

## Comparative analysis of guano from bats utilizing different dietary strategies

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### Abstract

Trophic cascades structure communities by facilitating the transfer of nutrients and organic matter between trophic levels. For example, bat guano serves as a resource base in isolated communities by supporting an assemblage of arthropods, fungi, bacteria, and lichen that varies depending on the diet of the bat producing it. These differences in community structure may result from differences in guano composition. The purpose of this study was to compare dry matter, organic matter, lipid, carbon, nitrogen, and phosphorus content for three species of bats that consume diets of blood, fruit, or insects. Guano from sanguivorous *Desmodus rotundus*, frugivorous *Pteropus rodricensis*, and insectivorous *Tadarida brasiliensis* was collected from captive and wild bats by spreading tarps below roosts (three roosts per species) and capturing feces for six consecutive days. Guano was homogenized using a hand blender, and homogenized samples were analyzed for dry matter content by drying at 105 °C and for organic matter content by combustion at 500 °C. Guano lipid content was determined by lipid extraction using petroleum ether. Guano carbon and nitrogen content were determined by elemental analysis, and phosphorus content was determined by persulfate digestion. We found no significant differences in guano dry matter, organic matter, lipid, or phosphorus content among species. Guano nitrogen content on a dry matter and organic matter basis and carbon content on an organic matter basis differed significantly among species. These differences may reflect differences in the diets consumed by each species.

## **Introduction**

Transfer of nutrients and organic matter occurs in many communities due to the redistribution of biomass by a consumer. For example, detritivorous fish release benthically-derived phosphorus and organic matter into the water column in lake food webs, and these nutrients benefit phytoplankton (Polis et al. 1997). Because of this cascade, consumers can sustain local food webs via the production of a resource base. In other cases, seabirds transfer large amounts of organic matter and nutrients between oceanic and intertidal or terrestrial communities by feeding on marine fish and subsequently depositing fish carcasses and guano and laying eggs on land (Polis et al. 1997). This nutrient input subsidizes otherwise isolated food webs and provides food for organisms such as macroinvertebrates in tidal pools and detritivorous beetles on islands used by roosting seabirds (Methratta 2004). Increased isolation of these communities causes a greater dependence on the resources contributed by the mobile consumer. In the Antarctic, bird guano promotes lichen growth, and this lichen in turn supports entire communities of arthropods and microorganisms (Polis et al. 1997). Similarly, bat guano supports a great diversity of organisms including arthropods, fungi, bacteria, and lichen that represent different trophic levels. Consumers in this system feed directly on guano or on bacteria and/or arthropods that live on guano (Ferreira and Martins 1998). Energy flow in these trophic cascades is therefore based on guano production by roosting bats (Hairston and Hairston 1993).

Interestingly, the diversity of organisms living on or in guano piles differs depending on the diet of the bat producing the guano. Guano from sanguivorous bats is typically inhabited by fly larvae, nematodes, springtails, and beetles. Guano of

insectivorous bats is inhabited by mites, pseudoscorpions, beetles, thrips, moths, and flies. Lastly, guano of frugivorous bats is inhabited by spiders, mites, isopods, millipedes, centipedes, springtails, barklice, true bugs, and beetles (Ferreira and Martins 1998). Although several studies have compared the impact of guano from different bat species on cave ecology (Trajano 1996, Ferreira and Martins 1998, Shahack-Gross et al. 2004), the composition of guano from bats consuming different diets has received little attention.

