# Prey Consumption of the Neotropical Frog *Engystomops pustulosus* (Anura: Leptodactylidae) in Northwestern Venezuela

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*Abstract*.—We describe the diet of *Engystomops pustulosus* (Túngara Frog) during the reproductive season. We tested the hypotheses of a relationship between frog mouth width (MW) and prey size, and between the lack of teeth and diet specialization in this genus. We also explored sexual differences in diet composition, and the relationship between female fecundity (number of ova and volume of the ova mass) and the total volume of prey in the stomach. We analyzed stomach contents, identified prey items according to class, subclass, order, or family level, and measured their maximum length and width. Three categories of arthropods dominated the diet: Termitidae (termites), Parasintengona (mites), and Formicidae (ants). Both sexes ate Termitidae and Parasintengona in larger proportions than expected by chance, but only females ate more Formicidae than expected. Males ate more items and more prey categories than females. We found no association between MW (all together and by sex) and prey size or between female fecundity and total volume of prey in the stomach. The diversity of the diet and the equitability were relatively low (numerically: Shannon-Wiener H´ = 1.29; $N_1$ = 3.62; Pielou´s J´ = 0.35), and the trophic niche was narrow (standardized Levin´s Ba = 0.04). These values correspond to those expected for a specialist forager. Our study population consumed more Termitidae than any other examined so far, and an extremely large amount of Parasitengona. Diet composition suggests that *E. pustulosus* is an active forager. We conclude that *E. pustulosus* is a dietary specialist in termites and mites, but eats other prey opportunistically.

*Key Words*.—diet; Formicidae; mites; Parasitengona; *Physalaemus*; termite specialist; Termitidae; Túngara Frog

## Introduction

The diet of a species affects its role in the energy flow in the ecosystem and its relationship with other species, including competition for resources and predation (Simon and Toft 1991; Cloyed and Eason 2017). The diet also provides crucial information to understand life-history traits and the consequences of habitat alterations (e.g., Lips et al. 2005). Most anurans mainly consume invertebrates, especially arthropods (Toft 1980a, 1980b, 1981; Menéndez-Guerrero 2001; Santos et al. 2004), but several species eat small vertebrates sporadically (Duellman and Lizana 1994; Measey et al. 2015) and fruit consumption has been documented in one species (da Silva and de Britto-Pereira 2006). Most anuran species are considered generalists, but diet specialization has been described too (e.g., Toft 1981; Parmelee 1999). The diet of a generalist reflects habitat prey abundance, whereas that of a specialist concentrates only on a few of the available foods regardless of their abundance. From available information, anurans have been classified into ant specialists, non-ant specialists (i.e., specialists in prey other than ants), and generalists (Toft 1980a). These categories should be viewed as the endpoints of a continuum that extends from extreme specialization to complete generalization.

Diet composition in anurans has been typically associated with mouth morphology, especially the presence/absence of teeth and mouth width (MW). Mouth width potentially limits maximum prey size (i.e., prey largest diameter and volume; Parmelee 1999). Ant specialists have relatively narrow mouths in relation to their snout-vent length (SVL; MW/SVL approximately 1/3), non-ant specialists have relatively wider mouths (MW/SVL approximately 2/5), and generalists have intermediate mouth widths (estimated from Toft 1980a, 1981). Consequently, based on the MW/SVL ratio, bufonids and dendrobatids are expected to be ant specialists whereas leptodactylids are expected to be non-ant specialists or generalists (Toft 1980a; Duré and Kehr 2004; Duré et al. 2009).

The presence of teeth hypothetically allows consumption of prey that are difficult to ingest (Lima and Moreira 1993). Strong support for this hypothesis

comes from an anuran community in south India, in which species that feed on ants and termites lack teeth, whereas those that feed on large prey have teeth that tend to show large secondary and tertiary cusps (Das and Coe 1994). The Neotropical genus *Engystomops* (foam frogs) is divided into two sister clades (unracked names; Ron et al. 2006), Duovox (with teeth) and Edentulus (without teeth). Several species in the Edentulus group are termite (soft-bodied prey) specialists: *E. petersi* (Peter's Dwarf Frog; Duellman 1978; Menéndez-Guerrero 2001), *E. freibergi* (no common name; Parmelee 1999), and *E. pustulosus* (Túngara Frog; Ryan 1985; González-Durán et al. 2012). By contrast, *E. pustulatus* (Guayaquil Dwarf Frog; a Duovox species) has a more generalist diet than the three other species that lack teeth (Narváez and Ron 2013). Despite the fact that termites were also present in the diet of *E. pustulatus*, other prey, larger and with harder exoskeletons than termites (e.g., Hymenoptera [mainly ants], Coleoptera, Hemiptera, Gastropoda, Chilopoda, and Isopoda), were well represented. Thus, there appears to be a general correlation between the lack of teeth and diet specialization in termites (soft-bodied prey) in the genus *Engystomops* (Narvaez and Ron 2013).

A few studies have documented sexual differences in diet probably related to sexual size dimorphism (Woolbright and Stewart 1987) and behavior (e.g., *Eleutherodactylus coqui*, Puerto Rican Coqui, Woolbright and Stewart 1987; *Hyla japonica*, Japanese Tree Frog, Hirai and Matsui 2000). Foraging habits and diet composition also correlate with each other. Active foragers consume large numbers of a narrow range of prey, whereas sit-and-wait foragers consume small numbers of a wide range of prey (Toft 1981). Therefore, diet composition allows predicting the foraging behavior of a species.

