



THE DEGREE OF FRUGIVORY OF BIRDS AS ESTIMATED FROM GASTRIC AND FECAL SAMPLES

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Abstract · The degree of frugivory (DF) has long been used to characterize the relative importance of fruits in the diet of a bird and, more recently, as a functional trait related to the role of birds in seed dispersal networks. Although quantitative estimations of DF are desirable, general, categorical classifications or coarse estimations of diet composition based in qualitative information are often used. Data on stomach, fecal, and regurgitation contents scattered in the literature or easily obtained in the field could be used to provide a quantitative, potentially more reliable assessment of DF. We compiled such data from the literature and our own fieldwork to obtain 12,576 samples from 985 Neotropical bird species, of which 489 species (49.6%) in 61 families had at least one fruit-containing sample. From this dataset we analyzed 250 species for which we got at least 10 samples to find that gastric (i.e., stomach plus regurgitation) and fecal samples provided similar estimates of DF despite potential differences in the degree of food digestion. The DFs we obtained were higher than those presented in the most frequently used source of quantitative DF estimates in the literature (the Elton Traits database). We further explored, with a few study cases, the utility of stomach, fecal and regurgitation samples to evaluate intraspecific geographic, sexual, and ontogenetic variations in DF, topics rarely investigated so far. We found that the range of intraspecific geographic variation in DF increased with increasing DF, and that sexual and ontogenetic differences may occur. We argue that stomach, fecal, and regurgitation sample data abundantly available in the literature or easily obtained from mist-netted birds may be used to produce quantitative assessments of DF likely more reliable than the estimates used so far and useful for a plethora of ecological studies.

Resumo · O grau de frugivoria de aves estimado a partir de amostras gástricas e fecais

O grau de frugivoria (GF) tem sido usado para caracterizar a importância relativa dos frutos na dieta das aves e, mais recentemente, como um traço funcional relacionado ao papel das aves nas redes de dispersão de sementes. Embora estimativas quantitativas do GF sejam desejáveis, classificações categóricas ou estimativas grosseiras da composição de dieta baseadas em informações qualitativas são frequentemente utilizadas. Dados de conteúdos estomacais, fecais e regurgitos dispersos na literatura ou facilmente obtidos em campo podem ser usados para fornecer uma avaliação quantitativa e mais confiável do GF. Nós compilamos esses dados da literatura e de nosso próprio trabalho de campo para obter 12.576 amostras para 985 espécies de aves neotropicais, das quais 489 espécies (49.6%) de 61 famílias tiveram pelo menos uma amostra contendo frutos. Destas, analisamos 250 espécies para as quais obtivemos ao menos 10 amostras e verificamos que amostras gástricas (i.e., estomacais e regurgitos) e fecais fornecem estimativas similares do GF, apesar das potenciais diferenças no grau de digestão do alimento. Os GF que nós obtivemos foram maiores do que aqueles apresentados na fonte de estimativas quantitativas de GF mais frequentemente utilizada na literatura (o banco de dados Elton Traits). Exploramos também, com alguns estudos de caso, a utilidade de amostras estomacais, fecais e regurgitos para avaliar as variações intraespecíficas geográficas, sexuais e ontogenéticas nos GF, tópicos raramente investigados. Descobrimos que a amplitude de variação geográfica intraespecífica no GF aumentou à medida que o GF aumenta, e que diferenças sexuais e ontogenéticas podem ocorrer. Nós argumentamos que os dados estomacais, fecais e de regurgitos abundantemente disponíveis na literatura ou obtidos de aves a partir de redes de neblina podem ser usados para produzir avaliações quantitativas de GF provavelmente mais confiáveis do que as estimativas usadas até agora e úteis para uma variedade de estudos ecológicos.

Key words: Diet · Functional traits · Intraspecific variation · Neotropical region · Ontogeny**INTRODUCTION**

The Degree of Frugivory (DF), loosely defined as the relative importance of fruits for the diet of a bird, has been used to characterize the reliance of birds on fruits, their responses to seasonal fluctuation in fruit abundance (Martin 1985, Wheelwright 1986) and habitat alteration (Moran et al. 2004), and their role in seed dispersal networks (Schleuning et al. 2011, Grass et al.

