UNIVERSIDAD NACIONAL AUTÓNOMA DE MÉXICO

POSGRADO EN CIENCIAS BIOLÓGICAS

CENTRO DE INVESTIGACIONES EN ECOSISTEMAS

Efectos de limitantes fisiológicas en la ecología y distribución geográfica en murciélagos nectarívoros

TESIS

QUE PARA OBTENER EL GRADO ACADÉMICO DE

DOCTOR EN CIENCIAS

PRESENTA

JORGE ISRAEL AYALA BERDÓN

TUTOR PRINCIPAL DE TESIS: Dr. Jorge E. Schondube Friedewold

COMITÉ TUTOR: Dr. Víctor Manuel Sánchez Cordero Dávila
Dr. Alberto Búrquez Montijo
Dr. Héctor Takeshi Arita Watanabe
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Morelia, Michoacán Septiembre de 2012

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UNAM
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COORDINACIÓN

Dr. Isidro Ávila Martínez
Director General de Administración Escolar, UNAM
Presente

Por medio de la presente me permito informar a usted que en la reunión ordinaria del Comité Académico del Posgrado en Ciencias Biológicas, celebrada el día 7 de mayo del 2012, se acordó poner a su consideración el siguiente jurado para el examen de DOCTOR EN CIENCIAS del alumno **AYALA BERDÓN JORGE ISRAEL** con número de cuenta **91075930**, con la tesis titulada: **"Efecto de las limitantes digestivas en la distribución geográfica en murciélagos nectarívoros"**, bajo la dirección del **Dr. Jorge Ernesto Schondube Friedewold.**

Presidente: Dr. Víctor Manuel Sánchez Cordero Dávila
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Sin otro particular, quedo de usted.

Atentamente
"POR MI RAZA HABLARA EL ESPIRITU"
Cd. Universitaria, D.F., a 20 de agosto del 2012.

M. del Coro Arizmendi

Dra. María del Coro Arizmendi Arriaga
Coordinadora del Programa

c.c.p. Expediente del interesado

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Al posgrado en Ciencias Biológicas de la UNAM

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RESUMEN

El papel de los murciélagos como prestadores de servicios ambientales está bien documentado. La eficiencia de estos organismos como polinizadores de las diferentes especies de plantas depende parcialmente de sus patrones de forrajeo y su preferencia por los recursos florales disponibles en su ambiente. Los patrones de forrajeo de estos animales están determinados tanto por los patrones fenológicos de las especies de plantas que visitan, como por los mecanismos fisiológicos que afectan su comportamiento, limitando la manera en que estos pueden utilizar la energía presente en el néctar. Para entender el papel que los animales nectarívoros juegan como polinizadores dentro de comunidades naturales requerimos no sólo entender la manera en la que estos responden a cambios en la disponibilidad de recursos en el ambiente, sino también describir el contenido nutricional de sus diferentes fuentes de alimento y conocer cómo su fisiología y necesidades energéticas pueden limitar o dirigir su comportamiento y su distribución espacial. La interacción entre la calidad nutricional del néctar (contenido de energía), su disponibilidad y la capacidad de los animales para procesar esta energía y satisfacer las necesidades de su metabolismo energético, limita los recursos florales que estos organismos pueden utilizar y determinan los retos ambientales que pueden enfrentar (temperaturas mínimas y humedad). De este modo, la interacción entre las capacidades digestivas y el gasto metabólico puede jugar un papel importante en determinar la amplitud de nicho ecológico y la distribución geográfica de murciélagos nectarívoros. El objetivo principal de este trabajo fue integrar el

papel que la fisiología digestiva ejerce sobre los patrones ecológicos de selección, uso de recursos, y distribución espacial de murciélagos nectarívoros. La información generada en este trabajo nos ayudará a entender la relación entre la fisiología y ecología de este grupo de animales y el papel que la fisiología tiene sobre su capacidad para adaptarse a diferentes condiciones ambientales. Adicionalmente podrá ser utilizada como información básica para el manejo y conservación de este importante grupo de polinizadores

ABSTRACT

The environmental services provided by nectar-feeding bats are well documented. The efficiency of these animals as pollinators of different plant species depends of their foraging patterns and their floral preferences. Their foraging patterns are determined by both the phenological patterns of plant species that they visit, and the physiological mechanisms that limit the form in which they can use the energy available in nectar. To understand the role that animals play as pollinators in natural communities, it is required not only understand the way in which they respond to changes in the availability of resources in their environment, but also to describe the nutritional content of their different food sources. This allows us to understand how their physiology and energy requirements may limit or direct their behavior and spatial distribution. The interaction between the nutritional quality of nectar (energy content), the nectar availability, and the ability of animals to process the energy content in food limits the floral resources that these animals can use and may determine the environmental challenges that animals are able to face (minimum temperature and humidity). Thus, the interaction between the digestive abilities and metabolic rate can play an important role in determining the extent of ecological niche and geographical distribution of nectar-feeding bats. The main objective of this work was to integrate the role of digestive physiology on ecological selection patterns, resource use, and spatial distribution of this group of animals. The information generated in this study will help us understand the relationship between their physiology and ecology, and the role that physiology has on its ability to adapt to

different environmental conditions. Also, the information can be used as background for management and conservation strategies for this important group of pollinators.

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Introducción

Diversos estudios de fisiología han sido utilizados para solucionar problemas de manejo y conservación de fauna silvestre en el pasado. Los estudios fisiológicos nos permiten determinar la forma en que los animales perciben su ambiente y los mecanismos que utilizan para responder a cambios en él. Sin embargo, el uso de estudios fisiológicos con fines de conservación y manejo de fauna es limitado, lo que es sorprendente si consideramos que las primeras respuestas que los animales presentan ante cambios en su hábitat son de tipo fisiológico (Figura 1).

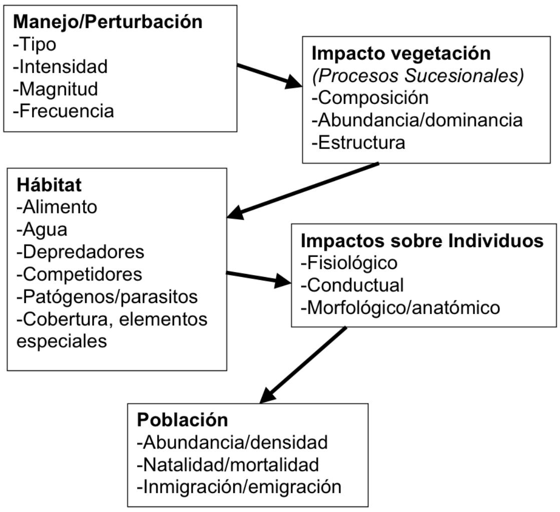

Figura 1. Modelo conceptual del manejo de fauna silvestre basado en Leopold (1986). El manejo o la perturbación de un sistema afecta su vegetación, lo cual a su vez genera cambios importantes a nivel de hábitat. La fauna responde a estas modificaciones de su ambiente primero a un nivel fisiológico, seguido por cambios conductuales, y a largo plazo (tiempo evolutivo) con modificaciones anatómicas o morfológicas. Los cambios a nivel fisiológico, sobre todo aquellos relacionados con el uso de recursos como alimento y agua, al limitar la adquisición de nutrientes esenciales y energía, afectan de manera importante los procesos poblacionales de las especies.

A pesar de que las principales contribuciones de la fisiología al campo de la conservación animal se han dado desde el área de la ecotoxicología, otras áreas de la fisiología, como la fisiología digestiva, la endocrinología, la fisiología ecológica, e incluso la fisiología evolutiva han tomado un papel más activo en este campo. Esto ha generado el desarrollo de una nueva disciplina llamada *fisiología de la conservación*, la cual ha sido definida por Wikelski y Cooke (2006) como "*el estudio de las respuestas fisiológicas de los organismos ante las alteraciones antropogénicas de su hábitat que puedan generar o contribuir a la reducción de poblaciones*". De manera similar a la fisiología ecológica tradicional, esta nueva disciplina estudia los mecanismos que permiten a las especies adaptarse a cambios en su ambiente que puedan afectar sus dinámicas poblacionales. La fisiología de la conservación va mas allá de describir patrones para incluir conocimiento mecanístico detallado de las causas de problemas de conservación (Stevenson et al., 2005). Aunque la fisiología que se utiliza en esta nueva disciplina incluye funciones de todo el organismo como el metabolismo, el control de temperatura, la nutrición y las respuestas endocrinas e inmunes a cambios en el ambiente, poco se ha hecho fuera del campo de la endocrinología del estrés y las respuestas a compuestos tóxicos (Buchanan, 2000; McEwen y Wingfield, 2003; Segerstrom, 2007; Svensson et al., 1998; Wingfield et al., 1995).

Un aspecto generalmente ignorado en la biología de la conservación son los detalles de la nutrición animal, en especial de cómo los mecanismos digestivos afectan la obtención y asimilación de nutrientes (Diamond, 1991; Karasov, 1990; Karasov y Diamond, 1988; Martínez del Rio et al., 1992). Aunque esto pueda parecer trivial, entender las preferencias y los patrones de uso de diversos recursos alimenticios depende de conocer las necesidades nutricionales y los mecanismos digestivos y de transporte de nutrientes que se dan a nivel del tracto digestivo (Diamond et al., 1986; Karasov y Diamond, 1983; Schondube y Martínez del Rio, 2003; Struempf et al., 1999). La falta de conocimiento de estos procesos reduce nuestra capacidad para entender como un animal responderá a cambios en su hábitat y las consecuencias que esto tiene sobre su sobrevivencia al limitar la adquisición de nutrientes esenciales y energía (Martínez del Rio, 1994; Ayala-Berdon et al., 2008). Esto es especialmente importante cuando los animales prestan servicios ecosistémicos claves, como es el caso de los vertebrados nectarívoros quienes actúan como importantes polinizadores (De Marco y Monteiro-Coelho, 2004; Kremen, 2005).

El objetivo general de este trabajo fue determinar el papel que la fisiología digestiva juega para entender los patrones ecológicos de selección, uso de recursos y distribución espacial de murciélagos que se alimentan de néctar. Este estudio permitió determinar cómo los mecanismos digestivos afectan e interactúan con el metabolismo para generar patrones ecológicos de uso de recursos y distribución espacial de murciélagos nectarívoros. La información generada podrá ser utilizada como información básica para el manejo y conservación de este importante grupo de polinizadores.

1. La calidad del néctar y su efecto en vertebrados

Los animales que se alimentan de néctar son sumamente importantes en las comunidades naturales (Allen-Wardell, 1998; Fleming y Sosa, 1994; Kearns y Inouye, 1997; Kearns et al., 1998). Se ha estimado que más de 2,000 especies de plantas dependen de ellos total o parcialmente para su reproducción (Dobat, 1985; Heithaus et al., 1975). Desde el punto de vista evolutivo, las plantas han desarrollado estrategias que sirven para atraer a sus polinizadores (Baker y Baker, 1973; Faegri y van der Phil, 1979; Freeman et al., 1991). Una de esas estrategias es la producción de néctar en las estructuras florales (de la Barrera y Nobel, 2004; Cruden et al., 1982; Simpson y Neff ,1983). El néctar es una solución acuosa compuesta principalmente por tres tipos de azúcares: el disacárido sacarosa y los monosacáridos glucosa y fructosa. Adicionalmente, el néctar presenta cantidades mínimas de aminoácidos, vitaminas y electrolitos (Baker y Baker, 1973; Baker y Baker, 1983; Baker y Baker, 1998; Nicolson y Thornburg, 2007).

Las características antes mencionadas han hecho que el néctar sea considerado como uno de los alimentos más simples y fáciles de asimilar presentes en la naturaleza. Sin embargo, este recurso presenta bastante heterogeneidad en su composición (Baker et al., 1998) y su concentración (Pyke y Waser, 1981; Rodríguez-Peña et al., 2007). La composición de azúcares presentes en néctares consumidos por murciélagos y aves presenta una gran variedad de combinaciones que usualmente están relacionadas el tipo de polinizador que visita las diferentes especies de plantas (Baker y Baker, 1998). Por ejemplo, los néctares de las plantas polinizadas por murciélagos del nuevo mundo presentan una mayor proporción de glucosa y fructosa con respecto a la cantidad de sacarosa, mientras que los néctares de plantas visitadas por aves pueden estar dominados por sacarosa (si son visitadas por colibríes o aves sol - Nectarinidae) o ser ricos en glucosa y fructosa (si son visitadas por aves paserinas del nuevo mundo o aves nectarívoras no especializadas en el viejo mundo; Baker et al., 1998; Rodríguez-Peña et al., 2007). Con respecto a la concentración de azúcares contenidos en el néctar de plantas polinizadas por vertebrados, se sabe que en los trópicos esta puede variar de un 3 a un 67% (Pyke y Waser, 1981; Rodríguez-Peña et al., 2007).

El gradiente de concentración de azúcares presentes en el néctar tiende a generar respuestas conductuales diferentes en los individuos que se alimentan de él. Diversas especies de animales nectarívoros disminuyen su consumo de alimento cuando la concentración de azúcares en este se incrementa (Collins, 1981; Downs, 1997; López-Calleja et al., 1997; McWhorter y Martínez del Rio, 2000; Schondube y Martínez del Rio, 2003; Ayala-Berdon et al., 2008 y 2009). La existencia de una relación negativa entre la densidad de nutrientes en el alimento y su tasa de consumo no es exclusiva de los animales nectarívoros y ha sido observada en diversas especies de animales (Castle y Wunder, 1995; Montgomery y Baumgardt, 1965). Esta relación entre la cantidad de alimento consumido y su calidad nutricional ha sido nombrada la “respuesta de consumo” (Castle y Wunder, 1995), y puede ser descrita utilizando una función exponencial negativa de la forma $V = aC^{-b}$, donde V es el consumo de alimento, C es la concentración de nutrientes en el alimento y el intercepto (a) y la pendiente (b) son constantes derivadas empíricamente (Figura 2; Martínez del Rio et al., 2001;

McWhorter y Martínez del Rio, 1999; 2000).

La respuesta de consumo tiende a ser considerada como el resultado de alimentación compensatoria (Simpson et al., 1989), esta explicación implica que los animales 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). Si la densidad de energía/nutrientes en el alimento disminuye, los animales compensan por medio de aumentar la cantidad de alimento que consumen. En aves nectarívoras se ha encontrado que la relación negativa entre volumen de néctar ingerido y concentración de azúcar en el néctar tiende a generar tasas de ingestión de azúcares relativamente constantes (Levey y Martínez del Rio, 1999; López-Calleja et al., 1997). Una hipótesis alternativa a la alimentación compensatoria para explicar la repuesta de consumo es que la ingestión de alimento está limitada por la habilidad de los animales para procesar los nutrientes contenidos en él (Levey y Martínez del Rio, 1999; Martínez del Rio et al., 2001). ¿Cómo podemos determinar si la alimentación compensatoria o limitantes fisiológicas están controlando la respuesta de consumo de un organismo? McWhorter y Martínez del Rio (2000) y Martínez del Rio et al. (2001) han propuesto un método analítico para hacer esto:

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 ($N = aC^{-b}$, $C = aC^{1-b}$). Por lo tanto, animales que presentan valores de b (pendiente), 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).

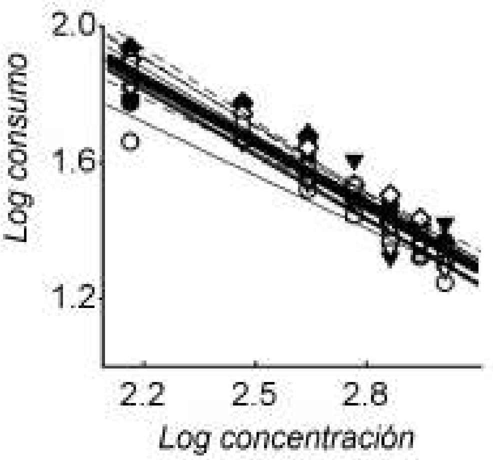

Figura 2. Respuesta de consumo observada en seis individuos de la especie de murciélago filostómido *Glossophaga soricina* en un bosque tropical caducifolio. Cada símbolo representa un individuo. Se observa que la cantidad de alimento consumido se incrementa cuando la calidad nutricional de este disminuye. La relación esta descrita por una función exponencial negativa que logarítmicamente se comporta como una recta del tipo $V = a - bC$. En esta relación el volumen de alimento consumido (V) esta inversamente afectado por la concentración (C). La ordenada al origen (a) y la pendiente (b) son derivadas empíricamente (tomado y modificado de Ayala-Berdon et al. 2008).

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 (Figura 2). 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 (Ayala-Berdon et al. 2008; 2009; Ayala-Berdon y Schondube 2011; ver McWhorter y López-Calleja, 2000 para una revisión más profunda sobre este tema).

Estudios previos realizados con murciélagos y aves nectarívoras han identificado que su capacidad digestiva para asimilar diferentes azúcares es lo que limita su consumo máximo de energía (McWhorter y Martínez del Rio, 2000; Martínez del Rio et al., 2001; Schondube y Martínez del Rio, 2003; Ayala-Berdon et al., 2008 y 2009). Se sabe que la glucosa y la fructosa son dos monosacáridos que son absorbidos de manera directa en el tracto digestivo a través de transporte activo (Karasov y Martínez del Rio, 2007). Sin embargo, la sacarosa es un disacárido que está compuesto por una molécula de glucosa y una de fructosa unidos por un puente de hidrógeno. Antes de ser absorbido, este azúcar tiene que ser hidrolizado por la sacarasa (enzima que degrada dichos puentes), limitando la cantidad de alimento que puede ser consumido y procesado por unidad de tiempo (McWhorter y Martínez del Rio, 2000). Un estudio previo desarrollado por nuestro grupo de investigación demostró que aunque varias especies de murciélagos filostómidos presentan limitantes digestivas, la tasa de hidrólisis intestinal de sacarosa esta acoplada con aquella de absorción de glucosa y fructosa (Ayala-Berdon et al., 2008). Nuestros resultados sugieren que el sistema digestivo de estas especies está diseñado de acuerdo al principio de simorfosis, el cual señala que los procesos fisiológicos en serie están diseñados para que un paso no sea más limitante que el otro (Weibel, 2000).

2. Reajustes conductuales para adecuar el gasto metabólico al consumo máximo de energía.

La relación entre las limitantes digestivas y las respuestas de consumo en animales nectarívoros está bien documentada (Schondube y Martínez del Rio, 2003; Schondube, 2003; Ayala-Berdon et al., 2008 y 2009). Sin embargo, las respuestas de consumo realizadas con murciélagos filostómidos han revelado que especies que presentan limitantes digestivas logran mantener un balance de masa corporal, aún cuando estos presentan déficits de obtención de energía cuando se alimentan de néctares diluidos. Esto sugiere que los gastos metabólicos de estos organismos están pareados con su consumo de alimento. Si esto es cierto, los animales ajustan sus gastos metabólicos a sus consumos energéticos. Una hipótesis es que los animales llevan a cabo reajustes importantes de sus patrones de forrajeo, utilizando mecanismos fisiológicos/conductuales para el ahorro de energía.

Estudios con aves han demostrado que cuando la concentración de alimento disminuye, los individuos incrementan el tiempo de alimentación y percha (Collins et al., 2004). Una explicación a este comportamiento estipula que el tiempo de permanencia en la percha es utilizado para vaciar el tracto digestivo para que pueda ser utilizado para ingerir una nueva comida (Diamond et al., 1986). Alimentarse de néctares diluidos debe llenar el tracto digestivo con una cantidad mínima de nutrientes. Sin embargo, la concentración baja de nutrientes debe obligar a los organismos a optimizar el presupuesto energético incrementando el tiempo de alimentación.

El primer capítulo de esta tesis está enfocado en determinar el efecto que tienen las limitantes fisiológicas en los patrones de forrajeo de dos especies de murciélagos filostómidos que presentan diferentes niveles de limitantes digestivas. Hipotetizamos que cuando los murciélagos sean incapaces de lograr una alimentación compensatoria, su balance energético dependerá de una compensación conductual que disminuya los costos del forrajeo. De esta manera, cuando la calidad nutricional del néctar disminuya, los murciélagos incrementarán sus tiempos de visita, alimentación y descanso, y disminuirán su tiempo de vuelo.

3. Especialización fisiológica y sus efectos sobre el uso de recursos alimenticios en campo.

Los exponentes de las respuestas de consumo tienden a ser variables tanto en murciélagos como en aves nectarívoras (McWhorter y Martínez del Rio, 2000;

Martínez del Rio et al., 2001; Schondube y Martínez del Rio, 2003; Ayala-Berdon et al., 2008 y 2009). Esta variación sugiere la existencia de diferentes niveles de especialización fisiológica que afecta la capacidad de los organismos para asimilar nutrientes y obtener energía, sobre todo cuando se alimentan de néctares a concentraciones bajas (Ayala-Berdón et al., 2008; Martínez del Rio et al., 2001; Ramírez et al., 2005).

Las diferencias en la capacidad de digerir y/o transportar azúcares a través de la pared intestinal no solo son importantes a nivel filogenético (Lotz y Schondube, 2006; Martínez del Rio, 1990b; Schondube et al., 2001), sino que pueden tener importantes repercusiones a nivel ecológico, al separar las capacidades digestivas de los miembros de una misma comunidad de organismos nectarívoros limitando la cantidad de energía que un animal requiere consumir

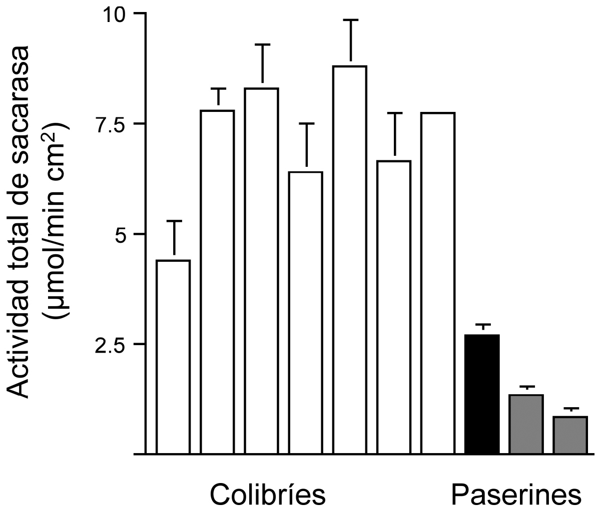

Figura 3. Diferencias en la actividad de sacarasa entre los miembros de la comunidad de aves nectarívoras del Nevado de Colima, Jalisco. Las diferentes especies de colibríes (barras blancas) presentan actividades de esta enzima que son entre 2 y 9 veces mayores a las que presentan los paseriformes nectarívoros (barras oscuras). *Diglossa baritula* (barra negra), un paseriforme que consume una dieta de colibrí, presenta valores intermedios entre los de los colibríes y los de los otros paseriformes. Estas diferencias digestivas sugieren la existencia de diferencias en el uso de recursos florales en campo asociadas a las capacidades digestivas de los animales, con los paseriformes debiendo consumir néctares con bajos contenidos de sacarosa (Schondube 2003).

para satisfacer sus necesidades metabólicas (Figura 3; Schondube, 2003).

