Title: Rapid GIT transit time in volant vertebrates, with implications for convergence in microbiome composition

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ABSTRACT:

Flying birds and bats have simplified gastrointestinal tracts (GITs) and low intestinal mass to facilitate flight. Previous work showed reduced GIT transit times in birds relative to other vertebrates—but GIT transit has never been collectively quantified for bats. Unique among mammals, bat GIT microbiomes are dominated by Pseudomonadota bacteria (previously Proteobacteria), which also dominate the microbiomes of flying birds – we hypothesized this convergence to result from rapid GIT transit times for both volant taxa. We conducted a meta-analysis of vertebrate GIT transit times which showed that bats and flying birds have significantly faster transit times relative to nonvolant vertebrates. Additionally, within the bat order (Chiroptera), we demonstrated decreasing transit times associated with increasing body mass, a pattern contrasting other vertebrates (including volant birds) and possibly influencing GIT microbiome composition. This inverted mass-transit association is likely driven by diet as fruit- and nectar-consuming Pteropodids are the largest of all bats.

BACKGROUND

Flying vertebrates (e.g., birds and bats) have unique gastrointestinal physiologies, likely evolved to facilitate powered flight [1]. As one such adaptation, flying birds are known to have rapid gastrointestinal tract (GIT) transit times [2,3], i.e., the time taken for food to pass through the stomach, small and large intestine, and colon prior to excretion [4]. Rapid GIT transit is hypothesized to help volant animals maintain low body masses for flight [2] while maximizing energy intake [5]. Faster GIT transit times also enable an increase in consumption rate during foraging [6]. GIT transit time influences the composition and abundance of the GIT microbiome; specifically, in humans, GIT transit is directly, positively correlated with total bacterial count [7,8] and positively associated with the presence of slow-growing gut microbes, such as methanogens [9,10]. In humans, the small intestine (where transit time ranges from 3-5 hours) has lower microbial diversity and abundance relative to the colon (where transit time can exceed 30 hours) [11]. Most mammalian GIT microbiomes that have been surveyed to date (chiefly those of Artiodactyla, Carnivora, Primates, and Xenarthra) are dominated by the phyla Firmicutes and Bacteroidota (formerly Bacteroidetes)[12]. By contrast, both bird and bat GIT microbiomes tend to be dominated by the phylum Pseudomonadota (previously called Proteobacteria) [13]. It is possible, therefore, that the convergence in composition of flying bird and bat GIT microbiomes could be explained by similarly rapid GIT transit. Though rapid GIT transit times are well-described for flying birds, GIT transit has never been collectively summarized for bats.

The passage of digesta through the GIT is affected by many factors, several of which converge in flying birds and bats. Anatomically, longer and more complex GITs (e.g., multiple stomach chambers such as those found in herbivorous ungulates) increase digesta passage time

[14], but shorter, simpler GITs (such as those found in small birds and bats) decrease transit time [15,16]. As previously reviewed [15], relative to non-flying mammals, both small birds and bats have short, simple digestive tracts, with reduced surface area and intestinal tissue [16,17]. Most bat GITs also lack a cecum and appendix, organs with specialized digestive properties in other taxa [17–20]. To compensate for the loss of intestinal absorptive surface area important for nutrient uptake in the GIT, both flying birds and bats have expanded villi in the GIT epithelium and exhibit greater reliance on paracellular absorption, the passive uptake of water soluble nutrients in the GIT, for meeting energetic demands [16].

In addition to anatomy, diet composition plays a key role in modulating GIT transit times. The water and fat content, consistency, and hardness of ingested food all impact passage time [21]. For instance, insectivorous bats have longer food retention times than frugivorous/nectivorous bats, likely due to a diet of harder, higher consistency food [5,22,23]. Evidence from pollen-eating bats indicates that the degree of dietary specialization is related to the efficiency of digestion, as well: for example, flower-specialist bats digest pollen content into protein resources more rapidly than generalist species [24,25]. Similarly, among insectivorous bats, the shortest GIT transit times have been recorded for trophic specialists [22]. It is hypothesized that the microbiome may play a larger role in nutrient extraction for diet specialist species and may explain some of the differences in the efficiency of digestion and gastrointestinal design [26].