In the present study, we aimed to describe the diet of the leptodactylid *Engystomops pustulosus* during the reproductive season at a locality in northwestern Venezuela. Two short communications have documented the diet of the species (Ryan 1985; González-Durán et al. 2012), but we believed it important to increase the detail and scope of the analysis. We tested the hypotheses of a relationship between frog MW and prey size, and between the absence of teeth and diet characteristics in *Engystomops* (Das and Coe 1994; Narváez and Ron 2013). We predict based on our first hypothesis an increase in prey size with frog MW. Based on the second hypothesis, we predict diet specialization on soft-bodied prey. We also examined sexual differences in diet composition. Finally, we analyzed the relationship between female fecundity and the volume of the stomach content. We expect the larger the ova mass, the less space available in the



**Figure 1**. A male *Engystomops pustulosus* (Túngara Frog) vocalizing in a pond at the study site (Hacienda La Guáqura, Venezuela). Notice the dorsal stripes characteristic of the stripped morph. (Photographed by Cesar Molina).

abdomen to hold a large meal, and, in consequence, the smaller the total prey volume.

## Materials and Methods

*Study subject.*—*Engystomops pustulosus* has a brownish-colored dorsum and has two morphs (Ryan 1985), striped and unstriped (Fig. 1). The skin is broadly pustulated, as in bufonids, but parotid glands are absent and the species lacks teeth (Ron et al. 2006). Average SVL is 30 mm, and females are slightly larger than males (Ryan 1985). The species is a prolonged breeder, and reproductive activity occurs during the rainy season, from mid-May to September, in Venezuelan lowlands (Tárano 2010). Males gather in choruses, and females actively search for males (Ryan 1985).

*Engystomops pustulosus* is a widespread Neotropical species ranging from Colombia (to the south of the Magdalena Valley), Venezuela, and Trinidad and Tobago, to northern Mexico (Frost 2019), although the records from Guyana are doubtful (Josefa Celsa Señaris, pers. comm.). The genus ranges from Mexico to the Amazonian basin and the lowlands of western Ecuador and northeastern Peru (Ron et al. 2006), but *E. pustulosus* is the only species reported in Venezuela (Barrio-Amorós et al. 2019). In Venezuela, it is widely distributed and abundant in the herbaceous lowlands north of the Orinoco River, but it also occurs in montane forests, dry tropical forests, and anthropogenic areas (Rivero-Blanco and Dixon 1979; Tárano 2010). The species has been classified as Least Concern by the International Union for Conservation of Nature in view of its wide distribution, occurrence in a broad range of habitats, presumed large population, and because it is unlikely to be declining (Santos-Barrera et al. 2010).

***Study site***.—We conducted the study at Hacienda La Guáquira (10°20'4''N, 68°39'17''W), in the montane complex Macizo de Nirgua, at the westernmost edge of the Coast Mountain Range in northern Venezuela. La Guáquira ranges from the margins of the Yaracuy River (100 m elevation) to the northwestern margin of Cerro Zapatero (1,400 m elevation). Vegetation varies from mist forest in the highlands (> 900 m elevation) to semi-deciduous tropical-humid forests in the lowlands (Lozkat 2007). Large areas of the lowlands (below 100 m elevation) have been turned into cattle ranching, and rice and corn fields. We collected the specimens in the lowlands, mainly in cattle ranching areas and along dirt roads adjacent to rice and corn fields, across an area of approximately 1,200 ha.

***Capture methods and diet analysis***.—We captured frogs by hand at night from 2000 to 0000, from June to September in 2012 and in June 2013 (3 d per month, 15 d total). These months correspond to the rainy season at the study site. We euthanasized frogs by soaking them in Xylocaine 5% (50 mg/g) anaesthetic gel immediately after capture, fixed each specimen in formalin 4% to stop digestion, and preserved them in ethanol 70%. We deposited the specimens at Laboratorio de Biología y Conservación de Anfibios y Reptiles (Instituto de Zoología y Ecología Tropical, Facultad de Ciencias, Universidad Central de Venezuela) for use in another study. We measured SVL and MW (from corner to corner, mouth closed) in the lab with a dial caliper (Vernier) to the nearest 0.1 mm. We removed the stomach through a ventral longitudinal incision and dissected it on a Petri dish. We determined age class by inspection of the gonads; individuals with undeveloped gonads we deemed subadults. For gravid females, we determined fecundity (number of ova in oviducts and total volume of the ova mass).

We observed the contents of each stomach under a stereoscopic microscope (AmScope SE306R-PZ, AmScope, Irvine, California, USA.), identified prey items (i.e., an item is an individual prey) according to class, subclass, order or family level (i.e., prey categories) following McGavin (2002). We counted the number of items in each category ($N_i$), and measured the maximum length and width of all items under the stereoscopic microscope with an ocular micrometer to the nearest 0.01 mm. For abundant categories ($N_i > 100$), we only measured 50 items chosen at random; we did not measure broken or partially digested items. We calculated the volume of prey items by using the equation of a prolate spheroid (Dunham 1983)

$$Vi = 4/3\,\pi(l/2)(w/2)^2$$

where $l$ represents the length of the item and $w$ its maximum width.

Using these variables we calculated: (1) the numeric composition of the diet as the number of items of a prey category ($N_i$), (2) the total volume per prey category per stomach ($\sum V_i$), (3) the absolute frequency as the number of stomachs with the prey category ($S_i$), and (4) the importance of a prey category as

$$I = \frac{\%F + \%N + \%V}{3}$$

where $\%N = \left(\frac{N_i}{N_t}\right) \times 100$; $\%F = \left(\frac{S_i}{S_t}\right) \times 100$; $\%V = \left(\frac{V_i}{V_t}\right) \times 100$

and $N_t$ is the total number of items of all preys, $S_t$ is the total number non-empty stomachs, and $V_t$ the total volume of prey in all stomachs. In addition, we estimated the diversity of the diet through the Shannon-Wiener index

$$H' = -\sum p_i \ln p_i$$

where $p_i$ corresponds to the proportion of stomachs with prey $i$, in number or frequency. We also used the Hill's $N_1$ ($N_1 = e^{H'}$; Hill 1973). We used the former indexes for comparison purposes because they are of common use. Diet equitability was estimated through Pielou's formula

$$J' = H'/H_{max}$$

where $H_{max} = \log R$, and R is prey richness. Niche breath was estimated through the standardized Levins' index following Hurlbert (1978)

$$Ba = \frac{B-1}{R-1}, \text{B is the Levins' index, } B = 1/\sum p_i^2$$

where $p_i$ is the proportion of stomachs with prey $i$, and R is the number of prey categories or richness. Index Ba ranges from 0 to 1; 0 corresponds to a strict specialist and 1 to a perfect generalist.

