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Trophic relationships in a neotropical bat community: a preliminary study using carbon and nitrogen isotopic signatures

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Abstract: We used stable isotope techniques to determine the nitrogen and carbon isotopic composition of 21 species of neotropical bats (17 phyllostomids, two mormopids, one molossid and one emballonurid) representing a diverse array of feeding habits (e.g. frugivory, nectarivory, insectivory, carnivory, and sanguinivory) as well as the isotopic composition of plants and insects presumably ingested by the bats. We found trophic enrichment of both ^{13}C and ^{15}N, although ^{15}N enrichment was lower than expected between frugivorous and insectivorous bats. Our data indicate that most species of frugivorous bats examined have a mixed diet of fruits and insects and only *Centurio senex, Artibeus lituratus* and *Dermanura watsonii* appear to be exclusively frugivorous. One species of insectivorous bat, *Tonatia evotis*, apparently relies on fruit as part of its diet. Although preliminary, our results indicate that stable isotope methods can be used to quantify the importance of different dietary classes in the diets of bats.

Résumé: Des techniques isotopiques ont été utilisées afin de déterminer la composition isotopique en carbone et azote de 21 espèces de chauves-souris néotropicales (17 phyllostomides, 2 mormopides, un molosside et un embalonuride) présentant un tableau diversifié de leurs tendances alimentaires (fructivore, nectarivore, insectivore, carnivore, et sanguinaire) ainsi que la composition isotopique des plantes et des insectes vraisemblablement ingérés par les chauvessouris. Nous avons trouvé un enrichissement trophique aussi bien en azote (N^{15}) que carbone (C^{13}) bien que la richesse en azote était plus basse que prévue entre les chauve-souris à tendance fructivore et insectivore. Nos données indiquent que la plupart des espèces de chauvesouris fructivores qui ont été examinées ont un régime alimentaire mélangé de fruits et d'insectes et seules *Centurio senex, Artibeus lituratus* et *Dermanura watsonii* semblent être exclusivement fructivores. Une des espèces de chauve-souris insectivores, *Tonatia evotis*, intègre apparemment les fruits dans son régime alimentaire. Quoique préliminaires, nos résultats montrent que les méthodes isotopiques stables peuvent être utilisées pour quantifier l'importance des différentes types d'alimentation dans les régimes alimentaires des chauve-souris.

Resumen: Se utilizaron técnicas de isótopos estables para determinar la composición isotópica de nitrógeno y carbono de 21 especies de murciélagos neotropicales (17 folostómidos, dos mormópidos, un molósido y un embalonúrido) con diversos hábitos alimenticios (frugívoros, nectarívoros, insectívoros, carnívoros y sanguinívoros), así como la composición isotópica de plantas e insectos ingeridas presumiblemente por los murciélagos. Encontramos enriquencimiento trófico tanto de ^{13}C como de ^{15}N, aunque éste último fue más bajo que lo esperado entre los murciélagos frugívoros e insectívoros. Nuestros datos indican que la mayoría de las especies de murciélagos frugívoros examinados tienen una dieta mixta de frutos e insecto y solamente *Centurio senex, Artibeus lituratus* y *Dermanura watsonii* parecen ser exclusivamente frugívoros. Una especie de murciélago insectivoro, *Tonatia evotis*, aparentemente toma frutos como parte de su dieta. Aunque preliminarmente, nuestros resultados indican que los métodos de isótopos estables pueden ser utilizados para cuantificar la importancia de diferentes tipos de dietas en los murciélagos.

