BRIEF COMMUNICATION

Intestinal Water Absorption Varies with Expected Dietary Water Load among Bats but Does Not Drive Paracellular Nutrient Absorption

Edwin R. Price[1,*]
Antonio Brun[2]
Manuel Gontero-Fourcade[2]
Guido Fernández-Marinone[2]
Ariovaldo P. Cruz-Neto[3]
William H. Karasov[1]
Enrique Caviedes-Vidal[2,4]
[1]Department of Forest and Wildlife Ecology, University of Wisconsin, Madison, Wisconsin 53706; [2]Laboratorio de Biología Professor E. Caviedes Codelia, Facultad de Ciencias Humanas, Universidad Nacional de San Luis, 5700 San Luis, Argentina; and Laboratorio de Biología Integrativa, Instituto Multidisciplinario de Investigaciones Biológicas de San Luis, Consejo Nacional de Investigaciones Científicas y Técnicas, 5700 San Luis, Argentina; [3]Departmento de Zoologia, Universidade Estadual Paulista, Avenida 24-A, 1515, CEP 13506-900 Rio Claro, São Paulo, Brazil; [4]Departamento de Bioquímica y Ciencias Biológicas, Universidad Nacional de San Luis, 5700 San Luis, Argentina

Accepted 7/8/2015; Electronically Published 8/14/2015

ABSTRACT

Rapid absorption and elimination of dietary water should be particularly important to flying species and were predicted to vary with the water content of the natural diet. Additionally, high water absorption capacity was predicted to be associated with high paracellular nutrient absorption due to solvent drag. We compared the water absorption rates of sanguivorous, nectarivorous, frugivorous, and insectivorous bats in intestinal luminal perfusions. High water absorption rates were associated with high expected dietary water load but were not highly correlated with previously measured rates of (paracellular) arabinose clearance. In conjunction with these tests, we measured water absorption and the paracellular absorption of nutrients in the intestine and stomach of vampire bats using luminal perfusions to test the hypothesis that the unique elongated vampire stomach is a critical site of water absorption. Vampire bats' gastric water absorption was high compared to mice but not compared to their intestines. We therefore conclude that (1) dietary water content has influenced the evolution of intestinal water absorption capacity in bats, (2) solvent drag is not the only driver of paracellular nutrient absorption, and (3) the vampire stomach is a capable but not critical location for water absorption.

Keywords: water absorption, flight, solvent drag, vampire, *Desmodus rotundus*, stomach.

*Corresponding author; e-mail: edwin.price@unt.edu.

Physiological and Biochemical Zoology 88(6):000–000. 2015. 1522-2152/2015/8806-4169$15.00. DOI: 10.1086/683114

Introduction

Water-soluble macronutrients such as glucose and amino acids can be absorbed in the intestine by two routes. The transcellular pathway involves membrane-bound carriers that transport nutrients into enterocytes. The paracellular pathway is not carrier mediated and involves the passive movement of nutrients through the tight junctions that bind adjacent enterocytes. Paracellular solute movements may result from solvent drag as water moves across the tight junctions or may occur by simple diffusion of the solutes (Pappenheimer and Reiss 1987). While the transcellular route represents the major pathway for nutrient absorption in most mammals, the paracellular route is particularly important to small flying birds and bats (Caviedes-Vidal et al. 2007; Lavin and Karasov 2008; Price et al. 2015). This has been hypothesized to be an adaptation that compensates for having smaller intestines, itself an adaptation to reduce the mass of the digesta carried by flying animals (Caviedes-Vidal et al. 2007; Price et al. 2015). Flying animals that consume watery foodstuffs, however, also need to rapidly eliminate water. Some nectarivorous birds, for example, may be able to avoid absorbing water (McWhorter et al. 2003; Purchase et al. 2013), while others, such as hummingbirds, absorb several times their body mass in water per day, which is then processed and eliminated by the kidney (McWhorter and Martínez del Rio 1999). In the intestine, water may be absorbed paracellularly via tight junctions or via transcellular routes such as aquaporins.