***Statistical analysis***.—We present results as means ± 1 standard deviation. We used non-parametric tests because transformation of data did not allow attaining normality (Shapiro-Wilk, $P < 0.04$ in all tests). We determined the association between SVL and MW, prey length (maximum length), and volume (maximum volume) using the Spearman rank correlation. In females, we also analyzed the relationship between the total volume of the ova mass and the total prey volume (total volume of prey in the stomach) using the Spearman rank correlation.

We also checked for sexual differences in diet by comparing the total number of prey categories per stomach, the number of prey items, and total prey

volume per category using a Mann-Whitney $U$ test. Additionally, we compared the diversity of the diet between sexes using the Hutchenson $t$ (Zar 1999) as

$$t = \frac{H\prime_1 - H\prime_2}{S_{(H\prime_1 - H\prime_2)}}$$

where 1 and 2 are the two sexes, and

$$S_{(H\prime_1 - H\prime_2)} = \sqrt{S^2_{H\prime_1} + S^2_{H\prime_2}}$$

where S is the variance of H′ for each sex estimated as

$$S^2_{H\prime} = \frac{\sum f_i ln f_i^2 - (\sum f_i ln f_i)^2/2}{\sum f_i^2}$$

where corresponds to $N_i$. The degrees of freedom of $t$ were estimated through

$$v = \frac{(S^2_{H\prime_1} + S^2_{H\prime_2})^2}{\frac{(S^2_{H\prime_1})^2}{N_1} + \frac{(S^2_{H\prime_2})^2}{N_2}}$$

where N is the total number of prey items.

We determined prey preferences per sex through contingency tables and the Chi-square test, based on $N_i$. We excluded prey categories with $N_i < 5$ to fulfill the requirement of at least 80% of the cells with $N_i > 5$ (Quinn and Keough 2002). Because this test was significant, we used the standardized residuals analysis to detect the significant preferences using

$$Z = \frac{(O - E)}{\sqrt{E}}$$

where $O$ is the observed value and $E$ the expected (random). Residuals were compared with $Z_{crit} = \pm 1.96$ ($\alpha = 0.05$) and $Z_{crit} = \pm 2.56$ ($\alpha = 0.01$). Positive significant residuals indicate prey preference and negative ones prey rejection (avoidance). We performed all the analyses with Statistica v.10 (Statsoft Inc., Tulsa, Oklahoma, USA) with $\alpha = 0.05$.

## RESULTS

***Morphology and fecundity.***—We captured 240 individuals (188 males, 48 females, four subadults), of which 60 individuals (30 pairs) were in amplexus. Mean SVL was 13.94 ± 1.68 mm (n = 4; range, 12.70–16.35 mm) in subadults, 25.79 ± 1.79 mm (n = 188; range, 21.40–29.90) in adult males, and 27.73 ± 1.59 mm (n = 48; range, 23.65–30.50) in adult females. Females were significantly larger than males ($U = 4.15$, df = 234, $P < 0.001$). Mean MW was 4.56 ± 0.125 mm (n = 4; range, 4.40–4.70) in subadults, 7.66 ± 0.63 mm (n = 188; range, 6.00–9.10) in males, and 7.87 ± 0.63 mm (n = 48; range, 6.70–9.25) in females. Despite the fact that females were significantly larger than males, MW did not differ between sexes ($U = 1.55$, df = 234, $P = 0.122$). As in many anurans, there was positive

correlation between SVL and MW at the population level (all individuals, subadults included; $rs = 0.78$, $P < 0.001$), and by sex (males: $rs = 0.79$, $P < 0.001$; females: $rs = 0.81$, $P < 0.001$); therefore, we performed subsequent morphometric analyses using MW. Mean female fecundity was 321 ± 124 ova (n = 30 females; range, 164–690), ovum diameter was 1.29 ± 0.09 mm (n = 8,832; range, 1.05–0.70), and total volume of the ova mass was 418.3 ± 153.2 mm³ (n = 30; range, 225.2–859.8).

***Diet composition.***—Of 240 frogs we captured, 42 individuals (17.5%) had empty stomachs or only contained parasites (35 males, seven females), and 28 stomachs (11.7 %) had only sand or plant material (22 males, six females). Hence, we based diet analysis on 170 non-empty stomachs. We identified all prey items and classified them into 40 prey categories (28 families, eight orders, one infraorder, two classes, one subclass; Table 1); most stomachs (92.7%) also contained sand and plant material. Most items were insects of the family Termitidae (termites), arachnids of the infraorder Parasitengona (mites; subclass Acari, order Trombidiformes), and insects of the family Formicidae (ants). Males consumed 33 categories whereas females consumed only 13, but Termitidae, Parasitengona, and Formicidae were the most important in both sexes (Appendices 1 and 2). Females consumed two categories not found in males: Dolichopodidae and Theridiidae. Subadults consumed nine categories of prey, and Isotomidae (Collembola) and Formicidae were the most important; however, we treat these data with caution because of the small sample (Appendix 3).

As a whole, numerically, Termitidae, Parasitengona, and Formicidae represent 94.5% of the diet, whereas, volumetrically, Termitidae alone represents 93.1% (97.4% in females; 91.6% in males). Prey preference analysis performed with the data from adult frogs and prey categories with $N_i > 5$ (Termitidae, Parasitengona, Formicidae, Staphylinidae, and Gastropoda; Table 1) indicated uneven consumption of prey ($X^2 = 60.8$, df = 4, $P < 0.001$). The residual analysis indicated that two categories were consumed at a higher proportion than that expected by chance in both sexes: Termitidae (male residual: +2.21, $P = 0.041$; female residual: +4.72, $P < 0.001$) and Parasitengona (male residual: +2.57, $P = 0.014$; female residual +5.50, $P < 0.001$); but only females consumed Formicidae at a higher proportion than that expected by chance (residual +2.40, $P = 0.021$).

