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INSIGHTS

Preference for *Collpa* Water by Frugivorous Bats (*Artibeus*): An Experimental Approach

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ABSTRACT

Several species of stenodermatine bats congregate in large numbers at *collpas* in southeastern Peru to drink water. We conducted the first experimental tests of preference for *collpa* water by representative bats. *Artibeus* species preferred mineral-rich *collpa* water over water from other sources, supporting the hypothesis that they seek resources (especially sodium) at *collpas*.

Abstract in Spanish is available at http://www.blackwell-synergy.com/loi/btp.

Key words: licks; Peruvian Amazon; Phyllostomidae; sodium; Stenodermatinae.

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In western Amazonia, large numbers of frugivorous bats congregate at *collpas* to drink water that has accumulated in soil depressions (Voigt *et al*. 2007; Bravo *et al*. 2008, 2010). The puddles are created and maintained by geophagous animals that repeatedly expose soil while consuming it (Brightsmith & Aramburú 2004, Montenegro 2004, Tobler 2008). Two main explanations for *collpa* visitation by bats have been proposed: (1) detoxification of plant secondary metabolites by clay (*e.g*., Voigt *et al*. 2008) and (2) supplementation of limited mineral resources (*e.g*., Tuttle 1974; Bravo *et al*. 2008, 2010). Based on exceptionally high and seasonally consistent sodium concentrations in *collpa* water ingested by frugivorous bats in southeastern Peru, Bravo *et al*. (2010) suggested that *collpas* chiefly function as sodium sources for these bats.

Although *collpas* are activity hotspots where bats drink *collpa* water (Ascorra & Wilson 1991; Voigt *et al*. 2007; Bravo *et al*. 2008, 2010), experimental evidence is lacking on whether bats can differentiate *collpa* water from other water sources. We completed a cafeteria-style choice experiment to test the hypothesis that frugivorous bats can discriminate between and prefer to drink *collpa* water over water from non-*collpa* sources.

METHODS

We conducted our experiments at Los Amigos Biological Station (12°34′09″ S, 70°06′01″ W, Department of Madre de Dios, SE Peru) in the lowland Amazonian forests between the Madre de Dios and the Los Amigos Rivers. From 2005 to 2007, the average annual temperature ranged from 23.9°C to 24.1°C and annual rainfall was 2152–2682 mm, unevenly distributed between the wet (October–April) and dry (May–September) seasons (Atrium 2008).

In April and August 2008, we experimentally tested the preferences of *collpa*-visiting bat species for *collpa* water compared with non-*collpa* water. We chose robust, medium-to-large-sized bat species that were common at *collpas* (Bravo *et al*. 2008, 2010) and easy to maintain in captivity: *Artibeus lituratus* (*ca* 70 g), *Artibeus planirostris* (*ca* 60 g), and *Artibeus obscurus* (*ca* 40 g). Although reproductive females are the most common bats at *collpas* (Bravo *et al*. 2008, 2010), we did not use reproductive females, to avoid causing them unnecessary stress. Nonreproductive females and males of the three focal species also visit *collpas* in mixed-species aggregations and drink water from them in the region (Bravo *et al*. 2008, 2010).

Experiments were conducted in a flight cage of 6.1 × 4.7 × 2.4 m built in *terra firme* forest under tree-canopy shade, to avoid overheating during the day. For ventilation, the sides of the cage were made of greenhouse shade-net and the top of the cage was covered with a tarp to protect bats from rain. We dug two identical rectangular holes 0.5 m apart in the center of the flight cage. In each hole, we fitted a 0.5 × 0.3 × 0.05 m aluminum pan to offer bats the opportunity to choose between two drinking-water sources: *collpa* and non-*collpa* water.

Although large numbers of bats visit *collpas* in the study area (Bravo *et al*. 2008, 2010), we chose to capture experimental bats at least 3.5 km from the nearest *collpa* to minimize biases in their responses to the experiment. We mist-netted *Artibeus* bats along trails previously established in the floodplain and *terra firme* forests from dusk (1745 h) to midnight. After capture, each individual was identified and placed in a clean cotton bag to be transferred to the flight cage. Bats that did not meet the criteria for the experiment were immediately released.