Finally, digesta passage rate is also modulated by interactions between the host diet and GIT microbial community [15]. For example, increased fiber intake can decrease passage time as its breakdown by microbes produces short chain fatty acids (SCFAs), which stimulate intestinal motility to reduce transit time. High SCFA concentrations are also typically associated with

lower GIT pH, which is, in turn, associated with reduced transit time [27]. Nonetheless, the interaction among SCFA-producing bacteria, SCFAs, and pH is complex: lower pH environments are associated with reductions in *Bacteroides* spp., typically one of the most abundant SCFA-producing genera in the mammalian GIT, and decreased production of the two most abundant SCFAs (Acetate and Propionate). By contrast, low pH environments favor some bacteria (e.g. *Eubacterium spp., Roseburia spp., and Faecalibacterium spp.*) that produce greater amounts of another SCFA, Butyrate [28] [29,30]. In humans, the more rapidly transiting small intestine is additionally dominated by microbial 'pioneer species' in the phylum Pseudomonadota, which have high growth rates to facilitate rapid colonization of new substrates [31,32]. For the same reason, Pseudomonadota are also some of the earliest microbes to establish in human neonate GIT microbiomes [33] and manifest at higher proportions in the GIT microbiomes of human hosts experimentally induced to rapidly transit food [34]. Pseudomonadota are highly diverse and, as a result, are thought to be responsible for considerable functional variation in the tasks performed by the human GIT microbiome, despite being lower in abundance than Firmicutes and Bacteroidetes [35,36].

We compiled GIT transit times from the literature for a variety of food types across a wide range of volant (bat and bird) and non-volant (carnivores, ungulates, rodents, primates, reptiles) vertebrate taxa. We aimed to compare digesta passage times to elucidate their possible role in structuring differences in the GIT microbial community across these taxa. We demonstrate that flying birds and bats do indeed have significantly faster gut transit times than non-volant vertebrates, offering one possible explanation for their uniquely congruent GIT microbiomes.

METHODS

Literature survey and statistical approach

We collected GIT transit times from the primary literature across a wide range of vertebrate taxa to assess the impact of flight on the duration of time required for food passage in the GIT. To compile our database, we searched Web of Science for publications which cited the following search criteria in the title or abstract: “gut OR gastrointestinal OR food” AND “transit OR passage” AND “rate OR time.” We restricted our search using the “NOT” function to exclude studies that cited the following off-target terms: human[s], [wo]men, obesity, diabetes, adult, child[ren], patient[s], volunteer[s], disease, mice/mouse, rat[s]. This exclusion thus focused our study on GIT transit in non-model animal systems.

This search returned 1169 results, which excluded human and laboratory mouse and/or rat studies. We then individually reviewed each of the 1169 studies, including any study that reported “minimum gut transit” (MGT), “mean retention time” (MRT), “transit time”, or “gut passage time” for any vertebrate taxon, in our final database. We examined each study to confirm that the value reported corresponded to the duration of time passed from consumption to first passage in feces to ensure these values were comparable. Though mean retention time (MRT), corresponding to the average period of time food is retained in the gut, offers an arguably more accurate representation of the gut passage rates relevant to the establishment of GIT microbial communities [37], we found MGT to be more widely reported in the literature across multiple vertebrate taxa, offering a larger pool for comparison. As a result, we report results from MGT records in the main analysis and results for MRT in the supplemental analysis (appendix 1). Most of the studies returned from the search were irrelevant to our research agenda; we identified 107 unique publications that reported MGTs or equivalent for 112 unique

species (MRT for only 36 unique species) for inclusion in our final database (https://github.com/brooklabteam/git-transit-time/tree/main). Some studies reported MGT or MRT for multiple taxa, leading to multiple entries in the database, which we summarized prior to statistical analyses.

