Reproduction and ontogenetic diet shifts in *Leptodactylus natalensis* (Anura, Leptodactylidae) from southeastern Brazil

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ABSTRACT: We studied ecological attributes of *Leptodactylus natalensis* in a coastal lagoon in the state of Espírito Santo, southeastern Brazil, based on specimens taken from November 1995 to March 1996. We collected 103 individuals: 24 males, 6 females, and 73 juveniles. Sex ratio was 4:1, biased toward males. We observed sexual size dimorphism, females being much larger than males. Males ranged in snout-vent length (SVL) from 25.0 to 35.7 mm, and females ranged from 34.5 to 38.1 mm. The number of mature oocytes varied from 992 to 1721, and it was positively correlated to SVL. We found four prey item groups in the stomachs of *L. natalensis* (Insecta, Arachnida, Crustacea, and Amphibia), belonging to seventeen prey categories. Arthropods were the most diverse food item. In terms of frequency, the most common food item was Formicidae, followed by Isoptera, which predominated also in terms of number of prey ingested. Small frogs predominated in terms of wet prey mass. Individuals smaller than 30.0 mm SVL fed mainly on Formicidae, Isoptera, and Coleoptera (adults and larvae), whereas individuals larger than 30.0 mm SVL fed mainly on small anuran species, indicating strong trophic ontogeny in *L. natalensis*.
Key words: fecundity, sex ratio, sexual dimorphism, trophic ontogeny.

RESUMO: **Reprodução e mudanças ontogenéticas na dieta de *Leptodactylus natalensis* (Anura, Leptodactylidae) do sudeste do Brasil.** Os atributos ecológicos de *Leptodactylus natalensis* foram estudados em uma lagoa litorânea do estado do Espírito Santo, sudeste do Brasil, baseado em amostras feitas nos meses de novembro de 1995 a março de 1996. Foram coletados 103 indivíduos: 24 machos, 6 fêmeas e 73 juvenis. A razão sexual foi de 4:1, inclinada para os machos. Houve dimorfismo sexual em tamanho, sendo que as fêmeas foram muito maiores

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que os machos. Os machos variaram em comprimento rostro-anal (CRA) de 25,0 a 35,7 mm e as fêmeas de 34,5 a 38,1 mm. O número de ovócitos maduros variou de 992 a 1721, sendo positivamente correlacionado ao CRA. Foram encontrados quatro grupos de presas nos estômagos de *L. natalensis* (Insecta, Arachnida, Crustacea e Amphibia), os quais representaram dezessete categorias de presas. Artrópodes compuseram o item alimentar mais diverso. Em termos de freqüência, o item mais comum foi Formicidae, seguido por Isoptera, que predominou também em termos do número de presas ingeridas. Os anuros pequenos predominaram em termos de massa úmida de presas. Os indivíduos menores de 30,0 mm de CRA alimentaram-se principalmente de formigas, cupins e besouros (adultos e larvas) e os indivíduos maiores de 30,0 mm de CRA alimentaram-se principalmente de anuros pequenos, indicando uma forte ontogenia trófica em *L. natalensis.*
Palavras-chave: dimorfismo sexual, fecundidade, ontogenia trófica, razão sexual.

Introduction

Anurans exhibit greater diversity of reproductive modes than any other tetrapod vertebrates (Haddad & Prado, 2005). In leptodactylids, there is a trend in the reproductive modes from foam nests on water toward increasing terrestriality (Gibson & Buley, 2004). Despite several reproductive modes, the decision on how to allocate resources to reproduction and growth can have important effects on associated life-history parameters as well as on population dynamics (Lardner & Loman, 2003). Energetic components to allocated resources to be used in growth or reproduction of most anurans are provided mainly by small arthropods, but diet composition of most anuran species depends upon prey availability (Duellman & Trueb, 1994).

Leptodactylus natalensis Lutz, 1930 is a medium-sized leptodactylid occurring in Brazil from the state of Rio de Janeiro, in the southeast, to Rio Grande do Norte, in the northeast, usually in muddied places of plain lands (Izecksohn & Carvalho-e-Silva, 2001). There is an extensive literature dealing with the ecology and systematics of leptodactylid species, especially because many are widely and very commonly distributed in South America (e.g., Langone, 1994; Cardoso & Sazima, 1977; Gallatti, 1992; Hirai & Matsui, 1999; Wogel *et al*., 2002; Teixeira & Vrcibradic, 2003; Maneyro *et al*., 2004; Prado *et al*., 2004). Calls and calling behavior of *Leptodacytlus natalensis* were studied by Heyer & Carvalho (2000). The diet of this

leptodactylid was studied by Santos *et al.* (2004), and parental care by Santos & Amorim (2006).