Individual bats were used only once in the experiments and they were kept for no more than two nights in captivity. On the night of capture, we placed a group of bats in the flight cage for acclimation with *ad libitum* food (bananas placed on a shelf) and creek water in one randomly determined pan. Food and water were

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TABLE 1. *Species, abundance, and sex of bats used in experimental trials, numbers of times bats drank water from the* collpa *or non-*collpa *water sources, and* P*-values for the binomial exact tests.* **Significant departure from equal probabilities between treatments.*

Date	Species, abundance and sex	*Collpa* water	Non-*collpa* water	*P*-values
04/4/08	*Artibeus lituratus* (3♂, 2♀)	29	4	< 0.01*
08/06/08	*A. lituratus* (2♂, 1♀)	8	5	0.58
08/10/08	*A. lituratus* (1♂), *Artibeus planirostris* (1♀)	19	12	0.28
08/13/08	*Artibeus obscurus* (2♂), *A. planirostris* (1♀)	32	12	< 0.01*
08/15/08	*A. lituratus* (1♂), *A. obscurus* (2♂)	15	3	< 0.01*
08/26/08	*A. lituratus* (1♂), *A. obscurus* (2♂)	25	19	0.45
08/28/08	*A. lituratus* (3♂)	51	20	< 0.01*
08/30/08	*A. lituratus* (2♀), *A. obscurus* (2♂)	34	25	0.29
09/03/08	*A. obscurus* (3♂), *A. planirostris* (1♀)	15	4	0.01*
09/06/08	*A. obscurus* (3♂)	29	1	< 0.01*

removed early the following day. On the second night, we conducted an experimental trial with that group of bats, which was released as soon as the trial ended. We conducted a total of ten trials with ten different groups from two to five bats each (Table 1). Based on preliminary trials when a single individual was placed in the flight cage it did not drink water from the pans. When more than one bat was in the flight cage, however, bats would drink water from the pans. Owing to the low numbers of conspecific bats captured per night during the dry season (August), some trials grouped two species of bats. No aggressive interactions were recorded whether or not more than one species was used in the trial.

To establish whether bats distinguish between and prefer *collpa* water vs. non-*collpa* water, we offered them a choice between two water sources: *collpa* vs. creek water/rainwater. *Collpa* water was collected from a *collpa* frequently visited by bats (*Collpa*/Lick No. 1 in Bravo *et al.* 2008, 2010). For the non-*collpa* source, we used rainwater for one trial (24 April 2008) conducted during the rainy season. We used creek water for the other nine trials, because rainwater was scarce during the dry season (August). We refer to creek water/rainwater as non-*collpa* water. The availability of water in the *collpas* depended on the amount of rainfall (28 mm for 1–28 August 2008). Thus, we used the same *collpa* water for two consecutive trials on a single occasion (26 and 28 August 2008). Although creek water was always available, we changed it at the same time that we changed the *collpa* water, to maintain standardized conditions between the two treatments. For each trial, we randomly assigned the two pans to one of the two water types.

DRINKING TRIALS.—All trials were conducted with the same protocol. At 1745 h, we filled each clean pan with either *collpa* or non-*collpa* water, as randomly assigned. We systematically rubbed ripe banana pulp once around the edges of both pans to initially attract bats to the pans (banana pulp was not applied to water pans during the acclimation period). Preliminary tests had shown that by rubbing banana along the edges of the pans, the likelihood that bats in the flight cage would visit the pans dramatically increased during the course of a night's trial (A. Bravo, unpubl. data). We recorded the behavior of bats with a video camera (Sony MiniDV night-shot) and an external infrared light (IR Lamp 6 by http://www.irlight.com). At 1800 h, we turned on the video camera and the infrared light and left the cage, leaving the video camera to record the bats' behavior for an hour. At 1900 h, we collected the video equipment and gave the bats *ad libitum* access to bananas. We then left them for 1.5–2 h, after which we captured each bat with an entomological net, fed it some sugar-water from a disposable syringe, and released it.

DATA ANALYSIS.—Following each trial, we carefully watched the video and counted the number of times bats sipped water from the *collpa* vs. non-*collpa* treatments. Bats drink on the wing, so each pass over the water surface with a sip was clearly distinguishable. Even though we individually marked bats, we could not identify individual bats in the video recordings, so drinking events were grouped for all bats in a given trial. We analyzed the preference for a water source with a binomial exact test for each experiment, and then we combined the probabilities of each independent test using Fisher's method (Sokal & Rohlf 1995).

WATER ANALYSIS.—We measured the mineral concentrations of each batch of *collpa* and non-*collpa* water used for the trials from 20-mL samples extracted from each water type with a sterile disposable syringe. We filtered samples with a 0.45-µm Nalgene syringe filter into a sterile centrifuge tube. We kept samples in a dark and cold place until analysis. The Soil Testing and Plant Analysis Laboratory at Louisiana State University Agricultural Center (http://www.lsuagcenter.com) measured the concentrations in parts per million (ppm) of calcium, magnesium, potassium, and sodium for all samples. We compared the mineral concentrations between *collpa* and non-*collpa* water using a Hotelling–Lawley test in a multivariate analysis of variance (MANOVA) (Everitt & Hothorn 2006). Because *collpa* water was derived from the same single source for all trials and all but one pan of non-*collpa* water was derived from the same single source (the exception being rainwater on 24 April 2008), the model treated each replicate as a repeated measure.

