# Ant Morphology Mediates Diet Preference in a Neotropical Toad (Rhinella alata)

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# Ant Morphology Mediates Diet Preference in a Neotropical Toad (*Rhinella alata*)

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Despite the widespread occurrence of myrmecophagy in anurans, it is unclear whether ant-specialists feed on ants opportunistically or whether they prefer species with specific morphological, ecological, or nutritional traits. We flushed 105 stomachs of a lowland neotropical toad, *Rhinella alata*, and identified each consumed ant to species level. We calculated linear selectivity to determine predator preference for ants by comparing the abundances of consumed species to their relative abundances in the leaf litter community on Barro Colorado Island, Panama. We conducted linear regression models to test whether linear selectivity or general predator preference related to seven morphological characteristics and two measurements of nutritional content. *Rhinella alata* preferentially ate 24 ant species. Other species were either avoided ($n = 34$) or were eaten opportunistically ($n = 26$). Preferred ant species were large and textured with hair and/or rugosity. We found that prey preference did not relate to prey nutritional content, that small ants were avoided even if they were superabundant in the environment, and that chemically defended and aggressive ants were preferred if they were large enough. We propose that *R. alata* prefers large ants because they represent a more efficient prey item in terms of prey handling time and because they are easier to see than are smaller ants. Furthermore, we hypothesize that predation attempts are more successful when prey are textured because microstructures on the tongue and prey surface may increase prey adhesion. The ant specialist *R. alata* is not specializing on any particular ant species but rather maximizing prey quantity over quality by only eating the largest ants, despite their scarcity in the environment.

MYRMECOPHAGY has evolved multiple times across mammals (Luo, 2007), reptiles (Pianka and Pianka, 1970; Pianka and Parker, 1975), and amphibians (Toft, 1980, 1981; Simon and Toft, 1991; Daly et al., 1997; Savitsky et al., 2012). The repeated evolution of ant-eating is relatively unsurprising given that ants are conspicuous in both terrestrial and arboreal environments, may constitute 20% of total tropical animal biomass (Fittkau and Klinge, 1973), and perform a myriad of ecological and ecosystem functions (Del Toro et al., 2012; Roslin et al., 2017; Tiede et al., 2017). While some predator–ant interactions evolve towards highly specialized interactions (e.g., dart frogs, whose aposematic colors may reflect ant-harvested toxins [Caldwell, 1996; Santos et al., 2016]), most predators are thought to select ants opportunistically (Redford, 1986; Griffiths et al., 1990). However, this consensus may stem from the lack of diet studies that identify prey taxa to species level (but see Konopik et al., 2014). Though it is well established that ant-eating anurans partition prey based on size (Toft, 1980; Pimentel, 1998; Menendez, 2001), it is unknown if frogs can choose for specific species of ants or for certain ant traits that are available in the surrounding ant community. This uncertainty is particularly evident for tropical anurans whose diets are potentially derived from highly diverse and taxonomically unresolved ant communities.

Tropical leaf litter ant communities are part of a complex 'brown food web' (BFW, Coleman and Crossley, 2003). The nutrients in BFWs are derived from detritus, although the processes are poorly understood (Kaspari et al., 2017). In BFWs, most nematodes, mites, and collembolans harvest wood-decomposing microbes, and in turn these groups are fed upon by invertebrates higher in the food web such as ants and millipedes. The abundance and diversity of litter animals can reach astounding numbers in areas as small as 1 m$^2$, are determined by environmental (Donoso and Ramón, 2009; Donoso et al., 2013), biogeochemical (Kaspari et al., 2017), and ecological factors (Moore et al., 2004; Donoso et al., 2010; Donoso, 2017), and are major drivers of ecosystem productivity (Endara et al., 2017; Tiede et al., 2017; Schuldt et al., 2018). It is in this context that many animals including anurans feed upon BFW productivity. However, few animal ecologists have incorporated BFW principles into their research, and we therefore know little of how BFWs regulate animal biomass (bottom-up regulation; Redford, 1986), and in turn how animals control BFW communities (top-down control; Morrison, 2018).

Ants are a morphologically, taxonomically, and ecologically diverse group (Del Toro et al., 2012). They provide anurans with potentially different nutrients (Kaspari et al., 2012) and aposematic alkaloids (Daly et al., 1994, 1997; Saporito et al., 2009; Santos et al., 2016; Moskowitz et al., 2018), and yet few studies have looked at the specific identities or traits of ants in frog diets. The first of such studies by Deyrup et al. (2013) found that the Narrow-Mouthed Toad, *Gastrophryne carolinensis*, fed on up to 43 species of mostly nocturnal ants. These results gave no evidence that frogs exhibit species-level specialization in the ants they eat. Importantly, the diet of *G. carolinensis* included the largest ants in the area (in the genera *Odontomachus* and *Camponotus*, known for their stings and formic acid, respectively), although in relatively low abundance. Konopik et al. (2014) compared the prey (ant) preference of the Borneo River Toad, *Phrynoidis juxtaspera*, between primary forest and oil plantations in Malaysia and found that prey species remained consistent across sites despite differences in ant communities composition. Finally, McGugan et al. (2016) used mitochondrial barcodes to identify ants and mites present in the diets of the dendrobatid frog *Oophaga sylvatica* from three localities in coastal Ecuador. Invertebrate alkaloids differed across geog-

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raphy and underlied the alkaloids that were sequestered by *O. sylvatica*. However, no study has looked at predator preference for specific ant traits.

*Rhinella alata* is a medium-sized, diurnal, and locally abundant leaf-litter toad in Panama. The species is an exceptionally polymorphic and cryptically colored toad (McElroy, 2016), with a diet almost entirely comprised of ants (Toft, 1980, 1981; Parmelee, 1999; Menendez, 2001; Fajardo-Martínez et al., 2013; Astwood-Romero et al., 2016). The toad is chemically defended, but unlike aposematic frogs, predation of ants by *R. alata* should not be influenced by toxin sequestration from their diet because *R. alata* synthesizes its own toxins (bufodianaloids) in paratoid glands (Lyttle et al., 1996). Therefore, *R. alata* provides a simplified opportunity to test how prey species identity, morphology, and nutritional traits influence predation. Specifically, we answer the following questions: 1) Which ant species does *R. alata* preferentially consume and avoid? and 2) How do prey species morphological and nutritional traits help explain their rates of consumption? We conducted this study in the Panama Canal, a tropical seasonal forest that harbors ∼400 species ant community.