From each study, we extracted the following information for each animal surveyed and included it in the final database (Supplementary Dataset 1): (1) Order/Family/Genus/Species/Common Name, (2) diet classification (classed as frugivore/nectarivore, insectivore, omnivore, folivore, carnivore), (3) mass (grams), (4) food included in feeding trial (broad description), (5) food type in feeding trial (specific classification among four possible categories: fruit/nectar/pollen, protein, fiber/foliage, or a label-solution typical to these studies, such as barium sulfate), (6) mean or median MGT (or MRT) and, when available, minimum/maximum, and standard error/deviation of reported MGT, and finally (7) number of individuals and number of trials included in survey. In cases where some of the species-level information (i.e., mass) was missing from the original study, we searched the literature to find averages of these values for the taxon in question. We recorded the source (if not the original study) and the method of data summarization for each entry in our database, adjacent to the metric in question.

We next compared GIT transit times across the different taxonomic groups surveyed. First, we used the unique transit time value for each entry in the database, preferentially selecting the median reported value when available, then the mean if median was not reported. We then calculated an average transit time value for each combination of unique species and diet type in our database, weighting database entries by the number of individuals surveyed and the number of trials conducted in each original study. In cases where this original sample size information

was not included in the study (n=9), we assumed a sample size and trial size of only one individual in the weighting. Once the dataset was collapsed to a single transit time metric for each combination of species and diet type (a total of 142 entries representing 109 unique species), we then compared gut transit across disparate taxa.

Due to small sample sizes, we elected to categorize all non-mammalian vertebrates taxonomically by Class (specifically, Birds, Fish, Amphibians, Reptiles) and all mammals by Order. We separated birds into “Flying” and “Non-Flying” categories, though only two non-flying birds (*Spheniscus demersus*, the African penguin, and *Strutheo camelus*, the common ostrich) were represented in the database. After assigning these groupings, we subset the database to compare only those taxonomic groupings where more than five entries were reported, thus reducing our final database for comparison down to 140 entries across 103 unique species (47 bats, 14 carnivores, 16 even-toed ungulates [order Artiodactyla], 16 flying birds [order Aves], 22 primates, 8 rodents, 17 reptiles; supplemental table 1).

Statistical Comparison of GIT Transit Times by Taxa

Using our most stringent data subset, we first asked the question: *What is the relationship between taxonomic grouping and digesta passage time?* To address this statistically, we fit a linear mixed effect model in the Gaussian family from the lme4 package in R (version 2023.9.1) [38], assuming a response variable of the $\log_{10}$ transit time (in hours), a single predictor variable of taxonomic grouping (modeled as a categorical factor) and a random effect of food type (Table 1). We used the “Rodent” grouping (which demonstrated the lowest median GIT transit outside the two volant taxa) as the reference level for comparison and computed p-values using Satterthwaite’s degrees of freedom method in the “lmerTest” package [39].

To elaborate on this interaction, we next asked the question: *What is the relationship between the interaction of host body mass, taxonomic grouping, and the rapidity of GIT transit time?* To address this question statistically, we fit another linear mixed-effects model in the lme4 package in R, this time querying the response variable of the $\log_{10}$ transit time (in hours) against the predicter variable of the interaction of the $\log_{10}$ of the host's body mass (in kg) with its taxonomic grouping and included a random effect of taxonomic grouping in this model (Table 2).

RESULTS

Relationship between taxonomic grouping and the rapidity of GIT transit time

We found strong evidence that each taxonomic grouping maintained a significantly different GIT transit time (as reported by MGT) compared to the reference level ("Rodent"; Table 1, Figure 1a). Carnivores, primates, ungulates, and reptiles had significantly greater GIT transit times relative to rodents, whereas birds and bats had shorter GIT transit times relative to rodents (Figure 2a).

Random effect of food type on the correlation between taxonomic group and GIT transit time

In the first mixed model (without inclusion of a covariate predictor of body mass), we found a significant random effect of food type on the relationship between taxonomic grouping and GIT transit time, with fruit/nectar/pollen diets associated with the most rapid gut transits (**Figure 2b**). There was no evidence to suggest that fiber/foliage or protein diets had an effect on MGT but labeled solutions (e.g., dye or barium-sulfate solution to monitor gastrointestinal transit time) were found to be associated with longer gut transit times (increased MGT).