La especialización fisiológica en caracteres digestivos, definida aquí como: 1) una alta actividad de sacarasa, 2) capacidad para lograr alimentación compensatoria, y 3) presentar tasas iguales de digestión de sacarosa y absorción/transporte de hexosas, debe permitir que un animal nectarívoro sobreviva adecuadamente consumiendo cualquier tipo de néctar independientemente de su composición de azúcares o concentración. Mientras que bajos niveles de especialización digestiva, representados por 1) una baja actividad de sacarasa, 2) incapacidad para lograr alimentación compensatoria, y 3) tasas de digestión de sacarosa menores a las tasas de absorción/transporte de hexosas, debe obligar a un animal nectarívoro a especializarse ecológicamente a visitar flores con néctares de hexosas que tengan concentraciones iguales o mayores que un15% (peso/volumen) de azúcar.

El segundo capítulo de esta tesis propone utilizar la fisiología digestiva como una herramienta para explicar la relación entre los procesos de adquisición de energía de los murciélagos nectarívoros y su ecología de comunidades, relacionando mecanismos fisiológicos (i.e. capacidad digestiva), con el uso y partición de recursos en campo. La predicción de trabajo es que los murciélagos nectarívoros que tienen la capacidad de lograr alimentación compensatoria tienen una ventaja fisiológica, ya que pueden utilizar una mayor cantidad de recursos en campo. De esta manera, ecológicamente se comportarán como generalistas. Sin embargo, las especies que presentan limitantes digestivas estarán más restringidas en el número de especies de plantas que pueden visitar, actuando como especialistas ecológicamente.

4. Capacidad para distinguir sabor en la selección de alimento en campo

A pesar de que las capacidades digestivas de los organismos les permiten tener acceso a ciertos recursos alimenticios presentes en su ambiente natural, las características que conducen la selección de plantas en campo son en general desconocidas. En aves paserinas la aversión por plantas que secretan néctares ricos en sacarosa está determinada por la falta de actividad de sacarasa en estos animales (Martínez del Rio et al., 1988; Martínez del Rio et al., 1989; Malcarney et al., 1994). Sin embargo, estudios realizados con murciélagos del viejo mundo y colibríes del nuevo mundo que no presentan esta limitante enzimática, han demostrado que estos prefieren alimentarse de sacarosa, aún cuando no hay una ventaja fisiológica aparente de consumir este azúcar sobre azúcares más simples como la glucosa o fructosa (Herrera, 1999; Herrera et al., 2000; Stiles, 1976; Martínez del Rio, 1990). Una hipótesis que explica este comportamiento estipula que los organismos tienden a preferir las características del néctar que predomina en las plantas que visitan en condiciones naturales (Baker and Baker, 1983; Martínez de Rio, 1990; Martínez del Rio et al., 1992; Herrera et al., 2000; Medina-Tapia et al., 2011). Esto implica que los animales tomarán decisiones basadas en el sabor, olor, u otras características de su alimento que pueden ser aprendidas y guardadas en su memoria sensorial. Con respecto a esta hipótesis, Medina-Tapia et al. (2011) demostraron que el colibrí de pico ancho (*Cynanthus latirostris*) es capaz de diferenciar el sabor de los tres principales azúcares presentes en los néctares que consumen (i.e. sacarosa, glucosa y fructosa). De acuerdo a los autores, los colibríes son capaces de seleccionar los diferentes azúcares por el grado de dulzura que son capaces de percibir, sobre todo cuando los colibríes se alimentan de néctares diluidos (~5%).

Estudios realizados con murciélagos del nuevo mundo (*Phillostomidae, Glossophaginae*), han determinado que la digestión de sacarosa y la absorción de glucosa y fructosa se encuentran pareadas, dejando claro que la preferencia por recursos alimenticios en campo no está dirigida por factores fisiológicos en estos organismos (Ayala-Berdon et al., 2008; Herrera y Mancina, 2008). Sin embargo, Herrera (1999) encontró que tres especies de murciélagos filostómidos tienden a preferir sacarosa con respecto a glucosa y fructosa a pesar de que los azúcares dominantes presentes en su dieta son las hexosas glucosa y fructosa (Baker y Baker, 1998; Rodríguez-Peña et al. 2007). Si los murciélagos no presentan limitantes digestivas para el consumo de los diferentes azúcares presentes en su dieta, ¿qué características dirigen su elección de alimento? El capítulo 3 de esta tesis está enfocado en tratar de explicar si la selección de alimento puede estar afectada por la capacidad de discriminar sabores en murciélagos. Para cumplir con este objetivo analizamos los umbrales gustativos para diferentes azúcares de dos especies de murciélagos nectarívoros del Nuevo Mundo: *Glossophaga soricina* y *Leptonycteris yerbabuenae*. La hipótesis generada en esta parte del trabajo fue que existen diferentes umbrales para los diferentes tipos de azúcares presentes en el néctar, y que los murciélagos son más sensibles para las hexosas glucosa y fructosa, azúcares que predominan en su dieta natural.

5. Efectos de las limitantes digestivas sobre la ecología y la distribución geográfica de los murciélagos que se alimentan de néctar

La integración de la información de capacidades digestivas, respuestas de consumo y datos de metabolismo, permitirá entender los patrones ecológicos de uso de recursos y los mecanismos fisiológicos involucrados en determinar la distribución espacial de algunas especies de murciélagos nectarívoros. La información sobre respuestas de consumo, metabolismo, comportamiento y su relación con las limitantes digestivas y la especialización ecológica ofrecerá una visión mecanística de la ecología de estos organismos. De esta manera el capítulo 4 de esta tesis se aborda la relación existente entre capacidades digestivas y necesidades energéticas para predecir la distribución espacial de estos organismos. Para cumplir con este objetivo, se analizaron las capacidades digestivas del murciélago *Leptonycteris nivalis,* una especie de murciélago filostómido que tiene la capacidad de vivir en ambientes muy fríos (Brown, 2008), en donde sus requerimientos energéticos son muy altos. Las capacidades digestivas se relacionaron con su capacidad para habitar ambientes con demandas energéticas altas. Con esta última sección se definieron los “nichos fisiológicos” de nuestra especie de estudio, los cuales nos permitieron entender e incluso hacer predicciones de las distribuciones espaciales de este importante grupo de polinizadores.

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CAPITULO 1. REAJUSTES CONDUCTUALES PARA ADECUAR EL GASTO METABÓLICO AL CONSUMO MÁXIMO DE ENERGÍA

Foraging behavior adjustments related to changes in nectar sugar concentration in phyllostomid bats.

Jorge Ayala-Berdon[1], Nelly Rodríguez-Peña[1], Mónica Orduña-Villaseñor[1], Kathryn E. Stoner[1], Detlev H. Kelm[2], Jorge E. Schondube[1].

[1]Centro de Investigaciónes en Ecosistemas, Universidad Nacional Autónoma de México, Antigua Carretera a Pátzcuaro No. 8701 C.P. 58190, Morelia, Michoacán, México. Telephone number (52)55-5623-27-64

[2]Leibniz Institute for Zoo and Wildlife Research, Alfred-Kowalke-Strasse 17, 10315 Berlin, Germany

Abstract

Nectar-feeding bats regulate their food ingestion in response to changes in sugar concentration as a way to achieve a constant energy intake. However, their digestive capability to absorb sugars can limit their total energy intake, particularly when sugar concentration in nectar is low. Our experimental study evaluated the effect that changes in sugar concentration of nectar have on the foraging behavior of the nectar-feeding bats *Glossophaga soricina* and *Leptonycteris yerbabuenae* in captivity. We measured foraging behavior and food intake when bats fed at different concentrations of sucrose (5, 15, 25 and 35% wt./vol.). To compensate for low-energy intake, both bat species reduced their flight time, and increased feeding time when sugar concentration decreased. Our results suggest that nectar-feeding bats in nature confront two scenarios with complementary ecological effects: 1) bats feeding on dilute nectars (i.e. ≤ 15% wt/vol.) should increase the number of flowers visited per night enhancing pollination, and 2) bats feeding on concentrated nectars could spend more time flying, including long- and short-distance-flights increasing food patch exploration for use during subsequent nights, and thus enhancing plant gene flow. Further studies on foraging behavior of nectarivorous bats under natural conditions are necessary to corroborate these hypotheses.

Key words: *Activity patterns, Phyllostomid bats, Behavioral compensation, Energy balance, Foraging behavior, Intake response.*

1. Introduction

Nectar-feeding bats face daily changes in the energy content of the nectar they consume (Heinreich, 1981; Baker and Baker, 1983; Pyke, 1991; Baker et al., 1998). These changes are related to the phenology of bat-pollinated plants (Sazima et al., 1999; Bustamante et al., 2010), the daily rhythms of flower anthesis (Sazima et al., 1999), the volume of nectar (Wolf, 2006), and the variation in sugar concentration ranging from approximately 3 to 33%, with an average concentration of 20% (Baker et al., 1998; Rodríguez-Peña et al., 2007). Bats regulate their food ingestion in response to changes in nectar sugar concentration, as a strategy to try to achieve a constant energy intake (Montgomery and Baumgardt, 1965; Slansky and Wheeler, 1992).

In addition to variation in energy content on nectar, physiological constraints (at the individual and population level), also can limit the amount of assimilable energy that nectarivores are able to acquire (McWhorter and Martínez del Rio, 2000; Martínez del Rio et al., 2001; Schondube and Martínez del Rio, 2003; Ayala-Berdon et al, 2008, 2009). Since nectar is basically sugar dissolved in water, bats feeding on dilute nectars or fleshy fruits consume very large quantities of water when trying to satisfy their energy budget (Korine et al., 2004; Ramírez et al., 2005). In contrast, bats feeding on concentrated nectars ingest much less water to cover the same energy needs (Carpenter et al., 1991). It seems that nectar-feeding bats' capacities to digest and absorb sugars are the main factors limiting the amount of energy they can acquire when feeding at different concentrations (Ramírez et al., 2005; Ayala-Berdon et al., 2008). The main digestive limitations to process sugars appear to be both the low affinity of the enzyme sucrose for its substrate, and the absorption rates of hexoses when bats feed on dilute nectars (Ayala-Berdon et al., 2008).

The physiological constraints affecting the energetic budget of bats control the amount of energy they can obtain when sugar concentration in nectar varies (Ramírez et al., 2005; Ayala-Berdon et al., 2008, 2009). Nectar-eating animals must maintain a positive or neutral balance between energy spent and energy obtained while foraging (Montgomery and Baumgardt, 1965; Wolf, 1972; Slansky and Wheeler, 1992; Winter et al., 1993; Winter, 1998). Ayala-Berdon et al. (2008) hypothesized that bats should regulate their activity patterns to compensate for negative energetic balances when they confront physiological constraints. They found that bats ingested less energy when feeding on dilute nectars, than when drinking on concentrated ones; however their body masses did not vary, suggesting a behavioral compensation (Ayala-Berdon et al., 2008, 2009).

Our study evaluates the effect of changes in sugar concentration of nectar on the foraging behavior of Pallas long-tongued bat *Glossophaga soricina,* and Saussure's--long nosed bat *Leptonycteris yerbabuenae*. Both species have been reported to exhibit physiological constraints when feeding at sugar concentrations ≤15% (wt/vol., ~ 438 mmolL^{-1}; Ramírez et al., 2006; Ayala-Berdon et al., 2008; 2009). To test the hypothesis that bats modified their behavior when their energy intake is controlled by a physiological limitation (Ayala-Berdon et al., 2008), we measured volumetric intake and foraging behavior when bats fed at different concentrations of sucrose solutions. We expected bats to exhibit compensatory feeding in response to changes in food quality, except at low concentrations (≤15% wt/vol.). We expected that bats confronted with dilute sugar solutions, would increase the time they spent drinking, and reduce their flight time as a strategy to increase their energy intake and save energy.

2. Materials and methods

2.1 Study site

Our study site was located in the Chamela-Cuixmala Biosphere Reserve, on the coast of Jalisco, México (19º22'-19º35'N, 104º56'-105º03´W; elevation from 0 to 500 meters). Vegetation is composed of lowland deciduous forest with small patches of riparian forest (Bullock, 1986). The climate is tropical sub-humid with a marked dry season (García-Oliva et al., 2002). There are about 22 species of plants that have been reported as pollinated by nectar feeding bats (Stoner et al., 2003). These plants secrete nectars that vary widely in sugar concentration (~3 to 33%; Rodriguez-Peña et al., 2007).

2.2 Study species

The study species were Saussure's long-nosed bat (*Leptonycteris yerbabuenae;* 21.04±1.67 g) and Pallas' long-tongued bat (*Glossophaga soricina*; 9.01±0.49 g). Both species feed mostly on nectars presenting 18% (wt/vol.) sugar concentrations but confront a wide variation in nectar sugar concentration in the field (Rodríguez-Peña et al., 2007; Stoner et al., 2003). Additionally, both species have been reported as organisms exhibiting physiological constraints that limit their food intake (Ramírez et al., 2005; Herrera and Mancina, 2008; Ayala-Berdon et al., 2008, 2009).

2.3 Bat care and housing

Because *L. yerbabuenae* is considered an endangered species by the Mexican government, our research permits (FAUT-0193) only allowed us to use 12 non-reproductive individuals of this species in our experiments. To have a balanced design we used the same number of individuals of *G.* soricina. Bats were captured using mist nets and transferred to semi-natural conditions at the study site. Individuals were kept during the day in colonies inside cages (0.6 x 0.6 x 0.6 m). Temperature and humidity were similar of those registered in bats' roosting sites (temperature 26–29°C and humidity 79–85%) in the region. During the experiments bats were transferred to flight cages (2.0 x 4.0 x 1.6 m), located inside the forest. During resting nights bats were fed a maintenance diet composed by 22.2% sucrose and 4.4% protein (Mirón et al., 2006), complemented with a mixture of vitamins and minerals (NEKTON-S®; Guenter, Enderle, Tarpon Springs, FL, USA). To identify changes in body condition, Individuals were marked with a numbered ring on the forearm and were weighed daily (± 0.01 g, OhausTM, Burlington, NC, USA). Captive bats maintained body mass during experimental trials and were released at their capture site upon completion of the study.

2.4 Intake responses

To analyze the capability of bats to obtain energy, we measured their food intake when fed from a range of sugar concentrations (see Ayala-Berdon et al., 2008 and Ayala- Berdon et al., 2009 for more details). Nectar solutions were made with sucrose at 146, 438, 730 and 1022 mmol L^{-1} [~5, 15, 25 and 35% (wt/vol.), respectively]. We chose these solutions because they simulate the range of concentrations consumed by bats at our study site (Rodriguez-Peña et al., 2007). Three bats received each one of the four solutions inside a flight cage (2.0 x 4.0 x 1.6 m) immersed in the forest from 20:00 to 06:00 hrs. This experimental design assured that bats confronted the same environmental characteristics present in their natural habitat (temperature and humidity). Each bat was used for a single trial and concentrations were assigned randomly. We quantified the total amount of solution consumed by weighing the solutions at the beginning and the end of each feeding trial (± 0.01 g; OhausTM, Burlington, NC, USA). To account for changes in volume or concentration caused by evaporation, we put a feeder outside each flight cage during experimental trials. Feeders were covered with mosquito mesh to prevent losses caused by consumption. No changes in volume or concentration were observed in control feeders.

2.5 Foraging behavior

To quantify foraging behavior, we recorded the behavior of bats during the intake response trials. Recordings were made using night vision cameras with a resolution of 30 frames per second (DIGIOP*G2*®, USA). Due to technical problems with the video recorder, we lost data from one individual of *G.* soricina feeding at 730mmol L^{-1}(25% wt/vol.). As a result of this, we obtained behavioral data from 11 *G. soricina* and 12 *L. yerbabuenae*. We recognized and quantified the time dedicated to each of four categories of behavior: 1) flying time, defined as the time bats flew without feeding, 2) feeding time, defined as the time bats drank from the feeder (both hovering in front and perching on the feeder), 3) hovering time, defined as the average of time used by bats while flying for drinking in front of the feeder on each visit and 4) resting time was obtained by adding feeding and flying time, and subtracting this value from the total experimental time (10 hours). For each behavioral category, we reported the total time as the number of seconds that the bats expend in each behavioral category during the experimental 10 h period (s $10h^{-1}$).

2.6 Data analysis

To determine the capability of animals to acquire energy, we used simple linear regressions on the log transformed data to establish the relationship between food intake (total volume ingested) and sugar intake with sugar concentration of nectar. To establish if bats were presenting compensatory feeding or physiological constraints, we tested the slopes of the relationships between sugar concentration and food intake against the compensatory value of -1 using a t test (see Ayala-Berdon et al. 2008 and 2009 for more details). Additionally we searched for a relationship between sugar intake and diet sugar concentration at the three highest diet concentrations to test for evidence of a constraint occurring on the most dilute diet using linear regressions. We expected that sugar intake would increase with concentration due the presence of physiological constraints. Total energy obtained by the bats during the night was calculated as the total sucrose ingested, making the assumption that 1g of ingested sugar renders 16.6 kJ (Judkin et al., 1971).

To analyze changes in behavior associated with sugar concentration and energy intake we used linear regressions on the individual log transformed data. We assumed that the relationship between flying and feeding time, and sugar concentration and food intake should fit power functions. We make these assumptions because activity patterns cannot be sustained indefinitely owing to the fact that animals depend on energy intake during exercise (Hammond and Diamond, 1997), and energy intake is limited by feeding time, gut volume, and kinetics of sugar assimilation (Carpenter et al., 1991). As a way to determine if concentration affected the relationship between time spent feeding and time spent flying, we conducted linear regressions between the ratio of flying and feeding time and sugar concentration on the individual log transformed data. Finally, to assess changes in average hovering time we compared the means of the values between concentrations using One-Way ANOVA.

3. Results

3.1 Intake responses

The two bat species significantly increased food intake when sugar concentration decreased (regression formulas: Log food intake = 3.70 - 0.85 Log concentration; and Log food intake = 3.62 - 0.76 Log concentration for *G.* soricina and *L. yerbabuenae* respectively). Similarly, both bat species reduced their food intake when sugar concentration increased ($F_{1,10}$=381.49 and $F_{1,11}$=148.02, $P<0.001$ for *G. soricina* and *L. yerbabuenae* respectively), and increased their total energy intake when sugar concentration increased (regression formulas: Log sugar intake = 0.23 + 0.14 Log concentration, F_{10}=11.07, P=0.008; and Log sugar intake = 0.2135874 + 0.206259 Log concentration, F_{11}=10.37, P=0.009 for *G. soricina* and *L. yerbabuenae* respectively). The slopes of the relationships between sugar concentration and food intake were different from the compensatory value of -1 (t_{11}=3.32, P= 0.006 and t_{11}=3.68, P=0.003 for *G. soricina* and *L. yerbabuenae* respectively). Additionally, energy intake was not affected by sugar concentration at the three highest diet concentrations (t_8=1.17, P= 0.27 and t_8=1.92, P=0.096 for *G. soricina* and *L. yerbabuenae* respectively), providing further robust evidence of constraints affecting both bat species on the most dilute diet.

3.2 Foraging behavior

Both Pallas' Long-tongued bat and Saussure's Long-nosed bat had the same behavioral responses to changes in sugar concentration in nectar. As predicted, bats decreased feeding time as sugar concentration increased (Log feeding time= 9.97-0.84 Log concentration, $F_{1,10}$=47.68, P<0.0001, r^2=0.84, β=0.99; and Log feeding time= 9.54-0.81 Log concentration, $F_{1,11}$=, P=0.0006, r^2=0.71, β=0.97; for *G. soricina* and *L.* yerbabuenae respectively; Fig. 1, middle panel). Feeding time decreased with energy intake, however this was not significant ($F_{1,10}$=2.02 , P=0.18 and $F_{1,11}$=2.61, P=0.13 for *G. soricina* and *L.* yerbabuenae respectively; Fig 1., bottom panel). We did not observe differences in the mean hovering time among concentrations ($F_{1,10}$=0.21, P=0.65 and $F_{1,11}$=2.37, P=0.15 for *G. soricina* and *L.* yerbabuenae respectively; Fig. 1, upper panel).

Flight time increased with sugar concentration, however this pattern was not significant ($F_{1,10}$=1.6, P=0.26 and F_{11}=1.18, P=0.3 for *G.* soricina and *L.* yerbabuenae respectively; Fig. 2, middle panel). However, we found a significant positive relationship between flight time and energy intake (Log flight time=7.06 + 1.9 Log sugar intake, $F_{1,10}$=9.72, P=0.012, r^2= 0.51, β=0.82; and Log flight time=6.41 + 1.9 Log sugar intake, $F_{1,11}$=10.49, P=0.008, r^2= 0.51, β=0.79; for *G. soricina* and *L.* yerbabuenae respectively; Fig. 2, bottom panel). Finally, the ratio between flight time and feeding time was positively related to sugar concentration (Log flight/feeding time=1.43+1.07 Log concentration, $F_{1,10}$=, P=0.0002, r^2=0.80, β=0.99; and Log flight/feeding time=1.94 +1.17 Log concentration, $F_{1,11}$=16.93, P=0.0021; 0.62, β=0.89; for *G. soricina* and *L.* yerbabuenae respectively; Fig. 2, upper panel).