The purpose of this study was to compare dry matter, organic matter, lipid, carbon, nitrogen, and phosphorus content of guano from bats consuming different diets. We chose *Desmodus rotundus*, *Pteropus rodricensis* and *Tadarida brasiliensis* for our study based on their diets of blood, fruit, and insects, respectively and due to their availability in American zoos and in the wild in Florida. *D. rotundus*, the common vampire bat, is a sanguivorous bat species native to Central and South America that feeds on mammalian blood. *P. rodricensis*, the Rodrigues flying fox, is a frugivorous megachiropteran native to the Rodrigues Island in the Indian Ocean. *T. brasiliensis*, the free-tailed bat, is an insectivorous bat common in the southern and southwestern United States that is known to roost in caves and man-made structures (Nowak 1994). We expected the results of this study to indicate that sanguivorous and insectivorous bat guano contained higher amounts of lipids, nitrogen, and phosphorus than frugivorous bat guano due to the content of their respective diets (Williams et al. 1941, Shahack-Gross et al. 2004). Previous studies have revealed substantial amounts of phosphorus (> 1000 mg/kg) and other minerals in most insects. These studies have also demonstrated high lipid (~ 35 – 60 %) and protein (~ 70 %) contents in insects (Finke 2002, Marconi et al.

2002). Cow's blood is a common captive sanguivorous bat diet and typically contains 57 % protein and 26 % lipids, 62 % of which are in the form of phospholipids (Williams 1941). Although nutrient content varies among different types of fruit, we expected frugivorous bat guano to contain low amounts of lipids and nitrogen relative to insectivorous or sanguivorous bat guano due to the typically low concentrations of proteins and lipids in fruit pulp (Johnson et al. 1985, Witmer and Van Soest 1998). All guano should contain moderately high organic matter and carbon contents because of the organic nature of each species' diet.

### **Materials and Methods**

Guano from three bat species was analyzed for dry matter, organic matter, lipids, carbon, nitrogen, and phosphorus content. *D. rotundus* guano was collected by the staff of the Sedgewick County Zoo, the Houston Zoo, and the North Carolina Zoo. These facilities feed their *D. rotundus* a diet consisting primarily of cow's blood. *T. brasiliensis* guano was collected from sites around central Florida including two roosts from bridges of the Florida Turnpike (C.R. 561 and S.R. 438). The species identity of bats within these roosts was confirmed by Jeff Gore of the Florida Fish and Wildlife Research Institute. We also collected *T. brasiliensis* guano from samples harvested previously at the University of Florida bat house by staff from the Environmental Health and Safety office. This bat house is home to a colony consisting largely of *T. brasiliensis*, although some southeastern bats (*Myotis austroriparius*) can also be found within this structure. These insectivorous bats consume a variety of flying insects found in Florida. *P. rodricensis* guano was collected by the staff of the Philadelphia Zoo and the Cleveland Metroparks Zoo and by us at the Lube Foundation in Gainesville, Florida. These

facilities feed their *P. rodricensis* a diet consisting of mixed fruits and dietary supplements. Plastic tarps were placed on the floor of each bat habitat for a minimum of two hours to collect guano from bats that had recently eaten. This process was repeated for six consecutive days. A spatula was used to scrape guano from the tarp for subsequent analyses. Bags of collected guano were frozen until the collection process was complete and then shipped frozen (if not obtained locally) to the Zoology Department at the University of Florida. All subsequent analyses were performed in the Archie Carr Center for Sea Turtle Research and in the Department of Geological Sciences Stable Isotope Laboratory at the University of Florida.

Thawed guano samples were rinsed from the collection bags into a glass beaker using deionized water. The resulting guano solution was homogenized using a Philips 4-speed hand blender (model HR1358) with an attached aluminum skirt. Homogenized guano was poured into a glass baking dish and dried in a Precision Mechanical convection incubator at 60 °C for a minimum of five days. Samples were then scraped from the baking dish and dried overnight in the incubator at 60 °C. Each analysis of nutrient content described below was performed in triplicate for each guano sample except in instances where insufficient sample mass was collected.