Relationship between the interaction of host body mass, taxonomic grouping, and the rapidity of GIT transit time

Four taxonomic groups demonstrated significant positive interaction effects with $\log_{10}$ body mass to modulate GIT transit time (as reported by MGT; Table 2): larger body size was associated with longer transit times in the case of all taxonomic groups, except for ungulates and bats (Figure 2c). By contrast, bats had marginally negative effects with $\log_{10}$ body mass to modulate GIT transit time – showing that larger body size was associated with shorter gut transit times.

Relationship between the interaction of host body mass, sub-grouping (e.g., family-level), and the rapidity of GIT transit time in the order Chiroptera

Given this reversal of the GIT transit time association with mass identified in the bat data, as well as the significant random effect of diet type in model 1 (Table 1), we next explored whether family-level groupings (associated with individual diet types) could further explain this relationship between host body mass and GIT transit time (as reported by MGT). We only considered data from bat families with more than one-entry (Phyllostomidae, Pteropodidae, and Vespertilionidae). While none of the family-level groupings had significantly different slopes from one-another, Pteropodidae – with a strictly fruit/nectar/pollen diet and the largest bodied bat family - was the only family with a non-negative slope (Figure 1C; Table 3).

Discussion

Understanding the factors influencing gastrointestinal transit (GIT) times across vertebrate taxa is crucial for unraveling the intricate relationship between digestive physiology,

diet, and evolutionary adaptations. In this study, we aimed to compare GIT transit times among volant (bats and birds) and non-volant vertebrates while considering the effects of body mass and diet. Our findings shed light on the unique digestive adaptations of flying vertebrates and their implications for GIT microbial communities. Consistent with previous research, our study reveals significant differences in GIT transit times among taxonomic groups. The taxonomic groupings of carnivora, primates, ungulates, and reptiles exhibited longer GIT transit times compared to rodents, whereas flying birds and bats demonstrated shorter transit times. This pattern of accelerated transit time was consistent for bats regardless of whether using the metric MGT or MRT as a proxy for GIT transit time. This highlights the diverse strategies employed by vertebrates to process and assimilate nutrients, allowing them to adapt to unique life histories and dietary preferences shaped by their evolutionary histories.

In our second analysis, host body mass emerged as a key determinant of GIT transit times across taxonomic groups. Generally, larger-bodied species exhibited longer transit times, consistent with expectations based on the scaling of gastrointestinal tract length [40]. Indeed, flying vertebrates have shorter intestines than non-volant mammals and reptiles to facilitate flight through reductions in body mass [41]. However, these anatomical changes in flying birds and bats have necessitated physiological adaptions for rapid nutrient absorption and water regulation which could, in turn, influence food passage times [16,42,43]. For example, we observed a reversal of this trend in Pteropodid bats, where larger body size was associated with shorter transit times (though this association was not statistically significant) [3]. Bats in the family Pteropodidae (Figure 1c), are on average, the largest of all bat species and maintain a fruit and nectar-based diet, which we showed to be associated with shorter GIT transit time. Nonetheless, while fruit and nectar-based diets were associated with more rapid gut transit than other diets in

our analysis (Figure 2b), taxonomic grouping remained a significant predictor of transit time even after accounting for diet—suggesting a unique digestive physiology in fruit bats independent of dietary differences. By contrast, label solutions were associated with longer transit times in our analysis, independent of vertebrate taxa, likely because these standard markers have foreign compositions that vertebrate guts are not optimized to digest.

The observed differences in GIT transit times among vertebrate taxa have significant implications for GIT microbial communities. Accelerated transit times in flying birds and bats may facilitate the rapid turnover of gut contents, shaping microbial diversity and composition; supported by the dominance of Pseudomonadota in volant species [13]. For many taxa, distinctive GIT microbial communities colonize different regions of the digestive tract and perform specific digestive functions [44]. By contrast, microbiome composition and abundance may be relatively consistent throughout regions of the bat GI tract (except in some frugivores, which demonstrate moderate shifts in relative abundance of different microbial taxa across the length of the GIT [45]). In general, consumption of difficult-to-digest, highly fibrous foliage is rare in both birds and bats [3]. Frugivorous bats disgorge most of the fibrous material (i.e., fruit pulp) in their diets and only consume the nectar and juice [46]. The increased importance of paracellular nutrient absorption in volant vertebrates may then negate the need for microbiota to enhance these processes in both birds and bats [15]. The convergence of GIT microbial communities between birds and bats, despite their phylogenetic distance, underscores the importance of GIT physiology in shaping microbial ecology.