4. Discussion

Both bat species presented slopes that were statistically different from the compensatory value of -1. This result indicated that physiological constraints are limiting the volumetric and energy intake of bats when they fed on low sugar concentrations. Additional support for this hypothesis comes from sugar intake being independent of sugar concentration in both bat species when the bats only fed at the three highest sugar concentrations. This condition prevented *G. soricina* and *L. yerbabuenae* to achieve the same energy intake when feeding at the lowest nectar sugar concentration (5% wt/vol.) compared to the intermediate and higher concentrations (15, 25 and 35 wt/vol.; ~30.5 and 37.5% less sugar respectively). However, bats maintained a constant body mass regardless of the sugar concentration at which they fed. Both *G. soricina* and *L. yerbabuenae* achieved this condition by compensating behaviorally in a similar fashion. As predicted, bats increased feeding time when sugar concentration decreased, but maintained constant their hovering times. Also bats showed a positive relationship between flight and energy intake. In this section we first discuss the effect of sugar concentration on feeding time. Second, we discuss the effects of energy intake on flight time. Finally we explore the relationship that physiology and behavior could have on the ecology of these bat species in the field.

4.1 Effects of sugar concentration on feeding time

The fact that both bat species presented physiological limitations that prevent them to ingest the same amount of energy when feeding on dilute nectars than when feeding on intermediate and concentrated nectars, while maintaining a constant body mass, indicates the existence of a behavioral compensation as suggested by Ayala-Berdon and collaborators (2008). Our behavioral data showed that both *G. soricina* and *L. yerbabuenae* increased time spent feeding when nectar concentration decreased. This was a consequence of bats increasing the number of visits to the feeder when concentration decreased. Similar patterns have been described for nectar feeding birds (Bakken et al., 2004, McWhorter et al., 2003). Since the volume of the gut could limit the amount of food that bats can ingest per visit when food is offered *ad libitum*, increasing total nightly volumetric intake requires visiting the feeder more times (Diamond et al., 1986).

Bats maintained constant the time they spent hovering during each visit to the feeder (mean value ± SD for all concentrations tested: 0.55±0.09 and 0.37±0.05 s^{-1} per visit for *G. soricina* and *L. yerbabuenae*, respectively). This suggests that they ingested similar amounts of nectar per visit, regardless of sugar concentration (0.124 ± 0.02 and 0.159 ± 0.04 ml per visit). This data supports the idea that the volume ingested per visit could be limited by the maximal volume of the receiving area of their guts (mouth, esophagus; Carpenter et al., 1991). This finding is similar to what has been reported for hummingbirds, in which the volume of the gut and the capacity to empty it, control food intake per feeding bout (Diamond et al., 1986). Our results also indicate that bats, by ingesting the maximum volume they can eat in each visit, are maximizing the amount of energy they can ingest per visit at the different sugar concentrations.

To establish the role that sugar assimilation plays in determining the time among feeding bouts, we estimated the time needed to process the total volumetric intake that each bat presented during our experiments using a gut function model (McWhorter and Martínez del Rio, 2000), and compared, using a t test, the predicted time with the actual time that the bats spent flying and resting (non-feeding time) derived from our experimental data. The model assumes that the intestine is analogous to a chemical reactor in which sucrase activity follows Michaellis-Menten kinetics, the food moves only in one direction, and the activity of the enzyme is homogeneously distributed along the gut (McWhorter and Martínez del Rio, 2000). In this model the time necessary to digest a given volume of food, can be estimated by knowing the assimilation efficiency, sugar digestion rates, and the volume of the bat's small intestine. Gut volumes and enzyme activity data were taken from Ayala-Berdon et al. (2008). Times to digest food predicted by the model did not differ from the non-feeding time values that we obtained in our experiments (t_{20}=0.841, P=0.41; and t_{22}=0.762, P=0.45 for *G. soricina* and *L. yerbabuenae* respectively). This result supports the idea that gut capacity to digest sucrose determines the time to empty the gut, and thus the time for a new visit to the feeder, as is the case in hummingbirds (Diamond et al., 1986).

The comparison we made provides some indirect evidence that bats are feeding at rates that could be determined by gut emptying. Using enzymatic data for the species we studied (Ayala-Berdon et al. 2008) we modeled the role that sucrose total activity and kinetics (affinity defined as 1/km) had on food intake. Our modeling results indicate that while sucrose total activity does not affect food intake significantly, the affinity of this enzyme for its substrate is the main factor affecting food intake while bats are feeding at low concentrations. This modeling suggests that sugar digestion when bats are feeding on sucrose-rich nectars, and/or sugar absorption when bats fed on hexose nectars (two processes that are paired; Ayala-Berdon et al., 2008), could impose limits to gut emptying, and affect feeding frequency. However this idea remains to be tested.

4.2 Effects of energy intake on flight time

Glossophaga soricina and *L. yerbabuenae* reduced flight time when they had a smaller energy intake. Welch et al. (2006, 2008), and Voigt and Speakman, (2007) found that hummingbirds and bats use endogenous supplies to maintain their energetic requirements when resting. However, when these animals are active, they fueled their daily activities directly from the sugars they ingested during their foraging time. This could make animals with sugar intakes physiologically limited to reduce activity to balance their energy budgets. When sugar intake is not limited physiologically, animals could present different behaviors, and under certain circumstances stored the additional energy that was not used during flying. In different species of nectar-feeding birds, this extra energy tends to be stored as fat to be utilized in the future (Blem, 1976; Ekman and Hake, 1990). However the bats in our experiments did not increase their body mass as a result of a higher energy intake.

Why bats did not gaining body mass while feeding on the most concentrated sugar solution? We have two possible explanations for this result: 1) the fact that bats did not gain weight could be caused by the short time they were exposed to the high sugar concentrations in our experiments (only one night). However, under natural conditions hummingbirds during one morning are able to increase their body mass by as much as 30% by building fat (Calder and Contreras-Martínez, 1995; Schondube et al., 2004). And 2) bats in our experiments showed a positive relationship between flight time and sugar intake. Kelm and collaborators (2011) proposed that hyperglycemic *Glossophaga soricina* individuals are unable to regulate their post-feeding blood glucose levels exclusively by insulin-triggered cellular glucose transport. The authors argue that despite this species may be tolerant to hyperglycemia; exercise performed by these animals may be regulating increases in blood glucose levels after food ingestion. Under this context, an increasing flight time in our studied bat species could help them to control blood glucose levels when individuals are feeding at high sugar concentrations (Kelm et al., 2011). However this hypothesis remains to be tested.

4.3 Ecological effects of bat's physiology and behavior

The results presented in this work were obtained from bats maintained under semi-natural conditions. These results suggest that nectar-feeding bats in the field may confront two scenarios with complementary ecological effects: a) feeding on dilute nectars and b) feeding on concentrated ones. *Glossophaga soricina* and *L. yerbabuenae* showed changes in volumetric intake in nectar solutions when individuals fed at the different experimental sugar concentrations. However, these two species were unable to achieve compensatory feeding and their energy intake was lower when they fed at the lowest sugar concentration (this study; Ramirez et al., 2005, Herrera and Mancina, 2008, Ayala-Berdon et al., 2008, 2009). Moreover, these differences in volumetric intake, and the reduction in energy intake observed, were closely related to the activity patterns of the bats. Bats increased their total nightly feeding time as a way to increase their volumetric intake when fed at the lowest sugar concentrations (5 and 15% wt./vol.). Nevertheless, as mentioned above, physiological and morphological capabilities of individuals appear to pose a limit to food ingestion per visit. This could limit nectar-feeding bats in the field if we consider that chiropterophilic plants secrete mostly dilute nectars (Pyke and Waser, 1981; Baker and Baker, 1983, Baker et al., 1998; Rodriguez-Peña et al., 2007).

Several authors have generated hypothesis to explain why plants pollinated by flying vertebrates (birds and bats) secrete dilute nectars (Pyke and Waser, 1981; Roces et al., 1993; Nicolson, 2002). One theory has proposed that this condition is the result of a selective pressure posed by plants to ensure multiple visits by animals to guarantee pollination (Heinreich, 1981). Despite the fact that secretion of dilute nectar may be the result of plants decreasing costs of nectar production (Heinrich, 1981; Pyke, 1991), the physiological limitations of bats to maintain a high energy budget forces them to increase the number of flowers visited per night while feeding on dilute nectars. This limitation should enhance pollen movement and pollination (as suggested by Heithaus et al., 1975; Fleming and Sosa, 1994; Kearns and Inouye, 1997; Allen-Wardell et al., 1998). However, because bats tend to reduce flight time when feeding on dilute nectars, pollen movement should occur only on a limited area.

The second scenario exists when bats feed on more concentrated nectars and obtain a higher energy intake per visit. In this situation, bats present a higher flight activity. The presence of a positive relationship between flight time and sugar intake may function as an adaptive trait increasing the capacity of animals to search for new feeding and roosting sites, and to fly long distances in one night (Kelm et al., 2011). Since nectar availability is not predictable form day to day (Heitahus et al., 1975; Lemke, 1986), flying long distances would allow nectar-feeding bats to explore more foraging patches that they could use in the following nights, and will also enhance gene flow among plant metapopulations.

Our study documents changes in nectar bat foraging patterns (i.e. feeding and flight time), as a consequence of differing nectar concentration. This is an important step for understanding nectar bat foraging patterns and start to unravel the complex relationship that exist between behavioral adjustments and physiological limitations. However, further studies are needed to understand the physiological and ecological consequences that feeding on food that differs in its energy content have on nectar-eating bats in the field.

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Figure legends

Figure 1. Upper panel: *Glossophaga soricina* (open circles, continuous line) and *L. yerbabuenae* (closed circles, dashed line) maintained constant their hovering time among sugar concentrations tested. Data are presented as means with their respective SD. Middle panel: Both bat species increased feeding time when nectar sugar concentration decreased. Bottom panel: Relationship between feeding time and energy intake. This relationship was not significant; however we present the line to show the positive tendency of the data. While we used individual log transformed data for the relationships between feeding time with sugar concentration and sugar intake, we present the figures using non-transformed data to simplify the biological interpretation of our results. Data points in middle and bottom panels represent individual independent values.

Figure 2. Upper panel: The ratio of flight and feeding time was positively correlated with sugar concentration in the nectar-feeding bats *Glossophaga soricina* (open circles, continuous line) and *L. yerbabuenae* (closed circles, dashed line). Middle panel: Effect of sugar concentration on flight time. This relationship was not significant; however we present the line to show the tendency of data. Bottom panel: flight time increased with energy intake in both bat species. In all cases we used individual log transformed data, however we decided present the figures using non-transformed data to simplify the biological interpretation of our results. All data represent individual independent values.

Figure 1

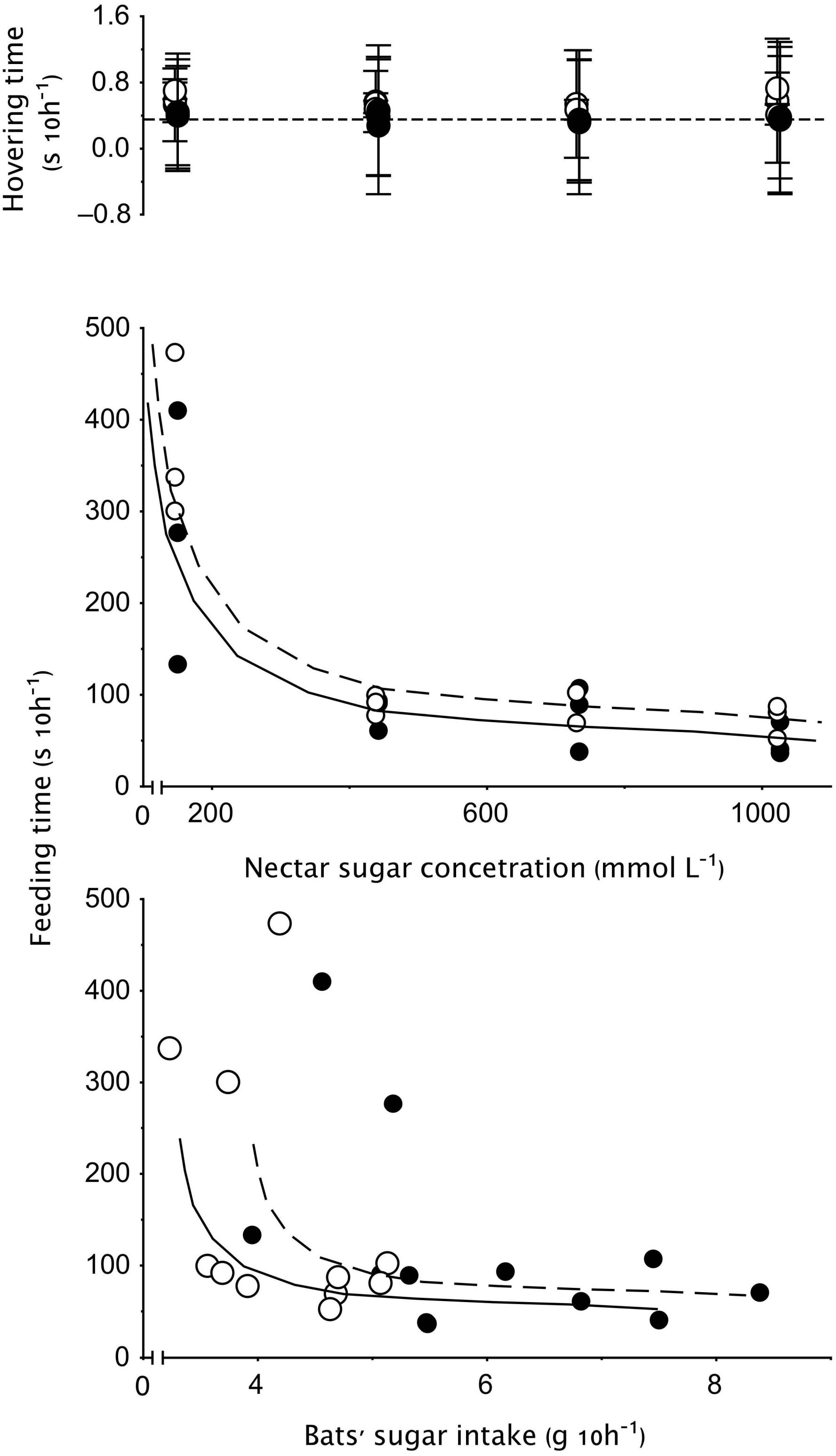
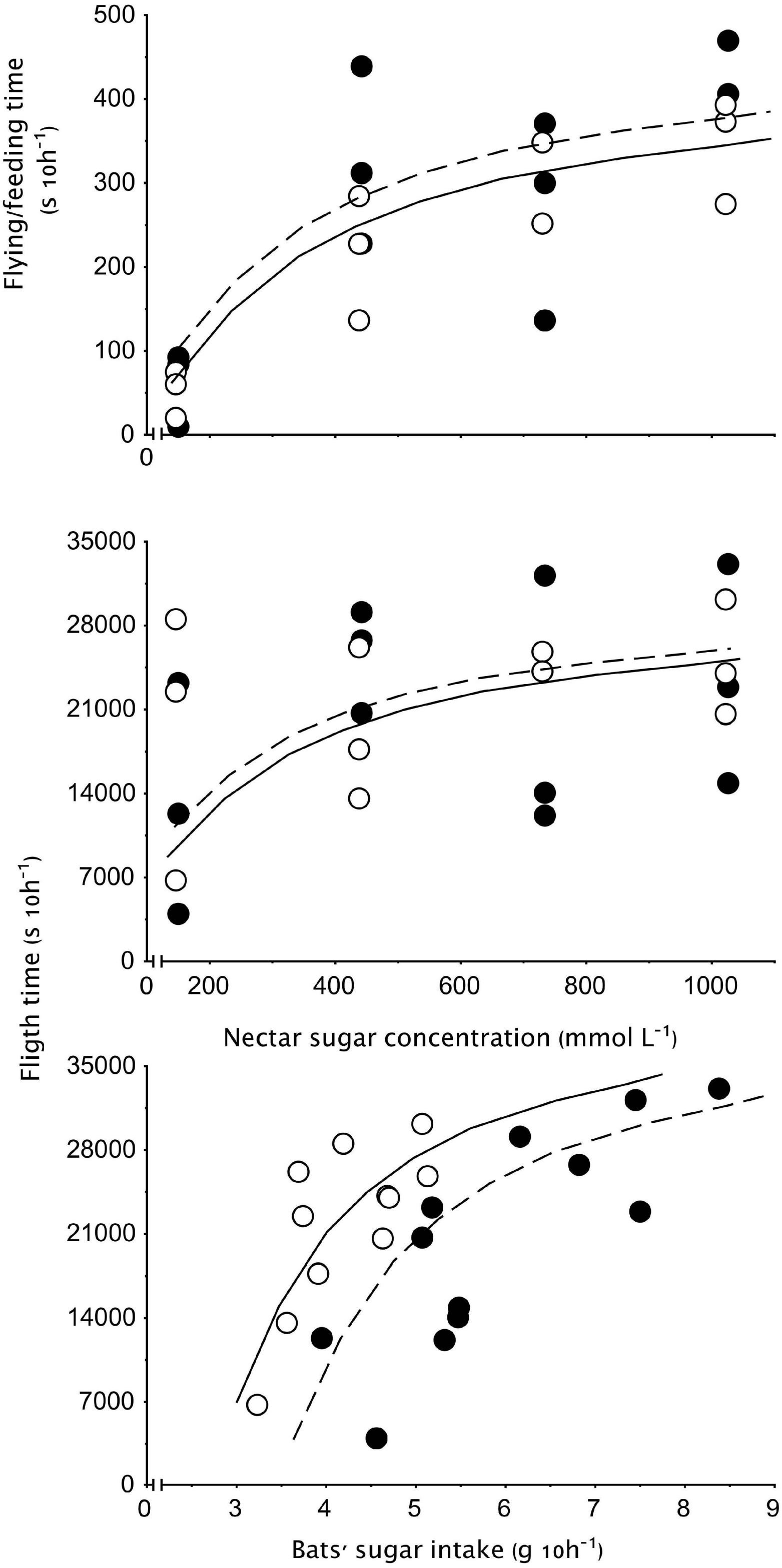

1.6
0.8
0.0
–0.8
Hovering time (s 10h^{-1})
500
400
300
200
100
0
0
200
600
1000
Nectar sugar concetration (mmol L^{-1})
Feeding time (s 10h^{-1})
500
400
300
200
100
0
0
4
6
8
Bats′ sugar intake (g 10h^{-1})

Figure 2.


500
400
300
200
100
0
0
Flying/feeding time (s 10h^{-1})
35000
28000
21000
14000
7000
0
0
200
400
600
800
1000
Fligth time (s 10h^{-1})
Nectar sugar concentration (mmol L^{-1})
35000
28000
21000
14000
7000
0
0
3
4
5
6
7
8
9
Bats' sugar intake (g 10h^{-1})

CAPITULO 2. ESPECIALIZACIÓN FISIOLÓGICA Y SUS EFECTOS SOBRE EL USO DE RECURSOS ALIMENTICIOS EN CAMPO

A physiological perspective on nectar-feeding adaptation in phyllostomid bats.

Jorge Ayala-Berdon and Jorge E. Schondube

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

Abstract

Nectar-feeding animals increase their food intake when nectar sugar concentration decreases. However, some species present physiological constraints that limit their energy intake when nectar is diluted. We hypothesized that the digestive capacities of bats affect their ability to acquire and store energy, modifying how they use food resources in the field. We measured the food intake and changes in body mass of the members of an assemblage of nectar-feeding bats (*Choeronycteris mexicana*, *Leptonycteris yerbabuenae* and *Glossophaga soricina*), feeding at different sucrose solutions (146, 292, 438, 584, 730, 876 and 1022 mmol L^{-1}). The three bat species presented differences in their food intake and their capacity to store energy. While *C. mexicana* was able to maintain a constant energy intake at all concentrations tested, *G. soricina and L. yerbabuenae* decreased their sugar/energy intake at the lowest concentrations. *C. mexicana* also increased body mass independent of sugar concentration, while *G. soricina* and *L. yerbabuenae* did not. Based on our results we generated a model relating digestive capacities and the use of food resources in the field. Our model's predictions and field data supports the idea that digestive traits affect the way these animals use the food resources present in their environment.

Keywords: Body mass, Digestive capacities, Food intake, Nectar-feeding adaptation, Phyllostomid bats.

1. Introduction

Nectar produced by plants varies widely in sugar concentration (from ~3 to 67%; Pyke and Waser, 1981; Baker et al., 1998; Rodríguez-Peña et al., 2007). Variation in nectar sugar concentration affects the amount of energy that nectar-feeding animals obtain while feeding. As a response to changes in sugar concentration, these animals tend to modify their food intake rate, increasing the volume of food they ingest when nectar energy density decreases (Montgomery and Baumgardt, 1965; Slansky and Wheeler, 1992). This behavioral response, named “intake response” by Castle and Wunder (1995), is caused by animals attempting to maintain a constant energy intake when nutrient concentration of food varies (named compensatory feeding; Montgomery and Bumgardt, 1965; Slansky and Wheeler, 1992).

In the last decade numerous studies have described the intake responses of a wide range of nectar-feeding animals (Collins, 1981; Slansky and Wheeler, 1992; López-Calleja et al., 1997; Josens et al., 1998; McWhorter and Martínez del Rio, 1999; McWhorter and Martínez del Rio, 2000; Martínez del Rio et al., 2001; von Helversen and Winter, 2003; Shondube and Martínez del Rio, 2003; Ramírez et al., 2005; Ayala-Berdon et al., 2008; Herrera and Mancina, 2008; Ayala-Berdon et al., 2009). These studies have shown that while some organisms are able to achieve compensatory feeding (Levey and Martínez del Rio, 1999; López-Calleja et al., 1997; von Helversen and Winter, 2003), others present physiological constraints that prevent them from maintaining a continuous energy intake when feeding on dilute nectars (<15 % sugar; Ayala-Berdon et al., 2008). There are three aspects that can constraint food intake when animals feed on dilute nectar: First, the size of the gut that limits the volumetric food intake of animals. Second, the rate of sugar assimilation, affected at low concentrations by the low affinity of the enzyme sucrase for its substrate (Martínez del Rio, 1990; Hernandez and Martínez del Rio, 1992). And third, the large volumes of water that are ingested while animals try to compensate for the low energy content of their food (Herrera and Mancina, 2008). In this situation the rate at which the kidneys get rid of the ingested water may regulate the volumetric intake (Karasov, 1990; Martínez del Rio, 1990; Diamond, 1991; Karasov and Hume, 1997). Ayala-Berdon et al (2008), using a mathematical model, identified that sugar digestion, absorption and gut size impose an upper limit to volumetric food ingestion in nectar-feeding phyllostomid bats, even when they are feeding on dilute nectars (5%; Ramírez et al., 2005; Ayala-Berdon et al., 2008; Ayala-Berdon et al., 2009).