## MATERIALS AND METHODS

*Toad diet collections.*—Individual toads were sampled from August to September 2010, on Barro Colorado Island (BCI; 09°09′N, 79°51′W) in Panama, which receives approximately 2600 mm of annual rainfall, with nearly 90% of it falling between May and November (Leigh, 1999). We opportunistically sampled toads and recorded GPS localities at the point of capture (McElroy, 2016). We recorded snout–vent length (SVL) and removed each individual's stomach contents using non-lethal gastric lavage (Solé et al., 2005). Stomach contents were stored in 95% ethanol, and individual toads were released at the point of capture the following day. We identified individual ant prey to species level based on morphology. Non-ant prey items (e.g., mites and Coleoptera) were identified to class or family level.

*Sampling the BCI ant community.*—We collected 216 leaf litter samples (1 m²) from six sites at BCI. We brought samples to the laboratory and used Berlese funnels to separate invertebrates from the leaf litter. The sampling was designed and implemented as part of broader studies looking at patterns of morphological trait and phylogenetic dispersion across spatial scales in the island (Donoso, 2014) and the world (Gibb et al., 2017; Parr et al., 2017). More information on methods to survey the leaf litter ant community can be found in Donoso (2014). To reduce noise in our analyses, we excluded species that were captured in fewer than five Berlese funnels and that were eaten by fewer than two toads. We retained ant species that were eaten frequently but that were not captured in traps, or vice versa, as these potentially represent prey species that *R. alata* highly prefers or avoids. We also excluded army ant species, such as those in the genera *Labidus* and *Eciton*, because they are swarming species with high spatial and temporal variation in abundance. We used generalized linear models (GLMs) with Poisson error to relate the abundance and diversity of consumed ants to toad sex and SVL.

*Prey trait datasets.*—Our final dataset comprised 84 ant species and included five continuous and two morphological variables: Weber's length, head width, head length, pilosity (i.e., hairiness), number of spines, sculpture (smooth,

moderate, rough), and head color (yellow, yellow-brown, red-brown, brown, black, blue). We chose these morphological traits because ants are likely to rely on them (size, speed, camouflage) as defense (Parr et al., 2017), as well as because frogs could use them as visual cues. Specific information about morphological traits can be found in table 1 of Parr et al. (2017). To test the hypothesis that prey nutritional content and trophic position are associated with predator preference, we compiled a smaller dataset of 40 species for which nutritional data are available (Kaspari et al., 2012). Nitrogen composition (high %N = low C:N ratio) is a general proxy for the nutritional value of ants because %N is positively correlated with protein content and negatively correlated with chitin, an indigestible molecule for most vertebrates (Sullivan et al., 2014). Stable isotope ratios of nitrogen represent a measure of ant trophic position. We report our isotopic data using standard delta (δ) notation, where δ values represent the ratio of the rare to common isotopes. The abundance of δ15N of a consumer is typically enriched by 3.4‰ relative to its diet.

*Selectivity analysis.*—To determine the preference of *R. alata* for each ant species, we used a linear selectivity index (Strauss, 1979; Palkovacs and Post, 2008; Zandonà et al., 2011), which is defined as:

$$L_i = r_i - p_i$$

for a prey species i, where $L_i$ is the measure of selectivity, $r_i$ is the relative abundance in the stomach, and $p_i$ is the relative abundance in the environment. To calculate selectivity, we pooled the data for stomach contents and subtracted the proportion of a prey species in the environment from the proportion of that prey species in the stomachs. One benefit of the metric is that it enabled us to retain prey species that were absent from either the stomachs or from the environment. After using simulations to determine a range of $L_i$ values that represent neutral preference, linear selectivity is easy to interpret; prey species with values greater than a positive threshold are considered 'preferred' and species with values below a negative threshold are considered 'avoided.' To assess significance of linear selectivity values, we replicated our analysis through simulation. We started with the environmental proportions of ant species and generated 1000 matrices by randomly sampling between 3000–5000 ants with replacement. From the simulated matrices, we calculated linear selectivity and generated a null distribution of values for each prey species. The null distributions are species-specific, as they depend on each species' proportion in the environment. We classified ants as preferred if their selectivity values were greater than the null distribution and avoided if their selectivity values were less than the null distribution. We considered species with real selectivity values that fell within the null distribution to be predated upon at a rate that is proportional with their environmental abundance (i.e., 'neutral').

*Principal components analysis.*—We characterized the phenotypes of ant species by conducting principal component analysis (PCA) of continuous and categorical trait data. We used the function dudi.mix from the package ade4 because it allows for analysis of both continuous and ordered categorical variables (Dray and Dufour, 2007). The PCA was done with a correlation matrix of centered and normalized variables. The function dudi.mix transformed the ordinal

**Table 1.** Poisson GLM analysis of counts of prey diversity and abundance according to frog gender, frog size (snout–vent length, SVL), and their interactions. Significant values are depicted in bold.

| | Diversity | | | | Abundance | | | |
|---|---|---|---|---|---|---|---|---|
| | Est. | SE | z | P | Est. | SE | z | P |
| Intercept | 1.22 | 0.77 | 1.58 | 0.11 | **1.30** | **0.31** | **4.24** | **<0.001** |
| Sex | −0.64 | 1.40 | −0.46 | 0.65 | −1.05 | 0.55 | −1.90 | 0.06 |
| SVL | 0.01 | 0.02 | 0.74 | 0.46 | **0.05** | **0.01** | **8.44** | **<0.001** |
| Sex * SVL | 0.02 | 0.03 | 0.47 | 0.64 | **0.02** | **0.01** | **1.94** | **0.05** |

variables "trait.L" and "trait.Q" into linear and quadratic variables. In the PCA plot, the "trait.L" vector points toward increasing values of the trait, while the "trait.Q" vector points in the direction of moderate values for the trait. We grouped ant species in the PCA by their preference category to explore which prey traits influence predation.

***Stepwise model selection.***—Prior to conducting stepwise model selection, we used z-scores to standardize the continuous traits. Head width, head length, and Weber's length were >95% correlated with each other, so we only retained Weber's length as our proxy for size. We used backwards model selection to determine the best model that explains linear selectivity. We started from a full 5-parameter model and used the function 'dropterm' in the R-package MASS to iteratively remove parameters from our model. We then compared AICc values for each model, calculated Akaike weights to determine our support for each model, and compared coefficients to assess the importance of each trait. Because *Ectatomma ruidum* was an extremely highly preferred prey item (i.e., represents an outlier; see below), we performed a second analysis excluding *E. ruidum* and checked that its inclusion did not skew the results.

