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PHYSIOLOGICAL ECOLOGY - ORIGINAL RESEARCH

Seasonal reliance on nectar by an insectivorous bat revealed by stable isotopes

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Abstract Many animals have seasonally plastic diets to take advantage of seasonally abundant plant resources, such as fruit or nectar. Switches from insectivorous diets that are protein rich to fruits or nectar that are carbohydrate rich present physiological challenges, but are routinely done by insectivorous songbirds during migration. In contrast, insectivorous bat species are not known to switch diets to consume fruit or nectar. Here, we use carbon stable isotope ratios to establish the first known case of a temperate bat species consuming substantial quantities of nectar during spring. We show that pallid bats (*Antrozous pallidus*) switch from a diet indistinguishable from that of sympatric insectivorous bat species in winter (when no cactus nectar is present) to a diet intermediate between those of insectivorous bats and nectarivorous bats during the spring bloom of a bat-adapted cactus species. Combined with previous results that established that pallid bats are effective pollinators of the cardon cactus (*Pachycereus pringlei*), our results suggest that the interaction between pallid bats and cardon cacti represents the first-known plant-pollinator mutualism between a plant and a temperate bat. Diet plasticity in pallid bats raises questions about the degree of physiological adaptations of insectivorous bats for incorporation of carbohydrate-rich foods, such as nectar or fruit, into the diet.

Keywords *Antrozous pallidus* · Insectivory · Mutualism · Nectarivory · *Pachycereus pringlei*

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Introduction

Many plants offer nutrient rewards to animals (e.g., nectar, pollen, fruit) in exchange for the chance of pollen or seed dispersal to achieve successful reproduction. Mutualisms between plants and animals have led to a diversity of specialized diet strategies in a range of animal taxa, including mammals, birds and arthropods. In addition to specialized nectarivores or frugivores, some species opportunistically use plant resources during periods of seasonal availability (Wolf and Martínez del Rio 2000, 2003) or during particular events (e.g., migration) (Afik and Karasov 1995; McWilliams and Karasov 2001). Seasonal diet plasticity is common in birds, particularly in migratory songbirds, which can use digestive plasticity to cope physiologically with radically different diet sources, such as insects, seeds, and fruit or nectar (Afik and Karasov 1995; Karasov 1996; Murphy 1996).

Dietary switches from protein-rich insects to carbohydrate-rich nectar and fruits are physiologically challenging (Afik and Karasov 1995; Karasov 1996). Although numerous insectivorous bird species seasonally consume nectar or fruit (Herrera 1984; Levey and Martínez del Rio 2001; McWilliams and Karasov 2001; Parrish 2000; Pierce and McWilliams 2005; Wolf and Martínez del Rio 2003), diet switching from insects to fruit or nectar is essentially undocumented in the other primary group of insectivorous volant endotherms: bats (Frick et al. 2009). Although globally there is a wide diversity of diet strategies in bats, the occurrence of facultative nectar-feeding and diet switching from primarily insectivorous diets to supplementation with plant rewards is extremely rare in bats (Frick et al. 2009). Here, we present a novel case of seasonal facultative nectar-feeding in a temperate insectivorous bat and use stable carbon isotopes to demonstrate dependence on this seasonally abundant food source.

Many tropical nectar-feeding bats supplement nectar or fruit diets with insects for protein (Herrera et al. 2001b, 2002; Voigt et al. 2008b, 2011), but the converse strategy of insect-eating bats supplementing with nectar is known only in two species: the short-tailed bat (*Mystacina tuberculata*) and the pallid bat (*Antrozous pallidus*) (Arkins et al. 1999; Frick et al. 2009). The short-tailed bat is one of two bat species found in New Zealand and has unusual ground-foraging habits and an omnivorous diet strategy that includes pollen and nectar. Lack of terrestrial mammalian predators has been suggested in promoting unusual foraging and diet behavior in this species (Arkins et al. 1999). The pallid bat is a temperate species in the family Vespertilionidae, the largest family of bats (approximately 318 species) composed entirely of obligate insectivores. Insectivory is the ancestral condition in echolocating bats and remains the most common diet strategy across bat lineages (Arita and Fenton 1997; Simmons et al. 2008).

Pallid bats are thought to be primarily insectivorous with a diet composed of large arthropods, such as scorpions or crickets (Bell 1982; Hermanson and O'Shea 1983). Recently, this species was discovered to visit and pollinate flowers of the cardon cactus (*Pachycereus pringlei*) on the Baja California peninsula in northwestern Mexico (Frick et al. 2009, 2013). Pallid bats are successful and reliable pollinators of the cardon on the Baja peninsula (Frick et al. 2013) and have been observed actively lapping nectar from cardon flowers (Frick et al. 2009), yet the relative importance of nectar or pollen in their diet remains unknown (Herrera et al. 1993). By quantifying consumption and reliance on nectar or pollen by pallid bats, we aim to clarify whether the interaction between pallid bats and cardon cacti constitutes a mutualism, which may provide insight into the evolutionary trajectories and adaptations of both bat and plant. Furthermore, estimating nectar consumption by pallid bats raises new questions about the physiological flexibility of this bat species to cope with different diet sources and about constraints on digestive plasticity in volant mammals compared to birds.