For dry and organic matter content analyses, approximately 0.2-g samples of dried, homogenized guano were placed into tared crucibles, dried at 105 °C for 16 hours, weighed, combusted at 500 °C for three hours, and re-weighed. Dry matter content was calculated as dry mass after 16 hours at 105 °C divided by initial sample mass. Organic matter content was calculated as dry matter mass minus ash mass divided by dry matter mass. For lipid analyses, 0.1 to 0.2-g samples of dried, homogenized guano were placed

into cellulose thimbles. Lipids from each sample were then extracted in a Dionex ASE300 Accelerated Solvent Extractor using 100% petroleum ether, and the resulting lipid-free sample mass was determined using a Mettler AE200 electronic balance. For carbon and nitrogen analyses, 3 to 5-mg samples of dried, homogenized guano were weighed into tin capsules which were subsequently folded into a ball and placed into an autosampler carousel on a Carlo Erba NA 1500 CNS Elemental Analyzer. Samples were flash combusted inside an oxygen-rich quartz column containing chromium oxide and silvered cobaltic oxide at 1040 °C. The oxygen was removed through a reduction column containing elemental copper as the sample gas was transported in a helium carrier stream. The N<sub>2</sub> and CO<sub>2</sub> gases were separated as the stream passed through a 1.5 meter gas chromatographic column and then through a thermal conductivity detector. For phosphorus analyses, approximately 4-mg samples of dried, homogenized guano were subjected to persulfate digestion and measured as orthophosphates (Schelske et al. 1986).

Guano lipid, carbon, nitrogen, and phosphorus contents were calculated as percentages of initial sample mass on both dry and organic matter bases. These percentages were then tested for statistical significance using analysis of variance (ANOVA). When statistically significant differences among species were found, single-factor ANOVA was used for pairwise comparisons.

## **Results**

A summary of nutrient contents from each guano source and from each species can be found in Table 1. Guano dry matter and organic matter content did not differ significantly among the three bat species studied (Figure 1; ANOVA, Dry Matter –  $F = 1.734$ ,  $P > 0.5$ ; ANOVA, Organic Matter –  $F = 0.6873$ ,  $P > 0.5$ ). Guano lipid content

also did not differ among the three species on either a dry matter or organic matter basis (Figure 2; ANOVA, Dry Matter –  $F = 0.1425$ ,  $P > 0.5$ ; ANOVA, Organic Matter –  $F = 0.2990$ ,  $P > 0.5$ ). Guano carbon content on a dry matter basis was not significantly different between groups (Figure 3; ANOVA –  $F = 4.111$ ,  $P > 0.05$ ). However, guano carbon content on an organic matter basis differed significantly among groups (ANOVA –  $F = 7.788$ ,  $P < 0.05$ ) with *D. rotundus* demonstrating a higher percent carbon than *P. rodricensis* (ANOVA –  $F = 11.51$ ,  $P < 0.05$ ). Guano nitrogen content differed significantly among the three species tested on both a dry and organic matter basis (Figure 4; ANOVA, Dry Matter -  $F = 5.708$ ,  $P < 0.05$ ; ANOVA, Organic Matter -  $F = 5.987$ ,  $P < 0.05$ ), with *Desmodus rotundus* and *T. brasiliensis* demonstrating higher guano nitrogen content than *P. rodricensis*. Guano phosphorus content on a dry and organic matter basis did not differ significantly among species (Figure 5; ANOVA, Dry Matter –  $F = 1.923$ ,  $P > 0.1$ ; ANOVA, Organic Matter –  $F = 1.926$ ,  $P > 0.1$ ).

## **Discussion**

The results of this study suggest that differences in the composition of guano produced by bat species consuming different food items may explain differences in species assemblages in guano-based communities. Our analyses of guano from three species of bats revealed significant differences in nitrogen and carbon content among species but no significant differences in dry matter, organic matter, lipid, or phosphorus content.

We did not expect a significant difference in dry and organic matter content because the compositions of the diets of all three bat species are primarily organic. Because organic matter content is calculated as initial sample mass minus ash mass after

combustion, we can conclude that guano ash content did not differ among species. This result indicates that all three species produced guano comparable in overall mineral content.