Linear selectivity is a continuous response variable that does not incorporate our null model framework. As such, large selectivity values (e.g., *E. ruidum*) may influence model selection. To solve this, we performed ordered logistic regression with ant traits as the independent variables and preference category as the response variable. We used backwards model selection and AICc values to determine the best model that explains preference category. To explore model accuracy, we trained the model on 70% of the ant species and tested it with the remaining species. We performed 1,000 bootstraps and calculated the percent of the time our model predictions matched the empirical dataset. When our model predictions for a species did not match the empirical dataset, we distinguished between 'misses' (i.e., mismatches incorporating a neutral preference) and 'fails' (i.e., mismatches between avoid–prefer or prefer–avoid).

***Nutrition analysis.***—We analyzed a subset of 40 ant species for which we had %N and δN data to determine whether prey nutrition represents an important but overlooked trait. We conducted ANOVA to determine whether toad preference related to ant size (WL), nitrogen content (%N), or trophic position (δN). We used a Tukey's *post hoc* test to explore significant differences between preferred, avoided, and neutral ants.

## RESULTS

***Toad diets.***—We found 3,645 prey items from 61 ant species sampled from 105 individual *R. alata*. Of these prey items,

3,462 were ants and 183 (5%) were other taxa. Scarabaeidae ($n = 47$), Curculionidae ($n = 36$), and Aranea ($n = 34$) were the most abundant non-ant taxa. We removed 28 ant species because they were either army ants or were consumed by fewer than two toads, resulting in a final dataset of 3,424 individual ant prey items. The most consumed ant species included *Ectatomma ruidum* ($n = 2,064$), *Pachycondyla harpax* ($n = 245$), *Odontomachus bauri* ($n = 236$), and *Solenopsis* sp. "lash4" ($n = 213$).

The community dataset comprised 26,234 ant specimens from 98 species. The most abundant species in the environment were *S.* sp. "lash4" ($n = 4,121$), *Wasmannia auropunctata* ($n = 3,647$), and *Solenopsis* sp. 'JTsp1' ($n = 1988$). More details of the survey can be found in Donoso (2014). After removing army ants and species found in fewer than five traps, our final community dataset consisted of 25,645 specimens from 84 ant species.

Neither the sex (Poisson GLM, Est. = –0.64, $P = 0.65$) nor size (Poisson GLM, Est. = 0.01, $P = 0.46$; Table 1) of individual toads related to the consumed ant diversity. Larger toads ate more ants (Poisson GLM, Est. = 0.05, $P \leq 0.001$). Though females were larger than males (males: $\bar{x} = 40.6 \pm 0.6$ mm, females = $46.1 \pm 0.9$ mm, $P \leq 0.001$), they did not eat significantly more ants than males (Poisson GLM, Est. = –1.05, $P = 0.06$; Table 1).

***Selectivity analysis.***—Based on our linear selectivity ($L_i$) analysis and simulations, we categorized 23 ant species as preferred, 27 as neutral, and 34 as avoided. Larger ant size was necessary but not sufficient to be preferred as a food by toads (Fig. S1; see Data Accessibility). *Ectatomma ruidum* was overwhelmingly the most preferred ant species ($L_i = 0.60$), while *P. harpax* ($L_i = 0.07$), *O. bauri* ($L_i = 0.07$), and *S.* sp. "lash4" ($L_i = 0.06$) represented a second tier of preferred prey items (Fig. 1). The first and second most avoided ant species were *Solenopsis* sp. "yellow" ($L_i = –0.16$; Fig. 1) and *W. auropunctata* ($L_i = –0.13$; Fig. 1).

***Principal components analysis.***—The first principal component accounted for 38.4% of the variation and represented size traits (Weber's length, head length, and head width), pilosity, and headcolor.L. The second principal component accounted for 16.8% of the variation and was primarily characterized by traits relating to texture (sculpture.L, spines, and headcolor.Q). Overlaying the preference categories on our PCA indicated that neutral and avoided ants largely overlap in trait space, but preferred ant species were larger, darker, and hairier (Fig. 2).

***Stepwise model selection.***—The AIC model 'Weber's length + pilosity' was almost three times more informative than the second best model ('Weber's length + pilosity + sculpture') at explaining linear selectivity (Akaike weights = 0.57 vs. 0.21). We found a steep drop off in the ability of subsequent models
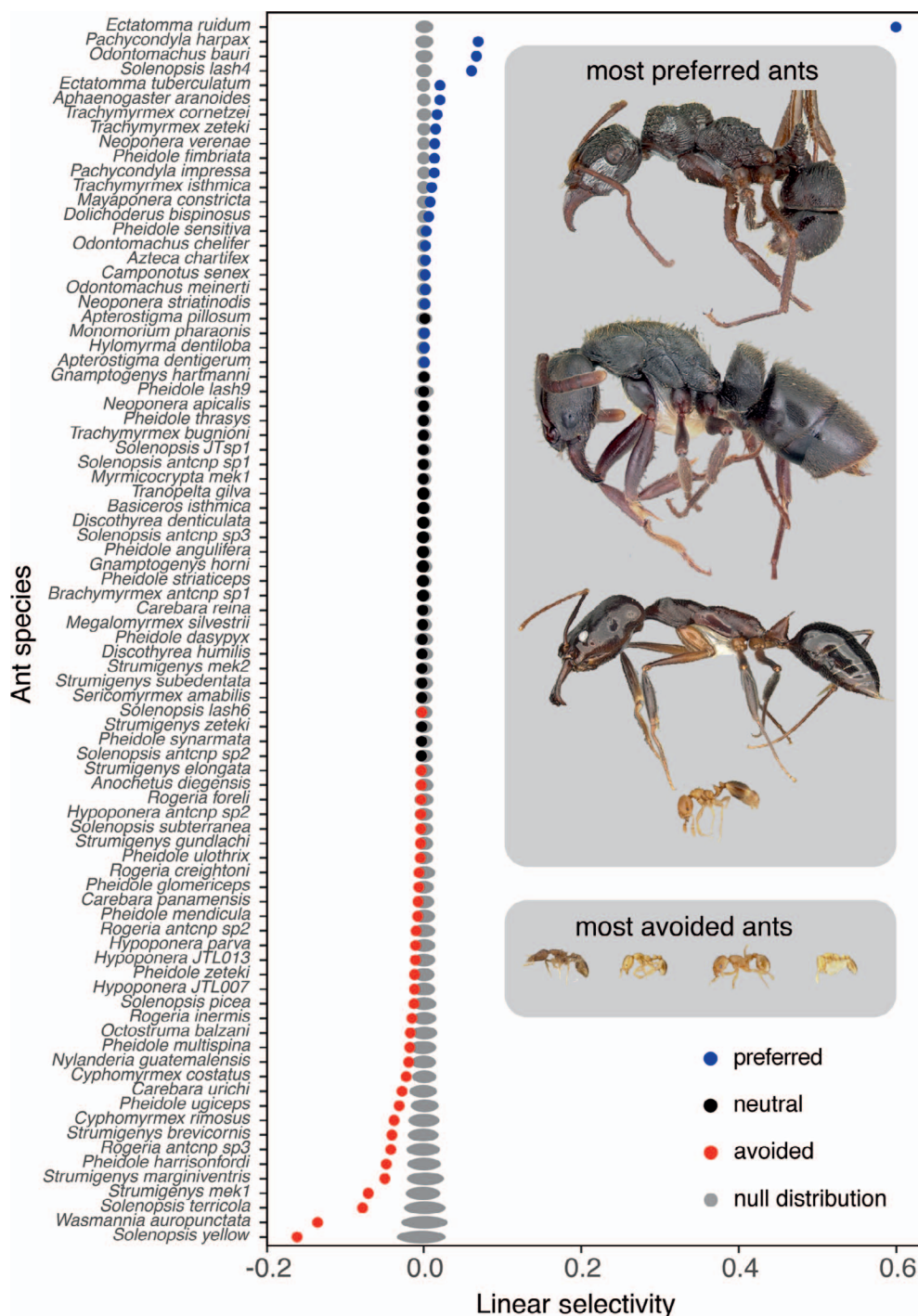
**Fig. 1.** The 84 ant species ordered by their linear selectivity value. Preference categories of ant species (preferred [blue], neutral [black], and avoided [red]) were established relative to a null model distribution (gray area). The cutoffs for null distributions are species-specific, which explains why preference categories are not perfectly ordered. Photographs of the top four and bottom four ants are size-scaled in relation to each other.

