

Diet of the recently introduced white-lipped frog *Leptodactylus fragilis* from a suburban ecosystem in western Cuba

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ABSTRACT - Diet and trophic interactions are essential for understanding aspects of the natural history of a species and evaluating its role within the ecosystem. A successful coloniser often has the ability to exploit a wide variety of prey during its establishment and may generate negative impacts due to competition with the local fauna. During both the dry and rainy seasons of 2019, in a suburban ecosystem in western Cuba, we analysed the diet of the recently introduced white-lipped frog *Leptodactylus fragilis*. Captured frogs were measured and stomach-flushed and released 24 h after analysis at the site of capture. Ninety-one *L. fragilis* were captured, 73 of them (80.2 %) had prey in the stomach. The most consumed prey were beetles, followed by spiders, and crickets. No difference was observed in prey consumption between seasons. This frog may change its foraging strategy according to the habitat it occupies. In this study, we observed high values of food niche breadth, which may imply a greater competition of *L. fragilis* for food resources with the native syntopic anurans in Cuba. Studies on temporal and spatial dynamics are needed to understand the possible competitive interaction with native amphibians in this area.

INTRODUCTION

A knowledge of diet and trophic interactions is essential for understanding aspects of the natural history, population dynamics, assessment of energy flow, and food webs in ecological communities (Anderson, 1991; Solé & Rödder, 2010). The most obvious potential impact of an invasive amphibian species is competition with native amphibians for food resources and this is likely to be greater when the introduced species occurs at high densities and with no apparent natural predators (Olson, 2011).

In the Caribbean region, 25 amphibian species have been introduced, five of them within the Cuban archipelago (Powell et al., 2011; Borroto-Páez et al., 2015). However, their impacts on the Cuban native fauna has yet to receive an appropriate evaluation. Recently, Rodríguez-Cabrera et al. (2018) reported the presence of an additional introduced amphibian, the white-lipped frog *Leptodactylus fragilis* (Brocchi, 1877) in two localities of western Cuba. These are the first observations of the species outside its native geographic range, which extends from southern-most Texas (USA), through Mexico and Central America, to northern Venezuela, up to the Venezuelan State of Sucre (de Sá et al., 2014). To date there is no evidence of any impacts of *L. fragilis* on native species in Cuba even though Del Castillo et al. (2021) predicted that this species could become successfully established, spreading mainly in open areas. The advertisement call of this species is loud and is audible over a large area, suggesting a potential invasion of the acoustic niche of native amphibian species (Del Castillo et al., 2021).

Leptodactylus fragilis is a generalist predator

(González-Durán et al., 2011), feedings on arthropods (Savage, 2002), especially spiders, beetles, bugs, ants, and cockroaches in the species' natural range (González-Durán et al., 2011; Méndez-Navárez et al., 2014; Arrieta, 2017). In that respect, understanding possible interactions with native amphibian species, and identifying other possible impacts such as resource competition or disruption of trophic webs, is of particular importance for conservation of native fauna. In this contribution, we provide the first analysis of the diet of the recently introduced *L. fragilis* from one of the known localities in Cuba, paying attention to the possible differences between dry and rainy seasons.

MATERIALS & METHODS

Fieldwork

The study was undertaken at Sandino town, Pinar del Río province (22° 4'43" N, 84° 12'10" W, WGS84). We collected frogs at four points along both sides of a 1.5 km stretch of road (from Sandino to Manuel Lazo). The frogs were collected in a suburban environment associated with ditches and ponds in the surroundings of this town (Fig. 1A). To detect the variation in diet with respect to seasons, we sampled in both the dry (April 2018 and February 2019) and rainy (June 2019) season. Frogs were captured by hand at 20:30 h to 22:00 h. Snout-vent length (SVL), the most common descriptor of body size in anurans, and head width (HW), which is related to feeding habits, as it limits maximum prey size (Emerson, 1985), were measured using a caliper to the nearest 0.2 mm. All frogs were stomach flushed (following Mahan & Johnson, 2007) and released at the site of capture 24 h afterwards. The

diet of individuals was determined from placing the stomach contents in vials, fixed with 70 % ethanol, and analysed (later) under a stereomicroscope. Prey items were classified at class or order level, or lower when possible. The investigation was undertaken under research permit P211PR900-003 and no further ethical approval was required for stomach flushing; no frogs died during the procedure.