Columnar cacti in Sonoran desert habitats function as keystone producers by providing spatio-temporally predictable and prolific resources of floral nectar, pollen and fruit during spring and summer months (Fleming 2002; Fleming et al. 2001; Wolf and Martínez del Rio 2003; Wolf et al. 2002). Nectar and fruit from columnar cacti in the Sonoran desert are an important dietary resource for a diversity of animals, including insects (Fleming et al. 1996), birds (Fleming et al. 1996; Wolf et al. 2002; Wolf and Martínez del Rio 2003), and several bat species (Arizmendi et al. 2002; Horner et al. 1998). For example, saguaro (*Carnegeia gigantea*) fruit provide both nutrient and water resources to doves (*Zenaida* spp.) and other bird species (Wolf et al. 2002; Wolf and Martínez del Rio 2003). The spring bloom of saguaro and cardon cacti along with several agave species (*Agave* spp.) provide a "nectar corridor" for migratory nectar-feeding bats that migrate from southern and central parts of Mexico to northwestern Mexico and into southwestern United States each spring (Fleming et al. 1993; Rojas-Martínez et al. 1999). Although visited by a variety of species, columnar cacti (particularly cardon) conform to a chiropterophilous pollination syndrome and depend heavily on bats for pollination (Fleming et al. 2001).

Cacti and agaves use the crassulacean acid metabolism (CAM) photosynthetic pathway, which produces a different ratio of stable carbon isotopes, $^{13}C/^{12}C$ [expressed in delta notation as $\delta^{13}C$ relative to the Pee Dee belemnite standard (VPDB)] compared to plants that use the C_3 photosynthetic pathway (e.g., trees, shrubs) (Griffiths 1992; O'Leary 1981). CAM plants tend to be much higher in ^{13}C than C_3 plants (Herrera et al. 2001b), producing mean $\delta^{13}C$ values from CAM sources of —12 ‰ compared to —27 ‰ from C_3 sources (Smith and Epstein 1971). Distinguishing between C_4 and CAM plants can be more challenging, but the paucity of C_4 plants in Sonoran desert habitats simplifies interpretation in our study region (Fleming et al. 1993; Wolf and Martínez del Rio 2003). Differences in $\delta^{13}C$ between C_3 and CAM or C_4 photosynthetic pathways can be used to determine the relative contribution of different plant sources in diets of consumers (Kelly 2000; Tieszen et al. 1983). For example, comparison of carbon stable isotope values can determine relative diet inputs of floral products (e.g., nectar) from cacti versus arthropods, which should generally reflect C_3 signatures from the dominant plant community (Herrera et al. 1993, 2006). However, if insects switch to CAM sources when available, insect-based diets could display a CAM signal during the cacti flowering season, obscuring interpretation (Herrera et al. 1993).

Carbohydrates from consumed nectar may be combusted directly to fuel metabolism and are not necessarily incorporated for tissue synthesis and storage (Voigt et al. 2008b; Voigt and Speakman 2007). However, these carbohydrates are present in exhaled breath, which can be sampled easily in a non-invasive and effective way (Hatch 2002; Perkins and Speakman 2001). Breath is particularly valuable for assessing current diet as it provides a near instantaneous measure of dietary items when compared to the integration time into tissues such as muscle or blood (Voigt et al. 2008a, b; Voigt and Speakman 2007). Turnover times in exhaled breath are generally on the scale of minutes (Voigt et al. 2008b; Welch et al. 2008), whereas turnover for muscle tissue or blood can range from days to months (Mirón et al. 2006; Voigt et al. 2003). Estimates of turnover rates in bat blood from laboratory studies have ranged widely from 100 to 134 days (Voigt et al. 2003) to 24–39 days (Mirón et al. 2006), and may depend on the quality of the diet (Mirón et al. 2006; Voigt and Speakman 2007). Comparing isotopic values in exhaled breath to values in other tissues, such as muscle or blood, may provide evidence of nutrient routing and help accurately describe diets of omnivorous animals (Voigt et al. 2008b).

We compare carbon stable isotope values in exhaled breath, blood, and wing tissue to determine seasonal variation in the diet of pallid bats compared to sympatric nectarivorous and insectivorous bat species. We hypothesized that pallid bats substantially supplement their diet with nectar during spring months when cactus flowers are widely available, but are primarily insectivorous during winter months when no cactus flowers are present. To test this hypothesis, we compared stable isotope values from pallid bats and sympatric bat species in two seasons: winter (January), when cactus nectar is unavailable, and spring (May), when cactus flowers are abundant on the landscape. We predicted that pallid bats would have similar carbon isotope ratios in exhaled breath to insectivorous bats during winter, but would have values intermediate between those of insectivorous bats and nectarivorous bats during spring, if they exhibit a seasonal shift in foraging dependence on cactus nectar when seasonally abundant. We also explored whether a shift from a primarily insectivorous diet to a diet dependent on nectar or pollen would be reflected in changes in nitrogen stable isotope ratio ($\delta^{15}N$) values, which are frequently used to infer trophic position (Kelly 2000).

We suspected that pallid bats may route carbohydrates from nectar to fuel metabolism while using proteins for tissue synthesis or storage, as has been shown in omnivorous phyllostomid bats (Voigt et al. 2008b; Voigt and Speakman 2007). If pallid bats employ nutrient routing or if integration times in blood and wing tissue are much longer than the time between sampling and the onset of nectar availability, carbon isotope values reflective of CAM sources may only be present in exhaled breath. We sampled during the mid to late flowering period (approximately 30 days after the onset of flowering) to increase the probability that changes in diet would be detectable in all tissues. We hypothesized that $\delta^{13}C_{breath}$ values would be higher (e.g., more CAM-like) than $\delta^{13}C_{blood}$ and $\delta^{13}C_{tissue}$ values during spring, if pallid bats route carbohydrates from nectar to fuel metabolism or turnover times in blood or wing tissue are slow relative to the onset of flower availability (Voigt et al. 2008b). Carbon isotope values consistent with a seasonal shift to substantial consumption of cactus nectar in pallid bats would document the first case of seasonal plasticity to directly utilize plant food resources in a temperate bat species.