Altogether, the diversity of the diet was H′ = 1.29 (numerically, Table 2) and the diet of males was more diverse than that of females (numerically: $t = 3.52$, df = 533, $P < 0.001$). $N_1$ was low due to the dominance of three prey categories ($N_1$ global = 3.62, $N_1$ males =

**TABLE 1.** Overall diet composition of *Engystomops pustulosus* (Túngara Frog) at Hacienda La Guáquira, northwestern Venezuela (St = n = 170 stomachs). Prey categories are shown in alphabetic order according to their class and order. Empty cells correspond to broken items not measured. Abbreviations are %N = $(N_i/N_t)\times100$, $N_i$ = number of items in category $i$, $N_t$ = total number of items, %F = $(S_i/S_t)\times100$, $S_i$ = number of stomachs with category $i$, %V = $(V_i/V_t)\times100$, $V_i$ = volume of category $i$ (mm³), $V_t$ = total volume of all categories, I = relative importance index (%N+%F+%V)/3, lv = larvae, ch = chrysalides, asterisk (*) denotes Subclass, and dagger (†) denotes Infraorder.

| Prey Category | | | Number | | Frequency | | Volume | | |
|---|---|---|---|---|---|---|---|---|---|
| Class | Order | Family | $N_i$ | %N | $S_i$ | %F | $\Sigma V_i$ | %V | I |
| Arachnida | Ixodida | Argasidae | 3 | 0.11 | 3 | 1.76 | 1.13 | 0.03 | 0.63 |
| | Trombidiformes | Trombidiidae | 4 | 0.15 | 2 | 1.18 | 1.02 | 0.02 | 0.45 |
| | | Parasitengona† | 804 | 29.48 | 49 | 28.82 | 148.87 | 2.97 | 20.42 |
| | Acari* | undetermined | 14 | 0.51 | 11 | 6.47 | 3.47 | 0.08 | 2.35 |
| | Araneae | Theridiidae | 1 | 0.04 | 1 | 0.59 | 0.17 | 0.00 | 0.21 |
| | Pseudoscorpionida | undetermined | 2 | 0.07 | 2 | 1.18 | 0.49 | 0.01 | 0.42 |
| Diplopoda | undetermined | undetermined | 4 | 0.15 | 4 | 2.35 | 1.50 | 0.04 | 0.85 |
| Entognatha | Collembola | Isotomidae | 19 | 0.70 | 4 | 2.35 | 1.68 | 0.04 | 1.03 |
| | | Sminthuridae | 25 | 0.92 | 7 | 4.12 | 0.67 | 0.02 | 1.69 |
| Insecta | Coleoptera | Anobiidae | 1 | 0.04 | 1 | 0.59 | 0.08 | 0.00 | 0.21 |
| | | Carabidae | 1 | 0.04 | 1 | 0.59 | 0.07 | 0.00 | 0.21 |
| | | Coccinellidae | 1 | 0.04 | 1 | 0.59 | 0.45 | 0.01 | 0.21 |
| | | Curculionidae | 1 | 0.04 | 1 | 0.59 | 0.32 | 0.01 | 0.21 |
| | | Elateridae | 7 | 0.26 | 3 | 1.76 | 7.48 | 0.18 | 0.73 |
| | | Lampyridae (lv) | 2 | 0.07 | 2 | 1.18 | 4.56 | 0.11 | 0.45 |
| | | Passalidae | 3 | 0.11 | 2 | 1.18 | 6.83 | 0.16 | 0.48 |
| | | Staphylinidae | 13 | 0.48 | 13 | 7.65 | 7.52 | 0.18 | 2.77 |
| | | Staphylinidae (lv) | 1 | 0.04 | 1 | 0.59 | 0.37 | 0.01 | 0.21 |
| | | undetermined | 1 | 0.04 | 1 | 0.59 | 0.73 | 0.02 | 0.22 |
| | | undetermined (lv) | 3 | 0.11 | 3 | 1.76 | 1.20 | 0.03 | 0.63 |
| | Diptera | Ceratopogonidae | 5 | 0.18 | 3 | 1.76 | 1.14 | 0.03 | 0.66 |
| | | Ceratopogonidae (lv) | 8 | 0.29 | 2 | 1.18 | 1.20 | 0.03 | 0.50 |
| | | Ephydridae | 1 | 0.04 | 1 | 0.59 | 0.22 | 0.01 | 0.21 |
| | | Ephydridae (lv) | 1 | 0.04 | 1 | 0.59 | 0.06 | 0.00 | 0.21 |
| | | Phoridae | 1 | 0.04 | 1 | 0.59 | 0.68 | 0.02 | 0.22 |
| | | Stratiomyidae (lv) | 1 | 0.04 | 1 | 0.59 | | | |
| | | Tephritidae | 2 | 0.07 | 1 | 0.59 | 0.58 | 0.01 | 0.22 |
| | | Dolichopodidae | 1 | 0.04 | 1 | 0.59 | 0.41 | 0.01 | 0.21 |
| | | undetermined (lv) | 3 | 0.11 | 2 | 1.18 | 4.70 | 0.11 | 0.47 |
| | | Sphaeroceridae (lv) | 2 | 0.07 | 2 | 1.18 | 0.31 | 0.01 | 0.42 |
| | Hemiptera | Miridae | 2 | 0.07 | 2 | 1.18 | 0.66 | 0.02 | 0.42 |
| | | Tingidae | 2 | 0.07 | 2 | 1.18 | 1.34 | 0.03 | 0.43 |
| | Hymenoptera | Formicidae | 271 | 9.94 | 75 | 44.12 | 52.97 | 1.07 | 18.38 |
| | | undetermined | 1 | 0.04 | 1 | 0.59 | | | |
| | Blattodea | Ectobiidae | 1 | 0.04 | 1 | 0.59 | 19.09 | 0.45 | 0.36 |
| | Isoptera | Termitidae | 1,475 | 54.09 | 66 | 38.82 | 4,667.54 | 93.13 | 62.01 |
| | Lepidoptera | undetermined (lv) | 1 | 0.04 | 1 | 0.59 | | | |
| | | undetermined (ch) | 1 | 0.04 | 1 | 0.59 | 0.23 | 0.01 | 0.21 |
| | Psocodea | Psocidae | 1 | 0.04 | 1 | 0.59 | 0.30 | 0.01 | 0.21 |
| Gastropoda | undetermined | undetermined | 14 | 0.51 | 12 | 7.06 | 13.56 | 0.32 | 2.63 |
| Malacostraca | Isopoda | undetermined | 23 | 0.84 | 5 | 2.94 | 58.08 | 1.37 | 1.72 |
| | | $N_t$ 2,727 | | | | | | | |