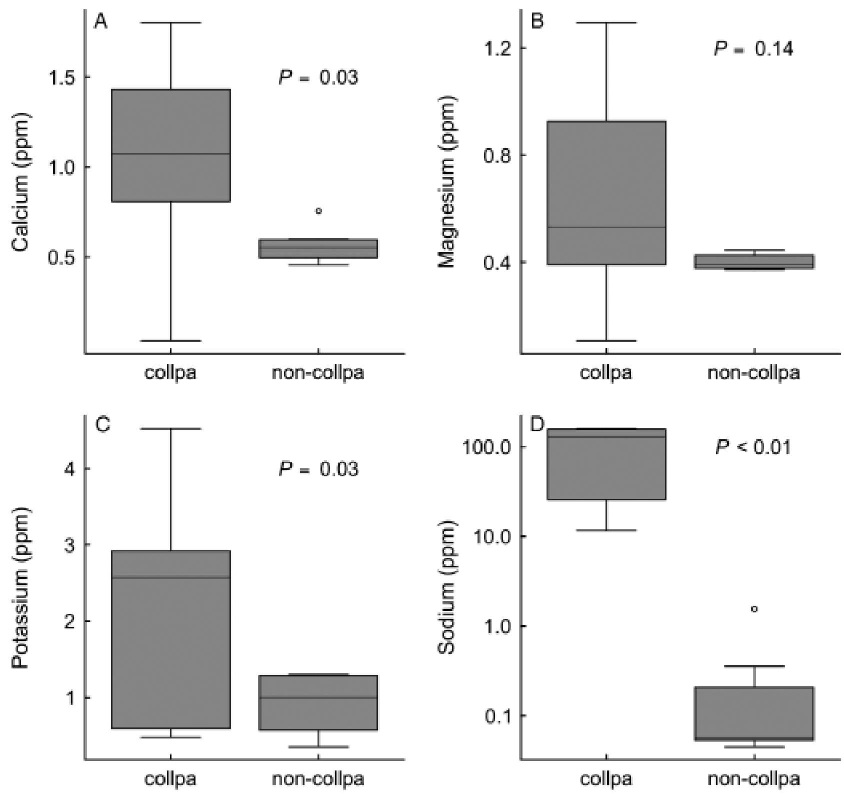

FIGURE 1. Concentrations of (A) calcium, (B) magnesium, (C) potassium, and (D) sodium in ppm of *collpa* and non-*collpa* water used in the choice experiments. Box plots show the median, upper, and lower quartiles, and highest and lowest data values. Note logarithmic scale for sodium concentrations.

All analyses were performed in R (Crawley 2007, R Development Core Team 2007).

RESULTS

A total of 33 *Artibeus* bats were used for the experiments: 17 *A. lituratus*, 12 *A. obscurus*, and 4 *A. planirostris* (Table 1). Of these, 25 were males and 8 were females. Independent results for each experiment showed that in small groups bats collectively preferred *collpa* water compared with non-*collpa* water. In all ten trials bats visited the *collpa* water source more often than the non-*collpa* water treatment, and in six, bats significantly preferred *collpa* water to non-*collpa* water (Table 1). In addition, the overall test using Fisher's method showed that the bats as groups significantly preferred *collpa* water ($P < 0.001$).

Concentrations of minerals differed between *collpa* water and non-*collpa* water ($F_{4,9} = 9.32$, $P < 0.01$). *Collpa* water contained significantly higher concentrations of calcium ($F_{1,12} = 5.33$, $P = 0.03$, Fig. 1A), potassium ($F_{1,12} = 5.72$, $P = 0.03$, Fig. 1C), and sodium ($F_{1,12} = 24.62$, $P < 0.01$, Fig. 1D) than non-*collpa* water. There was no significant difference in magnesium concentration between treatments ($F_{1,12} = 2.37$, $P = 0.15$; Fig. 1B).

