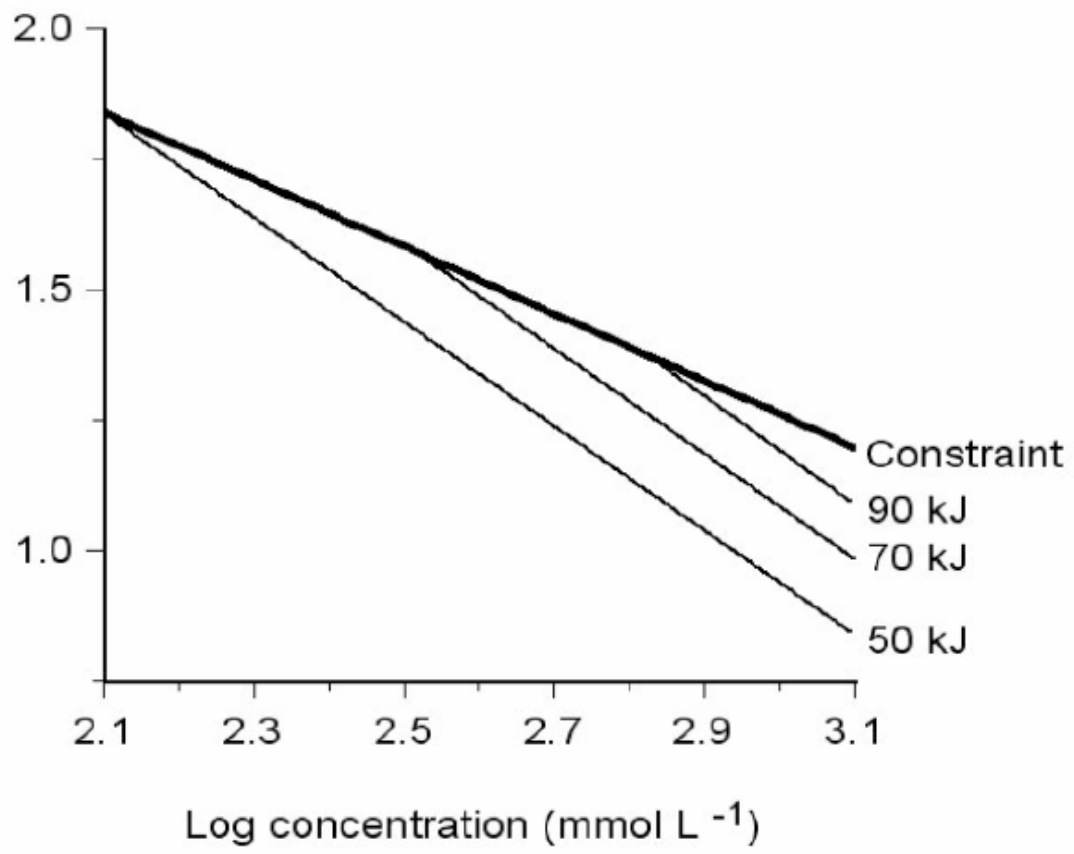**Figure 2.**

Figure 3.

Log concentration (mmol L $^{-1}$)

CONCLUSIONES GENERALES

En el presente estudio se encontró que en las tres especies de murciélagos un incremento en la concentración de azúcares presentes en el néctar causó una disminución en el consumo del mismo. Esta respuesta es similar a la encontrada para otros organismos, y ha sido denominada "respuesta de consumo". La similitud de las respuestas de consumo obtenidas para las tres especies de murciélagos (fig 1, capitulo 1), con las obtenidas para aves que se alimentan de néctar sugieren que las consecuencias osmorregulatorias se comparten entre estos grupos. Por ejemplo cuando las aves se alimentan a concentraciones bajas, deben eliminar de manera rápida una gran cantidad de agua. Esta condición se resuelve con las altas tasas de filtración renal de estos organismos. Sin embargo cuando las aves descansan deben de conservar el agua, situación que se agrava ya que los riñones de estos organismos son incapaces de concentrar orina. Los resultados sugieren que los murciélagos comparten este dilema con las aves. Los mecanismos usados por los murciélagos para resolver este dilema son desconocidos y necesitan ser investigados.