**Table 2.** Diversity, niche breath, and equitability of the diet of *Engystomops pustulosus* (Túngara Frog) at the Hacienda La Guáquira, northwestern Venezuela. Symbols are H′ = Shannon-Wiener diversity index, $N_1$ = Hill's number, J′ = Pielou's equitability, Ba = standardized Levins' niche breath.

|  |  |  | Males | Females | Total |
|---|---|---|---|---|---|
| Diversity | H′ | Numeric | 1.27 | 0.90 | 1.29 |
|  |  | Volumetric | 0.44 | 0.16 | 0.38 |
|  | $N_1$ | Numeric | 3.58 | 2.42 | 3.62 |
|  |  | Volumetric | 1.56 | 1.18 | 1.47 |
| Equitabilty | J′ | Numeric | 0.36 | 0.35 | 0.35 |
|  |  | Volumetric | 0.13 | 0.06 | 0.10 |
| Niche breath | Ba | Numeric | 0.04 | 0.05 | 0.061 |
|  |  | Volumetric | 0.004 | 0.006 | 0.004 |

3.58, $N_1$ females = 2.42). Equitability of the diet was low (J′ global = 0.35; males = 0.36; females = 0.35), and niche breath (Ba) was 0.04 (males = 0.05, females = 0.06). Volumetric index values were lower than numeric index values due to the extreme dominance of Termitidae (Table 2).

Each stomach contained, on average, 16.4 ± 24.1 items (range, 1–119) from 1.64 ± 1.1 prey categories (range, 1–9). The stomachs of males contained more items than those of females (males: 17.3 ± 24.3; females: 13.3 ± 23.4; $U = -2.42$, df = 164, $P = 0.017$), but not more prey categories (males: 1.7 ± 1.2; females: 1.43 ± 0.7; $U = -1.09$, df = 164, $P = 0.274$). On average, the maximum length of preys consumed by adults was 2.36 ± 1.21 mm (range, 0.50–6.35 mm) and their volume was 1.08 mm$^3$ ± 1.90 (range, 0.01–19.90). There was no sexual difference in ingested prey length (males: 2.31 ± 1.16 mm; females: 2.55 ± 1.39 mm; $U = -0.65$, df = 139, $P = 0.515$) or volume (males: 1.08 ± 2.06 mm$^3$; females: 1.06 ± 1.17 mm$^3$; $U = 0.43$, df = 139, $P = 0.665$). The latter results did not change when we only compared the preys consumed by both sexes (Termitidae, Parasitengona, Formicidae, Staphylinidae and Gastropoda). Total volume of the stomach contents was, on average, 27.1 ± 55.3 mm$^3$, and there was no sexual difference (males: 25.4 ± 54.1 mm$^3$, females: 33 ± 60.4 mm$^3$; $U = -0.88$, df = 139, $P = 0.372$). Additionally, there was no correlation between MW and the largest ingested prey item or the largest ingested prey volume, as a whole and by sex ($P > 0.200$ in both correlations). We found no correlation between the total volume of the ova mass and the total volume of prey in the stomach ($P = 0.983$).

### Discussion

The diet of *Engystomops pustulosus* in the population we studied was dominated by three prey categories, which represent more than 90% of the prey consumed. This indicates that the population is specialized on termites and mites (Parasitengona), and relies on ants secondarily. The dominance of termites in the diet of *E. pustulosus* has been reported before by Ryan (1985) for a population on Barro Colorado Island in Panamá (n = 16 stomachs), and by González-Durán et al. (2012) in Colombia (n = 78 stomachs). The extremely high consumption of mites (Parasitengona), however, has not been documented before.

Large numbers of termites are consumed by several species of *Engystomops* (*E. petersi*, Duellman 1978; *E. freibergi*, Parmelee 1999; *E. pustulatus*, Narváez and Ron 2013) and *Physalaemus* (*P. albifrons*, Bahia Dwarf Frog, Cruz 2000; *P. albonotatus*, Menwig Frog, Falico et al. 2012; *P. biligonigerus*, Weeping Frog, Attademo et al. 2007; Rödder 2008; de Oliveira 2015; *P.* cf. *cicada* [no common name], Santana and Juncá 2007; *P. cuvieri*, Barker Frog or Cuvier's Foam Froglet, Santos et al. 2004; Leivas et al. 2018; *P. ephippifer*, Steindachner's Dwarf Frog, Caldwell and Vitt 1999; Rodrigues and dos Santos-Costa 2014; *P. gracilis*, Graceful Dwarf Frog, Da Rosa et al. 2002; *P. henselii*, Hensel's Dwarf Frog, Farina et al. 2018; *P. santafecinus*, Helvetia Dwarf Frog, Duré 1998). In tropical ecosystems, termites and ants represent a high proportion of the arthropod biomass (Fittkau and Klinge 1973; Traniello and Leuthold 2000). Their predominance in the diet, however, does not necessarily mean a preference or specialization.