The relationship between volumetric intake and sugar concentration is well described by power functions of the form $V = aC^{-b}$. Where V equals volumetric intake, C equals sugar concentration, and the intercept (a) and the exponent (b) are empirically derived constants (McWhorter and Martínez del Rio, 1999; McWhorter and Martínez del Rio, 2000; Martínez del Rio et al., 2001). Because volumetric intake (V) decreases as a power function of concentration (C), the amount of sugar ingested (A) is also a power function of sugar concentration

($A = aC^{-b}C = aC^{1-b}$; Martínez del Rio et al., 2001). Animals exhibiting exponents equal to 1 show “perfect” compensation with sugar intake independent of concentration (1 – b = 0). In contrast, animals with values of exponents smaller than 1 will show a positive relationship between sugar ingested and sugar concentration in food (i.e. energy density).

The capacity of animals to achieve compensatory feeding and/or the presence of physiological constraints may have effects in the energy balance and resource use of animals in the field. Here, we hypothesize that the digestive capacity of nectar-feeding bats (i.e. sugar digestion and absorption rates and water management), affects their energy balance, and limits their ability to maintain body mass and store energy. To address this hypothesis, we measured the intake responses of the members of an assemblage of nectar-feeding bats. We predict the existence of a relationship between the value of the exponent of the intake response and the capacity of bats to maintain and/or increase their body mass (Δ_{bm}). Nectar-feeding bats that achieve compensatory feeding, and consequently experience a constant intake of energy, should be considered as physiologically adapted and should present Δ_{bm} independent of concentration. Alternatively those bats with values of exponent < 1, should present a positive relationship between Δ_{bm} and concentration, and could be energetically limited when feeding at low concentrations (≤15%). The intake responses of *L. yerbabuenae* and *G. soricina* have been studied in the past to determine the presence of physiological constraints to assimilate sugars and obtain energy

(Ramírez et al,. 2005; Ayala-Berdon et al., 2008; Herrera and Mancina, 2008; Ayala-Berdon et al., 2009). Ayala and collaborators (2008, 2009) suggested that sugar digestion and absorption play the main role constraining the intake response of these bat species. The goal of our study is to use intake responses as tools to compare the digestive capacities among the members of an ecological community and to understand their possible ecological implications.

2. Materials and methods

2.1 Study site.

Our study site was El Jorullo Biological Reserve, which is located in the south-east part of Michoacán state, Mexico (19° 11' 60 N, 101° 37' 60 W). Vegetation at the site is composed by lowland tropical deciduous forest with small patches of perennifolius tropical forest. Climate is tropical sub-humid with a marked dry season. Annual temperature varies between 22-28°C, and annual precipitation oscillates between 500-1000 mm (INEGI, 1985).

2.2 Study species.

We conducted our experiments focusing on three of the four species of phyllostomid bats that form the assemblage of nectar-feeding bats at our study site: 1) The Mexican Long-tongued bat (*Choeronycteris mexicana*), 2) Saussure’s Long-nosed bat (*Leptonycteris yerbabuenae*), and 3) Pallas’ Long-tongued bat (*Glossophaga soricina*). The fourth species present in this assemblage, that we were unable to capture, was the Banana bat (*Musonycteris harrisoni*).

2.3 Bat care and housing.

Four C*hoeronycteris mexicana* (mean weight ± SD= 13.34 ± 1.17g), five *Glossophaga soricina* (mean weight ± SD= 9.36 ± 0.9g) and six *Leptonycteris yerbabuenae* (mean weight ± SD= 21.40 ± 1.37) were captured at the study site using mist nests. All bats were adult non-reproductive. After capture, bats were transferred to captive conditions in where they were kept in maintenance colonies in cages (0.6 x 0.6 x 0.6 m). Bats were fed using the diet described by Mirón et al. (2006), supplemented with vitamins and minerals (Nekton-S®; Guenter Enderle, Tarpon Springs, FL, USA). Individuals were marked with numbered plastic collars and weighed daily at the beginning and the end of each trial (± 0.01 g, OhausTM, Burlington, NC, USA). Bats were captured and used in experiments with permission of the Oficina de Fauna Silvestre, Mexico to JES (FAUT-0193). Captive bats maintained good health for the duration of trials and were released at their capture sites when the experiments were finished.

2.4 Intake responses.

To establish the digestive capacities of bats, we measured the intake responses of each individual bat when they fed at seven different solutions of sucrose. We know from previous studies that digestive capacities of nectar-feeding bats are related to sucrose digestion rate, gut transit time, gut volume and water management by animals, and that these capacities are realistically represented by the food volumetric intake when individuals face changes in sugar concentration (Ayala-Berdon et al., 2008). Solutions were made at 146, 292, 438, 584, 730, 876 and 1022 mmol L^{-1} of sucrose (≈ 5, 10, 15, 20, 25, 30 and 35% sugar wt./vol.). We only used sucrose solutions because phyllostomid bats' food intake is not affected by sugar composition (Rodríguez-Peña et al., 2007; Ayala-Berdon et al., 2008; 2009). Individuals were transferred from maintenance colonies to flying cages (3 x 2 x 1.6 m) which had a feeder in their center. Flying cages were immersed in the forest to simulated natural conditions (temperature, humidity). Bats received one sugar solution per night. We used a modified latin-square design to present the different concentrations to the bats. Since we did not have the same number of bats per species as concentrations, we corrected sugar concentration assignment by ensuring that we had dilute (146, 292 mmol L^{-1}), intermediate (438, 584, 730 mmol L^{-1}) and concentrated sugar solutions (876 and 1022 mmol L^{-1}) being offered to different bats during the same night. Because experimental sugar solutions lacked nitrogen sources, our experiments consisted of three nights of experiments followed by one day of resting. During the resting day bats received the maintenance diet. Solutions were weighed at the beginning (W_i) and the end (W_f) of each feeding trial from 21:00 to 07:00. Food consumed was estimated by subtracting W_f of W_i. Data of 730 and 876 in *C. mexicana* trials were lost due to ants invading the feeders. Each night we placed a feeder of each sugar concentration outside the flight cages to control for changes in concentration and volume due to evaporation. These feeders were covered with a mosquito mesh, to prevent drinking by insects and other nocturnal animals. Control feeders were weighed at the beginning and end of each trial, and the concentration of the solution was measured using a hand-held refractometer (Reichert 10431 0–50°compen- sated Brix temperature, Leica, Buffalo NY, USA) to account for changes in concentration. No changes in volume or concentration were observed in our control feeders.

2.5 Data analysis.

We estimated the slopes and intercepts of the relationships between food intake and sugar concentration using regression analysis on the log-transformed data of each individual bat. We estimated the exponent and intercept of each individual bat for each species. We compared the values of the intake response exponents to the 1 value expected for compensatory feeding using a one-sample t test. We also compared the exponent values from the intake response of the three studied species using an ANOVA. In addition, we calculated Δ_{bm} (in g 10h^{-1}) experienced by the bats, by weighing each individual at the beginning and the end of each trial. We used simple linear regressions to see the effect of concentration on Δ_{bm}. Since we expected Δ_{bm} to correlate positively with their sugar intake (SI, in g 10 h^{-1}), we correlated Δ_{bm} against SI of each bat using Spearman's rank correlations (r_s) and tested whether the average r_s was significantly greater than 0 using a t test. This procedure is appropriate because it avoids the pseudo-replication that one would incur when estimating r_s for pooled data. The average r_s values for a sample of bats satisfy the central limit theorem and hence, one can make inferences about whether they are positive or negative (Stuart and Odd 1994). Finally, we assigned an alpha value of 0.05 to all tests performed to determine the existence of statistical differences.

3. Results

3.1 Intake responses

The three species of bats increased food intake when concentration decreased. In all cases the relationships between food intake and concentration were described by power functions (r^2= 0.86, r^2= 0.93 and r^2= 0.86 for *C. mexicana*, *G. soricina* and *L. yerbabuenae* respectively; Figure 1b). There were no differences in intake responses among individuals of each species (t_3=0.18, P=0.86; t_4= 0.19, P= 0.86; and t_5=0.65, P=0.54 for *C. mexicana*, *G. soricina* and *L. yerbabuenae* respectively). The slopes of the intake responses of the three species differed statistically (Figure 1a; $F_{2,14}$ = 11.45, P = 0.0017). The exponents of the individual intake responses of *C. mexicana* did not differ statistically from the compensatory value of 1 (mean exponent = 0.92, t_3= 1.79, P= 0.17). *G. soricina* and *L. yerbabuenae* presented exponent values that varied statistically from the compensatory feeding value of 1 (mean exponent = 0.75, t_4= 4.5, P= 0.01; and 0.63, t_5=0.63, P= 0.001 respectively). Individuals of *G. soricina* and *L. yerbabuenae* ingested 35 and 55.6% less sugar respectively, when they fed at the lowest sugar concentration (146 mmol L^{-1}) than when they fed at the highest one (1022 mmol L^{-1}; mean food intake ± SD for 146mmol L^{-1}= 2.70g ± 0.55 and 3.48g ± 0.62; and mean food intake ± SD for 1022 mmol L^{-1}= 4.20g ± 0.20 and 7.85g ± 1.04 for *G. soricina* and *L. yerbabuenae* respectively, Fig. 2. upper panel).

Individuals of the three species of bats always gained body mass. However, Δ_{bm} corrected by body mass increased with sugar concentration in *G. soricina* (t_4=4.16, P= 0.01), while it remained constant in *L. yerbabuenae* and *C. mexicana* (Figure 2 upper panel; t_5= 2.13, P= 0.08 and t_3= 1.91, P= 0.15 respectively). SI increased with sugar concentration in *G. soricina* and *L. yerbabuenae* (F_{33}=48.95, P=0.0001; F_5=10.30, P=0.02), but not in *C. mexicana* (F_{19}=1.22, P=0.28, Fig 2. bottom panel). Finally, Δ_{bm} was positively correlated with sugar intake in *G. soricina* and *L. yerbabuenae* (t_4= 14.17, P=0.001 and t_5=3.53, P=0.016 respectively) but not in *C. mexicana* (t_3= 2.97, P= 0.059).

4. Discussion

The three species of bats showed intake responses similar to those observed for nectar-feeding birds (Martínez del Rio et al., 2001; McWhorter and Lopez-Calleja, 2000) and the same bat species under different conditions (von Helversen and Winter, 2003; Ramírez et al., 2005; Ayala-Berdon et al., 2008; Ayala-Berdon, et al., 2009). The intake response slopes were statistically different among the three studied species. Sugar intake was positively correlated to sugar concentration in food in *G. soricina* and *L. yerbabuenae*, but not in *C. mexicana*, a species that was able to achieve compensatory feeding. Also Δ_{bm} was positively affected by sugar intake in *G. soricina* and *L. yerbabuenae*, but not in *C. mexicana*. In this section we discuss our findings regarding compensatory feeding and physiological constraints in the three bat species, and propose a conceptual model to describe the ecological implications of the different degrees of digestive capacities exhibited by the three members of the studied nectar-feeding bat assemblage.

4.1 Intake responses and changes in body mass.

The three species of bats we studied showed classical intake responses in which food intake increased when energy density decreased (Montgomery and Bumgardt, 1965; Slansky and Wheeler, 1992). These responses have been reported before for the nectar-feeding bats *G. soricina* and *L. yerbabuenae* (Ramírez et al., 2006; Herrera and Mancina, 2008; Ayala Berdon et al., 2008; Ayala-Berdon et al., 2009), and the fruit-eating bat *Artibeus jamaicensis* (Ayala-Berdon et al., 2008). The intake responses reported previously for *L. yerbabuenae* did not differ from the one we obtained in this study, while the previous intake responses of *G. soricina* deviate from our new data. This suggests that differences in digestive capacities limit how animals respond to changes in environmental conditions. While *G. soricina* seems to use digestive and behavioral plasticity (i.e. increasing sucrase activity and/or gut size, and or changing feeding behavior) to modify food intake in response to changes in ambient temperature or food availability (Winter, 1998, Ayala-Berdon et al., 2009), *L. yerbabuenae* seems to lack this flexibility. While changes in feeding behavior have been reported for nectar feeding bats (Winter, 1998, Ayala-Berdon et al., 2008), the existence and extent of digestive plasticity to process sugars in these animals remains to be explored.

C. mexicana presented compensatory feeding with an exponent value that did not differ statistically from 1, implying a constant sugar intake among all the tested concentrations. This is the first report of a phyllostomid bat achieving compensatory feeding under semi-natural conditions while feeding on a wide range of sugar concentrations (146 to 1022 mmol L^{-1}). von Helversen and Winter (2003) found that *G. soricina* was able to achieve compensatory feeding under laboratory conditions (controlled humidity and temperature), when its metabolic expenditures were approximately of 50 kJ day^{-1}. Ayala-Berdon and collaborators (2009) used a graphical model to predict that *G. soricina* could achieve compensatory feeding when its metabolic demands were inside the limit of this metabolic expenditure value. They conclude that an increase of metabolic demands due to a decrease in temperature should cause an increase in food consumption that could be limited by the digestive capacities of this species. Under this context, the compensatory feeding capacity of *C. mexicana* should confer this species the ability to maintain constant fluxes of energy over the wide range of sugar concentrations and/or temperatures present along their geographic distribution.

G soricina and *L. yerbabuenae* presented intake responses with exponent values smaller than one. This generates a positive relationship between sugar consumed and sugar nectar concentration, and indicates the existence of a physiological constraint limiting energy intake when feeding at the lower sugar concentrations (146-438 mmol L^{-1}; Ayala-Berdon et al., 2008; Ayala-Berdon et al., 2009; Figure 2 bottom panel). This limitation in the energy intake of bats when feeding on dilute nectars could be caused mainly by sugar digestion when feeding on sucrose nectars (Ayala et al., 2008), and water management when bats feed on hexose solutions (Winter, 1998, Herrera and Mancina, 2008). However the relative role that sucrose digestion, hexose absorption and water absorption and excretion could have on food ingestion when bats feed on dilute nectars remains to be determined (Winter, 1998, Ayala-Berdon et al., 2008). These physiological constraints impose limits to the lower nectar concentration bats could use without losing energy, or the minimal temperatures at which this organisms can live (Martínez del Rio et al., 2001; Herrera and Mancina, 2008; Ayala-Berdon et al., 2009).

The differences in Δ_{bm} related to concentration exhibited by the three studied species indicate that their energetic gains were affected differently by their digestive capacities. All species gained body mass during the experiments, however, Δ_{bm} corrected by body mass varied among them. *C. mexicana* gained body mass in a similar fashion at all the concentrations tested. However, the value of the relationship between its Δ_{bm} and sugar intake was marginally non-significant (0.059). This condition could be the result of sugar intake being constant at all the sugar concentrations tested for this species. Also, this was caused by two bats that gained less weight than expected while feeding on the highest sugar concentration (35%). This could be caused by changes in bat behavior associated to sugar intake. We have observed *G. soricina* and *L.*

yerbabuenae increasing their flight time when feeding at high sugar concentrations (Ayala-Berdon et al. 2011). We have attributed this behavior as a form of sugar hyperactivity that could be used by the bats to control for high glucose levels in blood (Kelm et al., 2011). When bats are more active while feeding on high sugar concentrations, they do not gain weight (Ayala-Berdon et al., 2008).

G. soricina, gained body mass at all concentrations, however it gained 33.3 % less weight when feeding at the lowest sugar concentration (146 mmol L^{-1}) than when feeding at the highest one (1022 mmol L^{-1}). Finally, *L. yerbabuenae* presented an erratic pattern of body mass gain, in which some individuals gained body mass at some concentrations and not at others. This species' sugar intake is more affected by concentration (exponent = 0.63), and gained only ~6% of their body mass at the lowest concentration. These results suggest the existence of a gradient of digestive specialization (capacity to achieve compensatory feeding) that has repercussions in the body condition of the different bat species. These differences in digestive capacities should affect the way in which the members of this nectar-feeding bat assemblage respond to changes in nectar quality and temperature, and use the food resources available in their environment. Even though the sugar composition in the bat's diet could affect their capacity to maintain or gain body mass, this needs further exploration.

4.2 Ecological effects of digestive adaptation.

Martínez del Rio and collaborators (2001) proposed several ecological effects of the intake responses of nectar-feeding animals. They hypothesized that nectar concentration should change the behavior of the animals visiting the flowers at different levels. Nectar concentration should change feeding rate, length of visit, and the time the animal needs to process the ingested food (Diamond et al., 1986; Carter and Grovum, 1990; Carpenter et al., 1991**;** Savory, 1999; Denbow, 2000; Schondube and Martínez del Rio, 2003). Additionally, Ayala-Berdon and collaborators (2009) indicated that the digestive capacity that shapes the intake responses of nectar-feeding bats poses an upper limit to energy intake, controlling the energetic budgets of these animals. If this is correct, bats that are able to achieve compensatory feeding would maintain a constant energy intake independently of nectar sugar concentration, and could use any nectar source available to them. On the other hand, bats with physiological limitations would need to be more selective, preferentially using nectar sources with higher energy densities (i.e. > ~20% wt./vol. sugar), and/or changing their behavior to save energy.

In this study, we found that the different species that form the assemblage of nectar-feeding bats in El Jorullo exhibited important differences in their digestive capacities. Thus, the digestive capacity of each bat species may determine diet breadth within the range of plant nectar sugar concentrations available to them in the field. If this is true, individuals of *C. mexicana* that are capable of achieving compensatory feeding should have a wider “nectar-niche” than the other studied species. Supporting this idea, *C. mexicana* consumes the nectar of what seems to be a greater diversity of plant species than those consumed by *G. soricina* and *L. yerbabuenae* (Villa-R, 1967; Álvarez and González, Q. 1969; Gardner, 1977; Hevly, 1979; Arrollo-Cabrales et al., 1987). *G. soricina*, exhibited a digestive constraint (exponent=0.75.) less severe than the one we found in *L. yerbabuenae* (exponent= 0.63), and we expect them to have different levels of nectar concentration use restrictions.

While we do not have data on the diet of these two species in El Jorullo, in another tropical dry forest site (Chamela, Jalisco, Mexico, 19°22’–19°35’N, 104°56’–105°03’W), *G. soricina* consumes a wider range of plant species than *L. yerbabuenae* (Stoner et al., 2003) and *L. yerbabuenae* tend to focus its diet on plant species within the Bombacaceae and Cactaceae families that present nectars with sugar concentrations close to, or higher than 20% (Rodríguez-Peña unpublished data). This is interesting if we consider that in Chamela most flowers pollinated by bats are large and have open corollas (Rodríguez-Peña, pers. Comm.), allowing bats free access to the nectar regardless of their differences in body size or the characteristics of their rostrums and tongues. Our data suggests that at Chamela, sugar assimilation and water management could play an important role to determine the diet of these species. However the relationship between body size and resource use by nectar-feeding bats needs further exploration in our studied bat community.

Based on this data we constructed a conceptual model relating physiological adaptation and ecological specialization (Fig. 3). In this model, nectar-feeding bats able to maintain constant energy intake when concentration varies (i.e. with higher rates of sugar digestion/absorption and gut volume and transit time that allow them to handle large volumes of dilute nectar), should be considered as physiologically more adapted to handle a diverse nectar diet (Karasov and Diamond, 1988; Karasov, 1990; Martínez del Rio and Karasov, 1990, Karasov and Hume, 1997, Matínez del Rio et al., 2001, Schondube and Martínez del Rio, 2003). Physiologically adapted nectarivorous birds (hummingbirds and sunbirds) have higher sucrase activity, and higher glucose transport rates than other birds less adapted to this diet (most passerines; Martínez del Rio, 1990). The high sucrose activity of these physiologically adapted birds allows them to achieve compensatory feeding over a wide range of sugar concentrations (Levey and Martínez del Rio, 1999; López-Calleja et al., 1997). Similarly to what have been described for birds, physiologically more adapted nectar-feeding bats (i.e. species with high rates of sugar digestion/absorption and gut volume and transit time allowing a constant energetic intake when feeding at different sugar concentrations) must exhibit intake responses in which the exponent = 1, and Δ_{bm} independent of sugar concentration and will have the capacity to maintain a constant flux of energy while feeding on all nectar concentrations. As a result of this, they will have a wider diet breadth (at least across the tested nectar sugar concentrations), and would thus act as ecological generalists. On the other hand, bats physiologically less adapted for nectar feeding (i.e. species with low rates of sugar digestion/absorption and gut volume and transit time preventing a constant energetic intake when feeding at different sugar concentrations), should present intake responses with exponent values < 1, and Δ_{bm} that increase with sugar concentration. These species will benefit from feeding on more concentrated nectars. By doing so, they should have more restricted diets, and act as ecological specialists. The existence of a digestive adaptation gradient among the species that compose this nectar-feeding bat assemblage, and the data related to their dietary breadth, suggests that the partition of plant resources among nectar-feeding bats could be directed by their digestive and water management capacities.

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Figure legends

Figure 1. (a) The intake response's exponents of the three species of phyllostomid bats integrating the nectar-feeding community of EL Jorullo, Jalisco, Mexico. Letters bellow mean values that are different represent statistical differences between the species. (b) Intake responses of the three bat species. Dashed lines represent each individual regression, while heavy lines represent the regression obtained from the average of all intercepts and slopes. Individuals of *C. mexicana* exhibited compensatory feeding (regression formula: Log intake sucrose= 3.77 - 0.92 Log concentration). Slopes of *G. soricina* and *L. yerbabuenae* were smaller than the exponent compensatory value of 1 (regression formulas: Log intake sucrose= 3.36 - 0.75 Log concentration and Log intake sucrose= 3.24 - 0.62 Log concentration respectively), indicating the presence of a physiological constraint in these species.

Figure 2. Relationships between changes in body mass (Δ_{bm}), energy intake and sugar concentration. Upper panel: Δ_{bm} corrected by body mass increased with sugar concentration in *G. soricina* (t_4=4.16, P= 0.01), while remained constant in *L. yerbabuenae* and *C. mexicana* (t_5= 2.13, P= 0.08 and t_3= 1.91, P= 0.15 respectively). Lower panel: Compensatory feeding is characterized by constant energy intake (*C. mexicana*, mean exponent= -0.92, t= 1.79, P= 0.17), while in *G. soricina and L. yerbabuenae*, physiological constraints limit their energy intake at lower concentrations (> 438 mmol L^{-1}).