Data Analysis

We calculated the number of items consumed (N) and percent occurrence of different types of prey ($N_{\%}$); the frequency of occurrence (F , number of stomachs in which a given prey category was found), and its percent ($F_{\%}$) for each prey category. We calculated the trophic niche breadth using Levins' index (B) (Krebs, 1989):

$$B = \frac{1}{\sum_{i=1}^n P_i^2}$$

where P_i = fraction of items in the prey type i . For standardisation of niche breadth (B_A), we use Hurlbert's (1978) proposal: dividing B by the total number of resource states after correcting for a finite number of resources; range = 0 (no diversity, exclusive use of a single prey type, specialist) to 1 (highest diversity, prey items of all categories, generalist).

Mann-Whitney U-Tests were used to compare number and type of prey consumed between seasons. Spearman Correlation tests were calculated for the relationships between SVL/HW, SVL/prey length, and SVL/number of prey per stomach. The Simpson diversity index (D hereafter) (Simpson, 1949) was calculated to compare the variation in diversity of diets among seasons.

RESULTS

We sampled 91 individuals of *L. fragilis* (Fig. 1b), ranging from 31.18 to 43.00 mm SVL (37.04 ± 2.25 ; mean \pm SD), 10.58 to 14.12 mm HW (12.46 ± 0.57). Seventy-three frogs (80.2 %) contained prey in their stomachs, and 19.8 % had empty stomachs. Positive correlation was observed between SVL and HW (Spearman 0.769, $p < 0.001$, $N = 91$). Larger *L. fragilis* consumed larger prey (Spearman 0.370, $p = 0.040$; $N = 58$). There was a negative correlation between SVL and number of prey per stomach (Spearman -0.298, $p = 0.019$, $N = 62$), but there was no significant difference in number of prey consumed between seasons (U-test = 432.0, $Z = -0.690$, $p = 0.490$; $N = 62$). Niche breadth (dry season $B = 8.24$, $B_A = 0.36$; rainy season $B = 8.32$, $B_A = 0.37$) and number of prey items per stomach were similar between seasons.

No significant difference between seasons was observed in type of prey consumed (U-test = 694.500, $Z = -1.041$, $p = 0.298$). In the dry season, 41 (91.1 %) frogs had prey in the stomach, and four had empty stomachs; while in rainy season, 32 frogs (69.6 %) had prey in the stomach and 14 (30.4%) had empty stomachs. We reported 15 prey orders in the stomach of *L. fragilis* and recorded 246 prey items (Table 1S, see Supplementary Materials). The number of prey