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2014). Birds with a high DF usually have a generalized fruit choice, consuming fruits of a wider variety of plant species than birds that rely more sporadically on fruits (Moermond & Denslow 1985, Schleuning et al. 2011, 2012, Grass et al. 2014). Consequently, highly frugivorous birds often form the core of seed dispersal networks, contributing more strongly to their maintenance and cohesion than less frugivorous species (Ruggera et al. 2016, Sebastián-González 2017); however, this does not mean that birds with a low DF are unimportant as seed dispersers, as they may play a pivotal role in the dispersal of certain plant species (e.g., Carlo et al. 2003, Almazán-Núñez et al. 2016).

Categorical and continuous classifications of birds' DF have been used. Snow (1981) used the categories *specialist* for species that have most of their diets composed of fruits, and *generalist* for species consuming mostly other food items, with fruits forming a relatively minor part of their diet. In ecological works, frugivorous birds have been classified as *obligate frugivores* for species with fruits as the major food item, *partial frugivores* for species that include other major food items in their diets, and *opportunistic frugivores* for species that only occasionally eat fruits as a supplementary food resource (Kissling et al. 2007, see Moran & Catterall 2010 for a variation of this classification). Lopes et al. (2016) proposed a refinement of the categorical classification by assigning birds to five categories, ranging from *insectivorous* for birds including less than 10% of fruits in their diet, to *frugivorous* for those eating more than 90% of fruits.

For continuous data, the most comprehensive and widely used source of DF is the Elton Traits database (Wilman et al. 2014), which provides the percentage of the diet composed by fruits and other food items for all the bird species of the world. Although invaluable as a source of information for multispecies studies, the data in Elton Traits came from coarse estimations of diet composition based on qualitative information gathered mostly from the species accounts in field guides, general treatises (e.g., Handbook of the Birds of the World series, Del Hoyo et al. 1992), or books dedicated to specific bird groups and families. In the words of Wilman et al. (2014), they “followed a defined protocol to translate the verbal descriptions [of diet] into standardized, semiquantitative information about relative importance of different [diet] categories.” As an alternative to assessing the DF of birds in a more accurate, quantitative way, one may rely upon detailed diet studies, which unfortunately are available for only a few species (e.g. Gaiotti et al. 2017). What is fairly available but sparse in the literature—including the old and grey literature—are data on stomach, fecal and regurgitation contents, not necessarily collected with the purpose of formally describing diets, but which might be used to assess DF in a quantitative way. Fecal samples have already been used to infer DF, but rarely and only for a few bird species (Jordano 1987). Therefore, our primary goal was to gather such widespread information to provide quantitative estimates of DF for Neotropical birds. By incorporating the composition of several stomach, fecal or regurgitation samples taken from wild birds, we provide a different approach (hopefully more accurate) to estimate the DF than that provided in Elton Traits. In addition, we used stomach, fecal, and regurgitation samples to show the utility of such samples to evaluate intraspecific variation (geographic, sexual, and ontogenetic) in DF in a few case studies. Contrary to the tem-

poral dimension of intraspecific variation in DF that usually occurs in response to seasonal fluctuations in the availability of fruits (Carnicer et al. 2009, Naoe et al. 2018), geographic, sexual, and ontogenetic (i.e., from nestlings to adults) variations in DF are poorly explored in the literature.

Because different methods of diet sampling (i.e., stomach contents, fecal and regurgitation samples) may have advantages and deficiencies related to their adequacy for DF assessment, we asked if each methodology provided similar DFs. For instance, stomach (or crop) and regurgitation contents provide less digested food items than fecal samples, which makes it easier to identify food items (Rosenberg & Cooper 1990).

METHODS

We searched the literature for studies reporting the contents of stomach, regurgitation, and fecal samples collected from wild birds in the databases Google Scholar, Scopus, and Web of Science using the terms “bird”, “diet”, “stomach”, “fecal”, “regurgitation”, “Neotropic”, and their combinations in English, Portuguese, and Spanish. We did not attempt to include direct observations of foraging birds in the analyses because this method tends to inflate DF estimates by favoring the record of conspicuous food items (e.g., fruits in detriment of insects; Rosenberg & Cooper 1990), although it has already been used to assess DF (Greenberg 1981, Martin 1985). As seed dispersal is the focus of most of the ecological studies for which DF estimates are useful, we focused on all birds feeding on fleshy fruits. We included data on birds in the families Psittacidae and Columbidae normally taken as seed predators, but that may disperse seeds (Tella et al. 2015, Checon 2020), as well as birds for which the relative role as seed predators or seed dispersers is largely unknown (e.g., Tinamidae, Odontophoridae). We, however, did not consider typical granivorous birds, specifically species in the genera *Carduelis*, *Spinus* (Fringillidae), *Ammodramus*, *Arremon*, *Zonotrichia* (Passerellidae), *Amaurospiza*, *Cyanoloxia* (Cardinalidae), *Sicalis*, *Rhopospina*, *Haplospiza*, *Sporophila*, and *Volatinia* (Thraupidae), and the non-native *Estrilda* (Estrildidae) and *Passer* (Passeridae). Although birds in these taxa may occasionally eat fleshy fruits, they are mostly interested in the seeds of dry fruits (e.g., achenes) that they supposedly destroy, being thus best-called seed predators than seed dispersers. Nevertheless, the possibility that these birds pass intact seeds through their guts needs further investigation.