Materials and methods

Sample collection

Bats were sampled in study locations near Loreto and the Sierra de la Giganta in Baja California Sur, Mexico where pallid bats have been observed visiting cardon flowers (Frick et al. 2009). Habitat vegetation conforms to the Sonoran Desert sarcocaulescent type dominated by columnar cacti (*P. pringlei*, *Stenocereus thurberias*) and desert trees (*Cercidium*, *Bursera*, and *Jatropha*) (Shreve 1951; Wiggins 1980). We collected samples of exhaled breath, blood, and wing tissue during winter (January 2011) and spring (May 2011) for pallid bats and insectivorous bat species (Table 1). Nectar-feeding bats were sampled during spring, but migrate away from the study area and were unavailable for sampling during winter (Fleming et al. 1993; Rojas-Martínez et al. 1999). Isotopic values of diet source material from C_3 plant material, C_3 insects, and CAM plant material were collated from the literature (Table 2). Values for C_4 were not included because these plants are rare in the study region (Wolf and Martínez del Rio 2003).

We captured bats using mist nets placed near night roosts, over water sources, or in stands of flowering cardon from sunset until midnight. Bats were held individually in cloth bags for 5–60 min and were examined for reproductive status, age, and identified to species (Racey 2009). To collect exhaled breath samples we placed each bat individually into a small sealed plastic container outfitted with a sealed needle at one end. The container was washed of CO_2 using air flushed by hand pump with NaOH before and after each bat was placed in the container. Each bat was sealed in the breath chamber for 5 min to allow exhaled CO_2 to accumulate. We connected evacuated Vacutainers (Labco, Buckinghamshire, UK) to the needle fused to the breath chamber to collect exhaled breath (Voigt et al. 2008b). Bats were monitored for signs of stress during

Table 1 Mean (±SE) carbon stable isotope ratio ($\delta^{13}C$) values in exhaled breath, blood, and wing tissue of sampled bat species by season

Species	Foraging guild	Season	Exhaled breath		Blood		Wing tissue	
			δ^{13}C (SE)	*n*	δ^{13}C (SE)	*n*	δ^{13}C (SE)	*n*
Antrozous pallidus	Omnivorous	Winter	−25.5 (0.8)	10	−17.5 (0.6)	7	−19.0 (0.5)	11
		Spring	−15.8 (0.4)	22	−14.7 (0.2)	26	−15.5 (0.2)	24
Corynorhinus townsendii	Insectivorous	Winter	–	–	–	–	–	–
		Spring	–	–	–	–	−20.9 (–)	1
Eptesicus fuscus	Insectivorous	Winter	–	–	–	–	–	–
		Spring	−24.0 (0.2)	2	−20.2 (0.3)	2	−19.2 (0.3)	2
Lasiurus xanthinus	Insectivorous	Winter	–	–	–	–	–	–
		Spring	–	–	–	–	−16.7 (–)	1
Myotis californicus	Insectivorous	Winter	−22.3 (3.0)	3	−18.2 (1.0)	2	−20.26 (0.2)	4
		Spring	−21.6 (1.5)	4	−21.2 (0.7)	4	−19.4 (0.4)	6
Parastrellus hesperus	Insectivorous	Winter	–	–	–	–	−19.7 (0.4)	2
		Spring	–	–	−21.5 (0.3)	5	−20.5 (0.3)	5
Nyctinomops femorosaccus	Insectivorous	Winter	−23.9 (0.3)	3	−19.9 (0.3)	3	−21.0 (0.4)	3
		Spring	–	–	–	–	−19.2 (–)	1
Tadarida brasiliensis	Insectivorous	Winter	–	–	–	–	−18.1 (–)	1
		Spring	–	–	–	–	–	–
Mormoops megalophylla	Insectivorous	Winter	−20.5 (3.7)	3	–	–	−20.8 (0.3)	3
		Spring	–	–	–	–	–	–
Macrotus californicus	Insectivorous	Winter	−23.5 (0.7)	3	–	–	−19.3 (0.1)	4
		Spring	−21.6 (1.1)	7	−19.5 (0.4)	13	−19.3 (0.3)	14
Choeronycteris mexicana	Nectarivorous	Winter	–	–	–	–	–	–
		Spring	−13.2 (0.01)	3	−10.4 (0.3)	2	−11.6 (0.2)	2
Leptonycteris yerbabuenae	Nectarivorous	Winter	–	–	–	–	–	–
		Spring	−12.3 (0.1)	2	−12.5 (0.7)	11	−12.1 (0.6)	14

Table 2 Mean (±SE) δ^{13}C values from diet sources taken from the literature

Diet source type	*n* species	Mean δ^{13}C (SD)	Locality	References
C_3 plant material	10	−26.4 (0.5)	Sonora, Mexico	Fleming et al. (1993)
C_3 fruits	42	−27.2 (1.8)	Dry season; Jalisco, Mexico	Herrera et al. (2006)
C_3 fruits	24	−27.7 (2.0)	Rainy season; Jalisco, Mexico	Herrera et al. (2006)
C_3 seeds	12	−24.9 (0.2)	Southern Arizona, USA	Wolf and Martínez del Rio (2000)
C_3 fruits	9	−24.4 (1.5)	Jalisco, Mexico	Shipley (unpublished data)
C_3 plant summary		−26.12 (1.4)		
C_3 insects	60	−24.9 (2.8)	Dry season; Jalisco, Mexico	Herrera et al. (2006)
C_3 insects	39	−25.8 (2.1)	Rainy season; Jalisco, Mexico	Herrera et al. (2006)
C_3 insect summary		−25.35 (0.6)		
CAM plant material[a]	21	−12.6 (0.3)	Sonora, Mexico	Fleming et al. (1993)
CAM fruits	8	−13.7 (0.6)	Dry season; Jalisco, Mexico	Herrera et al. (2006)
CAM fruits	12	−13.7 (0.7)	Rainy season; Jalisco, Mexico	Herrera et al. (2006)
CAM nectar[b]	1	−12.7 (0.4)	Southern Arizona, USA	Wolf and Martínez del Rio (2000)
CAM fruit[b]	1	−13.1 (0.3)	Southern Arizona, USA	Wolf and Martínez del Rio (2000)
CAM plant summary		−13.2 (0.5)		

[a] Cactus and agave species

[b] Saguaro (*Carnegiea gigantea*) fruit

containment and released on site. We drew approximately 50 µl of blood by venipuncture with a small sterile needle in an uropatagial vein and collected it using a capillary tube (Voigt and Cruz-Neto 2009). Blood was kept refrigerated or frozen until analyzed. Wing tissue was collected with a sterile 3-mm wing biopsy punch and preserved by desiccation (Simmons and Voss 2009).