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Resumo: Para determinar a composição isotópica de azoto e carbono de 21 espécies de morcegos neotropicais (17 phyllostomidios, dois mormópidios, um molóssidio e um embalonurido) com hábitos alimentares diversos (frutívoros, nectarivoros, insectívoros, carnívoros e sanguinívoros): bem como a composição isotópica das plantas e insectos presumivelmente ingeridos pelos morcegos usaram-se técnicas de isótopos estáveis. Encontrou-se um enriquecimento trófico quer no ^{13}C quer nol ^{15}N se bem que o enriquecimento em ^{15}N tenha sido menor do que o antecipado entre os morcegos frutívoros e os insectívoros. Os resultados obtidos indicam que muitas das espécies de morcegos frutívoros examinadas têm uma dieta mista de frutos e insectos e só a *Centurio senex*, *Artibeus lituratus* e *Dermanura watsonii* parecem ser exclusivamente frutívoras. Uma das espécies de morcegos insectívoros, *Tonatia evotis* está, aparentemente, depende dos frutos como parte da sua dieta. Os resultados, embora preliminares, indicam que uso de métodos com recurso aos isótopos estáveis podem ser utilizados para quantificar a importância das diferentes classes de dieta dos morcegos.

Key Words: carnivorous bats, feeding habits, frugivorous bats, nectarivorous bats, insectivorous bats, stable isotopes, trophic levels, vampire bats.

Introduction

Dietary studies in bats have traditionally relied on the examination of feces, pellets, fur, and stomach contents (Alvarez & Gonzalez Quintero 1970; Thomas 1988; Whitaker 1988). More recently, stable isotope techniques have been used in bats to determine trophic patterns as well as migratory movements (DeMarais *et al.* 1980; Fleming *et al.* 1993; Herrera *et al.* 1993). While these techniques do not provide detailed information about the identity of food consumed by animals, they allow long-term examination of dietary trends. For example, the examination of the isotopic composition of muscle tissue reflects the diet of the animal 1-2 months before the tissue was collected (Tieszen *et al.* 1983). Carbon and nitrogen are the most commonly used isotopes in animal studies and, because they show trophic fractionation, they can be used to separate trophic levels in animal communities (Hobson *et al.* 1994; Mizutani *et al.* 1992). In general, there is an enrichment of 1‰ in ^{13}C and of 2-4‰ in ^{15}N from one trophic level to the next (DeNiro & Epstein 1978; Ehleringer *et al.* 1986).

Here we examine the trophic structure of a neotropical bat community using both carbon and nitrogen stable isotope signatures. We focus on bats in the family Phyllostomidae because its members exhibit a broad array of feeding habits (e.g., nectarivory, frugivory, insectivory, carnivory, sanguinivory; Gardner 1977). Bats in this family have been traditionally separated into different trophic guilds although some frugivorous and nectarivorous species may consume insects and leaves to fulfill their nitrogen requirements (Kunz & Díaz 1995; Thomas 1984). On the other hand, some insectivorous phyllostomids also ingest fruits (Gardner 1977). The primary goal of this study was to determine the extent to which bats that are traditionally assigned to the above-mentioned categories rely on plant and/or animal sources to meet their nutritional requirements. We also examined bats in other families that are considered strictly insectivorous and plants and insects that are probably consumed by bats. We expected to find an enrichment in ^{13}C and ^{15}N of at least +1‰ and +3‰, respectively, from lower to higher trophic levels if bats fit into distinct feeding categories and do not include a mixture of animal and plant items in their diet.

Materials and methods

Tissue samples were collected in April 1994 at the end of the dry season during a faunal survey of the Calakmul Biosphere Reserve (19-18° N, 89-90° W) conducted by the Instituto de Ecología in the state of Campeche, Mexico. Pectoral muscle samples were collected from bat specimens originally sacrificed for a museum reference collection. We collected samples from one to three individuals of nine species of frugivorous and nectarivorous bats (Phyllostomidae : *Centurio senex* Gray, *Artibeus lituratus* Olfers, *A. intermedius* Allen, *Vampyressa pusilla* Wagner, *Carollia brevicauda* Schinz, *C. perspicillata* Linnaeus, *Dermanura watsonii* Saussare, *Sturnira lilium* Geoffroy, *Glossophaga soricina* Pallas), 9 species of insectivorous bats (Phyllostomidae : *Tonatia brasiliensis*