We also included in the analyses unpublished data that we gathered from fecal samples of 18 bird species mist-netted at the campus of the University of São Paulo State at Rio Claro, Brazil (22°23'38.65"S, 47°32'25.70" W; see Potascheff et al. 2010 for a description of the campus vegetation), and also from 13 species sampled at the km 41 Farm in the area of the Biological Dynamics of Forest Fragments Project (BDFFP) near Manaus, Brazil (2°27'2.94"S, 59°46'3.54"W; see Laurance et al. 2002 for a description of the reserve vegetation).

Upon inspecting the samples under a stereomicroscope, we classified them as having only fruit remains (e.g., intact or broken seeds, pulp), only arthropods, a mix of fruits and arthropods, or other food items (e.g., mollusks, leaves, vertebrate remains). We calculated the frequency of occurrence

Table 1. Average degree of frugivory (DF) obtained from a literature review and field data for Neotropical families having at least one species with ≥ 10 samples (gastric and fecal samples pooled). N refers to the number of species included in each family (see Supplementary Table 1 for species details). Families are arranged in taxonomic order following IOC World Bird List - <https://www.worldbirdnames.org/>.

Family	N	Mean DF \pm SD	DF Range
Tinamidae	6	63.7 \pm 9.5	50.9 – 75.0
Anatidae	2	85.4 \pm 14.7	75.0 – 95.8
Cracidae	1	91.2	-
Odontophoridae	1	75.0	-
Nyctibiidae	1	0.0	-
Caprimulgidae	4	0.9 \pm 1.8	0.0 – 3.6
Apodidae	2	0.0 \pm 0.0	0.0 – 0.0
Trochilidae	4	1.7 \pm 1.9	0.0 – 3.5
Cuculidae	6	10.7 \pm 7	0.0 – 20.0
Columbidae	7	94.4 \pm 5.6	87.0 – 100
Rallidae	1	65.4	-
Charadriidae	1	2.8	-
Jacaniidae	1	23.3	-
Scolopacidae	1	15.6	-
Accipitridae	3	0.0 \pm 0.0	0.0 – 0.0
Strigidae	3	3.6 \pm 3.2	0.0 – 6.3
Trogonidae	9	44.9 \pm 24.4	20.4 – 95.5
Momotidae	4	24.0 \pm 16.9	1.8 – 42.9
Galbulidae	3	0.0 \pm 0.0	0.0 – 0.0
Bucconidae	3	6.1 \pm 10.5	0.0 – 18.2
Capitonidae	2	72.0 \pm 22.3	56.3 – 87.8
Ramphastidae	9	93.2 \pm 8.6	73.1 – 100
Picidae	8	20.4 \pm 21.5	0.0 – 50
Falconidae	5	0.7 \pm 1.7	0.0 – 3.7
Psittacidae	4	94.8 \pm 5.9	86.4 – 100
Furnariidae	23	3.7 \pm 10.4	0.0 – 50
Thamnophilidae	15	3.8 \pm 3.3	0.0 – 10.5
Conopophagidae	2	1.9 \pm 2.6	0.0 – 3.7
Rhinocryptidae	2	18.2 \pm 25.8	0.0 – 36.5
Tyrannidae	41	24.0 \pm 26.2	0.0 – 95
Cotingidae	1	56.3	-
Pipridae	10	77.2 \pm 26.8	10.0 – 100
Tityridae	3	23.0 \pm 8.7	13.9 – 31.3
Vireonidae	4	20.6 \pm 21.9	0.0 – 50.7
Corvidae	2	62.4 \pm 8.1	56.7 – 68.2
Troglodytidae	1	0.0	-
Poliophtilidae	1	7.1	-
Turdidae	6	65.5 \pm 7.6	57.1 – 79.2
Mimidae	2	50.6 \pm 10.3	43.3 – 57.9
Passerellidae	2	6.1 \pm 8.6	0.0 – 12.1
Icteridae	6	32.7 \pm 21.8	9.4 – 62.5
Parulidae	8	3.5 \pm 3.6	0.0 – 11.1
Cardinalidae	2	29.3 \pm 11.6	21.2 – 37.5
Thraupidae	28	46.2 \pm 25.8	0.0 – 90.9