Stable isotope analysis

Vacutainers containing exhaled CO_2 samples were analyzed on a GV Instruments MultiFlow coupled to a GV Instruments IsoPrime isotope ratio mass spectrometer at the Boston University Stable Isotope Laboratory (http://www.bu.edu/sil/). Gas calibration standards, as well as air samples from Boston, Massachusetts were analyzed for both quality control and data correction. Each Vacutainer was sampled once (single peak sampling) and several were sampled multiple times as a check on isotopic variability. Each sample isotope ratio was compared to NBS 20 (Solnhofen limestone), an in house gas standard, whose isotope ratio is calibrated to international standards. International standards were obtained from the National Bureau of Standards in Gaithersburg, Maryland.

Blood and wing tissue samples were prepared and analyzed at the stable isotope facility at the University of Oklahoma. Wing tissues were cleaned by immersing them in 2:1 chloroform:methanol within an Eppendorf tube and shaking the tube for 30 s (Paritte and Kelly 2009). We drained the solvent and allowed the sample to air dry for 24 h under a fume hood. We cleaned the samples in detergent once with 1 L of a 1:30 (detergent: deionized water; v/v) solution of Fisher Versa-Clean (catalog number 04-0342; Fisher Scientific), then rinsed the samples three times in three 1-L baths of deionized water, and allowed the samples to air dry for 24 h under a fume hood. Blood samples were prepared by drying to constant mass.

We weighed tissue samples of 350 ± 10 µg and loaded them into 3.5-mm × 5-mm tin capsules. Samples were organized into runs of 49 that contained 40 samples and nine standards. Standards comprised seven replicates of an in-house standard (finely powdered feathers from a brown-headed cowbird) (Kelly et al. 2009) and one USGS-40 and one USGS-41 purchased from the National Institute of Standards and Technologies. Isotope ratio data were processed with a ThermoFinnigan Delta V isotope ratio mass spectrometer connected to a Costech elemental analyzer. Samples were automatically dropped from a 50-position zero-blank autosampler with standards spaced evenly through the run to allow correction for instrumental drift. Corrections follow methods described by Sharp (2007).

Ratios of $^{13}C/^{12}C$ and $^{15}N/^{14}N$ are expressed in δ notation as the relative difference (per mil ‰) between the samples and international standards (VPDB for C and air for N). For example, the $\delta^{13}C$ values are calculated as:

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

Analysis

We used two-way ANOVA to test whether mean $\delta^{13}C$ and $\delta^{15}N$ values differed among feeding groups (insectivorous bats, pallid bats, nectarivorous bats) and season (winter, spring) ($\delta^{13}C$ ~ feeding group × season). We performed an ANOVA for each of three tissue types: exhaled breath, blood, and wing tissue. The design was not fully factorial because nectar bats do not occur in the study area in winter. We applied Tukey's honest significant difference (HSD) method to adjust for type I errors in post hoc pairwise comparisons among groups. We compared $\delta^{13}C$ values for breath versus blood and wing tissue samples for pallid bats during spring using *t*-tests to determine whether there were significant differences in $\delta^{13}C$ values that may indicate nutrient routing of carbohydrates to fuel metabolism. We compared spatial variation in $\delta^{15}N$ values for pallid bats in spring among site locations ($n = 6$) using ANOVA with site locality as a fixed effect.

Isotopic mixing models

To determine relative contributions to diet from C_3 and CAM sources, we used a Bayesian mixing model approach implemented in the stable isotope analysis in the SIAR package (Parnell et al. 2010) in program R (Team 2012). The SIAR mixing model uses multiple dietary sources and incorporates uncertainty in input parameters to produce potential dietary solutions as true probability distributions (Parnell et al. 2010). We used a carbon-only mixing model with means and SDs of C_3 (−25.9 ± 0.75) and CAM (−13.5 ± 1.6) sources derived from the literature (Table 2). Our mixing model does not resolve whether CAM-insects contribute to the diet, but we rely on behavioral observations that show pallid bats consume nectar from flowers regularly and rarely predate on nectar-feeding moths (Frick et al. 2009). Trophic enrichment factors (TEFs) were estimated as 3.4 ‰ $\Delta^{13}C$ for C_3 and 2.7 ‰ $\Delta^{13}C$ for CAM for blood and wing tissue based on laboratory studies on two nectarivorous phyllostomid bats (*Leptonycteris curasoae*; *Glossophaga soricina*) (Voigt et al. 2003) and two insectivorous vespertilionid bats (*Myotis myotis*; *Myotis nattereri*) (Siemers et al. 2011). TEFs for breath were estimated as 1.0 ‰ $\Delta^{13}C$ for C_3 and −1.6 ‰ $\Delta^{13}C$ for CAM based on values reported from laboratory studies on nectar-feeding bats (*Carollia perspicillata; Glossophaga soricina*) (Voigt

et al. 2008b; Voigt and Speakman 2007). Bayesian mixing models are sensitive to trophic discrimination factors (Bond and Diamond 2011) and TEFs that are not species or tissue specific have been criticized (Caut et al. 2009). SIAR can incorporate uncertainty in TEFs and we chose empirically estimated TEFs from species that are phylogenetically related and with similar dietary habits that were available in the literature and assigned a SD of 1.5 to account for additional uncertainty. Results from Bayesian mixing model are reported as mean proportions with 95 % credible intervals (CIs), which are a Bayesian equivalent of a confidence interval and are based on the posterior probability distribution.