Peters, *T. evotis* Davis & Carter, *Micronycteris schmidtorum* Sanborn, *M. brachyotis* Dobson, *Mimon bennettii* Gray; Mormoopidae : *Pteronotus parnellii* Gray, *P. davyi* Gray; Molossidae : *Molossus rufus* Miller; Emballonuridae : *Peropteryx macrotis* Wagner); one carnivorous species (Phyllostomidae : *Vampyrum spectrum* Linnaeus) and two species of vampires (Phyllostomidae : *Diphylla ecaudata* Spix and *Desmodus rotundus* Geoffroy). We also collected nocturnal insects and tissue (leaves or fruits if available) from plants that are presumably ingested by bats. Because fractionation can occur in plant parts (Handley & Raven 1992), tissue actually ingested by the animals was collected whenever possible preferentially fruit, although there is evidence of leaf consumption in some frugivorous bats (Kunz & Díaz 1995). We collected one individual of one unidentified species of insect in each of the orders Coleoptera, Lepidoptera, and Hemiptera along with one individual of eight species of plants (Leguminosae : *Bauhinia* sp. (leaf); Piperaceae : *Piper amalago* Linnaeus (leaf); Moraceae : *Ficus* sp. (fruit), *Brosimum alicastrum* Berg (leaf); Sapotaceae : *Manilkara sapota* van Royen (leaf), *Chrysophyllum* sp. (fruit), *Pouteria campechiana* Baehni (leaf); Combretaceae : *Bucida buceras* Linnaeus (fruit).

The nitrogen and carbon composition of pectoral muscle collected from individuals of the 21 species of bats was assessed using the methodology described by Sealy *et al.* (1987). In brief, 12-15 mg of ground muscle samples were combusted at 800°C in Vycor ampules with 1 g of cupric oxide, 1 g of copper and a small amount of silver foil (ca. 50 mg). Nitrogen and carbon were cryogenically purified from the combustion products in a vacuum system. Purified nitrogen and carbon were analyzed in a PRISM micromass spectrophotometer. Isotope ratios were expressed using the delta notation in parts per thousand (per mil) where

$$\delta^{15}N = [\frac{(^{15}N/^{14}N)_{sample}}{(^{15}N/^{14}N)_{standard}} - 1] \times 1000,$$

and

$$\delta^{13}C = [\frac{(^{13}C/^{12}C)_{sample}}{(^{13}C/^{12}C)_{standard}} - 1] \times 1000.$$

A similar procedure was used with the insect and plant samples, except that we used 20 mg of plant tissue per sample. The standard for $\delta^{13}C$ values was the Peedee belemnite marine limestone (PDB), and it was atmospheric nitrogen for $\delta^{15}N$. Precision was ± 0.175‰ (S.D.) and ± 0.1‰ (S.D.) for nitrogen and carbon, respectively.

Statistical analysis

Mean $\delta^{13}C$ and $\delta^{15}N$ values were compared between trophic levels (i.e., plants vs. frugivorous bats, plants vs. insects, frugivorous bats vs. insectivorous bats, insects vs. insectivorous bats, insectivorous bats vs. carnivorous bats) using Mann-Whitney's U tests. When more than one individual per species was analyzed, we used the mean value for the species. Bats were separated into trophic guilds for the analysis according to traditional classifications (Gardner 1977). We also conducted a linear regression analysis using mean $\delta^{13}C$ and $\delta^{15}N$ values of all the species of bats and followed the above-mentioned procedure when one species was represented by more than one individual.

Results

Mean values of $\delta^{13}C$ and $\delta^{15}N$ increased (i.e., exhibited enrichment of ^{13}C and ^{15}N) from lower to higher trophic levels (Fig. 1). The enrichment in ^{13}C was +3.110 between plants and insects, +3.667 between plants and frugivorous bats, +1.716 between frugivorous and insectivorous bats, and +1.027 between insectivorous and carnivorous and vampire bats. The enrichment in ^{15}N was -0.400 between plants and insects, +2.109 between plants and frugivorous bats, +0.750 between frugivorous and insectivorous bats, and +2.227 between insectivorous and carnivorous and vampire bats.