Figure 3. Conceptual model relating physiological and ecological adaptation in the nectar-feeding bat community of El Jorullo. Darker color represents a higher level of adaptation. Bats physiologically more adapted (intake responses with exponent = 1 and Δ_{bm} independent of sugar concentration) should be able to maintain a constant energy intake while using nectars of a wide range in sugar concentrations (146-1022 mmol L^{-1}). These species should be acting as ecological generalists visiting all flowers from which they could extract nectar. In the other extreme of the physiological adaptation gradient, bats unable to perform compensatory feeding (intake responses with exponent values < 1 and Δ_{bm} that increase with sugar concentration), will tend to fed on flowers with more concentrated nectars, acting as ecological specialists.

Figure 1

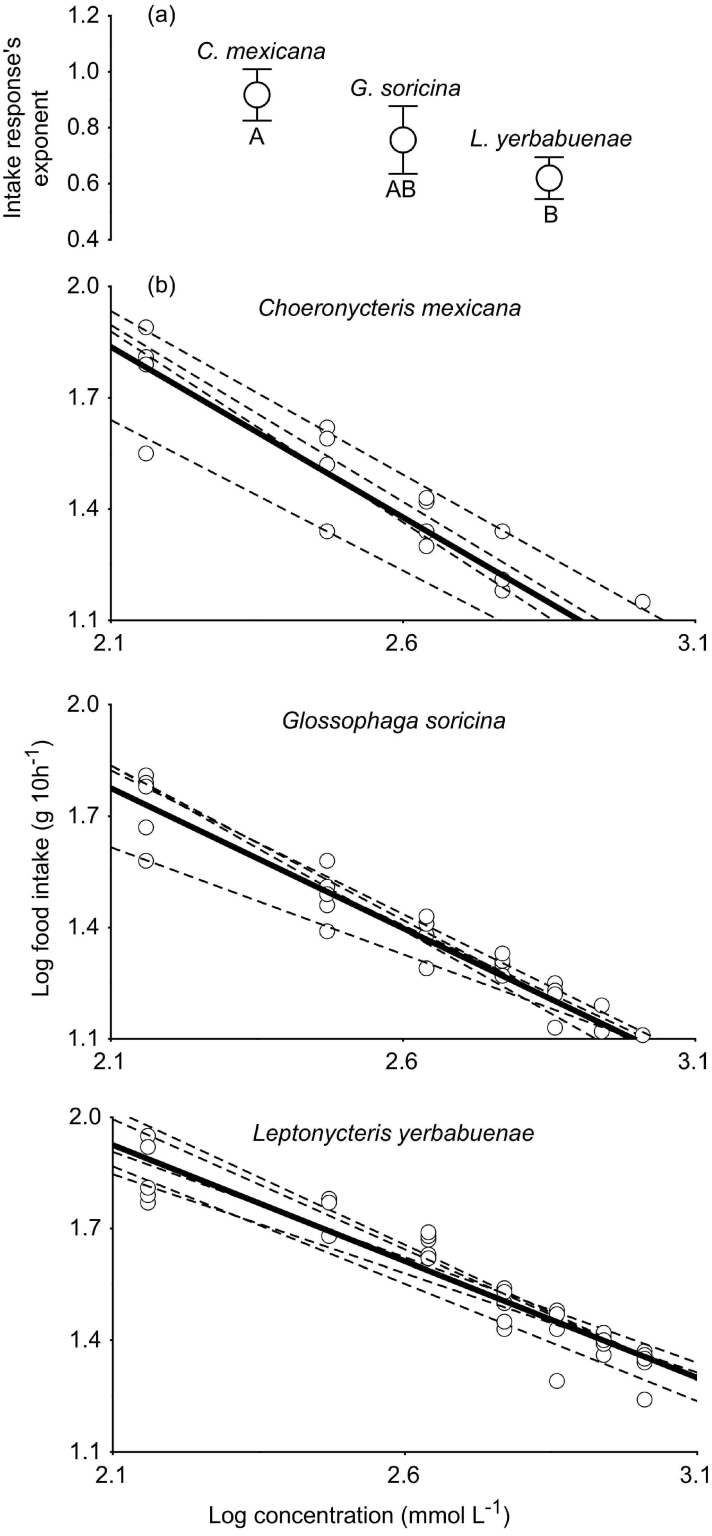

(a)
Intake response's exponent
1.2
1.0
0.8
0.6
0.4
C. mexicana
A
G. soricina
AB
L. yerbabuenae
B
(b)
Choeronycteris mexicana
Glossophaga soricina
Leptonycteris yerbabuenae
Log food intake (g 10h^{-1})
2.0
1.7
1.4
1.1
2.1
2.6
3.1
Log concentration (mmol L^{-1})

Figure 2.

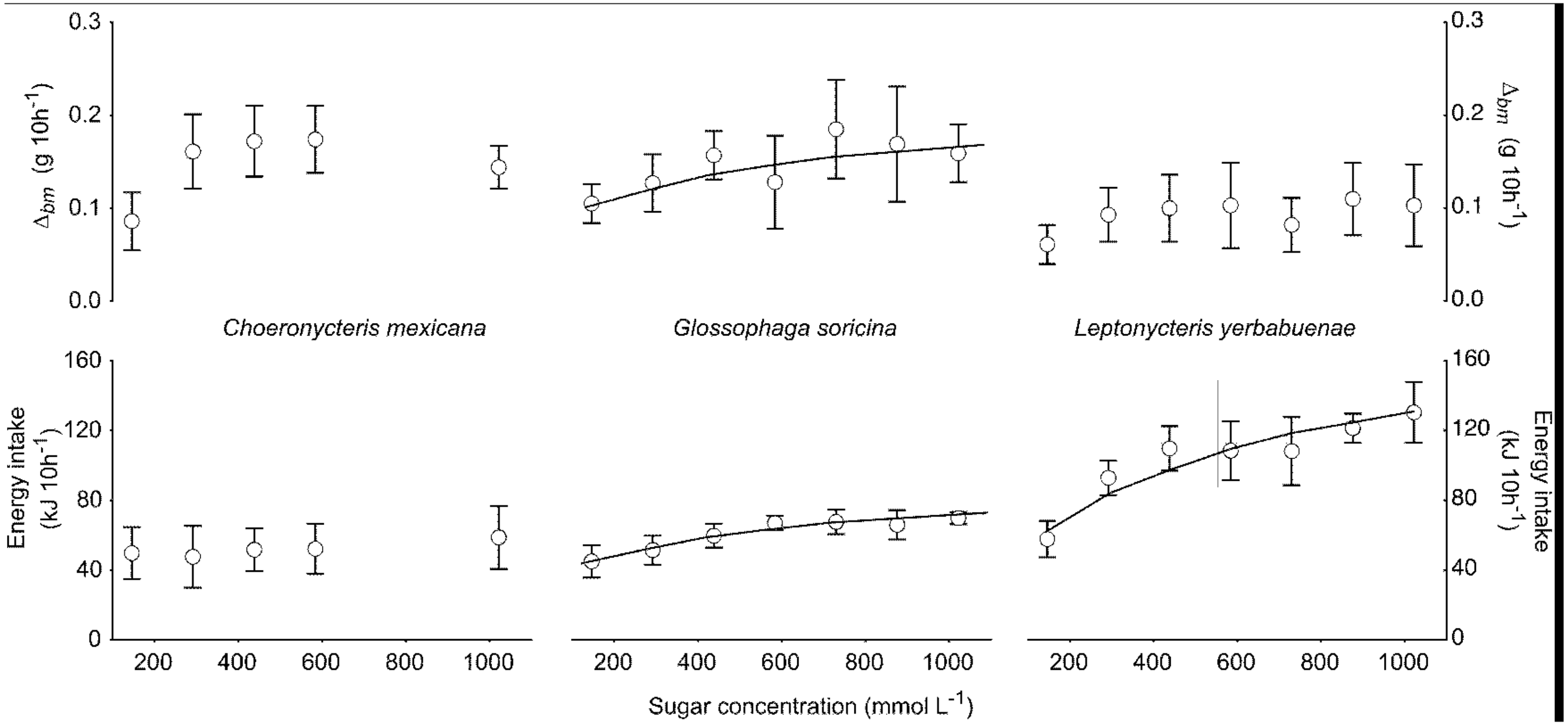
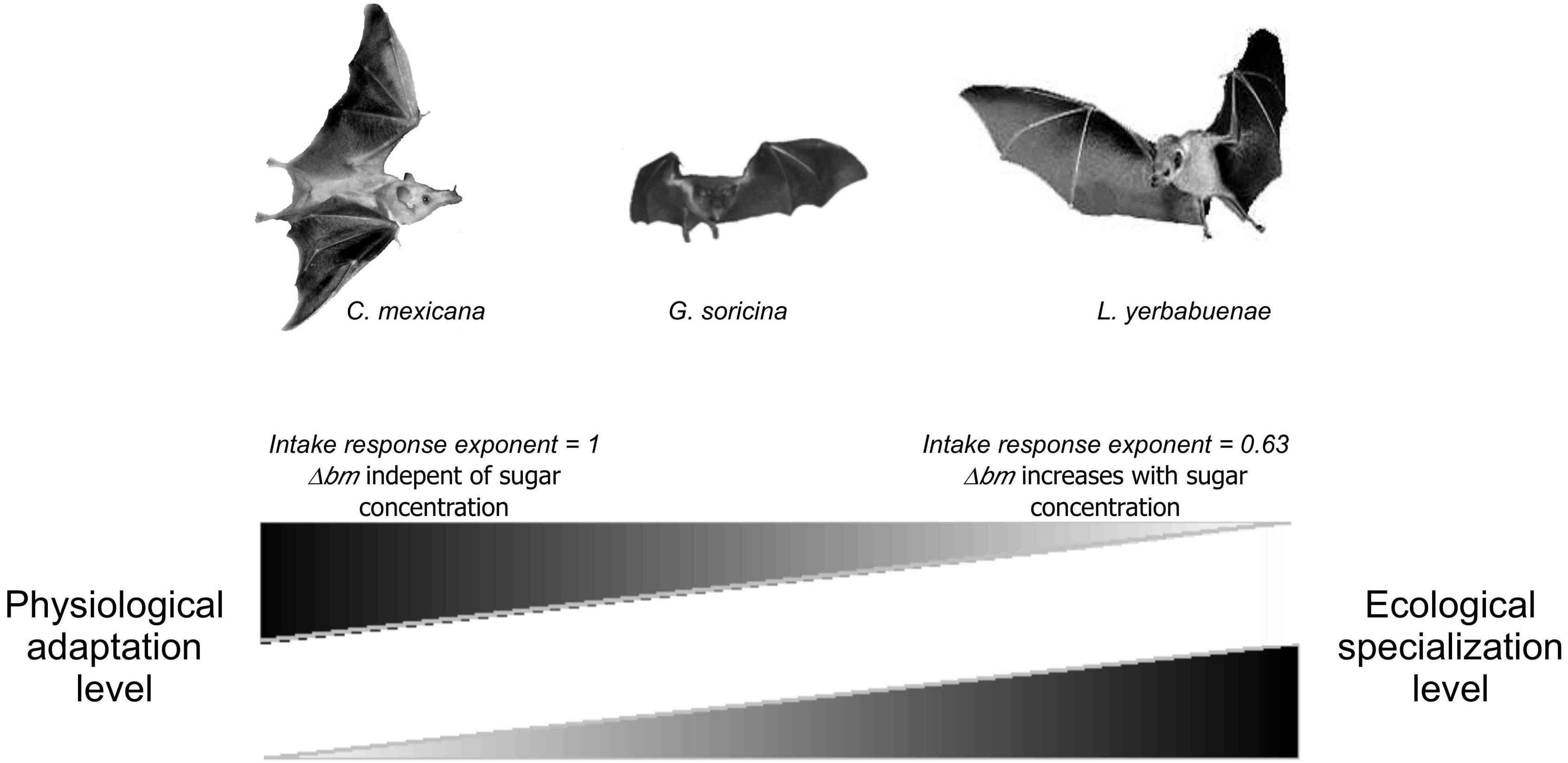

0.3
0.2
0.1
0.0
Δ_{bm} (g 10h^{-1})
Choeronycteris mexicana
Glossophaga soricina
Leptonycteris yerbabuenae
160
120
80
40
0
Energy intake (kJ 10h^{-1})
200
400
600
800
1000
Sugar concentration (mmol L^{-1})

Figure 3


C. mexicana
G. soricina
L. yerbabuenae
Intake response exponent = 1
Δbm indepent of sugar concentration
Intake response exponent = 0.63
Δbm increases with sugar concentration
Physiological adaptation level
Ecological specialization level

CAPITULO 3. *CAPACIDAD PARA DISTINGUIR SABOR Y SELECCIÓN DE ALIMENTO EN CAMPO*

Sugar gustatory thresholds and sugar selection in two species of Neotropical nectar-eating bats

Jorge Ayala-Berdon, Nelly Rodríguez-Peña, Cristian García Leal, Kathryn E. Stoner and Jorge E. Schondube*

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

Abstract

Nectar-feeding bats play an important role in natural communities acting as pollinators; however the nectar characteristics that conduct their food selection are unclear. Here we explored the role that sugar gustatory thresholds and sugar concentration could play on the sugar selection patterns of G*lossophaga soricina* and *L. yerbabuenae*. We expected 1) bats presenting different gustatory thresholds for the sugars present in their natural diet and 2) sugar preferences to be affected by sugar concentration in a similar fashion to what have been found in other vertebrates. Both bat species showed differences in their sugar gustatory thresholds and sugar preferences when confronted with solutions at different concentrations, and tended to prefer the sugar present in their diet. Contrary to our expectations, gustatory thresholds did not match sugar preferences at low sugar concentrations, and the main sugar composition in the nectar of the plants these species feed on. The results we found suggest bats would focus on the more abundant plants and learn to prefer the composition of its nectar. This could confer an advantage by allowing the bats to focus on the most common food resource present on their environment.

Key words: *Gustatory thresholds, nectar-feeding bats, nectar sugar composition, nectar sugar concentration, sugar preferences.*

1. Introduction

Nectar feeding animals tend to show specific preferences for different nectar sugar compositions (Baker and Baker, 1983; Martínez de Rio, 1990; Martínez del Rio et al., 1992; Herrera et al. 2000). These preferences seem to be the result of the close evolutionary interaction between the pollinators and the plants they visit (Martínez del Rio et al., 1992; Lotz and Schondube, 2006; Jhonson and Nicolson, 2008). While Martínez del Rio (1990; 1992) proposed the existence of digestive mechanisms underlying the sugar preferences of pollinators, several nectar-feeding animals have sugar selection patterns that are unexpected based on their digestive characteristics.

Two non exclusive mechanisms are known to affect food selection in nectar-feeding vertebrates: 1) the physiological capacities of animals to assimilate the main sugars present in nectar (sucrose, glucose and fructose; Martínez del Rio and Stevens, 1989; Martínez del Rio et al., 1992). Studies done with birds have demonstrated that some passerines avoid nectar containing sucrose, and the aversion for this sugar is associated with low or null activity of the enzyme sucrase (Martínez del Rio et al., 1988; Martínez del Rio et al., 1989; Malcarney et al., 1994; Lotz and Schondube, 2006). And 2) their capacity to detect and perceive the flavor of the sugars they ingest (Simmen et al., 1999; Herrera, 1999; Medina-Tapia et al., 2011). In some animals that have high sucrase activity, but do not show preferences for sucrose, sugar flavor has been related to their food selection patterns (Herrera 1999; Medina et al. 2011). For example, Neotropical frugivorous and nectarivorous bats, selected sucrose nectars, while this sugar is not common in the food they ingest in the field.

Herrera (1999) suggested that this result could be caused by the capacities of these bats to perceive the relative sweetness of this sugar; however this hypothesis has remained untested.

In the last decade many studies have shown that sugar preferences can be affected also by sugar concentration (Schondube and Martínez del Rio, 2003; Fleming et al., 2004; Lotz and Schondube, 2006; Brown et al., 2010b; Odendaal et al., 2010). These studies have demonstrated that animals tend to change their sugar preferences from sucrose when nectar is concentrated to hexoses when nectar is dilute. Two physiological mechanisms have been suggested to affect the preferences of these animals. By one hand, differences in osmotic pressures exercised by the different sugars have an effect on their sugar preferences (Karasov and Kork, 1996). Since sucrose is a disaccharide, a solution of this sugar exerts half of the osmotic pressure than a solution with a mixture of the hexoses glucose and fructose with the same energy content. Animals feeding at high concentrations may be expected to feed on sucrose as a way to increase transit times of food in response to this osmotic pressure difference (Karasov and Martínez del Rio, 2007). By other hand, the low affinity of the enzyme sucrase for its substrate could have an effect on the sugar preferences of the animals. Under this scenario sucrose processing at low concentrations may be deficient and animals should benefit from eating hexoses at low concentrations (Schondube and Martínez del Rio, 2003).

Neotropical nectar-feeding bats play an important role in natural communities acting as pollinators (Allen-Wardell, 1998; Fleming and Sosa, 1994; Kearns and Inouye, 1997; Kearns et al., 1998). However the nectar characteristics that conduct their food selection are unclear. Studies done with tree species of phyllostomid bats showed that rates of sugar digestion and absorption are similar for sucrose, glucose and fructose (Ayala-Berdón et al., 2008; Herrera and Mancina, 2008) and provided the support that physiological traits do not drive their food selection. Additionally, no preferences have been observed when these species of bats confronted artificial nectars with different sugar compositions (Rodríguez-Peña et al., 2007), contrary to a previous study where some frugivorous and nectarivorous bats of the same family preferred sucrose (Herrera, 1999). In this paper we explored the role that sugar gustatory thresholds and sugar concentration could play on the sugar selection patterns of two species of Neotropical bats. We expected that 1) If these species present different gustatory thresholds for the sugars present in their natural diet (sucrose, glucose and fructose), their sugar preferences when feeding on dilute nectar solutions should follow their capacities to detect the differences in sugar flavor intensity, and 2) we expected sugar preferences to be affected by sugar concentration in a similar fashion to what have been found in birds (Schondube and Martínez del Rio, 2003; Fleming et al., 2004, 2008; Brown et al., 2010a).

2. Materials and Methods

2.1 Bat care and housing

Pallas's Long-tongued bats (*Glossophaga soricina*) and Lesser long-nosed bats (*Leptonycteris yerbabuenae*) were captured using mist nets in the Cuixmala-Chamela Biosphere reserve that is located in the coast of Jalisco, Mexico (19°22'–19° 35'N, 104°56'–105°03'W). Six non-reproductive adults of each species were selected and transferred to the animal facility of the Centro de Investigaciones en Ecosistemas (CIECO, UNAM –www.oikos.unam.mx-). CIECO is located in Morelia, Mexico (19° 38' 53.91'' N, 101° 13' 44.31'' W). The experimental colonies of these two bat species were maintained in cages (0.6 x 0.6 x 0.6 m) under laboratory conditions (30% of humidity, 27°C temperature and a 12:12 dark:light regime). Bats were fed with the maintenance diet described by Mirón et al. (2006). The diet consisted of milk protein, cereal, banana, an equicaloric mixture of sucrose, glucose and fructose and vitamins and minerals (Nekton-S®; Guenter Enderle, Tarpon Springs, FL, USA). To evaluate changes in body mass, individuals were marked with collar rings and weighed daily before and after each feeding trial (± 0.01 g, OhausTM, Burlington, NC, USA). Bats maintained good health during the experimental trials.

2.2 Gustatory thresholds

Several methods have been proposed to assess sugar thresholds in animals. According with Brown et al. (2008), equimolar solutions of sugars is the best technique to determine the role that taste can have on food preferences because it allows for the same amount of molecules to interact with the gustatory receptors. However, this technique can have several confounding effects: 1) It may mask the effect of food choice derived by the energetic content in the different solutions offered, 2) ignore the effect that learning has on food choice, and 3) pay no attention to differences in the speed at which the different sugars are assimilated in the gut once they are ingested. To avoid this confusion, the utilization of equicaloric solutions has been suggested. Nevertheless, this technique generates a problem if animals are able to identify differences between similar sugar concentrations when confronted with diluted sugar solutions as in the case of our study species (Rodriguez-Peña et al., 2007).

To avoid these problems, and to test the capacity of bats to detect the flavor of a sugar, we presented animals to paired feeders containing either a sugar solution or pure water. We designed our experiment so that we could measure the gustatory thresholds of the bats for different sugars. We used the gustatory threshold definition of the American Society for Testing and Materials: "A concentration range exists bellow which the taste of a substance will be not detectable under any practical circumstances, and above individuals with a normal sense of taste would be readily detect the presence of the substance". In other words, the lowest physical energy level of a stimulus or lowest concentration in the case of a chemical stimulus that is perceivable by the sense of taste (Lindemann, 1996). To test for the gustatory thresholds of our bat species we used a design that has been used several times in the past to detect the capacity of animals to perceive sugar flavors. This design assumes that a sugar that is detected at a lower concentration has a stronger flavor than other sugar that is detected at a higher concentration, when both sugars are present in equimolar solutions to an animal (Simmen et al., 1999; Herrera, 2000; Laska et al., 2001; Li et al., 2009 among others). Sugar solutions were made with pure sucrose, glucose or fructose (≥0.99.5%; Sigma®). Sugar concentrations were tested first at 9 and 3% (wt./vol.). These numbers were the minimum differences that *G. soricina* and *L. yerbabuenae* were able to differentiate when confronted with sugar solutions with different concentrations (Rodríguez-Peña et al., 2007). We started using these concentrations, and when the bats were able to identify the sugar solution from the pure water we divided the concentration by two, and conducted a new trial. We repeated this procedure until the bats were unable to differentiate between the pure water and the sugar solution. Feeders were weighted at the beginning (W_i) and the end (W_f) of each trial (0.01g, OhausTM, Burlington, NC, USA) from 1900 to 0500. To avoid biases due feeder position (Jackson et al., 1998), we shifted the feeders at the middle of each trial. Because bats received very dilute solutions during trials, we allowed animals to rest one night for each experimental night. During the resting night, bats were kept in the cages and fed with the maintenance diet.