We used published data to explore further the preference of *E. pustulosus* for termites. We qualitatively compared ant and termite abundance in the diet of *E. pustulosus* on Barro Colorado Island (Ryan 1985), with their occurrence in litter in Gamboa (near Barro Colorado, Toft 1981). Ants and termites represented 32% of the invertebrate prey in the litter but almost 60% of the diet, which suggests that ants and termites are preferred by this frog. In another study, Santana and Juncá (2007) demonstrated that *P.* cf. *cicada* avoided the most abundant prey in the forest (ants) and preferred termites. Termites have a high content of carbohydrates and less chitin than ants (Marconi et al. 2002), which may make them more attractive as prey.

Diet specialization is usually related to prey morphology (size, stiffness, defensive structures) and habits (gregarious or solitary), and predator morphology (teeth, cranial structure, tongue, jaws; Emerson 1985; Das and Coe 1994). In several anuran species, the presence of teeth is correlated with the consumption of large and hard-bodied prey, whereas teeth absence is correlated with small and soft-bodied prey, such as ants and termites (Das and Coe 1994; Lima and Moreira 1993). In addition, an association between diet specialization and the absence of teeth in the genus *Engystomops* has been documented (Duellman 1978; Parmelee 1999; Ryan 1985; González-Durán et al. 2012; Narváez and Ron 2013). Our results are consistent

with these patterns: *E. pustulosus*, a toothless species, exhibited a specialized diet consisting mainly of soft-bodied prey. Toothed species of *Engystomops* and of the sister taxon *Physalaemus*, however, also consume high numbers of termites (e.g., *P. ephippifer*, Caldwell and Vitt 1999; Rodrigues and dos Santos-Costa 2014; *E. pustulatus*, Narváez and Ron 2013), and the toothed species, *P. biligonigerus* and *P. gracilis*, rely mainly on large soft-bodied coleopteran larvae (de Oliveira et al. 2015).

Mites are abundant in soil and litter but typically represent a small fraction of the diet of most anurans (Toft 1981). This is probably because they are a poor source of nutrients, have a high content of chitin, and their rate of assimilation and passage through the gut is low (Simon and Toft 1991). Several authors have rendered the consumption of mites in *Physalaemus* and *Engystomops* as incidental (e.g., Caldwell and Vitt 1999; Santos et al. 2004; Attademo et al. 2007; Narváez and Ron 2013; Rodrigues and dos Santos-Costa 2014). Given that there is an ecological association between social prey (such as ants and termites) and mites (Eickwort 1990), the latter can be consumed jointly with ants and termites. Conversely, other authors have suggested that mites are actively selected (Simon and Toft 1991; Da Rosa et al. 2002; Valderrama-Vernaza et al. 2009; Agudelo-Cantero et al. 2015). For instance, oribatid mites are of great importance in poisonous frogs because they represent the principal dietary source of alkaloids (e.g., *Oophaga pumilio*, Strawberry Poison-dart Frog, Saporito et al. 2007). Mite (mainly Parasitengona) consumption in our study population was remarkably larger than that reported for a population in Colombia by González-Durán et al. (2012), both numerically (29.6% versus 9.6%) and volumetrically (2.99% versus 0.52%), and for *P. gracilis,* (29.6% versus 15.9% numerically, Da Rosa et al. 2002). We lack data on mite abundance in our study site to conclude firmly that there is a preference for mites in *E. pustulosus*.

Anurans swallow their prey whole and MW has been hypothesized to limit the maximum prey size (Lima and Moreira 1993). In many species, however, there is no association between MW and prey size (Sanabria et al. 2005; Cossovich et al. 2011). This probably results from the fact that frogs, both generalists and specialists, do not only forage for maximum prey size (Lynch and Duellman 1997). We did not find support for this hypothesis in *E. pustulosus* either, because relatively small preys (ants, termites, and mites) dominated the diet.

We found that females consumed fewer prey items than males. We believe that this difference resulted from the limited abdominal space in females due to the ova mass, and the load that it represents (approximately 20% of body mass; Ryan 1985) to female movement (i.e., distance travelled in search of food). We observed that the stomachs of gravid females were laterally constrained in the abdominal cavity. Nevertheless, we found no correlation between the volume of prey in the stomach and the volume of the ova mass. Only five of 35 females we caught were not gravid, limiting a comparison between gravid and non-gravid females. In other species, male engagement in vocal activity limits the time available for prey search and increases the likelihood of finding empty stomachs and less stomach content (e.g., *Eleutherodactylus coqui*, Woolbright and Stewart 1987; *Hyla japonica*, Hirai and Matsui 2000). This was not the case in our study; males consumed more items and had a more diverse diet than females, and there was no sexual difference in the likelihood of finding empty stomachs (males 18%; females 16%). The cause of the sexual difference found in our study remains to be explored.

We analyzed the stomach contents of four subadults, but the small sample precludes any analysis of ontogenetic variation in diet in *E. pustulosus*. Subadults, however, consumed a high proportion of Collembola (especially Isotomidae). Given that the exoskeleton of Collembola has less chitin than that of ants and, with a few exceptions, individuals lack toxins or defensive structures (e.g., strong mandibles), subadults can be actively selecting them; however, this idea remains to be tested.

We found sand and plant debris in most stomachs. Sand and plant material are common in the stomachs of many anurans (Evans and Lampo 1996; Anderson et al. 1999; Santos et al. 2004; Batista et al. 2011; Camera et al. 2014), but their relevance has not been established satisfactorily. Several authors have proposed that they are accidentally ingested during prey capture (e.g., Suazo-Ortuño et al. 2007), whereas others propose that their consumption is functional (Anderson et al. 1999).

We did not observe foraging behavior in *E. pustulosus,* but several authors have suggested that active foragers would consume large numbers of a narrow range of small, slow moving prey, whereas sit-and-wait frogs would consume small numbers of a wide range of large, mobile prey (Toft 1981). From our results, we postulate that *E. pustulosus* is an active forager. Because its preferred prey are social, large numbers of items are consumed when a colony is found.