to explain linear selectivity (Table 2). Removing *E. ruidum* from the analysis did not influence the top models. However, the one parameter model ('pilosity') dropped from 3$^{rd}$ (Akaike weight = 0.09; Table S1; see Data Accessibility) to 5$^{th}$ (Akaike weight = 0.01), likely because *E. ruidum* is very pilose. The model rankings for the ordinal logistic regression analysis were consistent with the linear regression analyses. The top three models explaining preference category (Table 3) and linear selectivity (Appendix S1; see Data Accessibility; excluding *E. ruidum*) include the ant traits Weber's length, pilosity, and sculpture, and have combined probabilities >90%. We utilized the second best model ('Weber's length + pilosity + sculpture') for predicting preference category from ant traits because it includes all the variables in the 90% confidence set. The chosen model correctly predicted the

preference category in our training set 70% of the time ($\bar{x}$ = 0.70; s.d. = 0.10; Fig. S2A; see Data Accessibility). When the model incorrectly predicted preference category it was due to 'misses' 93% of the time ($\bar{x}$ = 0.93; s.d. = 0.10; Fig. S2B; see Data Accessibility) and 'fails' only 7% of the time ($\bar{x}$ = 0.07; s.d. = 0.10; Fig. S2C; see Data Accessibility). Moderately sized ants that were textured were more likely to be preferred, and small ants that were textured were more likely to be neutral (Fig. S2; see Data Accessibility).

***Nutrition.***—Weber's length differed between preference categories (ANOVA: $F_{(2,37)}$ = 128, $P$ < 0.001). Preferred ants were two and a half standard deviations larger than avoided ants (Tukey HSD: $P$ < 0.001) and two standard deviations larger than neutral ants (Tukey HSD: $P$ < 0.001). Neutral ants were
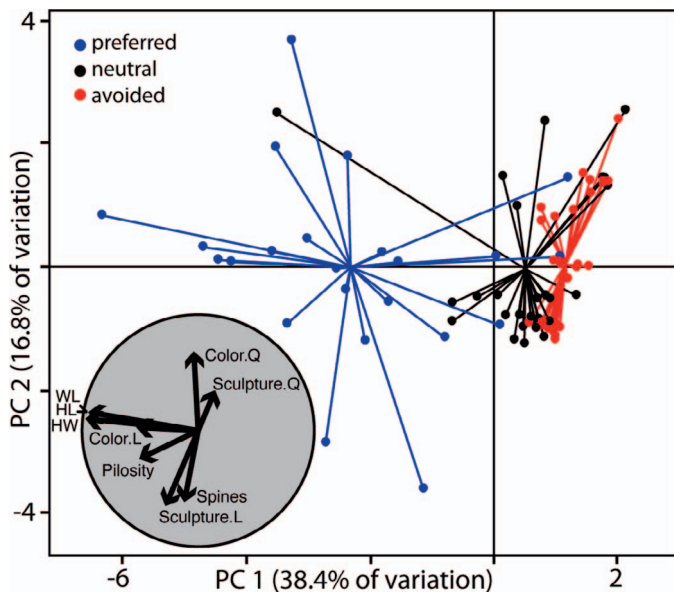
**Fig. 2.** Principal component analysis of seven ant traits. Ant species were plotted in the PCA and colored by preference categories (preferred [blue], neutral [black], and avoided [red]). The inset depicts the correlations between principal components and each ant trait. "trait.L" (linear) and "trait.Q" (quadratic) are representations of ordinal variables. The linear representation indicates the direction in PC space of increasing trait values, while the quadratic representation indicates the direction in PC space of moderate trait values.

slightly larger than avoided ants (Tukey HSD: $P = 0.03$). Neither %N (ANOVA: $F_{(2,37)} = 0.028$, $P = 0.97$) nor δN (ANOVA: $F_{(2,37)} = 1.011$, $P = 0.37$) differed between preference categories (Fig. 3).

## DISCUSSION

While it is well established that *R. alata* eats ants, we extend previous studies by identifying prey to species-level and comparing prey abundances with species abundances in the leaf litter habitat. We found that *R. alata* prefers to eat relatively rarer large ants and avoids eating common smaller ants. Generally speaking, optimal foraging theory predicts that an organism will behave and forage in a manner that maximizes fitness by reducing the energetic and opportunity costs associated with consuming poor-quality prey (Emlen, 1966). In this context, that *R. alata* prefers the largest (and more rare) ants suggests that the nutritional gain from

**Table 2.** Model rankings for general linear regression where model variables are ant traits and the response variable is linear selectivity ($L_i$). For each model, we report the number of parameters (K), AICc, differential AIC ($\Delta_i$), and the Akaike weight ($w_i$). The Akaike weight can be interpreted as the likelihood for a particular model.