2.3 Effect of concentration on sugar selection

To determine sugar selection, six *G.soricina* and six *L.yerbabuenae* were presented to paired feeders containing sugar solutions at three different concentrations (5, 25 and 35% wt./vol.). We applied this design to see if sugar selection changed with concentration. Sugar solutions were made from sucrose or a mixture of glucose-fructose. We presented the bats with a mixture of glucose and fructose because in the case of concentration-preference selection in nature, animals confront sugar mixtures instead of pure particular sugars (see Martínez del Rio et al., 1992 and the review done by Nicolson and Fleming, 2003 for more information on this topic). Trials were conducted from 1900 to 0500, and the feeders were shifted at the middle of each trial to avoid position selection biases (Jackson et al., 1998). Feeders were weighed at the beginning (W_i) and the end (W_f) of each trial to the nearest 0.01g (OhausTM, Burlington, NC, USA), and consumption was estimated by subtracting W_f from W_i.

2.4 Relation between sugar flavor and sugar selection

Bats feed mostly on hexose-dominant nectars (Baker et al. 1998). Because the flavor associated to the sugar composition of the current diet may influence the preferences of bats in the field, we examined whether shifting diets changed the preferences of bats. We fed six bats of each species an only sucrose maintenance diet for two weeks and obtained an estimate of their preference for sucrose over a hexose mixture (1:1 glucose-fructose). The same bats were then fed a maintenance diet made with an only hexose mixture for two more weeks and then they were retested. Both maintenance diets were tested at a sugar concentration of 20%. The procedures of these sugar selection trials were the same that those performed for the sugar thresholds and the effects of concentration on sugar selection (see above). We used one-tailed t-test to detect a change in sugar preference associated to the change of the main sugars present in the bats' diet.

2.5 Data analysis

Gustative thresholds, sugar selection patterns and the relation between sugar flavor and sugar selection were determined using a preference index (PI)

$$PI = \frac{solution\ A \text{ intake (g)}}{solution\ A \text{ intake (g)} + solution\ B \text{ intake (g)}}$$

In the gustative thresholds experiments, we interpreted the selection of sugar solution ($solution\ A$) over pure water ($solution\ B$) as evidence of bats detecting flavor. In the trials were we tested the effects of sugar concentration and sugar flavor habituation on sugar selection, we compared the consumption of one the sugar solution with respect to the other (sucrose – solution A; 1:1 glucose-fructose mixture – solution B). Values of PI ranged from 0 to 1. PI values statistically equal to 0.5 indicated an incapacity to detect sugar flavor in the gustatory threshold trials, and indifference to select between sugar solutions in the sugar preference trials. A PI value statistically higher than 0.5 represented a preference for solution A, while a value smaller than 0.5 represented a preference for solution B (Martínez del Rio, 1990a). For each species and each pairwise comparison of sugars, we obtained an estimate of mean preference using six bats and six replicated trials per bat. The inter-individual variance in preference (i.e. the variance among the six individual means) was used to construct a confidence interval for the mean preference of each species. We used this confidence interval to test the null hypothesis that preference was not significantly different from 0.5 (the indifference point where consumption of both solutions tested is equal) using one sample t-tests on arcsing $\sqrt{PI}$ preference values.

3. Results

3.1 Gustative sugar thresholds

The two species of bats differed on their gustative thresholds for sucrose, glucose and fructose (Fig. 1). The nectar-feeding bat *G. soricina* detected sucrose at a concentration of 0.138%. However the same individuals were unable to perceive this sugar at 0.136%. This result indicated that the threshold for this sugar was between these two concentration values. In this bat species, gustatory thresholds for both glucose and fructose were between 0.386 and 0.3435% (Table 1, Fig. 1).

Sugar gustatory thresholds for the bat *L. yerbabuenae* were between 0.562and 0.553% for sucrose, between 0.536 and 0.511% for glucose, and between 1.468 and 1.125% for fructose (Table 1, Fig. 1).

3.2 Sugar selection patterns

The two bat species had different responses in their sugar preferences when sugar concentration changed. *G. soricina* was indifferent to sugar composition at any of the three sugar concentrations tested (PI = 0.53 ± 0.2, t_{10}= 0.28, P= 0.78; PI = 0.52 ± 0.11, t_{10}= 0.36, P= 0.72 and PI = 0.51 ± 0.19, t_{10}= 0.059, P= 0.95 for 5, 25 and 35% respectively). *L. yerbabuenae* preferred hexoses at concentrations of 5 and 25% (PI = 0.63 ± 0.08, t_{10}= 3.37, P= 0.07 and PI = 0.72 ± 0.19, t_{10}= 2.63, P= 0.025 for 5 and 25% respectively) and was indifferent at a concentration of 35% (PI = 0.50 ± 0.23, t_{10}= 0.11, P= 0.91; Fig. 2).

3.3 Relation between sugar flavor and sugar selection

Both bat species had preferences for the sugars present in the maintenance diet they ingested in the two weeks period before their sugar preference trials. When bats fed with the sucrose only maintenance diet they showed a marked preference for sucrose (PI =0.957 ± 0.07, t=8.51, P<0.0001 and PI =0.978 ± 0.06, t=31.97, P<0.0001 for *G. soricina* and *L. yerbabuenae* respectively; Fig. 3), however they shifted their preferences for this sugar to a preference for the hexose mixture after feeding on the hexose mixture maintenance diet (PI =0.0532 ± 0.06, t=7.562, P<0.0001 and PI =0.0365 ± 0.05, t=-34.08, P<0.0001 for G. soricina and L. yerbabuenae respectively; Fig. 3).

4. Discussion

G. soricina and *L. yerbabuenae* showed differences in their sugar gustatory thresholds and sugar preferences when confronted with solutions at different concentrations. Additionally, both bat species tended to prefer the sugar present in their diet. Contrary to our expectations, gustatory thresholds did not match sugar preferences at low sugar concentrations, and the main sugar composition in the nectar of the plants these species include in their diet. In this section, we first discuss the significance of the differences in the gustatory thresholds of our study species and compare them with those of other mammals and birds. Second we focus on the sugar preferences of both bat species, and the effect that sugar concentration had on the sugar selection patterns of *L. yerbabuenae*. Finally, we explore the role that sugar composition in the diet of bats has on their sugar preferences.

4.1 Sugar gustatory thresholds

In this study we found that *G. soricina* and *L. yerbabuenae* differed on their sugar gustatory thresholds for the three most common sugars present in nectar. While *G. soricina* had a lower gustatory threshold for sucrose, followed by the thresholds for glucose and fructose, *L yerbabuenae* had a lower threshold for glucose followed by the thresholds for sucrose and glucose. Our results indicate that both bat species perceive the flavor of these three sugars differently. This is similar to what has been reported for other species of fruit-eating bats (Herrera, 1999) and nectar and fruit-eating birds (Martínez del Rio, 1990a; Medina-Tapia et al., 2011).

Many authors have addressed the study of sugar gustatory thresholds in a variety of organisms, including humans, insects, birds and mammals (Laska et al., 1999; Herrera et al., 2000; Smutzer et al., 2008; Mujagic et al., 2010; among others). These studies have shown that animals differ highly in their capacity to detect sugar flavors (see Medina-Tapia et al., 2011 for more information). Jiang et al. (2011) and Medina-Tapia and collaborators (2011), have suggested that the capacity to detect and differentiate among sugar flavors is strongly related to the animals' predominant diet. According with these authors, specialist nectar-eating animals should have lower sugar gustatory thresholds than organisms that feed on other diets (i.e. seeds, meat). In accordance with this hypothesis, our study species that feed mainly in floral nectar had sugar gustatory thresholds that were about one to two orders of magnitude lower than those presented by chickens, quails and turkeys, animals that feed mainly on seeds (from ~ 2 to 10% for the different sugars in poultry to

~ 0.1 to 1.0 for the different sugars we found for our study species; Medway, 1959; Harriman and Milner, 1969), and two carnivore species (*Mustela putorius furo,* and*Panthera leo persica* from ~ 20 to 90% for the different sugars respectively; Li et al., 2009).

However, sugar thresholds reported here for *G. soricina* and *L. yerbabuenae* were one order of magnitude higher than those reported for hummingbirds (~ from 0.03 to 0.1% for the different sugars; Medina-Tapia et al., 2011) and a nectar-feeding marsupial (*Caluromys philander*; Simmen et al., 1999). While hummingbirds show sugar preferences while feeding on dilute nectars that match their sugar gustative thresholds, suggesting that their capacities to perceive the relative sweetness of the different sugar affect they sugar preferences (Medina-Tapia et al., 2011), bats did not. *G. soricina* had sugar preferences that were independent of its sugar gustatory thresholds indicating that despite its capacity to perceive differences on the flavor and relative sweetness of different sugars, this generalist nectarivorous bat is not using this capacity to select food. Our results are not conclusive for the specialized nectarivore *L. yerbabuenae.* This species had a lower sugar gustatory threshold for glucose than for sucrose and fructose, and preferred to feed on hexoses at lower concentrations. Since we do not know how the receptors for the different sugars interact in this species, is impossible to know if the lower gustatory threshold for glucose is affecting sugar selection for hexoses when feeding on dilute nectars. However, bats species from both the Old World (*Epomophorus wahlbergi,* S*ynconycteris australis*) and the New world (*G. soricina* and *L. yerbabuenae*) did not show preferences for a particular sugar when confronted with different combinations of sugars at a concentration close to 20% (i.e. nectars dominated by sucrose or hexoses and balanced solutions of the three sugars; Law, 1999; Rodríguez-Peña et al., 2007; Coleman and Downs, 2012), suggesting that the role that sugar gustatory threshold have on sugar selection in this group of animals is not important.

4.2 Effect of sugar concentration on food selection

The two bat species showed differences on their sugar selection responses when they fed at the three different sugar concentrations tested. While individuals of *G. soricina* were indifferent at all the sugar concentrations, individuals of *L. yerbabuenae* preferred hexoses over sucrose at 5 and 25%, and did not show any preference when fed at a concentration of 35%. Similar patterns on the variation of sugar preferences with changes in sugar concentration have been found in old world frugivorous bats, and a variety of birds (Schondube and Martínez del Rio, 2003; Fleming et al., 2004; Downs and Perrin, 2008; Brown et al., 2010; Odendaal et al., 2010; Medina-Tapia et al., 2011; Coleman and Downs, 2012).

Similarly to our results for *L. yerbabuenae*, the Epauletted fruit bat (*Epomophorus wahlbergi*) showed a preference for hexoses at low concentration (5%) and became indifferent to sucrose, glucose or fructose when fed on intermediate concentrations (from 10 to 25%; Coleman and Downs, 2012). In birds, shifts in sugar preferences associated to changes in sugar concentration vary among groups. For example, Broadtailed (*Selasphorus platycercus*) and magnificent Hummingbirds (*Eugenes fulgens*), Cinnamonbellied Flowerpiercers (*Diglossa baritula*), and Whitebellied Sunbirds (*Nectarinia talatala*), changed their sugar preferences from hexoses when feeding at dilute nectars (5%), for a preference for sucrose or being indifferent at high concentrations (35%; Schondube and Martínez del Rio, 2003; Fleming et al. 2004, 2008; Brown et al., 2010b), while the Red lory (*Eos bornea*) preferred sucrose while feeding at low concentrations and became indifferent to sugar composition when feeding on a sugar concentration of 20% (Downs, 2007).

Schondube and Martínez del Rio (2003) proposed two complementary mechanisms driving sugar selection in nectar-feeding animals when they confront changes in the sugar concentration of their food. 1) Differences in osmotic pressures exercised by the different types of sugars (Karasov and Kork, 1996), and/or 2) the interaction between sugar concentration and the affinity of the enzyme sucrase for its substrate. The rate at which food is moved through the intestine is negatively related to its osmotic concentration (Karasov and Kork, 1996). Since sucrose is a disaccharide, this sugar generates a half of the osmotic pressure compared with what is exerted by an equicaloric solution of the hexoses glucose and fructose (Karasov and Martínez del Rio, 2007). Under this scenario, sucrose consumption may be expected at high concentrations, allowing high intestinal transit rates of food. However, the evidence of an osmotic mechanism affecting the sugar selection of nectar-eating animals feeding on concentrated sugar solutions is limited (but see Schondube and Martinez del Rio, 2003). On the other hand, the enzyme sucrase presents a low affinity for its substrate in most nectar-eating animals (Martínez del Rio, 1990b; Hernández and Martínez del Rio, 1992; Schondube et al. 2001; Schondube and Martínez del Rio 2007). This condition may constrain sucrose processing at low concentrations. In this situation, animals should benefit, and prefer hexose consumption at low concentrations when they become less efficient in assimilating sucrose (Schondube and Martínez del Rio, 2003). This idea was tested by Ayala-Berdon et al. (2011). By modeling sugar digestion, they found that a reduction in the affinity of the enzyme sucrase diminished the daily energy intake in two species of nectar-eating phyllostomid bats.

Our results show that the sugar selection patterns of *L. yerbabuenae* were affected by sugar concentration. We interpret the pattern of sugar selection *L. yerbabuenae* as a result of the low affinity of the enzyme sucrase for it substrate in this species when animals fed at the low and intermediate concentrations (19.23 μmol μL^{-1}; Schondube et al., 2001). As Ayala-Berdon and collaborators (2011) suggested, this could cause energy intake to be limited when bats feed on dilute sucrose solutions, forcing the bats to prefer a hexose mixture. Curiously, *G. soricina* did not followed the same pattern, even though it enzyme sucrase also had a low affinity for sucrose (22.72 μmol μL-1; Schondube et al., 2001). The pattern of sugar selection by this species could be explained by the fact that *G. soricina* has a better capacity to obtain energy when feeding on dilute nectars than *L. yerbabuenae*. Ayala-Berdon et al. (2008) found that *G. soricina* is less affected in its capacity to obtain energy when feeding on dilute nectars than *L. yerbabuenae*. This difference allows *G. soricina* to use a wider arrange of nectar sources in its diet and to act as an ecological generalist (Ayala-Berdon and Schondube 2011). The differences in digestive capacities of both species could explain their differences in sugar selection patterns when confronted with different sugar concentrations. However, this topic requires further exploration.

4.3 The role of diet composition on sugar selection by nectar-feeding bats

Many studies have shown that nectar-feeding animals tend to prefer the sugar compositions of the plants they visit (Baker and Baker 1983, Martínez de Rio 1990, Martínez del Rio et al. 1992, Herrera et al. 2000). These preferences have been related with the capacity of animals to assimilate sucrose (Martínez del Rio and Stevens, 1989; Martínez del Rio et al., 1992). Animals with low or null capacities to assimilate sucrose reject this sugar. Ingesting sucrose without having the capacity to assimilate it generates osmotic diarrhea and an aversion for this sugar (Martínez del Rio and Stevens, 1989).

Both *G. soricina* and *L. yerbabuenae* have digestive characteristics that allow them to assimilate sucrose, glucose and fructose with similar efficiencies (Ayala-Berdon et al., 2008; Herrera and Mancina, 2009). The high capacities of bats to assimilate different sugars have limited our capacity to understand the mechanisms that control sugar selection in these organisms (Herrera 1999; Rodriguez-Peña et al. 2007). Medina-Tapia et al. (2011) suggested that sugar flavor could play an important role in animals when food selection is not explained by their digestive capacities. The bat species we studied have the capacity to discriminate among the different flavors of the sugars they ingested, suggesting that bats could use nectar flavor to take food selection decisions.

If bats are not constrained by sugar assimilation, and were not following the sweetness of the sugars they were able to perceive to select their food, what factors determined the sugar selection in these species? One interesting result was that both bat species preferred the predominant sugars present in their maintenance diet (i.e. only sucrose diet and only hexoses diet). We found that after only two weeks of feeding on a specific sugar, bats got conditioned to it, and preferred the sugar solution containing it, and that they shifted their preferences when the main sugar in their diets changed. This result could help to understand why Herrera (1999) found a strong sucrose preference in phyllostomid bats, while Rodriguez-Peña and collaborators (2007) found a lack of preference in other species of this group.

Herrera (1999) found that the Neotropical bats *Artibeus jamaicencis*, *Sturnira lilium* and *Anoura geofroyi* preferred consistently the sugar sucrose over equicaloric solutions of glucose and fructose, despite they presented similar rates of assimilation for the three sugars and their diet in nature included predominantly glucose and fructose (Baker et al., 1998). However, the maintenance diet he used during the experiments was based on a commercial juice consisting that at time was added with sucrose (Jumex®). Rodriguez-Peña and collaborators (2007) offer bats a maintenance diet made in equal parts of sucrose, glucose and fructose and found a lack of preferences. Our results indicate that phyllostomid bats are influenced by the sugars present in their maintenance diets, suggesting that the differences among the sugar selection patterns reported in previous studies could have been the result of the experimental protocols and do not reflect real selectivity in the wild. However this topic requires further exploration.

The fact that bats responded in short periods of time to the sugar composition of their diets, and their capability to shift their sugar preferences when sugar type in the diet changes, may confer both bat species ecological advantages. Bats in the Neotropics feed mostly on plants that produced large numbers of flowers per plant, and have long flowering periods (Stoner et al., 2003). In this situation, bats would focus on the more abundant plants and learn to prefer the sugar composition of its nectar. This could confer an advantage by allowing the bats to focus on the most common food resource, and will make them better pollinators by increasing the probability of moving large pollen loads of the same species.

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Table 1. Gustatory sugar thresholds for sucrose, glucose and fructose exhibited for the bats *G. soricina* and *L. yerbabuenae*. We confronted six individuals of each species to paired feeders containing either sugar solutions or pure water and evaluated their preferences using a preference index (PI). We obtained the confidence intervals for each species and compared them wit an hypothetical value of 0.5 using a t test. The value assumed that bats consumed the same amount of both solutions and showed no preference. Values of PI and SD are presented as the mean of the six bats for each species.

	Sugar type	*Sugar concentration (%)*	*Mean PI*	*Mean SD*	*t value*	*P*
Glossophaga soricina						
	Sucrose	0.138	0.55	0.04	2.81	0.01
		0.136	0.52	0.07	0.87	0.4
	Glucose	0.386	0.63	0.14	2.24	0.04
		0.343	0.60	0.11	1.78	0.13
	Fructose	0.386	0.63	0.11	2.85	0.01
		0.343	0.55	0.09	1.37	0.20
Leptonycteris yerbabuenae						
	Sucrose	0.562	0.77	0.16	3.66	0.004
		0.553	0.60	0.12	1.39	0.19
	Glucose	0.536	0.61	0.10	2.73	0.02
		0.511	0.53	0.06	1.28	0.22
	Fructose	1.468	0.84	0.22	2.58	0.02
		1.125	0.66	0.22	1.85	0.94

Figure legends

Figure 1. Gustatory thresholds for sucrose, glucose and fructose for the nectar-feeding bats *G. soricina* and *L. yerbabuenae*. We confronted bats with paired feeders containing either pure water or sugar solutions. We constructed confidence intervals from PI's of individual variation and tested the result against the null hypothesis in which PI is equal to 0.5 using a t test. This analysis required arcsine transformation of the square root of the original data. To simplify the biological interpretation of our results, PI's are presented as means of the non-transformed data with their respective SD. Because bats always preferred the sugar solution when they showed their gustatory threshold, we only plotted two of the total concentrations tested.

Figure 2. Sugar preferences for sucrose or a mixture of glucose-fructose solutions for the nectar-feeding bats *G. soricina* and *L. yerbabuenae*. We confronted bats with paired feeders containing solutions of the different sugars. We constructed confidence intervals from PI's of individual variation and tested the result against the null hypothesis in which PI is equal to 0.5 using a t test. This analysis required arcsine transformation of the square root of the original data. To simplify the biological interpretation of our results, PI's are presented as means of the non-transformed data with their respective SD.

Figure 3. Changes in sugar selection associated to changes in the predominant

sugar in the diet experienced by *G. soricina* and *L. yerbabuenae*. We examined whether shifting diets changed the preferences of bats. We fed six bats of each species a only sucrose maintenance diet for two weeks and obtained an estimate of their preference for sucrose over an hexose mixture (1:1 glucose-fructose). The same bats were then fed a maintenance diet made with an only hexose mixture for two more weeks and then they were retested. We confronted bats with paired feeders containing solutions of the different sugars. We constructed confidence intervals from PI's of individual variation and tested the result against the null hypothesis in which PI is equal to 0.5 using a t test. This analysis required arcsine transformation of the square root of the original data. To simplify the biological interpretation of our results, PI's are presented as means of the non-transformed data with their respective SD.