Results

$\delta^{13}C$ values differed among feeding groups (insectivorous bats, pallid bats, nectarivorous bats) and seasons for all three tissue types (ANOVA: exhaled breath, $F_{4,37} = 37.62$, $p < 0.0001$, $R^2 = 0.71$; blood, $F_{4,70} = 68.17$, $p < 0.0001$, $R^2 = 0.78$; wing tissue, $F_{4,93} = 115.9$, $p < 0.0001$, $R^2 = 0.83$; Fig. 1). Across all tissue types, pairwise comparisons indicate no significant differences in $\delta^{13}C$ values between winter and spring for insectivorous bats and no significant differences between insectivorous bats and pallid bats in winter (Fig. 1). $\delta^{13}C$ values for pallid bats were significantly higher in spring than in winter for all tissue types, with the greatest difference observed in exhaled breath [breath, 9.7, 95 % CI 6.7–12.7, adjusted (adj.) $p < 0.0001$; blood, 2.9, 95 % CI 0.84–4.9, adj. $p < 0.0001$; wing tissue, 3.5, 95 % CI 2.0–4.9, adj. $p < 0.0001$]. In spring, $\delta^{13}C$ values in pallid bats were significantly higher than values for insectivorous bats (breath, 6.2, 95 % CI 3.4–8.9, adj. $p < 0.0001$; blood, 5.6, 95 % CI 4.3–7.0, adj. $p < 0.0001$; wing tissue, 4.0, 95 % CI 2.9–5.0, adj. $p < 0.0001$). $\delta^{13}C$ values in pallid bats in spring were significantly lower than in nectarivorous bats in blood and wing tissue (blood, −2.5, 95 % CI −4.1 to −0.85, adj. $p < 0.0004$; wing tissue, −3.5, 95 % CI −4.7 to −2.21), adj. $p < 0.0001$). There was no significant difference between pallid bats and nectarivorous bats in exhaled breath samples (adj. $p = 0.24$). Comparison of carbon isotope values across tissues to test for nutrient routing revealed there were no significant differences between carbon isotope values in exhaled breath and either blood (t-value = 0.45; $df = 22$; p-value = 0.66) or tissue (t-value = −1.28; $df = 26$; p-value = 0.21) for pallid bats during spring.

Analysis of $\delta^{15}N$ revealed no significant differences between insectivorous bats and nectarivorous bats in spring for either blood (−0.54, 95 % CI −2.2 to 1.1, adj. $p = 0.93$) or wing tissue (−0.77, 95 % CI −2.1 to 0.5, adj. $p = 0.50$), revealing that variation in $\delta^{15}N$ overlapped across trophic levels in this system (Fig. 2; Table S1). Mean values of $\delta^{15}N$ for pallid bats were higher than for both insectivorous bats and nectarivorous bats during spring months, although not always significantly (Fig. 2). An analysis of spatial variation in $\delta^{15}N$ for pallid bats, showed that bats captured at two sites over freshwater had significantly lower $\delta^{15}N$ values than at xeric sites (ANOVA: blood, $F_{5,27} = 9.7$, $p < 0.0001$, $R^2 = 0.64$; wing tissue, $F_{5,18} = 17.02$, $p < 0.0001$, $R^2 = 0.77$; Fig. S1; Table S2). This spatial

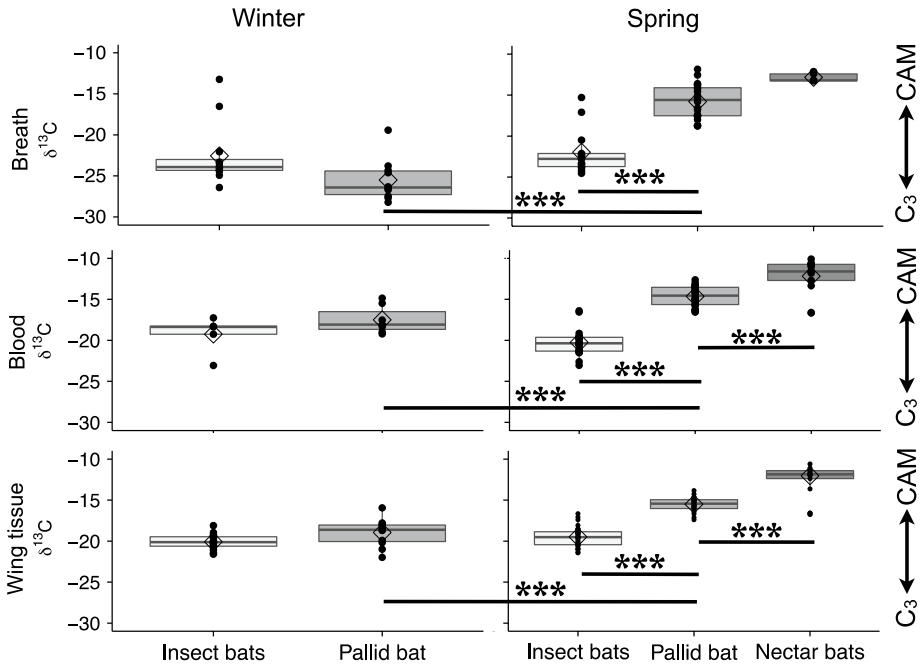

Fig. 1 Box plots of carbon stable isotope ratio ($\delta^{13}C$) values for each feeding group (insectivorous bats, pallid bats, nectarivorous bats) by season (winter, spring) for three different tissue types (exhaled breath, blood, wing tissue). *Lines* inside boxes represent medians and *open diamonds* display means. *Points* overlaid on box plots represent data values for each sample. *Black horizontal lines* indicate pairwise comparisons where group means were significantly different (***$p < 0.001$) after applying Tukey's honest significant difference (HSD) method. *CAM* Crassulacean acid metabolism