Carbon isotopic compositions were significantly different between plants and insects (U = 24, P = 0.018) and frugivorous bats (U = 72, P = 0.000), and between insectivorous bats and insects (U = 27, P = 0.01) and frugivorous bats (U = 71, P = 0.000). Significant differences in nitrogen isotopic composition were found between insects and insectivorous bats (U = 27, P = 0.016), and between insectivorous and carnivorous bats (U = 35, P = 0.010).

There was significant positive correlation between $\delta^{13}C$ and $\delta^{15}N$ values when the values for all the species of bats were pooled (r = 0.478, F = 5.64, P = 0.028, d.f. = 1, 19, Y = 0.655x - 29.313; Fig. 2). There was a substantial overlap between isotope values of insectivorous and frugivorous bats, particularly in $\delta^{15}N$ values (Fig. 2).

Discussion

In general, both carbon and nitrogen δ values discriminated among different trophic levels in the species of

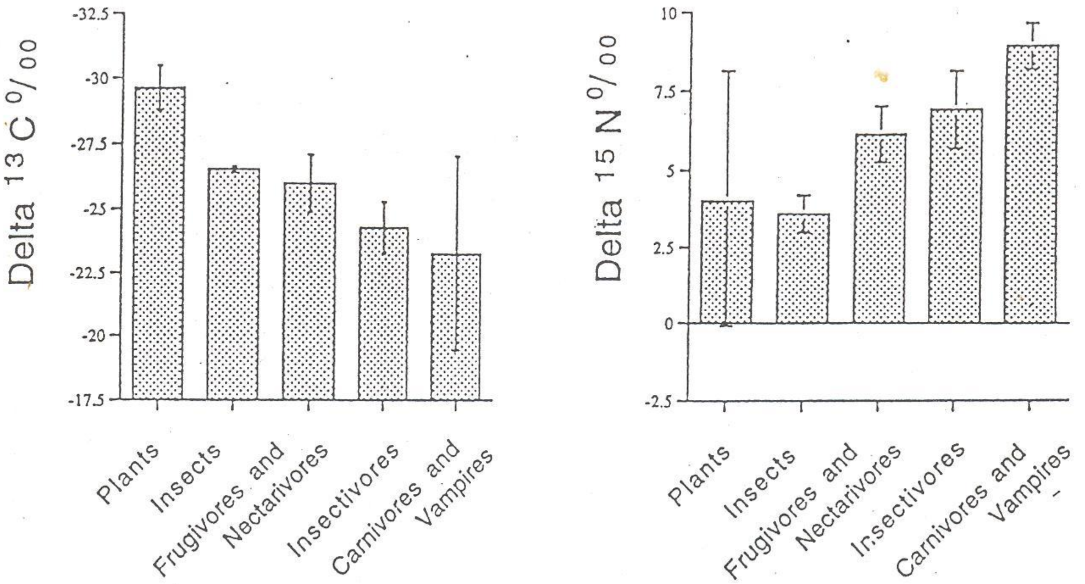

Fig. 1. Mean (±S.D.) δ^{13}C and δ^{15}N values for each trophic level in a neotropical bat community. Frugivores and nectarivores (n = 9), insectivores (n = 9), carnivores (n = 1) and vampires (n = 2) correspond to bat species. Sample sizes for plants and insects were 9 and 3, respectively. Mean values were calculated for species with more than one individual.

bats we studied, although some species did not fit their expected trophic level. The relationship between carbon and nitrogen isotopic signatures was significant although the correlation coefficient was not particularly high ($r = 0.478$); in some cases bats had low δ^{15}N and high δ^{13}C values (or otherwise) that prevented their categorization into a distinct trophic level (e.g. *Desmodus rotundus, Tonatia evotis, Sturnira lilium, Carollia brevicauda, C. perspicillata, Glossophaga soricina*).

^{13}C enrichment was higher between plants and primary consumers (insects and frugivorous bats) than between primary and secondary consumers (insectivorous bats) and between secondary and tertiary consumers (carnivorous and vampire bats). A similar pattern occurs in marine-terrestrial communities; carbon enrichment in primary consumers (e.g. euphausiids, mussels) is +3‰ relative to particulate organic matter but it decreases between higher levels (Hobson *et al.* 1994).