We have previously used an intestinal luminal perfusion technique in anesthetized animals to demonstrate differences in paracellular nutrient absorption between bats and nonflying mammals (Price et al. 2013, 2014; Brun et al. 2014). However, we have not previously compared intestinal water absorption among species in those experiments. Our first aim in this study was therefore to specifically examine intestinal water absorption to test evolutionary and physiological hypotheses. Based on the hypoth-

esis that the water absorption capacity of the intestine has evolved in response to the expected dietary preformed water load, we predicted that water absorption would be high in the intestines of sanguivorous, nectarivorous, and frugivorous bats. As food items, insects have lower water content (per dry mass or energy) than fruit, blood, and nectar (Morton 1973; Breidenstein 1982; Studier and Sevick 1992; National Research Council 2003; Rodríguez-Peña et al. 2007). Furthermore, we predicted that water absorption would be highly correlated with arabinose clearance across species (arabinose is a paracellularly absorbed carbohydrate), based on the hypothesis that paracellular absorption is driven primarily by solvent drag (Pappenheimer and Reiss 1987).

In conjunction with this analysis, we conducted a focused study on an extreme example of a vertebrate that consumes a watery diet, the vampire bat (*Desmodus rotundus*). Vampire bats take large, nutritionally dilute blood meals and begin urinating soon after commencing feeding in order to reduce enough mass to fly (Morton and Richards 1981). This requires rapid absorption of water, although the site(s) of water absorption is not certain. Vampire bats have a unique elongated, blind-ended stomach (Huxley 1865), which has been hypothesized to be the critical and primary site of water absorption (Wimsatt and Guerriere 1962). This hypothesis is based on the rapidity of urinary excretion following feeding, the concentration of ingested blood in the stomach in the hours following feeding, the high vascularity of the stomach, and high gastric Na^+K^+ ATPase activity (Wimsatt and Guerriere 1962; Mitchell and Tigner 1970; Morton and Richards 1981; Harlow and Braun 1997). However, many vertebrates are known to retain ingesta in the stomach while fluid passes to the intestine (Karasov and Hume 1997), and water absorption has never been measured directly in either the vampire stomach or the vampire intestine. Furthermore, if the stomach absorbs substantial water, it might be critical to this process, or, alternatively, water might be only opportunistically absorbed there, with greater water absorption occurring in the intestine. A second aim of this study was therefore to assess the water absorption capacity of the vampire stomach as well as its intestine using luminal perfusions.

Methods

Measurements of the absorption of arabinose during recirculating intestinal luminal perfusions (described more below) were previously reported for several species of bats and rodents (Price et al. 2013, 2014; Brun et al. 2014). These data were computed from two components: the change in buffer volume (water absorption) and changes in the concentration of arabinose in the buffer. Here, we analyze these components and also provide the previously reported arabinose clearance values for reference (fig. 1).

Vampire bats were captured by mist net and maintained in an aviary on the campus of Universidade Estadual Paulista in Rio Claro. Bats were provided bovine blood daily until experiments commenced. Procedures adhered to institutional animal use regulations and approved protocols (Universidade Estadual Paulista: A1-2013; University of Wisconsin: A01441).

Intestinal perfusions were conducted as in Brun et al. (2014). Briefly, seven vampire bats were anesthetized with isoflurane throughout the experiment while taped to a heating pad that maintained 37°C. The small intestine was cannulated ~1 cm from the stomach, while an exit cannula was placed 8.3 ± 0.83 cm distally. Prewarmed saline flushed the cannulated segment, and then a prewarmed buffer (10 mM D-glucose, 10 mM L-arabinose, 10 mM L-proline, 10 mM lactulose, 100 mM NaCl, 1.2 mM $NaHPO_4$, 20 mM $NaHCO_3$, 5 mM KCl, 1 mM $MgSO_4$, and 2 mM $CaCl_2$, pH 7.4; isosmotic based on composition) containing radiolabeled probes ([^{14}C]-L-arabinose and [^{3}H]-L-proline) was passed through the intestinal segment using a peristaltic pump (1 mL min^{-1}) and was recirculated to a tightly sealed reservoir. L-arabinose (M_r 150) is a somewhat smaller carbohydrate than glucose but has no affinity for intestinal transporters (Price et al. 2014) and thus allowed us to estimate the paracellular portion of glucose absorption. After 115 ± 6 min, the buffer was collected, and the animal was euthanized. The buffer was weighed before and after the experiment to determine net water loss or gain, and this information combined with the concentration of the probes—determined by scintillation counting of the buffer before and after the experiment—was used to determine disappearance (absorption) of the probes. D-glucose concentration was also measured in the buffer before and after the experiment using a commercial kit (Laborlab, Guarulhos São Paulo, Brazil), and this information was used to calculate glucose disappearance (absorption). The perfused section was cut longitudinally and laid flat to measure the nominal surface area perfused.