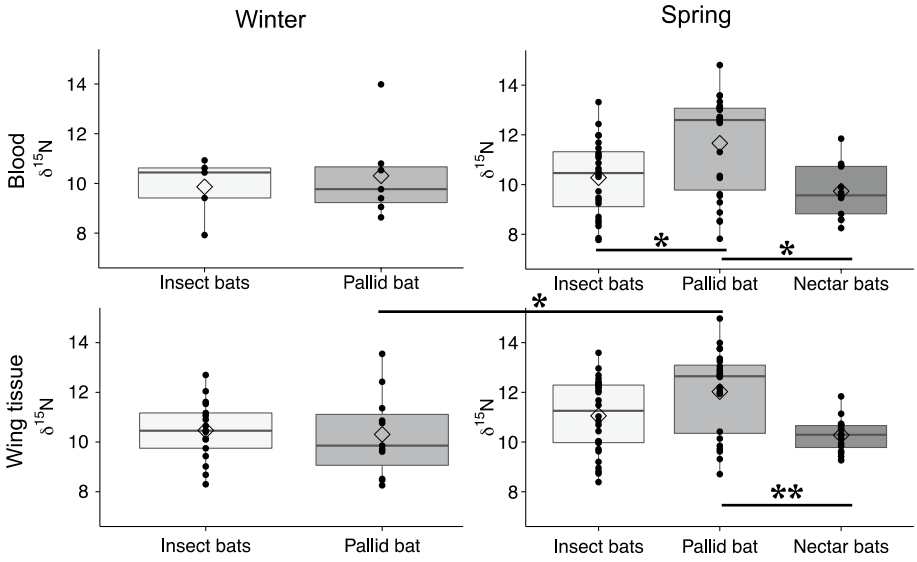

Fig. 2 Box plots of nitrogen stable isotope ratio ($\delta^{15}N$) values for each feeding group (insectivorous bats, pallid bats, nectarivorous bats) by season (winter, spring) for two tissue types (blood, wing tissue). *Lines* inside boxes represent medians and *open diamonds* display means. *Points* overlaid on box plots represent data values for each sample. *Black horizontal lines* indicate pairwise comparisons where group means were significantly different (**$p < 0.01$, *$p < 0.05$) after applying Tukey's HSD method

pattern was not evident in carbon data (ANOVA: blood, $F_{5,20} = 1.8$, $p = 0.17$, $R^2 = 0.13$; wing tissue, $F_{5,18} = 1.3$, $p = 0.3$, $R^2 = 0.06$). Unfortunately, capture rates of nectarivorous and insectivorous bats were not distributed evenly across capture locations, precluding an analysis to explore the relationships between location, habitat characteristics, and feeding preferences.

Mixing model results show pallid bats switch from a majority of source carbon from C_3 sources in winter to a majority of source carbon from CAM sources in spring across all tissue types (Fig. 3). In contrast to the *t*-test comparisons across tissues, the mean proportion of CAM carbon in the diet was considerably higher in exhaled breath compared to blood or wing tissue for pallid bats in spring (exhaled breath, 0.92, 95 % CI 0.85–1.0; blood, 0.67, 95 % CI 0.62–0.72; wing tissue, 0.60, 95 % CI 0.55–0.64; Fig. 3). Similarly, the mean proportion of C_3 carbon was much higher for exhaled breath compared to blood or wing tissue for pallid bats in winter (exhaled breath, 0.94, 95 % CI 0.85–1.0; blood, 0.57, 95 % CI 0.45–0.69; wing tissue, 0.69, 95 % CI 0.60–0.79; Fig. 3). Insectivorous bats showed no appreciable difference in source carbon across seasons and remained dominated by C_3 in spring (exhaled breath, 0.69, 95 % CI 0.51–0.85; blood, 0.80, 95 % CI 0.74–0.86; wing tissue, 0.73, 95 % CI 0.68–0.79; Fig. 3). Nectarivorous bats consumed a majority of CAM-based carbon in spring (exhaled breath, 0.90, 95 % CI 0.61–1; blood, 0.88, 95 % CI 0.78–0.98; wing tissue, 0.89, 95 % CI 0.80–0.98) (Fig. 3).

Discussion

Our results show that pallid bats employ diet plasticity to take advantage of a seasonal bloom of a keystone cactus species. During winter when cactus nectar is unavailable, the pallid bat diet reflects a strong signal of C_3 carbon sources, similar to sympatric insectivorous bat species (Figs. 1, 3). In spring, the diet of pallid bats displays a strong shift in carbon source pools to a majority of carbon derived from CAM-based sources and appears intermediate to insectivorous and nectarivorous diets (Figs. 1, 3). Pallid bats likely continue to forage on typical prey items, such as arthropods, but a significant portion of their diet appears to be fueled by nectar consumption in spring. Combined with our earlier work that established that the pallid bat is an effective pollinator of cardon (Frick et al. 2013), our results suggest interactions between the pallid bat and cardon represent the first known example of a plant-pollinator mutualism between a temperate bat and a plant.