Enrichment in ^{15}N was less pronounced than that of ^{13}C, especially between plants and insects and between frugivorous and insectivorous bats. Nitrogen composition in plants was highly variable (δ^{15}N values ranged from -2.2 to 9.9‰) and was not significantly different from that of insects and frugivorous bats. Furthermore, ^{15}N enrichment between plants and primary consumers was at the lower end of the expected range (DeNiro & Epstein 1978) and in the case of insects there was negative enrichment. Unless the insects in this study were feeding on items other than plants (rather unlikely for the insects included in the study), the expected values of plant δ^{15}N should be around 1.5 if there is a trophic enrichment of +3‰ (DeNiro & Epstein 1978) since the δ^{15}N average value for insects was 3.6. The limited number of species of plants included in this study is probably not fully representative of the nitrogen composition of the plant community in the area of study. In addition to this, because only one individual per species of plant was included in the analysis, we cannot estimate intraspecific variation in nitrogen composition. Similarly, the low sample size of insects in this study prevents any generalization for the isotopic composition of this group of animals.

Average ^{15}N enrichment among bat trophic guilds was lower than expected, especially between frugivorous and insectivorous bats. This suggests that some species of bats are not strict fruit - or insect-eaters. For example, *Tonatia evotis* had very low δ^{15}N values for an insectivore; members of the genus *Tonatia* feed on both insects and fruits (Gardner 1977), and our results support this. Several species of frugivorous bats (*Sturnira lilium, Carollia brevicauda, C. perspicillata*) had lower δ^{13}C values than