DISCUSSION

Our results suggest that bats of the stenodermatine genus *Artibeus* can discriminate between *collpa* water and water from other sources and that they prefer to drink *collpa* water. This preference supports the conjecture that bats purposely seek out and visit *collpas* to drink the water accumulated in soil depressions. Bats used for our drinking trials were not captured while visiting a *collpa* to drink. Moreover, bats used in trials were males and nonreproductive females, which visit *collpas* less frequently than do reproductive females (Bravo *et al.* 2008, 2010). Our experimental subjects therefore were biased, if anything, against *collpa*-visiting individuals. The clear tendency for temporarily captive bats to choose *collpa* water indicates a behavioral preference for *collpa* water.

Unlike insectivorous species, frugivorous bats usually can obtain all of their daily water requirements from their fruit diets (75–90% water; Fleming 1988, Studier & Wilson 1991). Thus it is unusual for them to drink water and it is likely that stenodermatine bats visit *collpas* seeking resources other than water (Bravo *et al.* 2008).

Collpa water contains high concentrations of selected minerals. Bravo *et al.* (2010) reported a consistent pattern of high concentrations of sodium in water from several *collpas* compared with creeks and oxbow lakes in southeastern Peru. Similarly, Izawa (1993) reported high sodium concentrations in *collpa* water from Colombia. In our study, three of the four key minerals analyzed were present in significantly higher concentrations in *collpa* water compared with the non-*collpa* water used in the experiments, particularly sodium (Fig. 1). Thus, as reinforced by Bravo *et al.* (2010), it is likely that *collpas* function as sources of limiting mineral nutrients for stenodermatine frugivorous bats in the Peruvian Amazon. Moreover, Bravo Ordoñez (2009) reported that figs (*Ficus* spp.) consumed by stenodermatine bats in the southeastern Peruvian Amazon contained lower concentrations of sodium compared with figs from other tropical regions. Bats in southeastern Peru likely face sodium constraints that may be overcome by drinking *collpa* water as a sodium source.

Sodium, one of the most important nutrients for animals, can be accurately detected by primates (including humans), rodents, and ungulates, but not by carnivores (Michell 1995). Unlike animals, plants require little sodium (Morris 1991) and they usually contain low amounts (Nagy & Milton 1979, O'Brien *et al.* 1998, Wendeln *et al.* 2000). So, in sodium-limited environments, species that feed primarily on plant tissues may need to search for salt (Denton 1982, Michell 1995, Roze 2009). The appetite for salt has been long suggested as a main driver for the intentional consumption of sodium-rich soil (Emmons & Stark 1979, Holdø *et al.* 2002, Ayotte *et al.* 2006, Powell *et al.* 2009, Roze 2009) and there is growing support for the hypothesis that sodium limitation also leads to bats' use of *collpas* (Bravo *et al.* 2008, 2010).

For frugivorous bats, we recommend the use of cafeteria-style experiments such as those performed here to identify experimentally whether they have a specific preference for one or more resources present in *collpa* water (minerals, clay, etc.). This approach should allow us to distinguish which minerals (especially sodium, *e.g.*, Bravo *et al.* 2010) or clay (*e.g.*, Voigt *et al.* 2008) are the principle resources drawing bats to *collpas*.

In contrast to other experimental studies (Giannini & Villalobos Brenes 2001, Korine & Kalko 2005, Hodgkison *et al.* 2007), we used groups of bats instead of single individuals per trial. In preliminary trials, single individuals may have been unresponsive to the water treatments due to reduced olfactory cues compared with food choice experiments or the social nature of bat visitation to *collpas*. We consequently added an olfactory cue (banana pulp) in an unbiased way to both treatments to increase the likelihood that bats would investigate the pans in the flight cages. The increased responses of groups of bats, compared with single individuals, indicate that social facilitation may be involved in *collpa* visitation as described for parrots in the Peruvian Amazon (Burger & Gochfeld 2003). Multispecies aggregation of large numbers of frugivorous bats, as occurs for parrots (Burger & Gochfeld 2003), suggests the presence of both inter- and intra-specific interactions. In the same way parrots at *collpas* seem to be highly vulnerable to predation (Burger & Gochfeld 2003), there is a risk of predation to bats (*collpas* attract predatory bats [*Phyllostomus hastatus* or *Chrotopterus auritus*], boas [*Corallus hortulanus*], felid predators [*e.g.*, *Leopardus pardalis*], and owls [L. Emmons and A. Bravo, pers. obs.]). Drinking water from *collpas* in a large bat flock may decrease the chance of predation per individual (Hamilton 1971). A preference for grouping to avoid predation may partly explain why in cages, no single individuals drank the water from any water treatment.

In conclusion, further choice experiments are needed to identify the exact constituents in *collpa* water that underlie bats' water preferences (as also advocated by Emmons & Stark 1979) and to determine degrees of individual preference by bats of different species, sexes, and reproductive conditions.

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