The switch from C_3 to CAM carbon was evident in all three tissues, suggesting that pallid bats are not just using nectar to fuel metabolism, but that carbohydrates from nectar are also incorporated into tissues (Voigt et al. 2008b). Pallid bats may also consume cactus pollen while visiting flowers, and carbon (and nitrogen) in tissues could result from both nectar and pollen consumption. However, we do not yet have any observational data of pallid bats grooming and consuming pollen. Results from the Bayesian mixing model suggest there may be some nutrient routing as the mean proportion of CAM sources in the diet in exhaled breath was 0.92 (95 % CI 0.85–1.0), but was lower for both blood and tissue (Fig. 3). This discrepancy between exhaled breath and blood and wing tissue values was also evident in winter, when there was a much higher proportion of C_3 sources in the diet based on exhaled breath versus blood or wing tissue (Fig. 3). These differences among tissues may suggest both differences in turnover times in the three tissues we sampled as well as nutrient routing (Voigt

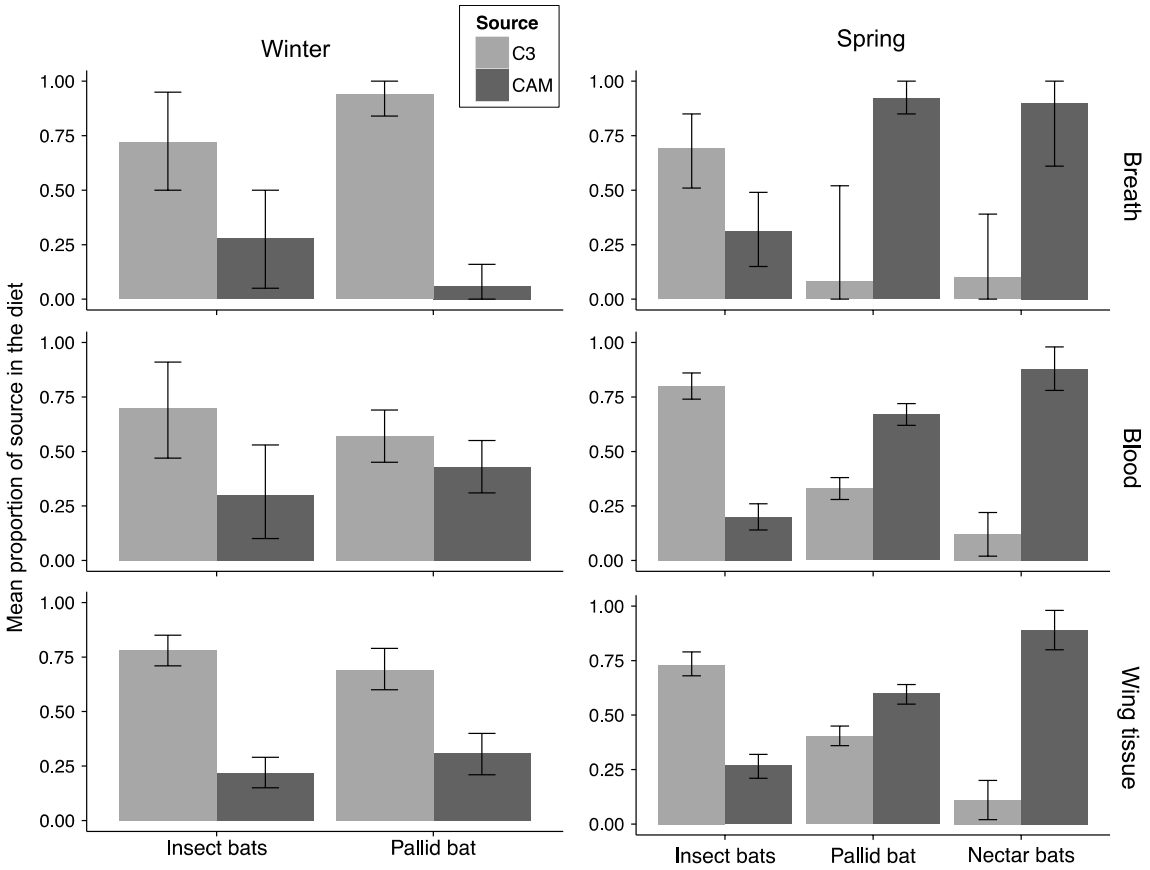

Fig. 3 Mean proportion of source in the diet for each feeding group (insectivorous bats, pallid bats, nectarivorous bats) by season (winter, spring) for three different tissue types (exhaled breath, blood, wing tissue) as estimated by the Bayesian mixing model. *Bars* represent means from the true probability distribution and *error bars* represent the 95 % credible interval values. Insectivorous bat diets are dominated by C_3 sources in winter and spring. Pallid bat diets shift from C_3 sources in winter toward CAM sources in spring. Nectar bat diets are dominated by CAM sources in spring. Mean proportions of C_3 in winter and CAM in spring are highest in exhaled breath for pallid bats

et al. 2008b). Exhaled breath should reflect the most immediate diet inputs and blood and wing tissue should represent longer temporal periods (days to months) (Mirón et al. 2006; Voigt et al. 2003).

Herrera et al. (1993) proposed that CAM-based carbon inputs in pallid bats may result from foraging for insects at cardon flowers given that pallid bats lack any obvious morphological adaptations for nectar feeding. However, Frick et al. (2009) showed that pallid bats directly consume cactus nectar from flowers and that bat-insect interactions at flowers are rare (<2 % of flower visits; Frick et al. 2009). Carbon isotope values from muscle tissue from museum specimens and live-captured pallid bats collected in areas with columnar cacti (Baja California and Sonora) during the flowering and fruiting season (April–July) were similar to those found for pallid bats in this study (Bahia Kino, Sonora, April, $\delta^{13}C = -16.77$ ‰ VPDB $\pm$ 0.44 SE, $n = 3$; Baja California Norte, June–July, $\delta^{13}C = -12.58$ VPDB $\pm$ 0.60 SE, $n = 4$) (Herrera et al. 1993). Data reported in Herrera et al. (1993) support our results that CAM-source carbon is important for pallid bats where they are sympatric with columnar cacti. By comparing carbon isotope ratios for pallid bats to those of sympatric insectivorous bats and nectarivorous bats, we show a seasonal shift in consumption of CAM-based carbon sources in pallid bats that appears intermediate between typical insectivorous diets that remain strongly dominated by C_3 sources in spring and nectarivorous diets that are strongly dominated by CAM-based carbon in spring.