of fruits in samples by summing 1 to fruit-only samples, 0.5 to mixed samples, and 0 for samples containing only arthropods or only other food items. The DF, ranging from 0-100%, was then calculated as the ratio between the total frequency of fruits in samples and the total number of samples analyzed for a given species. The same procedure was adopted for the literature data that often brings only qualitative information on the contents of samples, with no detailed assessment of the relative contribution of fruits, arthropods, or other food items, thus precluding a more sophisticated approach to convert qualitative into quantitative data.

We selected 31 species for which we have at least 10 samples gathered by each of at least two different methods to compare the DF they provided. As stomach and regurgitation contents are likely equivalent in terms of digestion of food items (Rosenberg & Cooper 1990), we pooled them for analyses under the name “gastric” samples. The same analytical procedure was adopted to compare the DF values we obtained with those available in the Elton Traits database.

The intraspecific geographic variation in DF was explored for 29 bird species for which we obtained DF from two to five different studies carried out in different localities. We used

samples of the Pale-breasted Thrush (*Turdus leucomelas*) captured in Rio Claro to explore the ontogenetic variation in DF by comparing the DF of adults and nestlings. Adult fecal samples came from mist-netted birds, while fecal samples from nestlings were obtained during banding activities when nestlings were 8–11 days old (nestlings stay in the nest for up to 14 days; M.A. Pizo unpubl. data). Manaus data on the White-crowned (*Pseudopipra pipra*) and White-fronted (*Lepidothrix serena*) manakins were used for testing for sexual differences in DF. Mist-netted birds were sexed through definitive plumage patterns typical of males or the presence of brood patches (exclusive to females in manakins), aided by inspection of skull ossification, gape, and molt lines (Ryder & Durães 2005, Johnson & Wolfe 2017). The sex of green-plumaged birds was confirmed by molecular markers coding for the sex-linked polymorphism of the chromo-helicase-DNA-binding genes, which is heterogametic in females (CHD-Z and W) and homogametic in males (CHD-Z), following the protocol described in Ito et al. (2003).

To account for at least part of the intraspecific variation in DF (see below), we only considered species with more than 10 samples, an arbitrary cutoff used for all the analyses.