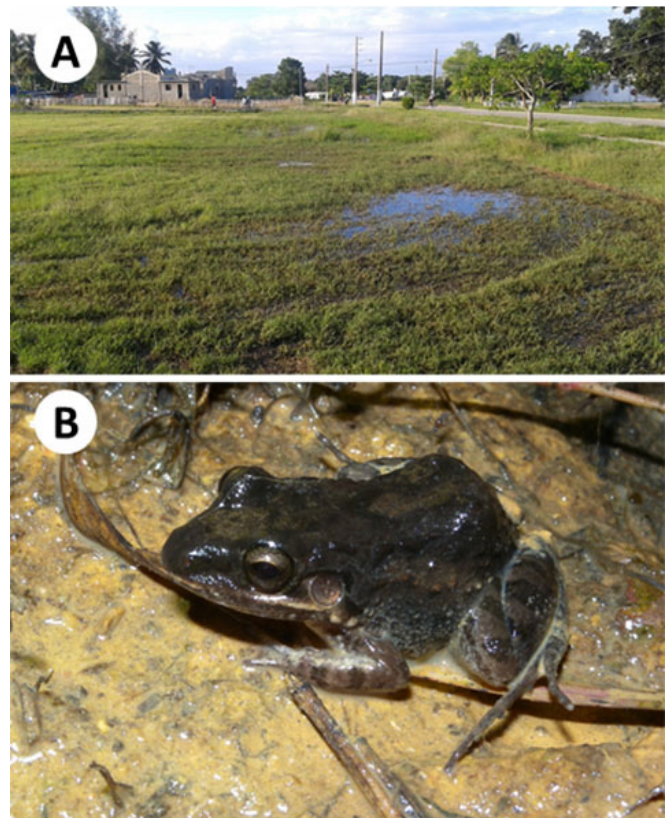


Figure 1. Typical habitat of the study area - **A.** Ditch in suburban environment of Sandino town, **B.** *Leptodactylus fragilis* in the study area

items per stomach in the dry season consumed by *L. fragilis* (mean = 3.3 ± 2.15 , range = 1–10) was similar to that in the rainy season (3.4 ± 3.08 , 1–16). Beetles (Coleoptera) were the most common prey in the stomach samples in this frog, observed in 26 individuals in the dry season ($F_{\%} = 25.2$, $N_{\%} = 22.1$), and in 19 in the rainy season ($F_{\%} = 27.5$, $N_{\%} = 33.0$), and represented by 14 families, followed by spiders (Araneae) ($F_{\%} = 16.9$, $N_{\%} = 16.3$), and crickets (Orthoptera) ($F_{\%} = 6.4$, $N_{\%} = 4.5$) (Table 1S). We identified two gastropod molluscs: the introduced semiaquatic snail *Hemisus brevis* (F. Thiaridae), and the land snail *Zachrysia* sp (F. Camaenidae). Many of the ants (Formicidae) consumed (70.6 %) corresponded to the introduced fire ant *Wasmannia auropunctata*. Other species identifiable to species level in *L. fragilis* stomach samples were the introduced cockroach *Periplaneta americana* and earwig *Carcinophora americana*. Inorganic materials (nylon and a fragment of plastic) were observed in two stomachs. The diversity of prey consumed by this species was only slightly higher during the rainy season ($D = 0.93$) than in the dry season ($D = 0.92$).

DISCUSSION

Prey size is directly correlated with body dimensions in some amphibians (i.e. SVL and HW) (Parmelee, 1999; González-Durán et al., 2011; Lunghi et al., 2018; this study), because a wide head and longer jaws contribute to larger gape (Emerson, 1985). In our study, individuals with larger SVL

tended to consume larger prey, which suggests that body size in *L. fragilis* influence the size of prey consumed (González-Durán et al., 2011; this study).

In our study, the most consumed prey by *L. fragilis* were beetles, observed in the stomach content in both seasons, followed by spiders and crickets. Nonetheless, Toft (1980) defined that wide niches and lower B_A values are distinctive of generalist predators in amphibians. In our study, the higher values of diversity of prey consumed and B_A in *L. fragilis* stomach samples reinforce the generalist/opportunistic predator classification (Taigen et al., 1982; González-Durán et al., 2011). This finding is consistent with previous studies of this species in its native range (González-Durán et al., 2011; Méndez-Narváez et al., 2014; Arrieta, 2017) independent of the habitat that this species temporarily occupies. Some invasive invertebrate species were also observed in the stomach samples of this frog: the semiaquatic snail *Hemisinus brevis* (F. Thiaridae), the cockroach *Periplaneta americana*, and the fire ant *Wasmannia auropunctata*; the latter represents the main content of Formicidae (70.6 %) samples. Other items, such as inorganic material (nylon and a fragment of plastic), were observed in two stomach samples of *L. fragilis*, possibly consumed accidentally while feeding.