| Model | K | AICc | $\Delta_i$ | $w_i$ |
|---|---|---|---|---|
| Weber's length + Pilosity | 2 | −212.2 | 0.00 | 0.57 |
| Weber's length + Pilosity + Sculpture | 3 | −210.1 | 2.02 | 0.21 |
| Pilosity | 1 | −208.3 | 3.81 | 0.09 |
| Weber's length | 1 | −208.0 | 4.17 | 0.07 |
| Weber's length + Pilosity + Sculpture + Spines | 4 | −207.8 | 4.40 | 0.06 |
| Weber's length + Pilosity + Sculpture + Spines + Color | 5 | −195.3 | 16.83 | <0.001 |

**Table 3.** Model rankings for logistic regression where model variables are ant traits and the response variable is predator preference (e.g., "preferred"). For each model we report the number of parameters (K), AICc, differential AIC ($\Delta_i$), and the Akaike weight ($w_i$). The Akaike weight can be interpreted as the likelihood for a particular model.

| Model | K | AICc | $\Delta_i$ | $w_i$ |
|---|---|---|---|---|
| Weber's length + Pilosity | 2 | 124.0 | 0.00 | 0.61 |
| Weber's length + Pilosity + Sculpture | 3 | 126.3 | 2.26 | 0.20 |
| Weber's length | 1 | 127.1 | 3.05 | 0.13 |
| Weber's length + Pilosity + Sculpture + Spines | 4 | 128.4 | 4.42 | 0.06 |
| Weber's length + Pilosity + Sculpture + Spines + Color | 5 | 137.5 | 13.47 | <0.001 |
| Pilosity | 1 | 163.3 | 39.25 | <0.001 |

consuming large ants outweighs the energetic cost of searching for relatively rare prey. However, it is also plausible that the apparent avoidance of small ants by *R. alata* is a result of their visual system and inability to see the smallest ants. Furthermore, we found no relationship between predator preference and nutrition content of prey. Thus, *R. alata* does not select for individual prey items that are high in nutritional value; instead they appear to increase energy intake by choosing large prey over more nutritious prey.

The best models explaining selectivity and preference include elements of texture, namely hairiness (pilosity) and rugosity (sculpture). This is the first anuran field study to find that prey traits other than size can explain predation rates. However, it is difficult to envision a scenario in which *R. alata* discerns between—and differentially preys upon—textured and untextured ants. Instead, we propose that *R. alata* preferentially selects for large ants and that predation success increases with increasing prey texture. Frog tongues are highly specialized for fast and reliable adhesion to prey items. Salivary glands located within the tongue increase the production and availability of highly viscous saliva and are instrumental in the tongues ability to adhere to prey items (Kleinteich and Gorb, 2015a; Noel and Hu, 2018). Micro-structures on the tongue are thought to increase the adaptability of the tongue to attach to uneven prey surfaces and facilitate the development of mucous fibrils which sustain prey adhesion during tongue retraction (Kleinteich and Gorb, 2015b; Kleinteich and Gorb, 2016). How variable prey texture (e.g., fur, hair, feathers) may influence tongue adhesion remains poorly understood (Kleinteich and Gorb, 2015a). However, microstructures of prey (pilosity and rugosity) may be analogous to microstructures on the tongue by promoting mucous fibrils and increasing the strength or length of tongue adhesion. Thus, we hypothesize that adhesion between a frog's tongue and a prey item is greater for textured ants than it is for untextured ants, which increases the probability of successfully handling and consuming pilose and rugous ant species. Understanding more about the general functional benefits of texture traits (e.g., pilosity and sensory capability; sculpture and desiccation resistance) and the potential trade-offs associated with predation deserves investigation.

*Ectatomma ruidum*, a common item in Neotropical frog diet studies (Weber, 1938; Lopez et al., 2007), had the highest selectivity value and was the most consumed species in our study representing 60% of all stomach items, despite it only representing 0.32% of the ant community. An underlying
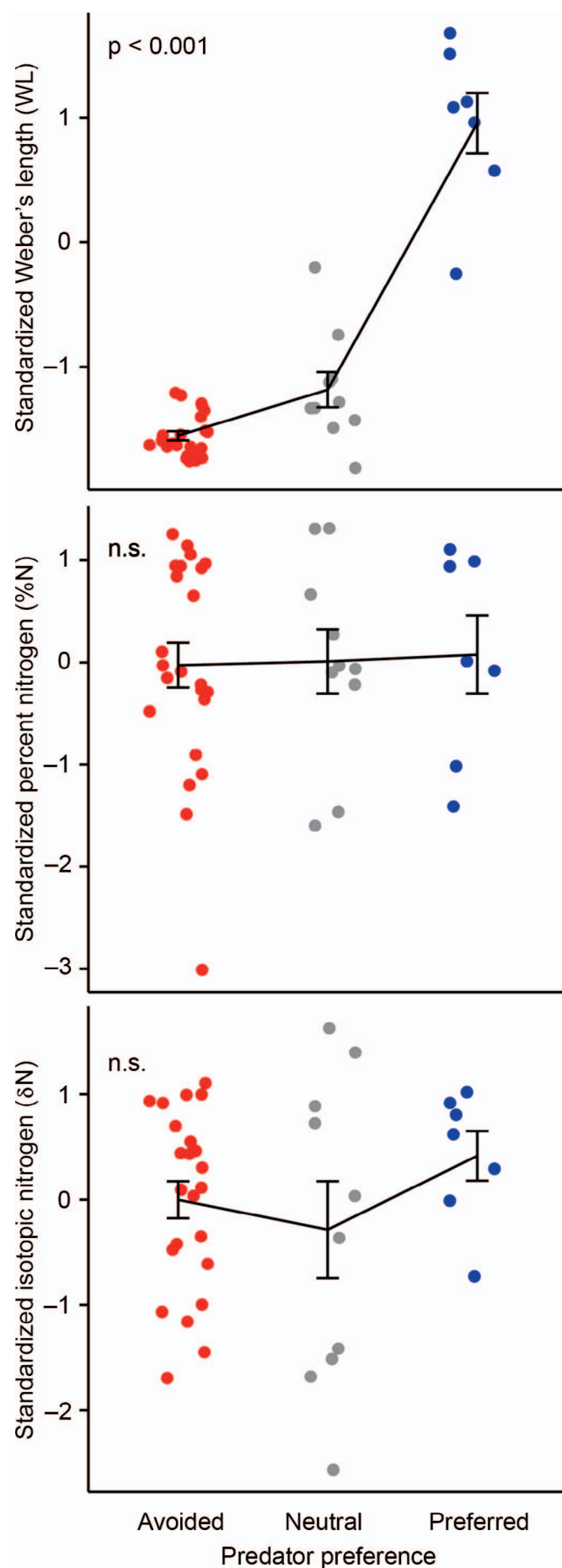
assumption while calculating linear selectivity is that the environmental community has been accurately sampled. As such, any trapping bias against certain species or phenotypes (e.g., size) may skew our findings. *Ectatomma ruidum*, while low in terms of abundance, was collected in 27% of the samples, indicating that it is well sampled and commonly encountered in the leaf litter, which gives us confidence in our environmental estimation. In fact, the next three most preferred ant species were all well represented in our sampling procedure and occurred in 21%, 12%, and 11% of traps, indicating that elevated selectivity values for those species is unlikely to result from environmental sampling bias (Fig. S1; see Data Accessibility). *Ectatomma ruidum* has the characteristics we expect in a preferred species: it is one of the largest ants on the island, and it is among the most textured species in our dataset. Even though nutrition and trophic position were not good predictors of preference of *R. alata*, it is intriguing that *E. ruidum* is one of the more nutritious ant species in terms of %N and δN. Importantly, size and pilosity predict predation of *R. alata* even when *E. ruidum* is removed from the analyses or when we analyze preference categories (as opposed to linear selectivity). Therefore, the extraordinary presence of *E. ruidum* in the diet of *R. alata* provides a natural validation of our result.