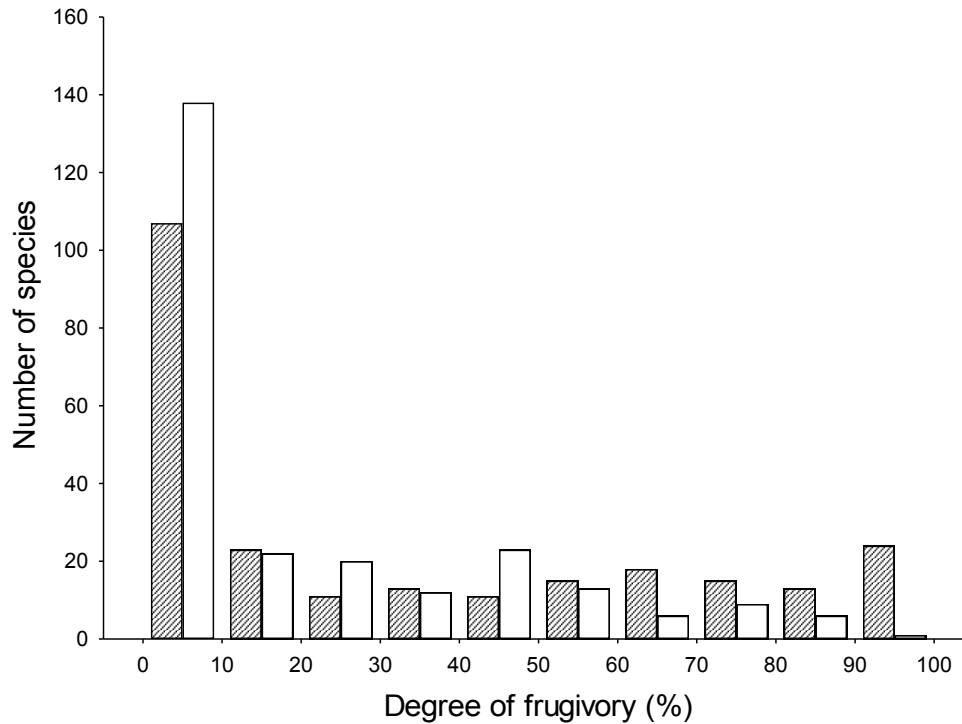


Figure 1. Frequency distributions of the degree of frugivory (DF) of 250 Neotropical bird species obtained in this study (hatched bars), and the DFs of the same species from the Elton Traits dataset. Only species with ≥ 10 samples (considering gastric and fecal samples pooled) were considered.

We used paired t-tests to compare the DFs obtained with different sampling methods and also to compare the DF estimates we obtained with the DFs provided in Elton Traits. Absolute deviations between ours and Elton Traits' DFs were correlated with the sample sizes of each species, a proxy for the reliability of our DF estimates. The larger the sample sizes the greater is the chance that our estimates encompass and reliably represent the intraspecific variability in DF. For this, we used Pearson's correlation applied to log-transformed sample sizes and $\log(n+1)$ absolute deviations. A positive correlation would indicate that the more reliable our DF estimates the more they differ from DF estimates provided in the Elton Traits dataset. To test for ontogenetic and sexual differences in DF we used chi-square tests applied to the frequencies of occurrence of fruits and arthropods in samples. We considered Elton Traits DFs as the percentages of fruits in the diets of birds as reported in column "Diet-Fruit" of Wilman et al.'s (2014) dataset. We also did the analyses considering the sum of the fruit and seed (column "Diet-Seed") contents as DFs. As both sets of analyses rendered similar results, we only report the results obtained with the "fruit-only DF", leaving the "composite DF" (i.e. summing the fruit and seed content) for the supplementary material. All the statistical tests were performed with STATISTICA 6.0 (StatSoft 1999). Bird species classification followed the South American Classification Committee (Remsen et al. 2021).

RESULTS

Based on 54 published and unpublished sources, we obtained 12,576 samples, comprising 6,558 (52.1%) stomach samples, 3,556 (28.3%) regurgitated samples, and 2,467 (19.6%) fecal samples. In total, we obtained data for 985 Neotropical bird species from 73 families, of which 489 species (49.6%) from 61 families (83.6%) had at least one sample

containing fruit remains. A subset of 250 species (25.5%) belonging to 45 (61.6%) families had more than 10 samples and were used in the analyses (Table 1 and Supplementary Table 1).

The mean values of DF did not differ between gastric and fecal samples (mean \pm SD = $31.3 \pm 27.4\%$ and $34.0 \pm 31.6\%$, respectively, $N = 31$ species in both cases; $t = -0.92$, $df = 30$, $P = 0.36$). We thus pooled all samples, irrespective of the method used, in the following analyses.

The DFs we obtained across the 250 study species were higher than the DF provided in Elton Traits ($33.3\% \pm 34.5\%$ and $21.5\% \pm 27.0\%$, respectively, $N = 250$ species; $t = 7.69$, $df = 249$, $P < 0.001$). The greater the sample size, the higher the absolute deviation between our DF estimates and Elton Traits values ($r = 0.25$, $N = 250$, $P < 0.001$). Assuming that a larger sample size increases the reliability of the estimates, this result indicates that the most reliable DF estimated from fecal and gastric samples were the ones that deviated most from the DF values provided in Elton Traits. Likewise, absolute deviations correlated positively with DF ($r = 0.56$, $N = 250$, $P < 0.001$), indicating that the deviations between ours and the DF estimates provided in Elton Traits tend to be greater for the most frugivorous species. Most (52.0%) of the species had $DF \leq 20\%$ (vs. 64.0% from the Elton Traits dataset), whereas 14.8% (vs. 2.8% in Elton Traits) had $DF \geq 80\%$ (Figure 1).