In conclusion, the studied population relies strongly on two prey categories, Termitidae and Parasitengona, with the former the most important, numerically and volumetrically, in both sexes. This dietary skew resulted in a narrow food niche, and low equitability and diversity, which characterize specialist foragers. Given that the preferred prey category is social (Termitidae), or live in association with social species (Parasitengona), we propose that *E. pustulosus* is an active forager that opportunistically eats other prey

found while searching for its preferred ones.

Finally, we acknowledge that to label an anuran as specialist or generalist confidently, we need estimations of food abundance in its habitat; nonetheless, most studies on anuran diet lack these data (but see Simon and Toft 1991; Hirai and Matsui 2000; Cloyed and Eason 2017; Cogălniceanu et al. 2018). Invertebrate-prey abundance, however, is often difficult to estimate and prey-sampling methods have their own biases and limitations (Candia 1997). Consequently, most diet studies simply rely on stomach contents, and researchers assume that an even distribution of the items describes a generalist diet, whereas a highly skewed distribution corresponds to a specialized one.

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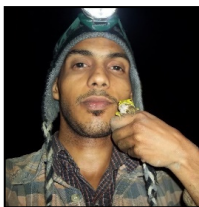
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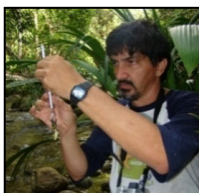


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**APPENDIX 1.** Diet of males of *Engystomops pustulosus* (Túngara Frog) at Hacienda La Guáquira, northwestern Venezuela ($S_t$ = n = 131 males). Prey categories are shown in alphabetic order according to their class and order. Empty cells correspond to broken items not measured. Abbreviations are %N = ($N_i/N_t$)×100, $N_i$ = number of items in category *i*, $N_t$ = total number of items, %F = ($S_i/S_t$)×100, $S_i$ = number of stomachs with category *i*, %V = ($V_i/V_t$)×100, $V_i$ = volume of category *i* (mm³), $V_t$ = total volume of all categories, I = relative importance index (%N+%F+%V)/3, lv = larvae, ch = chrysalides, asterisk (*) denotes Subclass, and dagger (†) denotes Infraorder.

| | Prey category | | Number | | Frequency | | Volume | | |
|---|---|---|---|---|---|---|---|---|---|
| Class | Order | Family | $N_i$ | %N | $S_i$ | %F | $\Sigma V_i$ | %V | I |
| Arachnida | Ixodida | Argasidae | 3 | 0.16 | 3 | 2.68 | 1.13 | 0.04 | 0.96 |
| | Trombidiformes | Trombidiidae | 4 | 0.21 | 2 | 1.79 | 1.02 | 0.03 | 0.68 |
| | | Parasitengona† | 725 | 32.76 | 40 | 30.53 | 141.6 | 3.97 | 22.42 |
| | Acari* | undetermined | 11 | 0.58 | 9 | 8.04 | 2.37 | 0.08 | 2.90 |
| | Pseudoscorpionida | undetermined | 2 | 0.09 | 2 | 1.53 | 0.57 | 0.02 | 0.54 |
| Diplopoda | undetermined | undetermined | 3 | 0.16 | 3 | 2.68 | 1.13 | 0.04 | 0.96 |
| Entognatha | Collembola | Isotomidae | 2 | 0.11 | 2 | 1.79 | 0.02 | 0.00 | 0.63 |
| | | Sminthuridae | 22 | 1.16 | 4 | 3.57 | 0.59 | 0.02 | 1.58 |
| Insecta | Coleoptera | Anobiidae | 1 | 0.05 | 1 | 0.89 | 0.08 | 0.00 | 0.32 |
| | | Carabidae | 1 | 0.05 | 1 | 0.89 | 0.07 | 0.00 | 0.32 |
| | | Coccinellidae | 1 | 0.05 | 1 | 0.89 | 0.45 | 0.02 | 0.32 |
| | | Curculionidae | 1 | 0.05 | 1 | 0.89 | 0.32 | 0.01 | 0.32 |
| | | Elateridae | 6 | 0.32 | 2 | 1.79 | 6.63 | 0.22 | 0.77 |
| | | Lampyridae (lv) | 2 | 0.11 | 2 | 1.79 | 4.56 | 0.15 | 0.68 |
| | | Passalidae | 3 | 0.16 | 2 | 1.79 | 6.83 | 0.23 | 0.72 |
| | | Staphylinidae | 8 | 0.42 | 8 | 7.14 | 3.99 | 0.13 | 2.57 |
| | | Staphylinidae (lv) | 1 | 0.05 | 1 | 0.89 | 0.37 | 0.01 | 0.32 |
| | | undetermined (lv) | 2 | 0.11 | 2 | 1.79 | 1.91 | 0.06 | 0.65 |
| | Diptera | Ceratopogonidae | 5 | 0.26 | 3 | 2.68 | 1.14 | 0.04 | 0.99 |
| | | Ceratopogonidae (lv) | 8 | 0.42 | 2 | 1.79 | 1.20 | 0.04 | 0.75 |
| | | Phoridae | 1 | 0.05 | 1 | 0.89 | 0.68 | 0.02 | 0.32 |
| | | Stratiomyidae (lv) | 1 | 0.05 | 1 | 0.89 | | | |
| | | Tephritidae | 2 | 0.11 | 1 | 0.89 | 0.58 | 0.02 | 0.34 |
| | | undetermined (lv) | 3 | 0.16 | 2 | 1.79 | 4.70 | 0.16 | 0.70 |
| | Hemiptera | Miridae | 1 | 0.05 | 1 | 0.89 | 0.30 | 0.01 | 0.32 |
| | | Tingidae | 2 | 0.11 | 2 | 1.79 | 1.34 | 0.04 | 0.65 |
| | Hymenoptera | Formicidae | 223 | 10.26 | 57 | 43.51 | 36.49 | 1.02 | 18.26 |
| | | undetermined | 1 | 0.05 | 1 | 0.76 | | | |
| | Blattodea | Ectobiidae | 1 | 0.05 | 1 | 0.89 | 19.09 | 0.63 | 0.53 |
| | Isoptera | Termitidae | 1,132 | 51.15 | 55 | 41.98 | 3,264.12 | 91.58 | 61.57 |
| | Lepidoptera | undetermined (lv) | 1 | 0.05 | 1 | 0.89 | | | |
| | | undetermined (ch) | 1 | 0.05 | 1 | 0.89 | 0.23 | 0.01 | 0.32 |
| | Psocodea | Psocidae | 1 | 0.05 | 1 | 0.89 | 0.30 | 0.01 | 0.32 |
| Gastropoda | undetermined | undetermined | 8 | 0.42 | 6 | 5.36 | 8.38 | 0.28 | 2.02 |
| Malacostraca | Isopoda | undetermined | 20 | 1.06 | 4 | 3.57 | 51.99 | 1.72 | 2.12 |
| | | $N_t$ | 2,213 | | | | | | |