Perfusions of the vampire bat's tubular stomach followed similar overall procedures. In five bats, we selected a cannulation site distal in the stomach and cannulated an exit site (4.9 ± 1.7 cm) proximally. For comparison with the vampire bats, we also perfused the stomachs of laboratory mice (*Mus musculus*, strain ND4). Mice were obtained from a commercial vendor and kept at the University of Wisconsin–Madison with standard chow and water provided ad lib. In five mice, we tied off the esophagus with suture and then placed an entrance cannula in the fundus, and the exit cannula was placed via the duodenum while suturing the stomach around the cannula proximal to the pylorus. We determined significant differences ($P < 0.05$) using ANOVA with Tukey's HSD tests and *t*-tests.

Results and Discussion

Water absorption in the vampire intestine, normalized to the surface area perfused, was 7.9 ± 0.77 μL min^{-1} cm^{-2}, which was higher than all other bats and rodents previously tested (fig. 1). Among bats, the vampires and the nectarivore had the highest water absorption, and insectivores had the lowest (see fig. 1 for statistical results). This pattern aligns well with the expected dietary preformed water load of these species. In our analysis, we have not controlled for phylogenetic relationships among species because the dietary groups largely separate

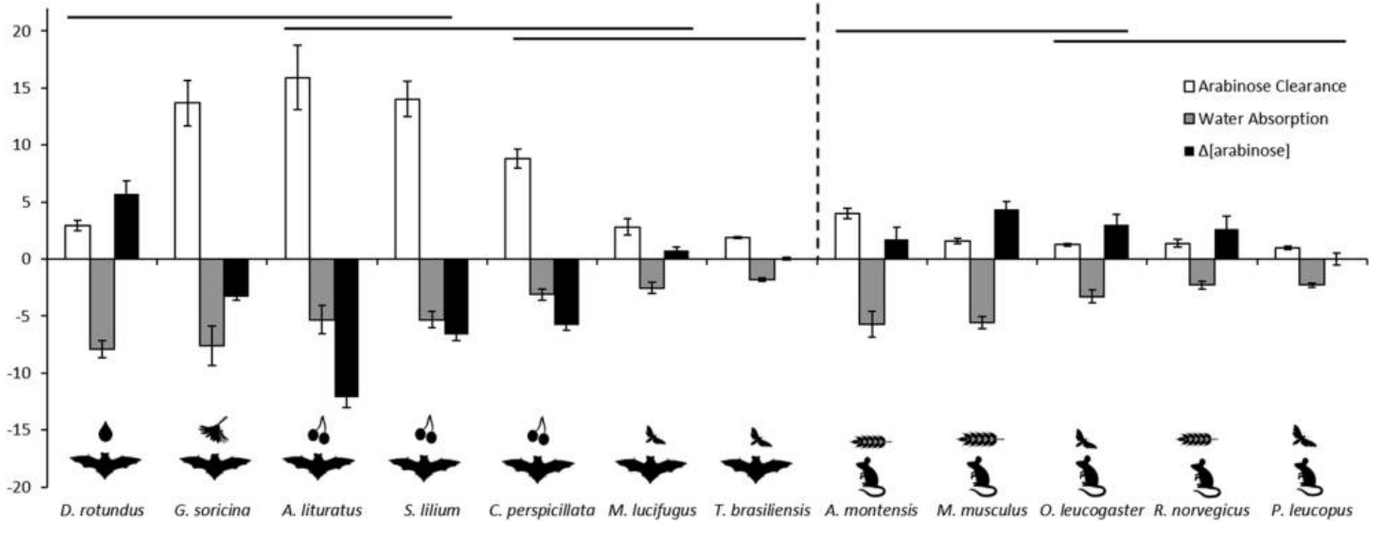

Figure 1. Water and paracellular probe absorption in the intestines of bats and rodents in recirculating luminal perfusions. L-arabinose clearance (μL min^{-1} cm^{-2}) was determined from both the water absorption (μL min^{-1} cm^{-2}; negative values represent net absorption from lumen to tissue) during the experiment and the change in concentration of L-arabinose in the buffer (here shown as [actual % change in concentration]/5 for ease of presentation). Positive values for Δ[arabinose] indicate that arabinose became more concentrated in the buffer because arabinose was not absorbed as quickly as water. Icons indicate group and primary diet: blood, nectar, fruit, insects, and seeds. Lines at the top represent homogeneous subsets for water absorption (Tukey HSD; ANOVA: $F_{11,68} = 12$, $P < 0.001$) for bats and rodents separately. Data are means ± SE and come from this study, unpublished data; some arabinose clearance values were previously reported (Price et al. 2013, 2014; Brun et al. 2014). Species are *Desmodus rotundus*, *Glossophaga soricina*, *Artibeus lituratus*, *Sturnira lilium*, *Carollia perspicillata*, *Myotis lucifugus*, *Tadarida brasiliensis*, *Akodon montensis*, *Mus musculus*, *Onychomys leucogaster*, *Rattus norvegicus*, and *Peromyscus leucopus*.