The range of geographic, intraspecific variation in DF increased with increasing DF (Pearson's $r = 0.70$, $N = 29$, $P < 0.001$, Figure 2). For *T. leucomelas*, we found that adults had a higher DF than nestlings (63.9%, $N = 187$ and 44.3%, $N = 79$, respectively; $\chi^2 = 8.76$, $df = 1$, $P = 0.003$). Regarding sexual differences in DF, males of *P. pipra* had marginally higher DF than females (86.2%, $N = 29$, and 69.8%, $N = 11$, respectively; $\chi^2 = 3.15$, $df = 1$, $P = 0.07$), whereas in *L. serena* the DFs of males and females were similar (71.2%, $N = 40$, and 75.0%, N

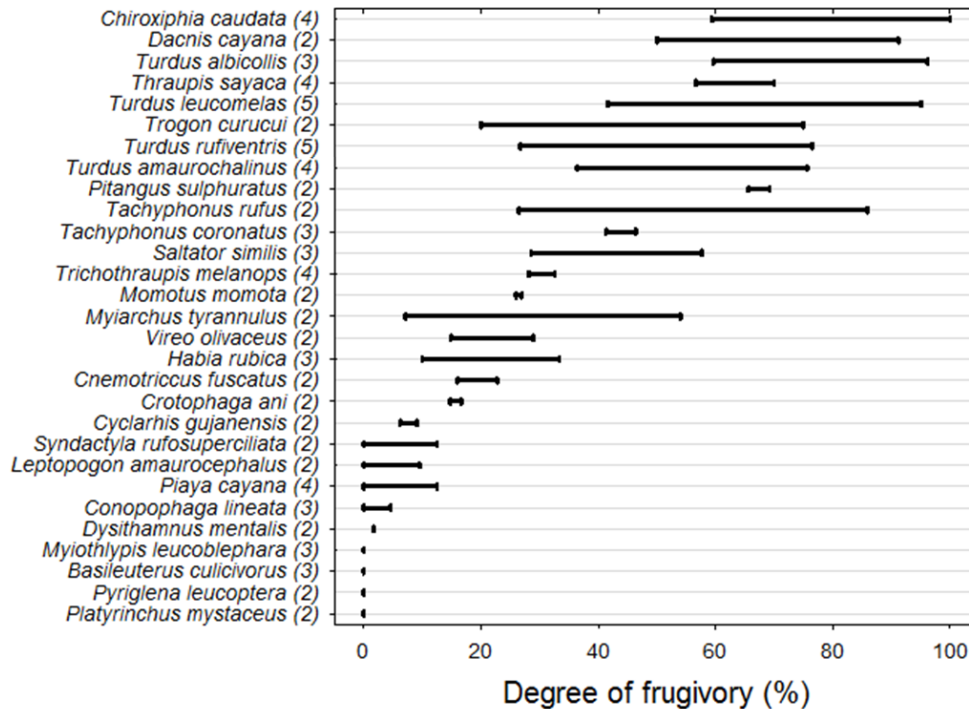


Figure 2. Intraspecific variation in the degree of frugivory (DF) of Neotropical birds. Bold lines encompass the DF ranges obtained from several studies (indicated in parentheses) conducted at different locations for each bird species. From bottom to top species are arranged in an increasing order of DF.

= 32, respectively; $\chi^2 = 0.13$, $df = 1$, $P = 0.72$).

DISCUSSION

We demonstrated that, despite potential differences in the degree of food digestion between gastric and fecal samples, both sampling methods provided similar estimates of DF according to our analytical approach. However, the ingestion of certain food types may lead to biases in DF estimates from gastric and fecal samples. For instance, large seeds in relation to bird size are usually regurgitated rather than defecated, and as such are less likely to appear in feces than in stomach or regurgitated samples (Jordano 2014). Likewise, certain birds, such as raptors, eat fruit pulp but do not ingest the seeds (Galetti & Guimarães Jr. 2004). In these cases, fruit-eating may be unnoticed unless other fruit remnants (e.g., fruit pulp) can be identified in fecal samples. Similarly, earthworms and other invertebrates with “soft” bodies are often not evident in fecal samples. Consequently, birds that frequently eat soft invertebrates (e.g., *Turdus* thrushes that typically eat earthworms) may have their DF overestimated if based solely on fecal samples. The same may be said about frugivores such as tanagers (Thraupidae) that occasionally drink nectar (Isler & Isler 1987). For these species, a combination of fecal samples with direct observations of foraging may provide a more reliable estimate of DF. At Rio Claro, for instance, our fecal samples resulted in a DF of 64.1% for *T. leucomelas*, while a study involving direct observations of foraging birds rendered a DF of 54.3% (Guzman 2014).