Although we initially predicted lipid content to be higher in sanguivorous and insectivorous bat guano, lipid content of guano samples was comparable among species. This lack of a statistically significant difference could result from efficient assimilation of dietary lipids by sanguivorous and insectivorous bats, from the feeding of fruits and/or supplements high in lipid content to captive frugivorous bats, or from contributions of intestinal and bacterial cell membranes to fecal lipids. Because we did not investigate differential digestive processing or composition of food consumed by each species in this study, we cannot address the lack of a difference in guano lipids further.

Guano carbon content as a percentage of dry matter did not differ significantly among the species tested. However, carbon content as a percentage of organic matter was significantly lower in guano produced by frugivorous bats than in guano produced by sanguivorous bats. Although fruit bats are incapable of digesting cellulose, they differentially ingest fruit pulp, juices, and seeds and spit out the more fibrous components of their diet after straining fruit against their palates (Wendeln and Runkle 2000). Fruit fiber carbon would therefore have been excluded from our guano samples and may explain the decreased carbon content of frugivorous bat guano. This difference could also be attributed to more efficient processing and assimilation of carbohydrates by fruit-eating bats adapted to a high-sugar diet than by carnivorous bats adapted to a more protein-rich diet. As stated above, however, we did not quantify nutrient content of food

items or examine digestive physiology so we therefore cannot infer anything further from our carbon data.

The nitrogen content of guano samples differed significantly among bat species. Nitrogen content was highest in sanguivorous and insectivorous bat guano and lowest in frugivorous bat guano. This difference can be explained by the presumed higher protein concentrations in animal tissues (i.e., blood and insects) than in plant tissues (i.e., fruits). Although the difference in nitrogen content between sanguivorous and insectivorous bat guano was not significant, insectivorous guano tended to be lower in nitrogen than sanguivorous bat guano. This trend might result from the potentially higher concentrations of indigestible diet components (i.e., chitin in insect exoskeletons) in an insectivorous diet than in a sanguivorous diet. No vertebrates are known to produce chitinases, enzymes capable of digesting chitin. While chitinase activity has been detected in insectivorous bats, these enzymes are presumably produced by symbiotic intestinal bacteria and do not appear to contribute significantly to digestion of chitin by these bats (Whitaker et al. 2004). Our results for nitrogen content of frugivorous bat guano coincide with the nitrogen content (1-2 %) of dry fruit pulp (Herbst 1986). Nitrogen is known to be a vital component of nutrient cycles in communities whose resource base consists of bat guano (Harris 1970), and differences in guano nitrogen content could therefore alter nutrient cycling or otherwise differentially structure guano-based communities.

Previous studies have found high phosphorus content in both insectivorous and sanguivorous bat guano due to phospholipids in blood and phosphates in insects (Williams 1941, Aldenius et al. 1983, Shahack-Gross et al. 2004). We therefore expected

phosphorus content to be higher in sanguivorous and insectivorous bat guano than in frugivorous bat guano based on diet composition. However, we found no statistically significant difference in phosphorus content of guano from the three species we studied. We may not have detected a significant difference between species due to the influence of dietary supplements fed to captive bats, as is typical in zoo animal husbandry.

In this study, we quantified the nutrient composition of guano from three species of bats consuming different diet items (blood, insects, and fruits). Although our results for most analyses were not statistically significant, we only analyzed guano composition quantitatively. It is possible that guano from bats utilizing different dietary strategies may support different communities of consumers as a result of qualitative differences in the types of fatty acids, amino acids, sugars, indigestible components, or toxins present in guano. However, our results do indicate that nitrogen and carbon may be of primary importance in structuring guano-based communities differently depending on the dietary strategy of the bats producing the guano. Bats are a diverse order with worldwide distributions, and an appreciation of bat guano composition is therefore vital to understanding the flow of nutrients in communities that include these animals. Additional research should be aimed at investigating the impact of varying nutrient levels on the productivity and diversity of communities that use bat guano as a resource base.

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