Like most leptodactylid frogs, *L. fragilis* is considered a sit-and-wait predator (Taigen et al., 1982; González-Durán et al., 2011). However, in some areas, this species may exhibit a bimodal foraging strategy, depending on which prey are available and abundant in a particular habitat (Méndez-Narváez et al., 2014). *Leptodactylus fragilis* seems to be able to change its foraging strategy according to the habitat it occupies: populations that live around ponds and open areas may exhibit the 'sit-and-wait' strategy (González-Durán et al., 2011, the present study); populations associated with forested areas, and/or livestock, and not necessarily nearby a body of water, may be active foragers (Méndez-Narváez et al., 2014). In addition, *L. fragilis* feeds more in open and certain anthropogenically disturbed areas than in forest-covered areas (Arrieta, 2017).

In our study, slightly higher diversity of prey was consumed by *L. fragilis* during rainy season; Arrieta (2017) obtained a similar result. The increase in diversity of prey in the stomach samples of this frog is probably related to prey availability during this season. However, we also detected the highest number of empty stomachs during that period. A plausible explanation could be related to reproductive behaviours. During this season, individuals spend more time in reproductive displays, and have less time to feed, especially at the time of capture (21:00 to 23:00 h). We suggest that this species, even though it is a generalist predator, in the dry season is slightly selective with its prey. In the rainy season, this species spends more time in reproductive activity, and feeds on every prey available in every non-reproductive moment it has (e.g. the 'intermission' of calling activity in males).

Werner et al. (1995) observed that similar body-sized species could overlap in diet, especially if they share the same microhabitat. These authors suggested that the potential strength of competitive interactions among species is likely mitigated by the habitat-related diet differences, and diet overlap declined with increasing body size disparity.

Leptodactylus fragilis has high feeding plasticity, and could become a competitor in newly colonised lands, like Cuba. According to the diversity of prey consumed by *L. fragilis* (Table 1S), the higher value of niche breadth ($B = 8.68$), and its morphological characteristics, we can assume that our studied population is a generalist/opportunistic 'sit-and-wait' predator. The species lives in syntopy with four native amphibian species (*Eleutherodactylus riparius*, *E. goini*, *Osteopilus septentrionalis*, and *Peltophryne empusa*) (Rodríguez-Cabrera et al., 2018). *Eleutherodactylus* frogs, juveniles and sub-adults of *O. septentrionalis* and *P. empusa* are similar in size to *L. fragilis* (Henderson & Powell, 2009; de Sá et al., 2014), and exhibit a similar type of prey consumption to this introduced species (García-Padrón, unpubl. data). Therefore, *L. fragilis* is likely to be in competition with this native species for trophic resources.

Leptodactylus fragilis has certain characteristics that are typical of successful invasive species: it is ecologically tolerant to a broad range of, and rapidly changing conditions; is highly tolerant of human presence (Perry et al., 2008; Powell & Henderson, 2008); is primarily a predator of arthropods (Powell & Henderson, 2008); and is relatively small and capable of rapid reproduction (Kolar & Lodge, 2001). Studies of temporal and spatial dynamics of this food-generalist species are needed to understand its possible competitive interaction with native amphibians in this area.

ACKNOWLEDGMENTS

This research was funded under the permits of the project 'Sistematización y actualización de información sobre el funcionamiento de ecosistemas de Pinar del Río, con énfasis en los forestales; su aplicación en la gestión del Sistema Nacional de Áreas Protegidas' code P211PR900-003. We are very grateful to Jorge Ferro and Sergio L. del Castillo for their support during the fieldwork. To the staff of Guanahacabibes National Park for the logistical support. Tom Kinsky helped us to improve the English language of the manuscript. J. Ferro, two other reviewers, and Professor Richard Griffiths provided useful suggestions to the manuscript.

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Accepted: 27 April 2022