Apart from such caveats, do gastric and fecal samples provide reliable estimates of DF in birds? Considering that the DF should reflect the contribution of fruits to the diet of an animal, gastric and fecal samples represent a way to estimate such a contribution with an indirect assessment of the frequency of fruit ingestion. As a single sample may contain fruits consumed in several foraging bouts, while fruits of a

single bout may be spread in several samples, the presence of fruit remains in gastric and fecal samples cannot be directly taken as the frequency of fruit-eating bouts. Notwithstanding, our approach to estimate the DF represents an alternative to the general, qualitative diet descriptions as in the Elton Traits database and other categorical classifications adopted in other studies (Kissling et al. 2007). Obviously, the accuracy of DFs based on gastric and fecal samples depends on the accuracy with which such samples are analyzed, which should vary depending on the aims of the study reporting the data and the experience of the person who processed the sample. Overestimation of DF in gastric and fecal samples may occur when fragments unrelated to fruits are misidentified as fruit remains. For instance, tiny arthropod fragments may easily be mistaken for small seeds. We suspect, however, that underestimation (i.e., failing to identify fruits in samples leading to underestimation of DF values) is more common, especially if samples were analyzed by people unfamiliar with the several ways that fruit remains appear in samples (e.g., fruit exocarp, pulp remains, very small and broken seeds).

Our results revealed a greater number of Neotropical bird families with frugivorous species than what is usually reported in the literature (Fleming & Kress 2013). These include unsuspected families such as Nyctibiidae, Strigidae, and Trochilidae. It is hard to assert if fruit consumption by species in these families was incidental (e.g., by preying upon frugivorous vertebrates in the case of owls) or not, or even if it is the result of misidentification of fruits in samples. However, the occasional consumption of fruits by highly insectivorous species has been confirmed by direct observations of foraging birds (e.g. Cuculidae, Thamnophilidae; Gomes et al. 2008). Likely, the presence of fruit remains in gastric and fecal samples taken from such “unsuspected” species only reflects a lack of knowledge on the subtleties of their diets. It is known, for instance, that hummingbirds may occasionally

drink fruit juices (Ruschi 2014), but apparently, they may also ingest entire fruits (Poulin et al. 1994, Manhães et al. 2010). Even though the consumption of fruits is rare, species in some of these families may have been underestimated as legitimate seed dispersers in the Neotropical region, as it likely happens with other bird families in other parts of the world where “unconventional frugivores” may have a really important but underappreciated role as seed dispersers (e.g., Ardeidae, Soons et al. 2016; Rallidae, Carpenter et al. 2018).

On the other extreme of the DF gradient (i.e., birds with DF > 90%; Supplementary Table 1) are birds often taken as seed predators (e.g., Columbidae, Psittacidae) and well-known seed dispersers (e.g., Ramphastidae, Pipridae). While recent studies have shown that psittacids occasionally disperse seeds (Tella et al. 2015), the role of Neotropical columbids as seed predators is taken for granted without a careful evaluation of their actual effects upon seeds. However, at least some columbids may pass seeds unharmed. For instance, captive *Patagioenas picazuro* defecated up to 76% of seeds intact, depending on the seed species (Danielle T. Ramos & Marco A. Pizo, unpubl. data). Toucans (Ramphastidae) are well known to occasionally prey on invertebrates and vertebrates, but their high DF was confirmed by observational studies (Galetti et al. 2000). Together with cotingids (Cotingidae) and euphonias (Fringillidae), manakins (Pipridae) are the quintessential Neotropical avian frugivores (Fleming & Kress 2013). The absence of the two former families from the top frugivores in our study (Supplementary Table 1) reflects the lack of data and, consequently, our ignorance about the basics of their diets (but see Pizo et al. 2002). Similarly, the high DF of the Rosy-billed Pochard (*Netta peposaca*, DF 95.8%, Supplementary Table 1) draws our attention to the general lack of information about the frequency of fruit consumption by Neotropical ducks in general (Silva et al. 2021). Members of Anatidae have been shown to be important dispersers of aquatic plants for wetlands around the world (Green et al. 2016).