along phylogenetic lines. Our data are nonetheless suggestive that the water content of the natural diet of bats has been a selective force on the permeability of the intestine to water. Additionally, the fact that vampire bats consume large, infrequent meals may place further pressure on their intestines to have a high capacity for water absorption. The pattern of water absorption in our bats could also reflect the diet history of the animals because they were not raised on a common diet. For example, water absorptive capacity can vary based on recent feeding (Fisher and Gardner 1976). To our knowledge, the effect of long-term acclimation to different diets on water absorptive capacity has not been experimentally tested.

The pattern of water absorption capacity in rodents was less clearly aligned with diet, although none of the rodents was expected to have a particularly high dietary water load and rapid absorption and elimination of water is likely to be less important to nonvolant mammals. Interestingly, the high capacity for intestinal water absorption in vampire bats did not translate into particularly high paracellular probe (L-arabinose) absorption, suggesting that much of the water absorption may occur via aquaporins instead of tight junctions. Arabinose clearance in vampire intestine (2.93 ± 0.43 μL min^{-1} cm^{-2}) was typical for bats relying on protein-rich diets ($P > 0.77$ vs. *Tadarida brasiliensis* and *Myotis lucifugus*), that is, higher than most rodents but substantially lower than frugivorous bats ($P < 0.001$ for all comparisons with frugivorous bats; fig. 1). Absorption of L-arabinose in vampire bats was 33.5 ± 5.0 nmol min^{-1} cm^{-2}, while D-glucose absorption was 59.7 ± 9.7 nmol min^{-1} cm^{-2} and L-proline absorption was 78.2 ± 11.5 nmol min^{-1} cm^{-2}. Paracellular absorption was thus estimated to represent 55% of total glucose absorption.

Paracellular nutrient flux can occur via diffusion or by solvent drag as water moves through tight junctions, the latter mechanism favored by Pappenheimer and colleagues (Pappenheimer and Reiss 1987). Several features of our data suggest that solvent drag is not the only driver of arabinose absorption. In these intestinal perfusion experiments, arabinose clearance is determined from both the absorption of water and the change in arabinose concentration in the buffer. One can see that water flux varies substantially between some similar species, even though arabinose clearance does not (fig. 1). For example, water absorption was high in *Mus musculus* ($P = 0.003$ vs. *Rattus norvegicus*), but this was offset by a rise in L-arabinose concentration in the buffer, resulting in intestinal arabinose clearance that was similar to *R. norvegicus* ($P = 0.98$). Likewise, the vampire bat intestine had an arabinose clearance that was similar to the insectivorous *M. lucifugus* despite several-fold higher water absorption. Arabinose clearance and net water absorption were not highly correlated when comparing all species ($r^2 = 0.16$) or just bats ($r^2 = 0.20$). Most notably, the L-arabinose concentration in the buffer decreased in the frugivorous bats (significantly different from 0; $P < 0.0001$ for all frugivorous bats), implying that arabinose absorption was faster than water absorption (fig. 1). Diffusion must therefore play a role in paracellular nutrient absorption in these species, perhaps even a dominant role, in comparison to solvent drag. This view is similar to that of Wood and Grosell (2012), who found that killifish intestinal paracellular solute flux was independent of water flux. Similarly, Napier et al. (2008) found that paracellular absorption of glucose was actually lower with dilute nectar diets in intact birds, although this was attributed primarily to changes in transit time.