Las respuestas de consumo obtenidas para las tres especies de murciélagos fueron iguales tanto para las soluciones de sacarosa como de hexosas (glucosa-fructosa; Fig 1, capitulo 1). Este resultado sugiere que los organismos perciben energéticamente igual los dos tipos de azúcares. Si esto es cierto entonces porque los néctares presentes en el nuevo mundo están dominados por hexosas?, cuales son los factores que determinan las

2

preferencias en campo? Una posible respuesta es que la elección de alimento esta basado en otras características tales como la forma de las flores, el olor, el sabor del néctar que producen o una combinación de todas las anteriores. Las causas que dirigen la elección de alimento deben ser investigadas.

La relación entre el consumo de alimento y la concentración obtenida indica que tanto *Leptonycteris curasoae, Glossophaga soricina* y *Artibeus jamaicensis* presentan respuestas de consumo en las cuales existen limitantes fisiológicas (fig. 1, capitulo 1). Esta condición se comprueba al obtener la correlación positiva entre la concentración y el consumo de azúcar (fig. 2, capitulo 1). Estudios previos con *G. soricina* sugieren que la hidrólisis de la sacarosa es uno de los pasos que limita la respuesta de consumo en esta especie. Las respuestas de consumo obtenidas en este trabajo no apoyan esa hipótesis y sugieren que el intestino de los murciélagos esta diseñado de acuerdo al principio de simorfósis, en el cual ningún paso limita mas que otro. Aparentemente los procesos de digestión, asimilación catabolismo y excreción limitan de manera homogénea la respuesta de consumo en las tres especies de estudio.

Los resultados del modelo de hidrólisis de sacarosa muestran que las respuestas de consumo pueden ser predecidas de una manera confiable (fig 4, capitulo 1). Ya que los datos enzimáticos y de morfología del intestino fueron tomados de la literatura, se sugiere que los parámetros sean medidos de las especies en campo. En *A. jamaicensis,* los resultados sugieren que limitantes

pre-ingestionales como la morfología de la boca y lengua, tienen una marcada importancia en organismos poco especializados para la dieta de néctar.

El entendimiento de la respuesta de consumo nos ayuda a conocer la cantidad de alimento que un organismo puede procesar, sin embargo, existen factores que dirigen los procesos fisiológicos que las limitan. Los resultados de la segunda parte de la tesis demuestran que las respuestas de consumo cambian de una estación de secas a una de lluvias (Fig. 1, capitulo 2). Estas diferencias pueden ser explicadas por procesos de gasto energético y manejo de agua por parte de los organismos. Cuando la temperatura decrece, los murciélagos incrementan su metabolismo y sus requerimientos energéticos, por lo que el consumo se incrementa (Fig. 2, capitulo 2). Además, a pesar de que la humedad relativa alcanza cerca del 100% en cualquier época del año, esta se mantiene constante a lo largo del día y la noche en época de lluvias. Esta condición debe reducir el coeficiente de evaporación de los murciélagos a través de la respiración y evapotranspiración por parte de las membranas de las alas, marcando las diferencias en las respuestas de consumo en las diferentes partes del año.

Los resultados muestran que existe un efecto de la temperatura en el consumo de alimento, sin embargo hay un efecto adicional de la concentración del néctar (Fig. 2, capitulo 2). Los resultados sugieren que los murciélagos tienen costos mayores cuando se alimentan de néctares de concentraciones

bajas cuando las temperaturas son bajas. Para comprobar esta hipótesis se

construyó un modelo en el cual interactúan la concentración, el gasto metabólico

y la capacidad máxima que tiene la especie para consumir alimento (Fig. 3,

capitulo 2). Los resultados muestran que cuando el gasto metabólico se

incrementa (debido a cambios en la temperatura), las respuestas de consumo

tienden a romperse a concentraciones cada vez más altas. Cuando este

fenómeno se presenta, los organismos deberán alimentarse de néctares mas

concentrados o estarán obligados a utilizar mecanismos como el torpor para

ahorrar energía. De esta manera las respuestas de consumo y las condiciones

ambientales que las dirigen pueden tener efecto en la distribución de los

organismos.