Though we did not formally test for avoidance of defensive traits in our analysis, we were unable to identify any patterns relating predator avoidance to other hypothesized defensive traits. While *W. auropunctata* and other weedy ants in the genus *Solenopsis* were the most avoided ant species, we attribute this primarily to their small size. Certainly, these species can be noxious; *W. auropunctata* is known to be invasive and to displace ants (Arnan et al., 2018) and other animals as well (reviewed in Wetterer and Porter, 2003). *Solenopsis* includes the fire ants with powerful stings. Nonetheless, *Solenopsis* ants are the preferred food items for the poison frog *Oophaga histrionica* in Colombia (Osorio et al., 2015), and in our analysis *Solenopsis* were preferred if they were large enough (e.g., *Solenopsis* sp. "lash4"). Furthermore, despite being removed from the analysis, we found army ants (subfamily Dorylinae) in stomachs of *R. alata,* indicating that aggressive ants with pincers were not necessarily a predator deterrent. *Rhinella alata* also preferred large ponerine ants (e.g., *P. harpax* and *O. bauri*) known for their painful stings (see Deyrup et al., 2013), and they preferred *Trachymyrmex* ants (e.g., *T. isthmica* and *T. zeteki*) that had the most spines (Parr et al., 2017). Taken together, these results indicate that ant traits that are traditionally thought of as anti-predator defenses are unlikely to play a large role in deterring *Rhinella alata*. Instead, predation is more likely to be influenced by prey size and foraging behavior than anti-predator defenses.

Arthropod declines can initiate bottom-up trophic cascades that affect insectivorous vertebrate predators, potentially resulting in localized extirpation at higher trophic levels (Lister and Garcia, 2018). Understanding the phenotypic traits that underlie trophic interactions allows for nuanced insights into food web dynamics (Spitz et al., 2014). *Rhinella alata* prefers large ants, which in general are expected to be negatively impacted by global climate change (Gibb et

**Fig. 3.** Species trait values for size (WL) and nutrition (%N, δN) grouped and colored by predator preference. Line graphs represent mean and standard error. The relationship between trait values and predator preference were tested with ANOVA.

al., 2015, 2018). If large ants are negatively affected by global change, vertebrate predators like *R. alata* may be forced to shift their diets towards smaller ant species. This study complements previous work showing that ant communities on BCI are structured by sets of phylogenetically similar ants of small size (Donoso, 2014). The preferential predation of large ant species may be partially responsible for producing this pattern (Abrams and Rowe, 1996; Roslin et al., 2017). It is difficult to draw general conclusions on the impact that prey traits have on myrmecophagous predator preference until more species-level diet studies accumulate. We provide one example for a non-alkaloid-sequestering myrmecophagous frog that clearly selects for large ants despite their scarcity in the environment. Our intriguing finding that pilosity and rugosity influence predation highlights that prey texture may be an overlooked factor in studies on the biomechanics of prey capture.

## DATA ACCESSIBILITY

Data are available at https://github.com/mattmcelroy/ant_buffet. Supplemental information is available at https://www.copeiajournal.org/ch-18-162.

## ACKNOWLEDGMENTS

## LITERATURE CITED

**Abrams, P. A., and L. Rowe.** 1996. The effects of predation on the age and size at maturity of prey. Evolution 50:1052–1061.

**Arnan, X., A. N. Andersen, H. Gibb, C. L. Parr, N. J. Sanders, R. R. Dunn, E. Angulo, F. B. Baccaro, T. R. Bishop, R. Boulay, C. Castracani, X. Cerdá, I. Del Toro, T. Delsinne** … **J. Retana.** 2018. Dominance–diversity relationships in ant communities differ with invasion. Global Change Biology 24:4614–4625.

**Astwood-Romero, J. A., N. Alvarez-Perdomo, M. F. Parra-Torres, J. I. Rojas-Peña, M. T. Nieto-Vera, and M. C. Ardila-Robayo.** 2016. Contenidos estomacales de especies de anuros en reservas naturales del municipio de Villavicencio, Meta, Colombia. Caldasia 38:165–181.

**Caldwell, J. P.** 1996. The evolution of myrmecophagy and its correlates in poison frogs (family Dendrobatidae). Journal of Zoology 240:75–101.

**Coleman, D. C., and D. Crossley, Jr.** 2003. Fundamentals of Soil Ecology. Third edition. Academic Press, Cambridge, Massachusetts.

**Daly, J. W., H. M. Garraffo, G. S. Hall, and J. F. Cover, Jr.** 1997. Absence of skin alkaloids in captive-raised Madagascan mantelline frogs (*Mantella*) and sequestration of dietary alkaloids. Toxicon 35:1131–1135.

**Daly, J. W., H. M. Garraffo, T. F. Spande, C. Jaramillo, and A. S. Rand.** 1994. Dietary source for skin alkaloids of poison frogs (Dendrobatidae)? Journal of Chemical Ecology 20:943–955.

**Del Toro, I., R. R. Ribbons, and S. L. Pelini.** 2012. The little things that run the world revisited: a review of ant-mediated ecosystem services and disservices (Hymenoptera: Formicidae). Myrmecological News 17:133–146.

**Deyrup, M., L. Deyrup, and J. Carrel.** 2013. Ant species in the diet of a Florida population of Eastern Narrow-Mouthed Toads, *Gastrophryne carolinensis*. Southeastern Naturalist 12:367–378.

**Donoso, D. A.** 2014. Assembly mechanisms shaping tropical litter ant communities. Ecography 37:490–499.

**Donoso, D. A.** 2017. Tropical ant communities are in long-term equilibrium. Ecological Indicators 83:515–523.

**Donoso, D. A., M. K. Johnston, N. Clay, and M. E. Kaspari.** 2013. Trees as templates for trophic structure of tropical litter arthropod fauna. Soil Biology and Biogeochemistry 6:45–61.