Future directions

Our study provides valuable insights into the factors which underpin variation in GIT transit times across vertebrate taxa. By elucidating the interplay between diet, body size, and

evolutionary history, we contribute to a deeper understanding of digestive adaptations in flying vertebrates and their implications for GIT microbial communities. Future research should explore the functional significance of these adaptations and their role in vertebrate ecology and evolution.

DECLARATIONS

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and material

The datasets generated and the code used for analysis for the present study are available at: https://github.com/brooklabteam/git-transit-time.git

Competing interests

The authors declare no competing interests.

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Author's contributions

ECR conducted the final data analysis and wrote the manuscript. KM collected data from the literature and helped with the data analysis. DNJ conceptualized the project, helped with the data collection, and wrote the manuscript. NAFR helped with the data analysis. NCD reviewed the data and wrote the manuscript. CJY helped with the data analysis. RKP conceptualized the project and provided funding. CEB conceptualized the project, collected the original data, helped with data analysis, funded the project, and wrote the manuscript. All authors reviewed and provided edits to the final manuscript.

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Table 1. Relationship between taxonomic grouping and the rapidity of GIT transit time.

Taxonomic Grouping	**Slope**	**Std. error**	**Degrees of freedom**	**t-value**	**p-value**
(Intercept)	0.5473	0.1353	40.5218	4.045	0.000228 ***
Flying Birds	-0.587	0.1492	139.7	-3.936	0.000130 ***
Bats	-0.427	0.1387	135.9	-3.080	0.002505 **
Carnivores	0.495	0.1635	133.1	3.028	0.002955 **
Primates	0.839	0.1435	13.85	5.849	3.34e-08 ***
Ungulates	1.017	0.1487	139.57	6.840	2.29e-10 ***
Reptiles	1.462	0.1488	139.97	9.827	< 2e-16 ***
random effect of food type: p-value = 0.02377*					
** at p <.05; ** at p < .01; ***at p < .001*					

Results of the mixed effects model of GIT transit times, as predicted by taxonomic grouping with a random effect of diet classification. *Model:* log_{10}*(transit time) ~ Class + (1| Diet), REML=F.*

Table 2. Relationship between the interaction of host body mass, taxonomic grouping, and the rapidity of GIT transit time.

log10(mass): taxonomic grouping	**Slope**	**Std. error**	**Degrees of freedom**	**t-value**	**p-value**
(Intercept)	0.89115	0.25511	7.13648	3.493	0.00978 **
$\log_{10}$(mass):Flying Birds	0.50171	0.08364	136.43675	5.998	1.69e-08 ***
$\log_{10}$(mass):Bats	-0.16267	0.08661	139.23271	-1.878	0.06244 .
$\log_{10}$(mass):Rodents	0.27406	0.11004	133.81241	2.491	0.01397 *
$\log_{10}$(mass):Carnivores	0.48411	0.19952	139.91864	2.426	0.01652 *
$\log_{10}$(mass):Primates	0.29365	0.13470	137.56043	2.180	0.03095 *
$\log_{10}$(mass):Ungulates	0.03099	0.09638	135.84425	0.322	0.74826
$\log_{10}$(mass):Reptiles	0.12151	0.06620	132.74398	1.836	0.06865 .
random effect of class: p-value = < 2.2e-16 ***					
** at p <.05; ** at p < .01; ***at p < .001*					

Results of the mixed effects model of GIT transit times, as predicted by the interaction of body mass and taxonomic grouping, with a random effect of taxonomic grouping. *Model:* $\log_{10}$*(transit time) ~* $\log_{10}$*(mass):Class + (1|Class), REML=F.*