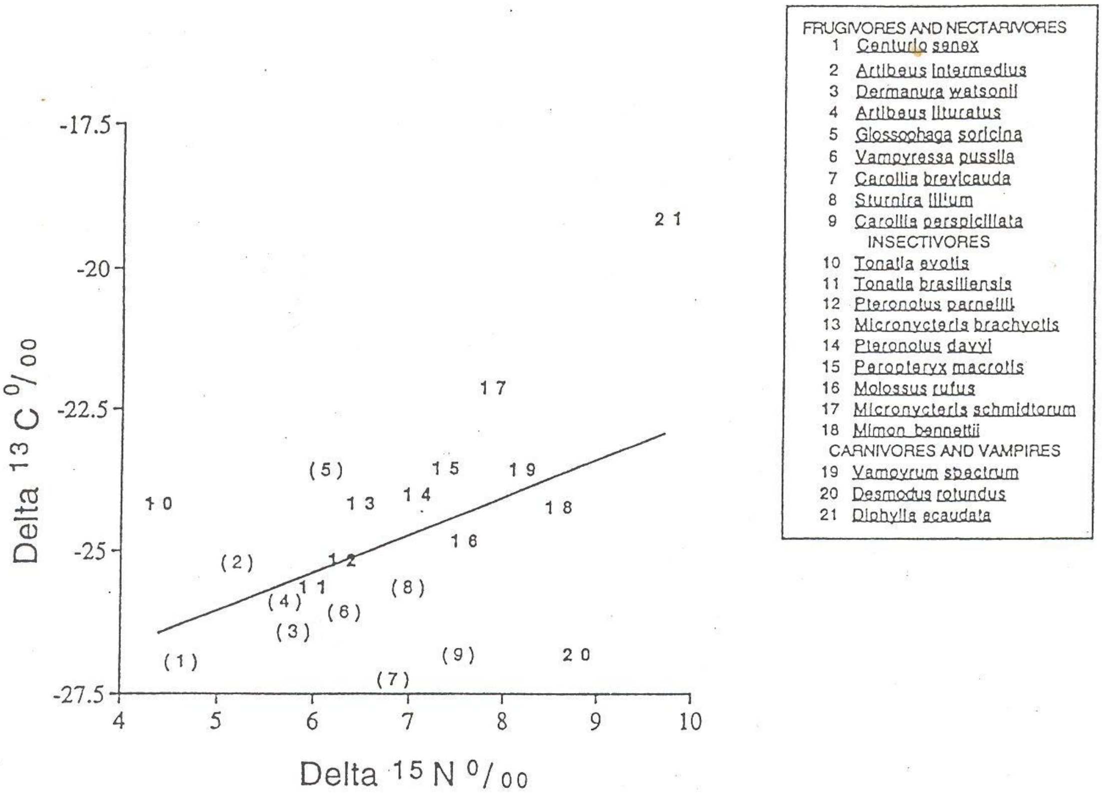

Fig. 2. ^{13}C and ^{15}N δ values for each species of bats in a neotropical community. Each point represents a species; mean values were used when a species was represented by more than one individual. Bolded numbers correspond to insectivorous, carnivorous and vampire species, and numbers inside a parenthesis represent frugivorous and nectarivorous species. Species identification of each number is illustrated in the figure. The line represents the least-squares linear regression of δ^{13}C and δ^{15}N species values.

insectivorous bats, but their δ^{15}N were not different than the mean value for insectivores. Similarly, *Glossophaga soricina* had low δ^{15}N values but high δ^{13}C values that suggest a mixed diet of fruits and insects. Based on the examination of stomach contents and feces (Fleming *et al.* 1972; Palmerim *et al.* 1989; Willig *et al.* 1993), the diets of these species are mostly composed of fruit with the proportion of insects ranging from none (*S. lilium*), to low (*C. brevicauda* and *C. perspicillata*) and large (*G. soricina*) amounts of insects. In contrast, *Artibeus lituratus Dermanura watsonii* and especially *Centurio senex* had low values of δ^{15}N and of δ^{13}C that suggest a diet of fruit. The diet of *A. lituratus* has been reported as consisting only of fruits (Palmerim *et al.* 1989; Willig *et al.* 1993) or a mixture of fruits and insects (Fleming *et al.* 1972), and *C. senex* is considered by Gardner (1977) to be an obligate frugivore. *A. intermedius* had a nitrogen composition similar to *C. senex* and a carbon isotopic signature intermediate between the mean values for frugivorous and insectivorous bats. Some insectivorous species (*Tonatia brasiliensis*, and *Pteronotus parnellii*) had δ^{15}N lower than the average for this group of bats although their δ^{13}C values were intermediate between insectivorous and frugivorous species. *P. parnellii* feeds mostly on insects but there is evidence that indicates that this species includes small amounts of fruits in its diet (Fleming *et al.* 1992). Pollen has also been reported in the diet of another species of *Pteronotus* (*P. quadridens* Gundlach; Rodríguez-Duran & Lewis 1987). The remaining insectivorous species seem to feed exclusively on insects, although some of them (*Mimon bennettii* and some individuals of *Micronycteris schmidtorum*) had slightly higher δ^{15}N values than the carnivorous species (*Vampyrum spectrum*). The diet of *V. spectrum* consists of small birds and mammals, insects and probably fruits (Gardner 1977) while *M. bennettii* and *M. schmidtorum*, although mostly insectivorous, may also include fruit in their diet (Gardner 1977). In general, the two species of vampire bats had nitrogen and carbon compositions that place them at the top of the trophic chain, except for the exceptionally low $\delta^{13}C$ value of *Desmodus rotundus*. We do not have an explanation for the carbon composition of *D. rotundus*.

In conclusion, isotopic examination of carbon and nitrogen composition offered a broad picture of the trophic structure in the bat community we studied. Our results show that it is rather difficult to place certain species of phyllostomid bats, especially frugivorous species, into distinct trophic guilds. Previous studies of feeding habits based on traditional methods support this view, but our study is the first attempt to quantify the relative importance of plant and animal items in the diet at a community level using stable isotope techniques. We consider this a preliminary study and hope to stimulate further use of stable isotopes in bat biology (also see Fleming 1995). In particular, future trophic studies should increase the number of species of plants and insects sampled and consider seasonal variations in the diet of the animals. Use of blood samples instead of collecting muscle tissue from dead animals would allow increased sample sizes without sacrificing animals. One of us (LGHM) is currently using blood to study food habits in bat communities.

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