**Donoso, D. A., M. K. Johnston, and M. E. Kaspari.** 2010. Trees as templates for tropical litter arthropod diversity. Oecologia 164:201–211.

**Donoso, D. A., and G. Ramón.** 2009. Composition of a high diversity leaf litter ant community (Hymenoptera: Formicidae) from an Ecuadorian pre-montane rainforest. Annales de la Société Entomologique de France 45:487–499.

**Dray, S., and A. Dufour.** 2007. The ade4 package: implementing the duality diagram for ecologists. Journal of Statistical Software 22:1–20.

**Emlen, J. M.** 1966. The role of time and energy in food preference. The American Naturalist 100:611–617.

**Endara, M. J., P. D. Coley, G. Ghabash, J. A. Nicholls, K. G. Dexter, D. A. Donoso, G. N. Stone, R. T. Pennington, and T. A. Kursara.** 2017. Coevolutionary arms race versus host defense chase in a tropical herbivore–plant system. Proceedings of the National Academy of Sciences of the United States of America 114:E7499–E7505.

**Fajardo-Martínez, X., A. Fajardo-Patiño, and J. de la Ossa.** 2013. Hábitos alimentarios del complejo *Rhinella margaritifera* (Laurenti, 1768) (Amphibia: Bufonidae), Amazon, Colombia. Revista Colombiana de Ciencia Animal 5:301–312.

**Fittkau, E., and H. Klinge.** 1973. On biomass and trophic structure of the Central Amazonian rain forest ecosystem. Biotropica 5:2–14.

**Gibb, H., R. R. Dunn, N. J. Sanders, B. F. Grossman, M. Photakis, S. Abril, D. Agosti, A. N. Andersen, E. Angulo, I. Armbrecht, X. Arnan, F. B. Baccaro, T. R. Bishop, R. Boulay** … **C. L. Parr.** 2017. A global database of ant species abundances. Ecology 98:883–884.

**Gibb, H., N. J. Sanders, R. R. Dunn, X. Arnan, H. L. Vasconcelos, D. A. Donoso, A. N. Andersen, R. R. Silva, T. R. Bishop, C. Gomez, B. F. Grossman, K. M. Yusah, S. H. Luke, R. Pacheco** … **C. L. Parr.** 2018. Habitat disturbance selects against both small and large species across varying climates. Ecography 41:1184–1193.

**Gibb, H., N. J. Sanders, R. R. Dunn, S. Watson, M. Photakis, S. Abril, A. N. Andersen, E. Angulo, I. Armbrecht, X. Arnan, F. B. Baccaro, T. R. Bishop, R. Boulay, C. Castracani** … **C. L. Parr.** 2015. Climate regulates the effects of anthropogenic disturbance on ant assemblage structure. Proceedings of the Royal Society B 282:20150418.

**Griffiths, M., P. J. M. Greenslade, L. Miller, and J. A. Kerle.** 1990. The diet of the Spiny-anteater *Tachyglossus aculeatus acanthion* in tropical habitats in the Northern Territory. The

Beagle. Records of the Northern Territory Museum of Arts and Sciences 7:79–90.

Kaspari, M. E., J. Bujan, M. D. Weiser, D. Ning, S. T. Michaletz, H. Shili, B. J. Enquist, R. B. Waide, J. Zhou, B. L. Turner, and S. J. Wright. 2017. Biogeochemistry and soil diversity: multiple elements shape richness of prokaryotes, fungi, and invertebrates in a Panama forest. Ecology 98:2019–2028.

Kaspari, M. E., D. A. Donoso, J. A. Lucas, T. Zumbusch, and A. D. Kay. 2012. Using nutritional ecology to predict community structure: a field test in Neotropical ants. Ecosphere 3:1–15.

Kleinteich, T., and S. N. Gorb. 2015a. Tongue adhesion in the horned frog Ceratophrys sp. Scientific Reports 4:5225.

Kleinteich, T., and S. N. Gorb. 2015b. Frog tongue acts as muscle-powered adhesive tape. Royal Society Open Science 2:150333.

Kleinteich, T., and S. N. Gorb. 2016. Frog tongue surface microstructures: functional and evolutionary patterns. Beilstein Journal of Nanotechnology 7:893–903.

Konopik, O., C. L. Gray, T. U. Grafe, I. Steffan-Dewenter, and T. M. Fayle. 2014. From rainforest to oil palm plantations: shifts in predator population and prey communities, but resistant interactions. Global Ecology and Conservation 2:385–394.

Leigh, E. G., Jr. 1999. Tropical Forest Ecology: A View from Barro Colorado Island. Oxford University Press, New York.

Lister, B. C., and A. Garcia. 2018. Climate-driven declines in arthropod abundance restructure a rainforest food web. Proceedings of the National Academy of Sciences of the United States of America 115:E10397–E10406.

Lopez, J. A., R. Ghirardi, P. A. Scarabotti, and M. C. Medrano. 2007. Feeding ecology of Elachistocleis bicolor in a riparian locality of the middle Parana River. The Herpetological Journal 17:48–53.

Luo, Z.-X. 2007. Transformation and diversification in early mammal evolution. Nature 450:1011.

Lyttle, T., D. Goldstein., and J. Gartz. 1996. Bufo toads and bufotenine: fact and fiction surrounding an alleged psychedelic. Journal of Psychoactive Drugs 28:267–290.

McElroy, M. T. 2016. Teasing apart crypsis and aposematism—evidence that disruptive coloration reduces predation on a noxious toad. Biological Journal of the Linnean Society 117:285–294.

McGugan, J. R., G. D. Byrd, A. B. Roland, S. N. Caty, N. Kabir, E. E. Tapia, S. A. Trauger, L. A. Coloma, and L. A. O'Connell. 2016. Ant and mite diversity drives toxin variation in the Little Devil Poison Frog. Journal of Chemical Ecology 42:537–551.

Menendez, P. A. 2001. Ecología trófica de la comunidad de anuros del Parque Nacional Yasuní en la Amazonía Ecuatoriana. Trabajo de grado Licenciado en Ciencias Biológicas. Pontificia Universidad Católica del Ecuador, Quito, Ecuador.

Moore, J. C., E. L. Berlow, D. C. Coleman, P. C. de Ruiter, Q. Dong, A. Hastings, N. C. Johnson, K. S. McCann, K. Melville, P. J. Morin, K. Nadelhoffer, A. D. Rosemond, D. M. Post, J. L. Sabo … D. H. Wall. 2004. Detritus, trophic dynamics and biodiversity. Ecology Letters 7:584–600.