Figure 1

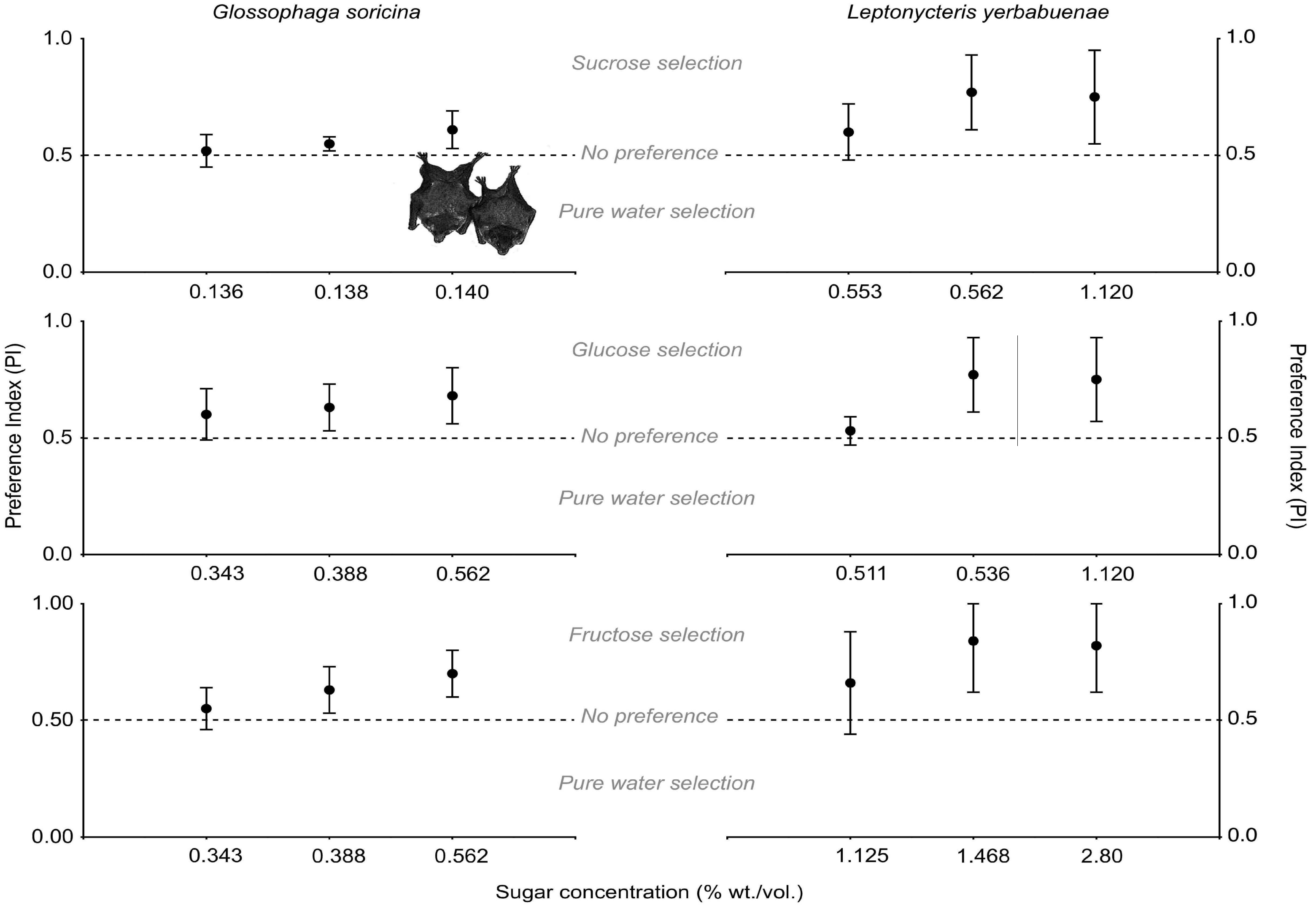

Glossophaga soricina
Leptonycteris yerbabuenae
Preference Index (PI)
Preference Index (PI)
Sucrose selection
No preference
Pure water selection
0.136
0.138
0.140
0.553
0.562
1.120
Glucose selection
No preference
Pure water selection
0.343
0.388
0.562
0.511
0.536
1.120
Fructose selection
No preference
Pure water selection
0.343
0.388
0.562
1.125
1.468
2.80
1.0
0.5
0.0
1.00
0.50
0.00
Sugar concentration (% wt./vol.)

Figure 2

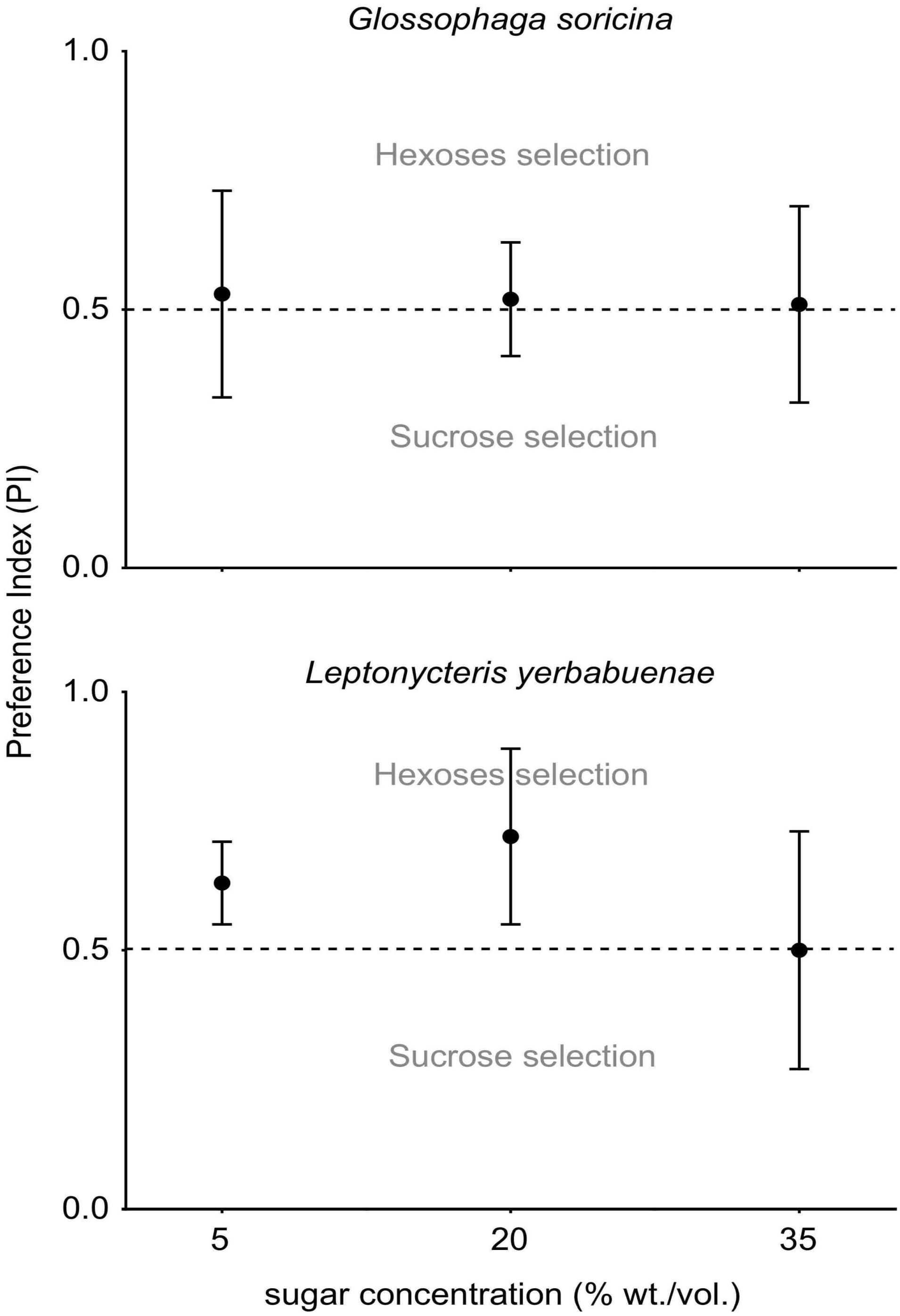
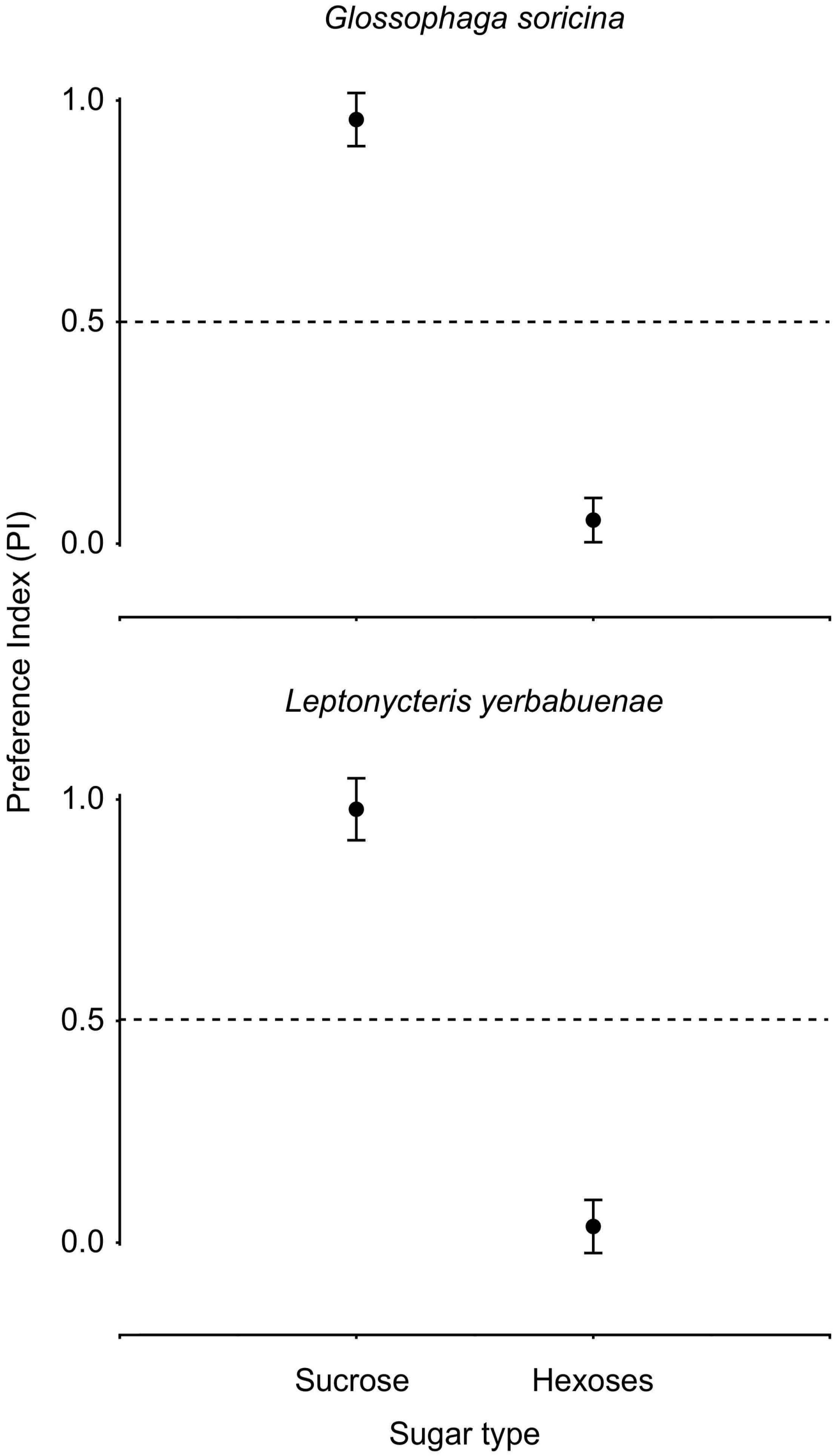

Glossophaga soricina
1.0
0.5
0.0
Hexoses selection
Sucrose selection
Preference Index (PI)
Leptonycteris yerbabuenae
1.0
0.5
0.0
Hexoses selection
Sucrose selection
5
20
35
sugar concentration (% wt./vol.)

Figure 3


Glossophaga soricina
1.0
0.5
0.0
Preference Index (PI)
Leptonycteris yerbabuenae
1.0
0.5
0.0
Sucrose
Hexoses
Sugar type

CAPITULO 4. *EFECTOS DE LAS LIMITANTES DIGESTIVAS SOBRE LA DISTRIBUCIÓN ESPACIAL DE MURCIÉLAGOS NECTARÍVOROS*

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

Jorge Ayala-Berdon[1], Ruben Galicia[2], Cesar Flores-Ortíz[3], Rodrigo Medellín Legorreta[2] and Jorge E. Schondube[1]

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

[2] Instituto de Ecología, Universidad Nacional Autónoma de México, Ap. Postal 70-275, 04510, Ciudad Universitaria, Distrito Federal, México.

[3] Laboratorio de fisiología vegetal, UBIPRO, Facultad de Estudios Superiores Iztacala, Universidad Nacional Autónoma de México, Avenida de los Barrios # 1, Los Reyes Iztacala, Tlanepantla, Estado de México, 54090, México.

Abstract

Digestive capabilities of nectar-feeding vertebrates to assimilate sugars affect their ability to acquire and store energy. These capabilities have repercussions in their feeding behavior and ecology and could determine the minimal temperatures at which these animals can survive, posing limits to their geographical and altitudinal distributions. In this study we described the sugar digestive capacity of *Leptonycteris nivalis* and related it with its capacity to live in cold environments. We measured the enzymatic activity of this species, and the intake response and changes in body mass of bats feeding at different sucrose concentrations (146, 438, 730 and 1022 mmol L-1, ≈ 5, 15, 25 and 35% sugar wt./vol.). We used a mathematical model based on enzymatic activity and gut morphology to predict food intake and compared them with the food intake of the bats. *L. nivalis* was able to obtain a constant energy intake regardless of the sugar concentration of their food. Also, body mass gain was independent of sugar concentration. The enzyme sucrase had a higher affinity for its substrate than those previously reported for other bat species, allowing this species to have a higher energy intake rate than other nectar-feeding bats. We propose that the ability to acquire energy conferred *L. nivalis* the faculty to invade cold environments avoiding ecological competition with its sympatric species *L. yerbabuenae*.

Key words: Altitudinal distributions, cold environments, digestive capabilities, geographic distribution, nectar-feeding bats, sucrase affinity.

1. Introduction

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

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

Ayala-Berdon and collaborators (2009) proposed that the bats' capability to acquire and store energy set an upper limit to the energy that these animals can use to fuel their metabolism. The interaction between gut capacity to acquire energy and the metabolic costs will determine the minimal temperatures at which these animals can survive, affecting their geographical and altitudinal distribution (Ayala-Berdon et al., 2009). The authors proposed that animals presenting compensatory feeding and the capacity to gain body mass independent of sugar concentration could have the capability to inhabit colder sites than those presenting physiological constraints (Schondube et al., 2003; Ayala-Berdon et al., 2009). For example, 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), while other species of nectar-feeding bats, like *Leptonycteris nivalis*, are able to survive in sites with minimum temperatures close to 0ºC (Arita, 1991; Brown, 2008; personal observations).

In this study we describe the gut capacity of *Leptonycteris nivalis*, a bat species that is able to live in sites colder than other nectar feeding bats. We explore the hypothesis that this bat has higher physiological capabilities to acquire energy than other nectar-feeding bats that live in sites at lower elevations or with warmer climates. To test our hypothesis we focused on the nectar-feeding bat *L. nivalis*. We measured the activity of the enzyme sucraseisomaltase in its gut, modelled its capacity to assimilate energy, and quantified the food intake and changes in body mass when feeding at different sugar concentrations. We expected this species to have higher activity of the enzyme than other nectar-feeding phyllostomids, and to be able to achieve compensatory feeding. Because nectar-feeding bats presenting compensatory feeding have shown increments in body mass independent of sugar intake in the past, we expected the same condition in our study species (Ayala-Berdon and Schondube, 2011).

2. Materials and methods

2.1 Study species

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

Segurajáuregui, 2010; Sánchez and Medellín, 2007).

2.2 Capture site

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

2.3 Bat care and housing

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

2.4 Intake responses

To establish the digestive capabilities of bats to acquire energy, we measured the intake responses and the capacity to gain body mass of each individual bat when they fed at four sucrose solutions with different concentrations (146, 438, 730 and 1022 mmol L-1 of sucrose; ≈ 5, 15, 25 and 35% sugar wt./vol.). We know from previous studies that the digestive capabilities to obtain energy of nectar-feeding bats are well represented by sucrose digestion rates, gut transit time, and gut volume (Ayala-Berdon et al., 2008). Also, these capabilities are realistically represented by the food volumetric intake when animals face changes in sugar concentration (Ayala-Berdon et al., 2008; 2009). . We only used sucrose solutions because phyllostomid bats' food intake is not affected by sugar composition (Rodríguez-Peña et al., 2007; Ayala-Berdon et al., 2008). Individuals were transferred from maintenance colonies to flying cages (3 x 2 x 1.6 m), which had a feeder in their center. Flying cages were located at the gardens of the university campus, a place that presented similar climate conditions to the capture site. This allowed us to simulated semi-natural conditions based on temperature and humidity. Bats received one sugar solution per night. We used a latin-square design (4 x 4) to ensure that we had dilute (146 mmol L-1), intermediate (438 and 730 mmol L-1) and concentrated sugar solutions (1022 mmol L-1) being offered to different individuals during the same night. Because experimental sugar solutions lacked nitrogen sources, our experiments consisted of one night of experiment followed by one day of resting. During the resting day bats received the maintenance diet and were kept under controlled conditions (see above). Solutions were weighed at the beginning (W_i) and the end (W_f) of each feeding trial from 20:00 to 04:00. This period of time corresponds to the normal foraging period of the bats at their capture site (R. Galicia, pers. Com.). Food consumed was estimated by subtracting W_f of W_i. Each night we placed a feeder of each sugar concentration outside the flight cages to control for changes in concentration and volume due to evaporation. These feeders were covered with a mosquito mesh to prevent drinking by insects and other nocturnal animals. Control feeders were weighed at the beginning and end of each trial, and the concentration of the solution was measured using a hand-held refractometer (Reichert 10431 0–50°compen- sated Brix temperature, Leica, Buffalo NY, USA) to account for changes in concentration. No changes in volume or concentration were observed in our control feeders.

2.5 Capability to store energy

To evaluate the capability of bats to store energy, we calculated the changes in body mass (Δ_{bm} in g 8h^{-1}) experienced by the bats, by weighing each animal at the beginning and the end of each trial. To assess if this capacity was ecologically realistic, we captured and weighted bats at the entrance of “La Cueva del Diablo” when they exited to feed at sunset (2000 h) and when they returned to the roost after foraging (0400 h).

2.6 Gut morphology and enzymatic activity

To assess gut morphology and enzymatic activity, three of our four experimental bats were euthanized. Duodenum, jejunum and ileum sections were dissected lengthwise to measure nominal surface area, and placed in 1.5 ml cryovials. Then guts were frozen immediately at -70°C and stored. Prior to conducting disaccharidase activity assays, guts were thawed at 5°C and homogenized (30 s, OMNI 5000 homogenizer at setting 6) in nine volumes of 350 mmol L^{-1} mannitol in 1 mmol L^{-1} Hepes/KOH, pH 7.5. Disaccharidase activities were measured following McWhorter (2009) modifications of Martínez del Rio et al. (1995) methodology. In brief, tissue homogenates (100 μl) diluted with 350 mmol L^{-1} mannitol in 1 mmol L^{-1} Hepes/KOH were incubated at 37°C with 100 μl of 56 mmol L^{-1} sugar (sucrose or maltose) solutions in 0.1 mol L^{-1} maleate/ NaOH buffer, pH 6.5. After 10–20 min of incubation, reactions were arrested by adding 3 ml of a stop/developing Glucose-Trinder (one bottle of Glucose-Trinder 500 reagent [Sigma, St. Louis, Mo.] in 250ml 1.0 mol L^{-1} TRIS/HCL, pH 7, plus 250 ml of 0.5 mol L^{-1} NaH2PO4/Na2HPO4, pH 7). Instead of reading each sample individually (McWhorter 2009), after 15 min at 20°C, the absorbances of the resulting solutions were measured simultaneously at 550 nm with a Spectra Elisa reader for 96-well micro plates (Oxford, USA).

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

for sucrose and maltose.

2.7 Data analysis

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

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

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

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

Where $S_{\max}$ is the rate of hydrolysis along the intestine (µmol min^{-1}µL^{-1}), K_m is sucrase's Michaelis-Menten constant (µmol µL^{-1}), and C_s is the concentration of sucrose (µmol µL^{-1}) down the intestine. The time (t) required to reduce the initial concentration of sucrose (C_{s0}) to a given final value (C_{sf}) can then be integrated from equation (1) to:

$$t = \frac{K_m \ln(C_{s0} / C_{sf}) + (C_{s0} - C_{sf})}{S_{\max}}, \text{ (2)}$$

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

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

We used gut morphology and enzymatic data from the collected individuals to fit the model. Gut parameters are presented in table 1. To compare observed intake of sucrose solutions with those predicted from the model, we used the coefficient of determination as a descriptor of goodness of fit (Anderson-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).

In addition, we calculated Δ_{bm} (in g 8h^{-1}) experienced by the bats, by weighing each individual bat at the beginning and the end of each trial. We used simple linear regressions to see the effect of concentration on Δ_{bm} against nectar concentration and sugar intake SI. We correlated Δ_{bm} against SI of each bat using Spearman's rank correlations (r_s) and tested whether the average r_s was significantly greater than 0 using a t test. This procedure is appropriate because it avoids the pseudo-replication that one would incur when estimating r_s for pooled data. The average r_s values for a sample of bats satisfy the central limit theorem and hence, one can make inferences about whether they are positive or negative (Stuart and Odd, 1994). Also we calculated Δ_{bm} for wild animals in the field using the data collected at "La Cueva del Diablo". We weighted bats when they were leaving the cave, and when they came back to roost after foraging. We captured a total of 75 bats (17 males and 58 females, when animals were exiting (2000 h; 36 individuals) and arriving from foraging (0400 h; 39 individuals) to "la cueva del Diablo". Since we weighted different individuals, the data we obtained represented a population mean. We compared this value with the mean Δ_{bm} for the experimental bats feeding at 438mmol L^{-1} (value close to the mean of the concentration of quiropterophilic plants, Rodríguez-Peña et al., 2007) using a t test. Finally, we assigned an alpha value of 0.05 to all tests performed to determine the existence of statistical differences.

3. Results

3.1 Intake responses and capacity to store energy

Leptonycteris nivalis increased food intake when sugar concentration on nectar decreased. The relationship between food intake and concentration was described by a power function (regression formula: Log food intake=2.7445-0.936 Log concentration, r^2= 0.94). There were no differences in intake responses among bats (t_3=0.18, P=0.86). Bats ingested 119.3 ± 20.8g of food when feeding at low concentrations (145 mmol L^{-1}), and reduced their intake to 18.5 ± 2.2 when feeding on the most concentrated diet (1022 mmol L^{-1}). The maximum volume of food ingested represented up to 5 times their body mass in nectar in one night. The exponents of the individual intake responses did not differed statistically from the compensatory value of 1 (mean exponent = 0.93, t_3= -1.45, P= 0.24). This implies that the changes in volumetric intake allowed bats to always obtained the same amount of sugar regardless of the sucrose concentrations tested (F_{14}=0.28, P= 0.60). Finally their Δ_{bm} was independent of sugar concentration (F_{14}=0.21, P= 0.64) and SI (F_{14}=0.38, P= 0.54).

We did not find statistical differences on Δ_{bm} of bats captured in the field at “La cueva del Diablo” respective to that exhibited by our experimental species feeding at 438mmol L^{-1} (Δ_{bm} field 3.57g ± 1.46 vs 2.57g ± 1.21 for the experiments; t_1=3.0, P=0.17).