Table 3. Relationship between the interaction of host body mass, sub-grouping (e.g. family-level), and the rapidity of GIT transit time in the order Chiroptera.

log10(mass): taxonomic grouping	**Slope**	**Std. error**	**Degrees of freedom**	**t-value**	**p-value**
(Intercept)	-0.114	0.148	45.000	-0.771	0.445
log_{10}(mass):Phyllostomidae	-0.143	0.099	45.000	-1.447	0.155
log_{10}(mass):Pteropodidae	0.002	0.137	45.000	0.017	0.987
log_{10}(mass):Vespertilionidae	-0.133	0.085	45.000	-1.574	0.122
***random effect of family: p-value* = 1**					
** at p <.05; ** at p < .01; ***at p < .001*					

Results of the mixed effects model of GIT transit times, as predicted by the interaction of body mass and family-level grouping, with a random effect of family-level grouping. *Model:* $log_{10}(transit\ time) \sim log_{10}(mass):Family + (1|Family), REML=F$.

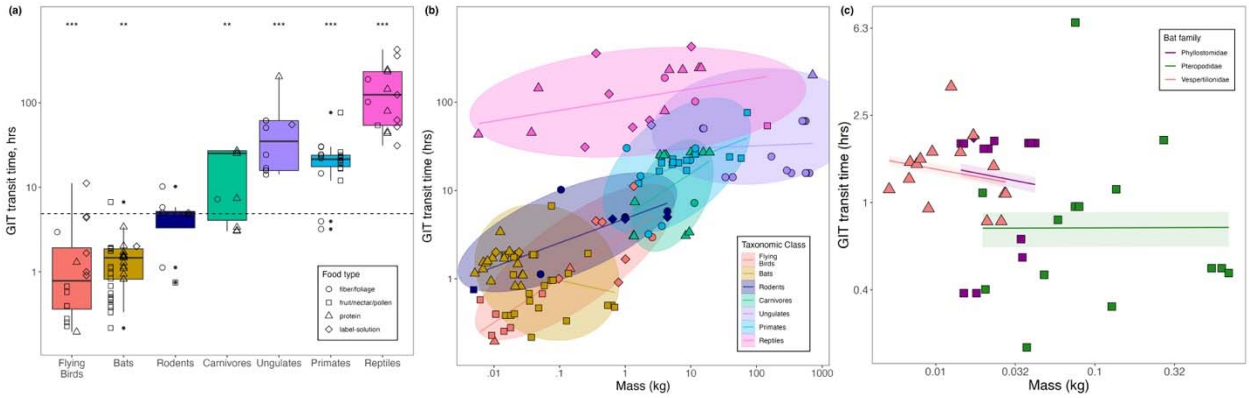

Figure 1: *Minimum GIT transit times (as reported by MGT) from experimental studies across volant and nonvolant vertebrates*. **(A)** Boxplots show the interquartile range of minimum GIT transit times (on a $\log_{10}$ scale) recovered from studies across diverse vertebrate taxa (x-axis). Shapes correspond to food type used in each study, according to the legend. Flying birds and bats have significantly faster GIT transit times relative to the reference taxon (rodents), and consequently, all other non-flying vertebrates (Table 1; linear mixed effects model; *=p-value<0.01, **=p-value<0.001, and ***=p-value< 0.0001). **(B)** Minimum GIT transit time (y-axis, $\log_{10}$ scale) for each taxon relative to their body mass (in kg; x-axis), with taxa distinguished via color and food type indicated by shape as highlighted in (A). Trend lines (Table 2; Linear mixed effects model) indicate that MGT increases with body mass in all taxa except bats, which show the opposite trend (p-value =0.06). Polygons encircle the full range of species surveyed in each taxa. **(C)** Bat-only data, showing the reversed direction of the relationship between mass and GIT transit for bats (from positive for other taxa to negative in bats). The largest-bodied Pteropodidae fruit bats show some of the most rapid transit times and appear to lack a relationship with body mass (Table 3).