Morrison, C. R. 2018. Predation of top predators: cane toad consumption of bullet ants in a Panamanian lowland wet forest. Journal of Tropical Ecology 34:390–394.

Moskowitz, N. A., A. B. Roland, E. K. Fischer, N. Ranaivorazo, C. Vidoudez, M. T. Aguilar, S. M. Caldera, J. Chea, M. G. Cristus, J. P. Crowdis, B. DeMessie, C. R.

Desjardins-Park, A. H. Effenberger, F. Flores … L. A. O'Connell. 2018. Seasonal changes in diet and chemical defense in the Climbing Mantella frog (Mantella laevigata). PLoS ONE 13:e0207940.

Noel, A. C., and D. L. Hu. 2018. The tongue as a gripper. Journal of Experimental Biology 221:jeb176289.

Osorio, D., L. Valenzuela, C. Bermudez-Rivas, and S. Castaño. 2015. Descripción de la dieta de una población de Oophaga histrionica (Athesphatanura: Dendrobatidae) en un enclave seco del Valle del Cauca. Colombia Revista Biodiversidad Neotropical 5:29–35.

Palkovacs, E. P., and D. M. Post. 2008. Eco-evolutionary interactions between predators and prey: Can predator-induced changes to prey communities feedback to shape predator foraging traits? Evolutionary Ecology Research 10:699–720.

Parmelee, J. R. 1999. Trophic ecology of a tropical anuran assemblage. Scientific Papers, Natural History Museum, The University of Kansas 11:1–59.

Parr, C. L., R. R. Dunn, N. J. Sanders, M. D. Weiser, M. Photakis, T. R. Bishop, M. C. Fitzpatrick, X. Arnan, F. Baccaro, C. R. F. Brandão, L. Chick, D. A. Donoso, T. M. Fayle, C. Gómez … H. Gibb. 2017. GlobalAnts: a new database on the geography of ant traits (Hymenoptera: Formicidae). Insect Conservation and Diversity 10:5–20.

Pianka, E. R., and W. S. Parker. 1975. Ecology of horned lizards: a review with special reference to Phrynosoma platyrhinos. Copeia 1975:141–162.

Pianka, E. R., and H. D. Pianka. 1970. The ecology of Moloch horridus (Lacertilia: Agamidae) in Western Australia. Copeia 1970:90–103.

Pimentel, A. 1998. The effects of size on the diets of six sympatric species of postmetamorphic litter anurans in central Amazonia. Journal of Herpetology 32:392–399.

Redford, K. H. 1986. Dietary specialization and variation in two mammalian myrmecophages. Revista Chilena de Historia Natural 59:201–208.

Roslin, T., B. Hardwick, V. Novotny, W. K. Petry, N. R. Andrew, A. Asmus, I. C. Barrio, Y. Basset, A. L. Boesing, T. C. Bonebrake, E. K. Cameron, W. Dattilo, D. A. Donoso, P. Drozd … E. M. Slade. 2017. Higher predation risk for insect prey at low latitudes and elevations. Science 356:742–744.

Santos, J. C., R. D. Tarvin, and L. A. O'Connell. 2016. A review of chemical defense in poison frogs (Dendrobatidae): ecology, pharmacokinetics, and autoresistance, p. 305–337. In: Chemical Signals in Vertebrates. B. A. Schulte, T. E. Goodwin, and M. H. Ferkin (eds.). Springer Science, New York.

Saporito, R. A., T. F. Spande, H. M. Garraffo, and M. A. Donnelly. 2009. Arthropod alkaloids in poison frogs: a review of the dietary hypothesis. Heterocycles 79:277–297.

Savitzky, A. H., A. Mori, D. A. Hutchinson, R. A. Saporito, G. M. Burghardt, H. B. Lillywhite, and J. Meinwald. 2012. Sequestered defensive toxins in tetrapod vertebrates: principles, patterns, and prospects for future studies. Chemoecology 22:141–158.

Schuldt, A., T. Assmann, M. Brezzi, F. Buscot, D. Eichenberg, J. Gutknecht, W. Härdtke, J. He, A. Klein, P. Kühn, X. Liu, K. Ma, P. A. Niklaus, K. A. Pietsch … H. Bruelheide. 2018. Biodiversity across trophic levels drives multifunctionality in highly diverse forests. Nature Communications 9:2918.

Simon, M. P., and C. A. Toft. 1991. Diet specialization in small vertebrates: mite-eating in frogs. Oikos 61:263–278.

Solé, M., O. Beckmann, B. Pelz, A. Kwet, and W. Engels. 2005. Stomach-flushing for diet analysis in anurans: an improved protocol evaluated in a case study in Araucaria forests, southern Brazil. Studies on Neotropical Fauna and Environment 40:23–28.

Spitz, R., V. Ridoux, and A. Brind'Amour. 2014. Let's go beyond taxonomy in diet description: testing a trait-based approach to predator-prey relationships. Journal of Animal Ecology 83:1137–1148.

Strauss, R. E. 1979. Reliability estimates for Ivlev's electivity index, the forage ratio, and a proposed linear index of food selection. Transactions of the American Fisheries Society 108:344–352.

Sullivan, M. L., Y. Zhang, and T. H. Bonner. 2014. Carbon and nitrogen ratios of aquatic and terrestrial prey for freshwater fishes. Journal of Freshwater Ecology 29:259–266.

Tiede, Y., D. A. Donoso, J. Bendix, R. Brandl, and N. Farwig. 2017. Ants as indicators of environmental change and ecosystem processes. Ecological Indicators 83:527–537.

Toft, C. 1980. Seasonal variation in populations of Panamanian litter frogs and their prey: a comparison of wetter and drier sites. Oecologia 47:34–38.

Toft, C. 1981. Feeding ecology of Panamanian litter anurans: patterns in diet and foraging mode. Journal of Herpetology 15:139–144.

Weber, N. A. 1938. The food of the giant toad, *Bufo marinus*, in Trinidad and British Guiana with special reference to the ants. Annals of the Entomological Society of America 31:499–503.

Wetterer, J. K., and S. D. Porter. 2003. The little fire ant, *Wasmannia auropunctata*: distribution, Impact and Control. Sociobiology 42:1–41.

Zandonà, E., S. K. Auer, S. S. Kilham, J. L. Howard, A. López-Sepulcre, M. P. O'Connor, R. D. Bassar, A. Osorio, C. M. Pringle, and D. N. Reznick. 2011. Diet quality and prey selectivity correlate with life histories and predation regime in Trinidadian guppies. Functional Ecology 25:964–973.