Seasonal northern distributions of migratory nectar-feeding bats are tightly coupled with availability of columnar cacti and agave species (Fleming et al. 1993) and represent the temperate limit of nectar-feeding behavior in New World bats (Rojas-Martínez et al. 1999). In contrast, the pallid bat is a non-migratory species with a primarily temperate distribution that extends from central Mexico into British Colombia (Hermanson and O'Shea 1983; Weyandt and Van Den Bussche 2007). Pallid bats occur year-round

in habitats on the Baja California peninsula (W. F. Frick, unpublished data). Results presented here suggest that pallid bats use diet plasticity to take advantage of a prolific nectar resource in the spring but are able to switch back to arthropod prey sources when nectar is unavailable. Columnar cacti which provide important nutrients and water to several non-nectar specialist bird species (Wolf and Martínez del Rio 2003), and support the northern-most extent of nectarivory in bats (Rojas-Martínez et al. 1999), seem to facilitate this opportunistic diet switching in pallid bats. The near absence of omnivorous habits and diet switching behavior in temperate bats is curious when compared to relative diet plasticity in many bird species (Herrera 1984; Levey and Martínez del Rio 2001; McWilliams and Karasov 2001; Parrish 2000; Pierce and McWilliams 2005; Wolf and Martínez del Rio 2003) and raises questions about evolutionary constraints that may limit diet plasticity in insectivorous bats.

The switch from protein-rich insectivorous diets to a carbohydrate-rich fruit/nectar diet is physiologically challenging (Afik and Karasov 1995; Karasov 1996) and studies have demonstrated that birds use digestive plasticity, such as changing gut size or enzyme activity, to efficiently process radically different food sources (McWilliams et al. 2004). Nectar-feeding glossophagine bats have digestive adaptations, such as large amounts of sucrase in their digestive tracts (Hernandez and Martínez del Rio 1992), that help process sugary diets rapidly (Voigt and Speakman 2007). Diets dependent on dilute nectar are devoid of protein and many phyllostomid bats supplement diets with pollen or insects for protein synthesis (Herrera et al. 2001b, 2002; Voigt et al. 2008b). Cardon nectar is dominated by hexoses, which may be easier to digest and favor transition from insect to nectar feeding in bats (Herrera 1999). Pallid bats likely consume some cardon pollen when visiting flowers for nectar, but whether they have digestive enzymes for breaking down the exine coating of pollen (Stanley and Linskens 1974) is not known. Herrera and Martínez del Rio (1998) showed that phyllostomid bats that are predominately frugivorous and visit flowers seasonally are less efficient than year-round flower-visiting bats at extracting pollen. Comparing the digestive function of pallid bats to omnivorous phyllostomid bats or insectivorous passerines may provide additional insights into the unusual diet strategy of pallid bats. Future work could also focus on determining whether pallid bats are capable of extracting nutrients from pollen by examining pollen grains in feces (Herrera and Martínez del Rio 1998).

One key physiological difference between phyllostomid nectar-feeding bats and insectivorous bats is kidney morphology and function (Herrera et al. 2001a). Nectarivorous diets produce copious dilute urine with a need for the concentration of salts, whereas insectivorous diets require kidneys that conserve water while excreting salts and urea (Herrera et al. 2001a). These competing needs are reflected in the dimorphism in kidney structure related to the ratio of thickness of the medulla and cortex in nectarivorous and insectivorous bats (Herrera et al. 2001a). The ability to maximize urine concentration is heightened in desert bats that are often water stressed due to limited availability of free water (Geluso 1978). Pallid bats have renal adaptations for maximizing urine concentration typical of bats adapted for desert environments that can sustain long periods without access to free water (Geluso 1975, 1978). These adaptations are in contrast to the renal structure and function typical of nectarivorous bats. Future comparisons of the renal structure of pallid bats in desert habitats within and outside of the range of flowering cacti could provide potential insights into the role of renal adaptations and diet in bats. Furthermore, differences in hydrogen isotopic ratio values in cardon nectar and surface water (Wolf and Martínez del Rio 2003), could be used to determine whether cactus nectar is an important source of hydration for pallid bats.

Analysis of nitrogen isotopes was not useful for interpreting trophic positioning in this arid terrestrial system, given that insectivorous and nectarivorous bats had overlapping nitrogen isotope values (Fig. 2). We found significant spatial variation in nitrogen isotope values for pallid bats in spring, with significantly lower nitrogen isotope values at sites where pallid bats were captured over freshwater than at xeric sites (Table S2). Our results are consistent with other studies that show that habitat moisture has a strong influence on nitrogen isotope ratios for terrestrial mammals (Kelly 2000). Future work could be done to disentangle the effects of access to freshwater and dietary habits in arid terrestrial systems.

Our results suggest that pallid bats consume significant amounts of nectar and pollen during the spring flowering season and use these carbohydrates to fuel metabolism as well as incorporate it into tissues. Earlier work demonstrated that pallid bats are effective pollinators of cardon in this same system (Frick et al. 2013). We conclude that the interaction between pallid bats and cardon cacti is likely mutualistic. Although pallid bats have probably been consuming cactus nectar for a long time, this interaction may be an example of an evolutionarily new mutualism that displays the early stages of a novel plant-animal interaction. Generalist diet strategies that employ plasticity to take advantage of seasonally abundant resources may be a key innovation for evolution of mutualisms between plants and animals. Future work is necessary to understand the physiological adaptations to support diet plasticity in bats and to determine the contribution of nectar feeding to reproduction or overall fitness, which could inform general theories on costs and benefits related to specific foraging behaviors (Pyke et al. 1977; Ricklefs 1996).

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