The high capacity for water absorption in the intestine of the vampire bat does not rule out the stomach as an important site. Gastric water absorption in the vampire bat was 4.6 ± 0.95 μL min^{-1} cm^{-2}, a rate several-fold higher than that in the mouse (0.83 ± 0.25 μL min^{-1} cm^{-2}; $t_5 = 3.8$, $P = 0.012$) but well shy of the vampire's intestinal water absorption rate (fig. 2; $t_8 = 2.7$, $P = 0.027$). A tighter epithelium in the stomach than the intestine might be expected because the vampire stomach likely produces HCl and pepsinogens based on the presence of oxyntic and chief cells (Rouk and Glass 1970; Kamiya et al. 1979). Arabinose clearance was high in the vampire stomach but also variable (23 ± 9.8 μL min^{-1} cm^{-2}), whereas gastric arabinose clearance in the mouse was similar to that in its intestine. In the stomach, the change in arabinose concentration was not significantly different from 0 for vampire bats ($t_4 = 1.65$, $P = 0.17$) or mice ($t_4 = 1.4$, $P = 0.23$), suggesting that arabinose clearance in the stomach may occur due to solvent drag.

In summary, our data suggest that the water absorption capacity of the intestines of bats has evolved in response to their natural dietary water load. Water absorption may have some influence on paracellular glucose absorption via solvent drag, but diffusion must also play a role. Additionally, vampires have the highest capacity for water absorption we have measured in mammalian intestine. Their capacity for gastric water absorption, while lower than that of the intestine, is still much higher than that of the mouse stomach. We propose that the unique

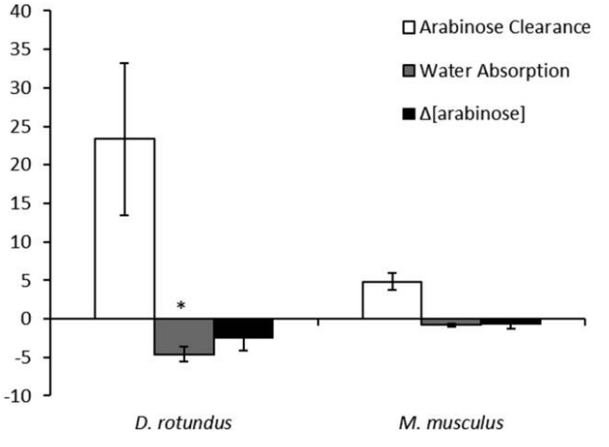

Figure 2. Water and paracellular probe absorption in the stomachs of vampire bats and mice. L-arabinose clearance (μL min^{-1} cm^{-2}) was determined from both the water absorption (μL min^{-1} cm^{-2}) during the experiment and the change in concentration of L-arabinose in the buffer ([% change in concentration]/5). An asterisk indicates that water absorption ($t_5 = 3.8$, $P = 0.012$) but not other variables ($P > 0.15$) differed among species. Data are means $\pm$ SE.

stomach of the vampire bat evolved primarily as a storage chamber, both for gastric digestion and for later regurgitation during social food sharing (Wilkinson 1984). Due to the stomach's enhanced permeability, substantial water and even some nutrient absorption can occur, but water absorption should increase as the meal is passed to the intestine.

Acknowledgments

Guilherme Ambar provided field assistance. The World Bat Library provided helpful bibliographic access. Funding was provided by Fundaçáo de Amparo á Pesquisa do Estado de São Paulo (2012/04610-5 to A.P.C.-N.), National Science Foundation (IOS-1025886 to W.H.K. and E.C.-V.), Agencia Nacional de Promoción Científica y Tecnológica (Fondo Nacional de Ciencia y Técnica PICT2007 01320 to E.C.-V.), the Company of Biologists (JEB Travelling Fellowship to E.R.P.), Universidad Nacional de San Luis (CyT 9502), and the University of Wisconsin–Madison Department of Forest and Wildlife Ecology.

Literature Cited

Breidenstein C.P. 1982. Digestion and assimilation of bovine blood by a vampire bat (*Desmodus rotundus*). J Mammal 63: 482–484.

Brun A., E.R. Price, M.N. Gontero-Fourcade, G. Fernandez-Marinone, A.P. Cruz-Neto, W.H. Karasov, and E. Caviedes-Vidal. 2014. High paracellular nutrient absorption in intact bats is associated with high paracellular permeability in perfused intestinal segments. J Exp Biol 217:3311–3317.

Caviedes-Vidal E., T.J. McWhorter, S.R. Lavin, J.G. Chediack, C.R. Tracy, and W.H. Karasov. 2007. The digestive adaptation of flying vertebrates: high intestinal paracellular absorption compensates for smaller guts. Proc Natl Acad Sci USA 104:19132–19137.

Fisher R.B. and M.L.G. Gardner. 1976. A diurnal rhythm in the absorption of glucose and water by isolated rat small intestine. J Physiol 255:821–825.