The use of gastric and fecal samples allowed us to evaluate intraspecific variation in DF, either geographic, ontogenetic, or sexual, which are barely known for birds in general and tropical species in particular (Herrera et al. 2005). The geographic intraspecific variation in DF revealed by the different studies may simply reflect the availability of fruits or the migratory and reproductive status of birds by the time these studies were conducted (Wheelwright 1986, Bairlein 2002, Guaraldo et al. 2016). It may also be indicative of intrinsic interpopulation differences in the relative contribution of fruits to the diet, especially for the most frugivorous bird species that also include a substantial proportion of invertebrates in their diets. Such species likely switch from arthropods to fruits, and vice-versa, more easily than species more restricted to insects (e.g., *Myiarchus* flycatchers) or fruits (e.g., euphonias).

The ontogenetic difference in DF we found for *T. leucomelas* likely results from the well-known higher demand of nestlings for protein, and as a result they are offered a higher proportion of animal prey than adults usually eat (Morton 1973, Lukhele et al. 2022). Much less known are sexual differences in DF (Gaiotti et al. 2017). Of the two manakins we analyzed, *P. pipra* showed greater tendency for sexual difference than *L. serena*. It is not totally surprising that males and females of lekking species, such as manakins,

differ in DF since males are more committed to lekking activities (e.g., attendance to their lekking territories) than females. As female visits to lek areas are unpredictable, mating success of males depends, among other factors (such as their display performance), on their presence at the displaying territories at leks, which poses a time constraint for foraging (Bosque 1996, Cestari et al. 2018). Males may thus rely on fruits more frequently than females, as fruits do not demand as much foraging time as arthropods do (Snow 1971, Cestari & Pizo 2013). Evidence so far indicates that adult males of *L. serena* apparently move considerably more among territories than *P. pipra* (e.g., Théry 1992, Endler & Théry 1996, Van Houtan et al. 2007, Uriarte et al. 2011). This may lead to differences in the time budget of males in relation to different commitments and lekking activities and, ultimately, in the contrasting sexual difference in DF between *L. serena* and *P. pipra*.

We arbitrarily chose the cutoff of 10 samples per species for the analyses, but researchers should be aware of the sample size necessary to account for intraspecific variation to obtain reliable central tendency and dispersion parameters in DF estimates. As we show here, adequate sample size likely increases with the DF of the species of interest, because geographic variation in DF tends to increase with DF. Therefore, depending on the relevant sources of variation and questions to be addressed, enough samples to account for the different sources of intraspecific variation (e.g. different geographic sites, individuals of different ages and sexes) should ideally be included in the analyses.

Whatever the reason, the intraspecific variation in DF revealed here calls attention to the risk of assigning a species to a specific category of DF (or functional group), which may vary from one population to another or from one period to the other. For instance, when adopting the detailed categorical classification proposed by Lopes et al. (2016) some species could be assigned to three different categories: from predominantly insectivorous to predominantly frugivorous, depending on the study considered (e.g., *Turdus rufiventris* whose DF varied from 26.7% to 76.5%). Our results thus expand to a lower taxonomic level the warning given by Remsen et al. (1993) that “blanket assignment of species to diet categories based solely on family membership, a practice widely used in current research on community ecology of Neotropical forest birds, is incorrect.”

In conclusion, we argued that stomach, fecal, and regurgitation sample data may be used to obtain quantitative assessments of DF, with advantages to the categorical classification often used in ecological studies and also to the qualitative information upon which the Elton Traits database (Wilman et al. 2014) is based on. Better than such alternatives, our approach allows for the assessments of intraspecific variation in DF to tackle largely unexplored questions (e.g., the intraspecific geographic variation in DF and its relation to habitat characteristics, such as food resource seasonality and vegetation structure). Moreover, the kind of data we used is abundantly available in the old and recent literature or, in the case of fecal samples, can be easily obtained from mist-netted birds. Such data indirectly reflect the importance of fruits in terms of the frequency of consumption. The frequency of occurrence of fruit in fecal and gastric samples is the most common way that data are reported in the literature, but volume-based estimates are also reported

(e.g., Witmer 1996) and may also be used, as the two measures of DF (frequency of fruit occurrence and mean proportion by volume of fruits per sample) have been shown to be highly and positively correlated (Parrish 1997). For DF estimates that reflect the energetic contribution of fruits to the diet or the assimilation of fruit nutrients relative to other food sources, mass estimation of fruit consumption (Nazaro & Blendinger 2017) and isotopic analysis (Herrera et al. 2005, Guaraldo et al. 2016) may be used respectively, although with much larger field effort and costs.

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