**APPENDIX 2.** Diet composition of females of *Engystomops pustulosus* (Túngara Frog) at Hacienda La Guáquira, northwestern Venezuela (Sᵗ = n = 35 females). Prey categories are shown in alphabetic order according to their class and order. Empty cells correspond to broken items not measured. Abbreviations are %N = $(N_i/N_t)\times100$, Ni = number of items in category $i$, Nt = total number of items, %F = $(Si/St)\times100$, Si = number of stomachs with category $i$, %V = $(Vi/Vt)\times100$, Vi = volume of category $i$ (mm³), Vt = total volume of all categories, I = relative importance index (%N, %F, %V)/3, lv = larvae, ch = chrysalides, asterisk (*) denotes Subclass, and dagger (†) denotes Infraorder.

| | Prey category | | Number | | Frequency | | Volume | | |
|---|---|---|---|---|---|---|---|---|---|
| Class | Order | Family | $N_i$ | %N | $S_i$ | %F | $\Sigma V_i$ | %V | I |
| Arachnida | Trombidiformes | Parasitengona† | 79 | 16.81 | 9 | 25.71 | 13.85 | 1.14 | 14.55 |
| | Acari* | undetermined | 1 | 0.21 | 1 | 2.86 | 0.07 | 0.01 | 1.03 |
| | Araneae | Theridiidae | 1 | 0.21 | 1 | 2.86 | 0.17 | 0.01 | 1.03 |
| Diplopoda | undetermined | undetermined | 1 | 0.21 | 1 | 2.86 | | | |
| Entognatha | Collembola | Sminthuridae | 1 | 0.21 | 1 | 2.86 | 0.07 | 0.01 | 1.03 |
| Insecta | Coleoptera | Elateridae | 1 | 0.21 | 1 | 2.86 | 0.81 | 0.07 | 1.05 |
| | | Staphylinidae | 5 | 1.06 | 5 | 14.29 | 3.62 | 0.30 | 5.22 |
| | | undetermined | 1 | 0.21 | 1 | 2.86 | 0.73 | 0.06 | 1.04 |
| | Diptera | Dolichopodidae | 1 | 0.21 | 1 | 2.86 | 0.41 | 0.03 | 1.03 |
| | Hemiptera | Miridae | 1 | 0.21 | 1 | 2.86 | 0.36 | 0.03 | 1.03 |
| | Hymenoptera | Formicidae | 30 | 6.38 | 15 | 42.86 | 7.76 | 0.64 | 16.63 |
| | Isoptera | Termitidae | 343 | 72.98 | 11 | 31.43 | 1,187.42 | 97.37 | 67.26 |
| Gastropoda | undetermined | undetermined | 5 | 1.06 | 5 | 14.29 | 4.19 | 0.34 | 5.23 |
| | | $N_t$ | 470 | | | | | | |

**APPENDIX 3.** Diet of subadults of *Engystomops pustulosus* (Túngara Frog) at Hacienda La Guáquira, northwestern Venezuela (Sₜ = n = 4). Prey categories are shown in alphabetic order according to their Class and Order. Empty cells correspond to broken items not measured. Abbreviations are %N = $(N_i/N_t)\times100$, Ni = number of items in category $i$, $N_t$ = total number of items, %F = $(S_i/S_t)\times100$, $S_i$ = number of stomachs with category $i$, %V = $(Vi/Vt)\times100$, $V_i$ = volume of category $i$ (mm³), $V_t$ = total volume of all categories, I = relative importance index (%N+%F+%V)/3, lv = larvae, ch = chrysalides, asterisk (*) denotes Subclass, and dagger (†) denotes Infraorder.

| | Category of prey | | Number | | Frequency | | Volume | | |
|---|---|---|---|---|---|---|---|---|---|
| Class | Order | Family | $N_i$ | %N | $S_i$ | %F | $\Sigma V_i$ | %V | I |
| Arachnida | Acari* | undetermined | 2 | 4.55 | 2 | 50 | 1.31 | 16.47 | 23.67 |
| Entognatha | Collembola | Isotomidae | 17 | 38.64 | 2 | 50 | 1.65 | 20.71 | 36.45 |
| | | Sminthuridae | 2 | 4.55 | 2 | 50 | 0.04 | 0.45 | 18.33 |
| Insecta | Coleoptera | undetermined (lv) | 1 | 2.27 | 1 | 25 | 0.12 | 1.45 | 9.57 |
| | Diptera | Ephydridae | 1 | 2.27 | 1 | 25 | 0.22 | 2.78 | 10.02 |
| | | Ephydridae (lv) | 1 | 2.27 | 1 | 25 | 0.06 | 0.77 | 9.35 |
| | | Sphaeroceridae (lv) | 2 | 4.55 | 2 | 50 | 0.31 | 3.85 | 19.47 |
| | Hymenoptera | Formicidae | 14 | 31.82 | 3 | 75 | 0.58 | 7.29 | 38.04 |
| Gastropoda | undetermined | undetermined | 1 | 2.27 | 1 | 25 | | | |
| Malacostraca | Isopoda | undetermined | 3 | 6.82 | 1 | 25 | 3.69 | 46.24 | 26.02 |
| | | $N_t$ | 44 | | | | | | |