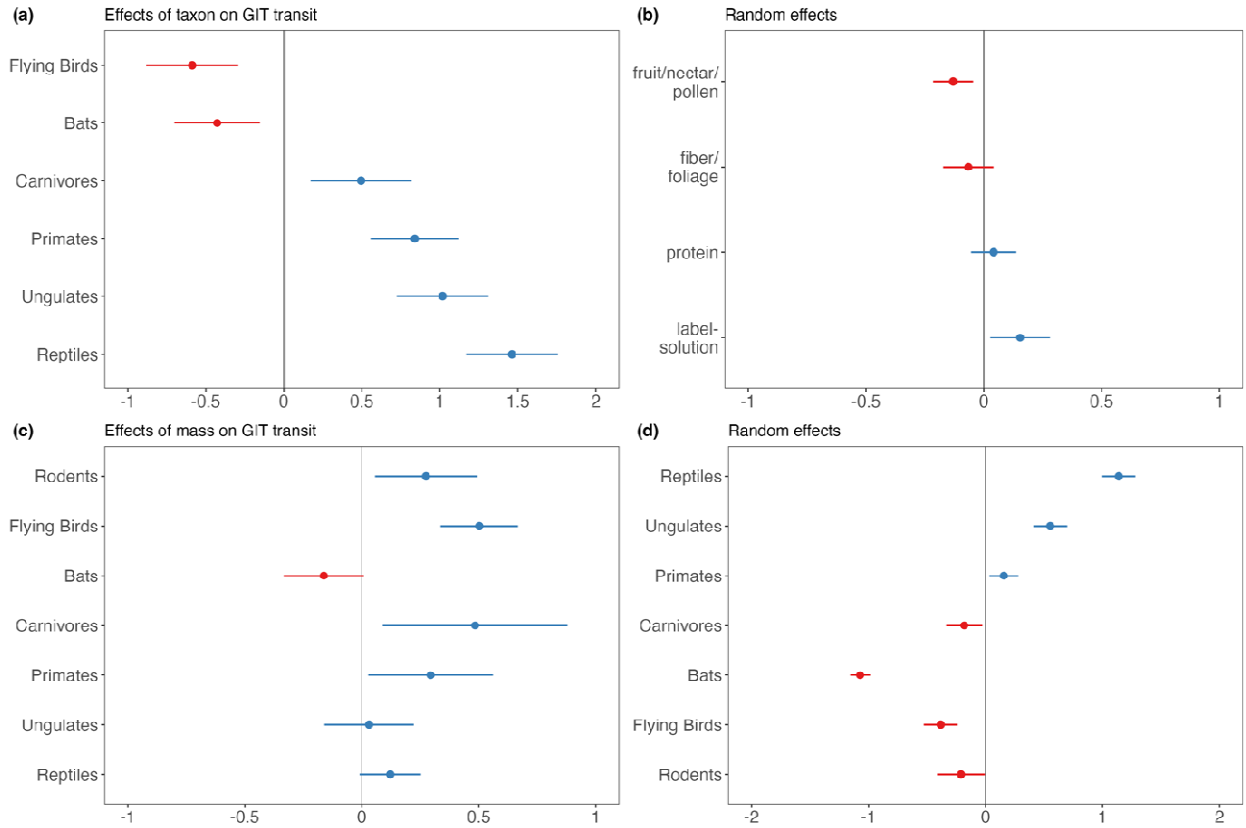

Figure 2: *Fixed and random partial effects on GIT transit time (as reported by MGT).* Fixed **(a)** and random **(b)** effects sizes from a linear mixed effects model with taxonomic class as a fixed effect and diet category as a random effect. Bats and flying birds show significant negative slopes, indicating more rapid GIT transit times than rodents, while all other taxa demonstrate significant positive slopes, indicating slower GIT transit times than rodents. Fruit/nectar/pollen diets show the most negative interaction with GIT transit, whereas label-solution (a medical solution marker, such as barium sulfate) shows a positive interaction. Fixed **(c)** and random **(d)** effects from a linear mixed effects model with log10(mass): taxonomic class as a fixed effect interaction and taxonomic class as a random effect. Bats are the only taxonomic group to show that mass has a marginally inverse effect on GIT transit.

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