Harlow H.J. and E.J. Braun. 1997. Gastric Na^+K^+ATPase activity and intestinal urea hydrolysis of the common vampire bat, *Desmodus rotundus*. Comp Biochem Physiol A 118:665–669.

Huxley T.H. 1865. On the structure of the stomach in *Desmodus rufus*. Proc Zool Soc Lond 33:386–390.

Kamiya T., P. Pirlot, and T. Matsubara. 1979. A note on the stomach of the vampire bat (*Desmodus rotundus*). Acta Anat Nippon 54:85–92.

Karasov W.H. and I.D. Hume. 1997. Vertebrate gastrointestinal system. Pp. 409–480 in W. Dantzler ed. Handbook of physiology. Vol. 13. Comparative physiology. Oxford University Press, New York.

Lavin S.R. and W.H. Karasov. 2008. Allometry of paracellular absorption in birds. Physiol Biochem Zool 81:551–60.

McWhorter T.J. and C. Martínez del Rio. 1999. Food ingestion and water turnover in hummingbirds: how much dietary water is absorbed? J Exp Biol 202:2851–2858.

McWhorter T.J., C. Martínez del Rio, and B. Pinshow. 2003. Modulation of ingested water absorption by Palestine sunbirds: evidence for adaptive regulation. J Exp Biol 206:659–666.

Mitchell G.C. and J.R. Tigner. 1970. The route of ingested blood in the vampire bat (*Desmodus rotundus*). J Mammal 51:814–817.

Morton D. and J.F. Richards. 1981. The flow of excess dietary water through the common vampire bat during feeding. Comp Biochem Physiol A 69:511–515.

Morton E.S. 1973. On the evolutionary advantages and disadvantages of fruit eating in tropical birds. Am Nat 107:8–22.

Napier K.R., C. Purchase, T.J. McWhorter, S.W. Nicolson, and P.A. Fleming. 2008. The sweet life: diet sugar concentration influences paracellular glucose absorption. Biol Lett 4:530–533.

National Research Council. 2003. Nutrient requirements of nonhuman primates. 2nd rev. ed. National Academy of Sciences, Washington, DC.

Pappenheimer J.R. and K.Z. Reiss. 1987. Contribution of solvent drag through intercellular junctions to absorption of nutrients by the small intestine of the rat. J Membr Biol 100:123–136.

Price E.R., A. Brun, E. Caviedes-Vidal, and W.H. Karasov. 2015. Digestive adaptations of aerial lifestyles. Physiology 30: 69–78.

Price E.R., A. Brun, V. Fasulo, W.H. Karasov, and E. Caviedes-Vidal. 2013. Intestinal perfusion indicates high reliance on paracellular nutrient absorption in an insectivorous bat *Tadarida brasiliensis*. Comp Biochem Physiol A 164:351–355.

Price E.R., K.H. Rott, E. Caviedes-Vidal, and W.H. Karasov. 2014. Paracellular nutrient absorption is higher in bats than rodents: integrating from intact animals to the molecular level. J Exp Biol 217:3483–3492.

Purchase C., K.R. Napier, S.W. Nicolson, T.J. McWhorter, and P.A. Fleming. 2013. Gastrointestinal and renal responses to variable water intake in whitebellied sunbirds and New Holland honeyeaters. J Exp Biol 216:1537–1545.

Rodríguez-Peña N., K.E. Stoner, J.E. Schondube, J. Ayala-Berdón, C.M. Flores-Ortiz, and C. Martínez del Rio. 2007. Effects of sugar composition and concentration on food selection by Saussure's long-nosed bat (*Leptonycteris curasoae*) and the long-tongued bat (*Glossophaga soricina*). J Mammal 88:1466–1474.

Rouk C.S. and B.P. Glass. 1970. Comparative gastric histology of five North and Central American bats. J Mammal 51:455–472.

Studier E.H. and S.H. Sevick. 1992. Live mass, water content, nitrogen and mineral levels in some insects from south-central lower Michigan. Comp Biochem Physiol A 103:579–595.

Wilkinson G. 1984. Reciprocal food sharing in vampire bats. Nature 308:181–184.

Wimsatt W.A. and A. Guerriere. 1962. Observations on the feeding capacities and excretory functions of captive vampire bats. J Mammal 43:17–24.

Wood C.M. and M. Grosell. 2012. Independence of net water flux from paracellular permeability in the intestine of *Fundulus heteroclitus*, a euryhaline teleost. J Exp Biol 215:508–517.