3.2 Enzymatic activity

Sucrase, and maltase activity in *L. nivalis* followed clasical Michaelis- Menten kinetics (Table 1). Enzyme activities standardized by intestinal nominal area

(cm^2) and wet mass of tissue (g) for the two enzymes were linearly and tightly correlated ($0.94 < r < 0.99$). Subsequently we standardize enzymatic activity by nominal area only. Intestinal nominal areas, intestinal lengths and enzymes activities of the four bats are presented in Table 2. Sucrase and maltase activities standardized by gut nominal area were positively correlated (Fig. 1). Optimal pH was 6.0 and 6.5 for sucrase and maltase respectively. Both sucrase and maltase activity decreased along the intestine (Table 1, Fig. 1).

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

Food intake predictions from the model were almost identical to the food intake exhibited by our study species (Table 3, Fig.2). Predictions from the model were tightly and positively correlated with the experimental data ($r^2=0.994$, $t_2 = 23.98$, $P = 0.001$). This tight relation between food intake predicted and observed suggest that the digestive capacity to process sucrose is responsible of the shape of the intake response in *L. nivalis*.

4. Discussion

The results obtained in this work demonstrated that the nectar-feeding bat *Leptonycteris nivalis* is able to achieve compensatory feeding when animals fed in the range of sugar concentrations tested. Also, we reported new data of enzymatic parameters for this species. The McWhorter and Martínez del Rio's (2000) model fitted our experimental data very well. Finally, Δ_{bm} was independent of sugar concentration when bats faced the different concentrations. In this section we first discuss the relative role that digestion and renal function play in shaping the intake responses of nectar-feeding bats.

Second, we related the enzymatic information with physiological capacities of bats and evaluated the efficiency of the gut function model to predict food intake in nectar-feeding bats. Finally we discuss the relationship between the capacity of *L. nivalis* to acquire energy and increase body mass with its aptitude to live in cold environments.

4.1 Digestive and renal limitations for energy acquisition

Studies conducted with birds have demonstrated that nectar-feeding vertebrates have different capacities to acquire the energy present in the nectar they consume (López-Calleja et al., 1997; Levey and Martínez del Rio, 1999; McWhorter and Lopez-Calleja, 2000; Martínez del Rio et al., 2001). These studies have shown that while some animals are able to achieve compensatory feeding (López-Calleja et al., 1997; Levey and Martínez del Rio, 1999), others present physiological constraints to acquire energy, especially when animals feed on dilute concentrations (Levey and Martínez del Rio, 1999; Martínez del Rio et al., 2001). Nectar-feeding bats respond to changes in the sugar concentration of nectar in a similar fashion than birds (Ramírez et al., 2005; Ayala-Berdon et al., 2008 and 2009). While the nectar-feeding bats *Choeronycteris mexicana* (Ayala-Berdon and Schondube, 2011) and *Leptonycteris nivalis* (this study) are able to achieve compensatory feeding and obtained the same amount of energy when feeding on concentrations ranging from 5 to 35% (wt./vol.), *Glossophaga soricina* and *Leptonycteris yerbabuenae* presented physiological constraints that limit their energy intake when animals feed on concentrations ≤15% (wt./vol.; Ramírez et al., 2005; Ayala-Berdon et al., 2008 and 2009). Under this context, nectar feeding bats differ in their digestive capacities to obtain energy with some species being able to satisfy their energetic budget by feeding at any nectar sugar concentration, while others exhibit energetic deficits when they feed on dilute nectars (Ayala-Berdon and Schondube, 2011).

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

4.2 Digestive characteristics of Leptonycteris nivalis

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

Ayala-Berdon et al. (2008) proposed that the affinity of sucrase for its substrate (K_m) and rates of hexoses assimilation in the small intestine may strongly affect the amount of food that an animal is able to ingest. According to this prediction, we found that the affinity of the enzyme sucrase of *L. nivalis* is one order of magnitude higher than the affinity presented by *L. yerbabuenae* (0.004 and 0.059 for *L. nivalis* and *L. yerbabuenae* respectively; Schondube et al., 2001). Under this context, the high affinity of sucrase for its substrate, may confer to *L. nivalis* the ability of achieve compensatory feeding when they confront different sugar concentrations. Furthermore, it has been shown that the rates of sugar digestion and absorption are paired in follostomid bats (Ayala-Berdon et al., 2008; Herrera and Mancina, 2008). If this is true for *L. nivalis*, this species should present paired sugar digestion and absorption and may have the capacity to feed from any source of nectar regardless of sugar composition or concentration on which they feed. However this hypothesis remains to be explored.

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

McWhorter and Martínez del Rio (2000) proposed that a Plug flow chemical reactor (Penry and Jumars, 1987) could be used as a method for modeling the food intake of nectar-feeding animals. The authors showed that this model predicts food intake accurately in Broad-tailed hummingbirds. Ayala-Berdon et al. (2008) applied this model on two species of nectar-feeding bats. Their results showed that the model explained the experimental data accurately (53 and 67% of the variation of the intake responses for *L. yerbabuenae* and *G. soricina* respectively). However, the power functions adjusted to their experimental data described the intake responses better (86 and 83% for *L. yerbabuenae* and *G. soricina* respectively). These authors assumed that the differences between the predictions of the model and their food intake observation were caused by the fact that the enzymatic data they used was from different individuals than their food intake data. In this study, we demonstrated that the gut function model explained remarkable well our food intake experimental data when enzymatic and gut morphology data comes from the same individuals than the food intake data (99.4% of the variation of the intake response explained by the model; fig. 3). Our results clearly shows that the model proposed by McWhorter and Martínez del Rio (2000), have a high accuracy to predict food intake in nectar-feeding bats.

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

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

Physiological capabilities limiting the maximum amount of energy that an animal can acquire should have important effects in the way they interact with their environment (Ayala-Berdon et al., 2009; Gaston et al., 2009; Kuo and Sanford, 2009; Rodriguez-Serrano and Bozinovic, 2009; Szathmary et al., 2009). Several studies have suggested that gut capabilities to acquire and store energy have effects on the behavior, ecology and geographical and altitudinal distribution of bats (Ayala-Berdon et al., 2009; 2011; Ayala-Berdon and Schondube, 2011). *Glossophaga soricina* and *Leptonycteris yerbabuenae* compensated for their incapacity to achieve compensatory feeding by reducing flight time and increasing feeding time when their energy intake was lower due the presence of a physiological constraint (Ayala-Berdon et al., 2011).

Additionally, differences in the selection and use of food resources by an assemblage of a nectar-feeding bat community appear to be driven by the physiological capabilities of these animals. Bats able to achieve compensatory feeding have the capacity to feed on any nectar resource present on their environment, acting ecologically as generalists and being able to invade broader geographical areas. On the other extreme, bats exhibiting physiological constraints would benefit from feeding on more concentrated nectars, becoming ecologically more specialists and having a narrower geographical distribution (Ayala-Berdon and Schondube, 2011).

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

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Table 1. Sucrase, and maltase activity parameters for the néctar-feeding bat *L. nivalis*. We presented enzyme activities standardized by intestinal nominal area (cm^2) and wet mass of tissue (g) for both enzymes. We used these parameters to fit the McWhorter and Martínez del Rio's model (2000) to our experimental data. Intestinal length, width and nominal areas of the four bats are presented in Table 2

	Sucrase	*Maltase*
pH optima	6.0	6.5
S max ($\mu mol\ min^{-1}\ L^{-1}$)	0.024	6.85
Km ($\mu mol\ \mu L^{-1}$)	0.068	10.17
Csf *	0.009	0.009
G (μL^{-1})	0.724	0.724

Smax: rate of hydrolysis along the intestine, *Km*: sucrase Michaelis–Menten constant, *Csf*: final concentration of sucrose after digestion, *G* volume of the intestine.

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 Martínez 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. Intestinal length, width and gut nominal area obtained from individuals of *L. nivalis* from which we acquired the enzymatic parameters.

Individual	*Intestinal Portion (cm)*	*Intestinal lenght (cm)*	*Intestinal width (cm)*	*Gut nominal area (cm^2)*
1	First	4	0.60	2.40
	Second	5.5	0.53	2.93
	Third	4	0.36	1.4
2	First	4	0.60	2.40
	Second	4	0.53	2.13
	Third	4	0.60	2.40
3	First	5	0.63	3.16
	Second	5	0.53	2.66
	Trird	5	0.43	2.16

Table 3. Experimental and moldeled predicitions of food intake of the néctar-feeding bat *Leptonycteris nivalis*. Predictions from the model were tightly and positively correlated with the experimental data. This tight relation between food intake predicted and observed suggest that the digestive capacity to process sucrose is responsible of the shape of the intake response in this néctar-feeding species.

	Food intake (g 8h^{-1})	
Sugar concentration (mmol L-1)	Experimental (mean ± SD)	Model
146	119.35 ± 20.8	136.22
438	48.5 ± 8.15	48.8
730	28.5 ± 6.01	29.8
1022	18.5 ± 2.2	21.4

Figure legends

Figure 1. a) optimal pH for both sucrase and maltase enzymes for the nectar-feeding bat *L. nivalis*. b) sucrase and maltase activity in the three portions of the bat's small intestine. Data are presented as averages with their respective SD.

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

Figure 1

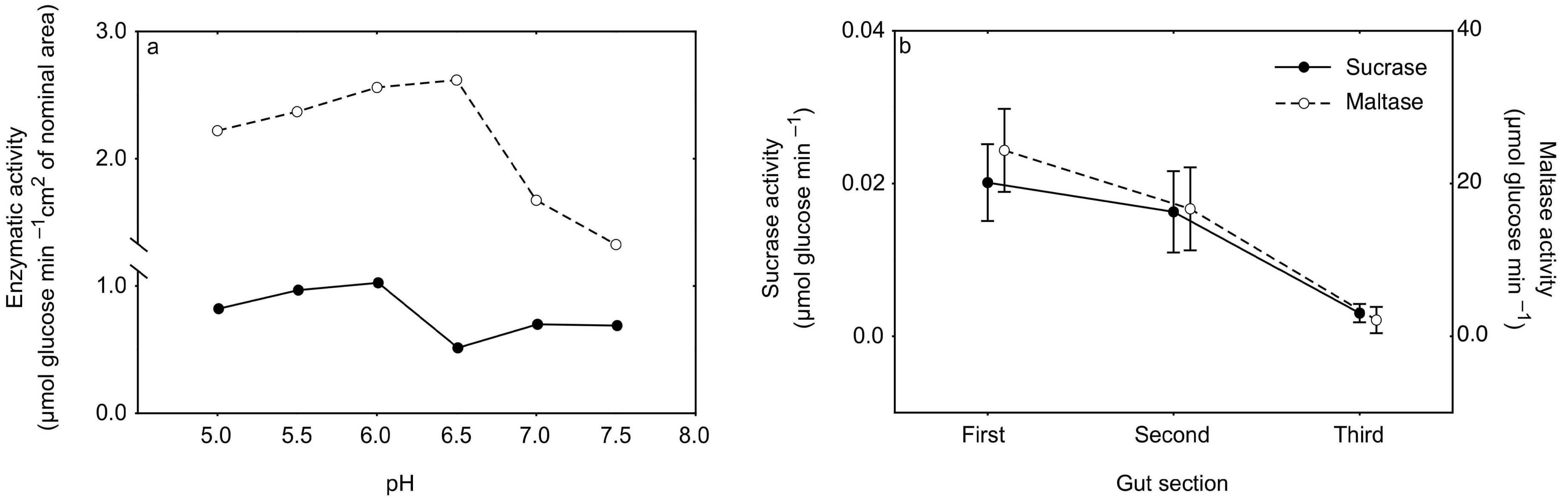
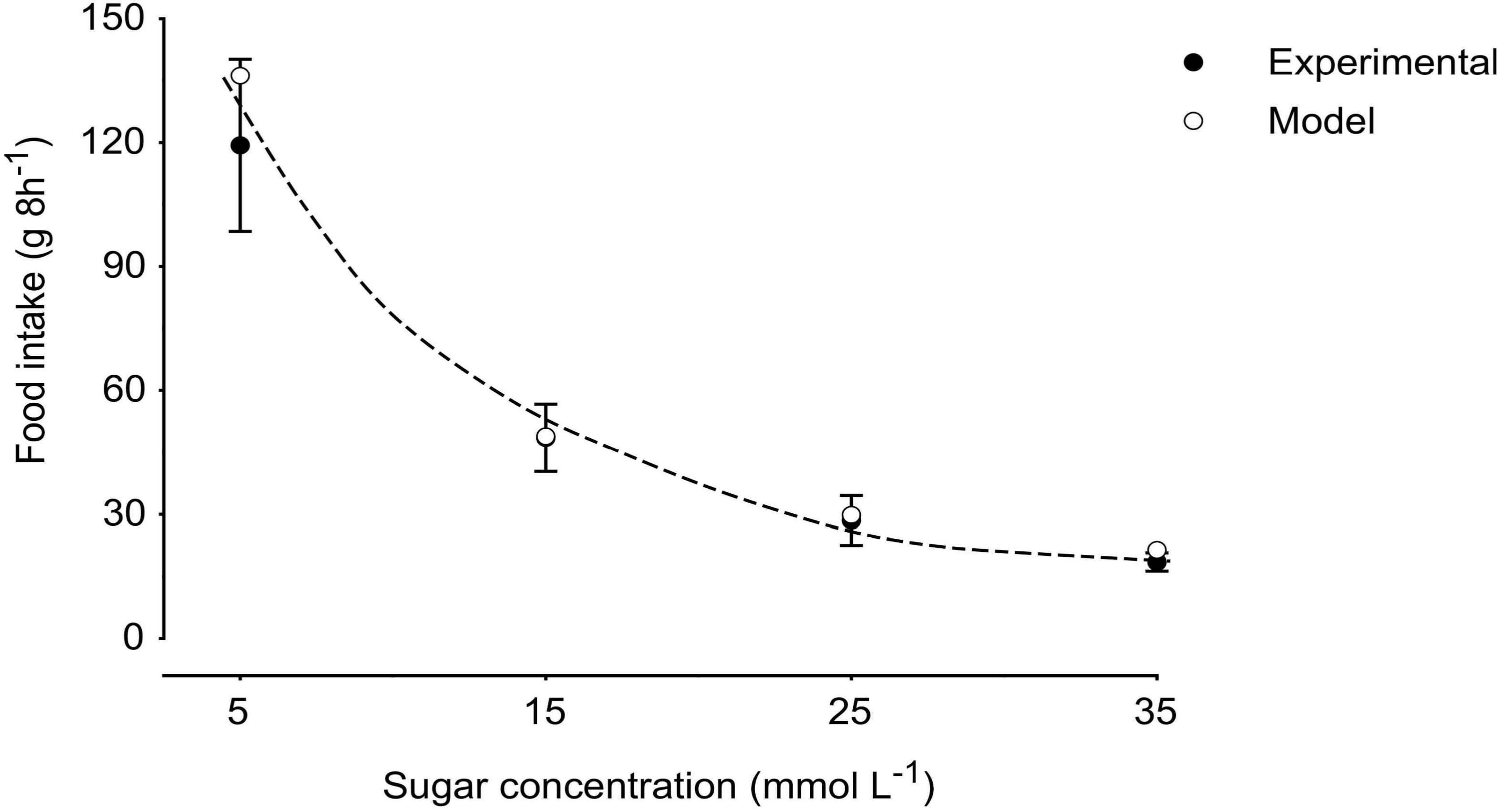

a
Enzymatic activity
(μmol glucose min −1cm2 of nominal area)
3.0
2.0
1.0
0.0
5.0
5.5
6.0
6.5
7.0
7.5
8.0
pH
b
Sucrase
Maltase
Sucrase activity
(μmol glucose min −1)
0.04
0.02
0.0
Maltase activity
(μmol glucose min −1)
40
20
0.0
First
Second
Third
Gut section

Figure 2


150
120
90
60
30
0
Food intake (g 8h^{-1})
5
15
25
35
Sugar concentration (mmol L^{-1})
● Experimental
○ Model

Conclusiones generales

Los resultados obtenidos en este trabajo demuestran que las capacidades digestivas para la obtención de energía de los murciélagos que se alimentan de néctar afectan su comportamiento, ecología y distribución geográfica. *G. soricina* y *L. yerbabuenae* son dos especies de murciélagos filostómidos que presentan limitantes digestivas para la obtención de energía (i.e. presentan déficits energéticos cuando se alimentan de concentraciones de azúcar iguales o por debajo de 15% -peso/volumen). Dichas especies modifican sus patrones de forrajeo cuando la concentración de su alimento cambia (ver Capitulo 1). Cuando los murciélagos se alimentan de néctares diluidos, ambas especies disminuyen su tiempo de vuelo e incrementan su tiempo de alimentación. Sin embargo, cuando se alimentan de néctares concentrados incrementan el tiempo que pasan volando. Esta respuesta parece estar asociada a otra limitante fisiológica, una incapacidad de regular los niveles de glucosa en sangre utilizando solo insulina, lo que los obliga a quemar el azúcar por medio del ejercicio. Estas respuestas conductuales a los cambios en la calidad del alimento tienen implicaciones ecológicas importantes para los organismos. Por un lado, murciélagos que se alimenten de néctares diluidos tendrán que aumentar el número de flores visitadas por noche, incrementando así la polinización cruzada. Sin embargo, cuando los animales consuman néctares concentrados tendrán la necesidad de pasar más tiempo volando. Esta respuesta conductual puede tener ventajas adaptativas al permitirles utilizar esta necesidad de movimiento para explorar nuevos parches florales que podrán utilizar para su alimentación en las noches siguientes. Esto es crucial si consideramos que el néctar floral es un recurso poco estable tanto en el tiempo como en el espacio. De manera adicional, la visita de estos organismos entre parches de vegetación que se encuentran separados por grandes distancias, incrementará el flujo genético entre diferentes poblaciones de plantas.

Las capacidades digestivas de los murciélagos no solo afectan su comportamiento sino también la capacidad que estos tienen para almacenar energía cuando se alimentan de diferentes concentraciones de azúcar. *Choeronycteris mexicana* es una especie de murciélago nectarívoro que es capaz de lograr una alimentación compensatoria (i.e. obtienen la misma cantidad de energía a cualquier concentración de azúcar a la que se alimente). A diferencia de *G. soricina* y *L. yerbabuenae* que presentan limitantes digestivas y una relación positiva entre la concentración del néctar del cual se alimentan, su obtención de energía y su ganancia de masa corporal, esta especie de murciélago tiene la capacidad de ganar peso de manera independiente a la concentración de la cual se alimenta (ver capitulo 2). Las diferencias entre las ganancias de peso entre las tres especies de murciélagos antes mencionadas nos indican que el manejo de energía por parte de estos organismos es distinto y puede tener implicaciones ecológicas importantes para las especies de murciélagos que se alimentan de néctar. *C. mexicana* al tener la capacidad de adquirir la misma cantidad de energía independientemente de la concentración a la que se alimente, puede utilizar cualquier fuente de néctar disponible para él, actuando ecológicamente como generalista en el consumo de néctar. Por otro lado, *G. soricina* y *L. yerbabuenae* se verán beneficiados al consumir néctares con más densidad de energía, para evitar así las restricciones impuestas por sus limitantes digestivas. Estas especies tenderán a ser más selectivas en los recursos alimenticios en campo, actuando más especialistas en el consumo de néctar. De acuerdo con nuestra hipótesis, varios estudios han encontrado que *C. mexicana* tiene una dieta más diversa consumiendo néctar de una mayor diversidad de plantas que *G. soricina* y *L. yerbabuenae*. Esto sugiere que las capacidades digestivas, y la forma en que estas determinan el consumo de energía afectan la forma en la que los miembros de una comunidad de murciélagos utilizan y se reparten los recursos florales presentes en los ecosistemas en los que habitan.

Aunque las capacidades digestivas de los murciélagos afectan la manera en que estos se comportan y la forma en que eligen su alimento en campo, algunos patrones de selección de alimento no pueden ser explicados únicamente por las características digestivas de estos animales. Algunos autores han sugerido que el sabor de los diferentes azúcares presentes en el néctar tiene un efecto en sus patrones de selección de alimento en campo. *G. soricina* y *L. yerbabuenae* presentan diferencias en la percepción del sabor de los tres principales azúcares presentes en el néctar (sacarosa, glucosa y fructosa; ver capitulo 3). Sin embargo, a pesar de tener diferentes sensibilidades para el sabor de estos azúcares, lo cual los hace percibirlos con diferentes grados de dulzura, los murciélagos no siguen sus umbrales gustativos (i.e. capacidad para distinguir el sabor de los diferentes azúcares), para elegir el alimento. Estas especies de murciélagos tienen la capacidad de detectar, y aprender la composición de azúcares presentes en su dieta, y tienden a elegir el sabor del azúcar más común en su alimento. Este resultado sugiere que ambas especies de murciélagos nectarívoros deben enfocarse en las especies de plantas más abundantes en su hábitat aprendiendo a elegir la composición de sus néctares, y prefiriéndola sobre el sabor del néctar de plantas menos abundantes. Esto puede tener un importante efecto sobre las plantas, ayudando a que los murciélagos tiendan a utilizar una especie de planta a la vez, lo que reduciría efectos negativos generados al depositar polen de otras especies en las flores que más visitan, mejorando su calidad como polinizadores.

Por último la capacidad digestiva de las diferentes especies de murciélagos, al afectar su capacidad para obtener energía, debe tener un efecto en las temperaturas mínimas en las que los organismos pueden vivir. *L nivalis* es una especie simpátrica de *L. yerbabuenae*. Sin embargo, esta especie tiene la capacidad de vivir en zonas más frías y más altas que *L. yerbabuenae*, capacidad que se ve representada en su nombre, ya que esta especie fue colectada por primera vez en sitios con nieve. Encontramos que esta especie no presenta limitantes digestivas y presenta alimentación compensatoria. Nuestros datos sugieren que la alta capacidad digestiva de *L: nivalis* para obtener energía de su alimento y su capacidad para ganar peso de forma independiente de la concentración del néctar del que se alimenta, le ha permitido habitar zonas que su especie hermana no puede utilizar debido a los altos costos energéticos asociados con habitar ahí (ver capitulo 4). Aparentemente la afinidad de la enzima sacarasa por su sustrato en *L. Nivalis*, la cual es 10 veces más alta que en *L. yerbabuenae* le ha dado la capacidad de incrementar el consumo de energía e invadir sitios fríos evadiendo así la competencia con su especie hermana.