Herein we present data on sex ratio, fecundity, and trophic ontogeny of *L. natalensis* from a coastal permanent lagoon in southeastern Brazil.

Material and Methods

Fieldwork was carried out in Lagoa de Maimbá, a coastal, permanent lagoon located between the municipalities of Anchieta and Guarapari, state of Espírito Santo, southeastern Brazil (20° 46'S, 40° 35'W). The lagoon has extensive vegetation at the margins and inside, represented mainly by *Typha dominguensis* (Typhaceae), *Nymphaea odorata* (Nymphaeaceae), *Eleocharis* sp., *Lagenocarpus rigidus* (Cyperaceae), and *Salvinia* sp. This site has gradually been deforested for the introduction of cattle.

Frogs were collected by hand at the margin of the lagoon from November 1995 to March 1996. We collected at night, between 20:00 and 23:00 h, sedated frogs with ether, killed them in a 10% ethanol solution, and transferred them to 10% formalin solution for preservation. After one day, we washed specimens and stored them in a 70% ethanol solution.

In the laboratory, we measured their snout-vent length (SVL) with a caliper to the nearest 0.1 mm, and weighed them with a spring scale (0.1 g precision). After these procedures, we dissected and sexed the animals. We counted the number of largest oocytes for sexually mature females to determine fecundity. The stomachs of all frogs were removed; food items were spread on a petri dish, and identified under a stereomicroscope. Prey items were identified to Order, counted, and had their length measured with a caliper and their wet mass taken with a digital balance (0.1 mg precision). We calculated frequency, number of prey items, and total prey mass for each prey category. To estimate the predominant hunting method of *L. natalensis*, we classified prey items into ecological guilds according to their predominant way of life. Specimens collected were deposited at Museu de Biologia Prof. Mello Leitão, Santa Teresa, Brazil (MBML).

Basic descriptive statistics provided include range and mean ± one standard deviation. Chi-square (X^2) analysis was used to test for differences in sex ratio. One-way analysis of variance (ANOVA) was used to test for sexual dimorphism in size. Homoscedasticity of variances was assessed using Levene's test, and normality was assessed using the Kolmogorov-Smirnov test, to meet ANOVA's assumptions (Neter *et al.*, 1990). The

relationship between fecundity and length of the largest prey item in the stomach against frog size (SVL) was tested using a simple regression analysis. We also performed a cluster analysis (UPGMA) based on Euclidean distances to detect possible qualitative differences in the diet according to size range of frogs. Only data on prey mass ($\log_{10}$ transformed) were used in this analysis.

Results

Male frogs produced sounds while hidden and immobile beneath the leaf litter of the muddy soil. We collected a few females moving around in the leaf litter, and found juveniles around several temporary pools formed during the rainy season when the Maimbá lagoon overflows. Other species coexisting with *L. natalensis* on the leaf litter and in low numbers were *Leptodactylus ocellatus*, *L. fuscus*, *Physalaemus crombiei*, *Chaunus crucifer*, and *C. granulosus*.

We collected 103 individuals of *L. natalensis*: 24 males, 6 females, and 73 juveniles. Sex ratio was 4:1, biased toward males ($X^2 = 10.8$, $p = 0.01$). Males varied in SVL from 25.0 to 35.7 mm (33.1 ± 2.8 mm), and females from 34.5 to 38.1 mm (37.2 ± 1.4 mm) (Figure 1). Mean SVL differed significantly between the sexes (ANOVA: $F = 9.5$, $p < 0.01$), with females being much larger than males. The number of mature oocytes varied from 992 to 1721 (1516.5 ± 267.9), and was positively correlated to SVL ($r^2 = 0.95$, $p < 0.01$) (Figure 2). We frequently observed foam nests of other leptodactylids, such as *Physalaemus* spp., and *Leptodactylus ocellatus* in the study area, but never found any foam nests of *L. natalensis*.

Most individuals (90.3%) had at least one type of prey in their stomachs. We found four prey groups in the stomachs of *L. natalensis*: Insecta, Arachnida, Crustacea, and Amphibia (Table 1). These belong to 17 prey categories, and arthropods were more diverse than any other food item. The most common food items were Formicidae, found in 40.9% of the stomachs, followed by Isoptera, found in 31.2%. Of the remaining prey categories, most were present in fewer than 10.0% of the stomachs. Formicidae and Isoptera also predominated in the number of prey ingested, together accounting for more than 66.0% of prey found in the stomachs. But small frogs (two hylids and one leptodactylid) predominated in terms of wet mass, accounting for more than 78.0% of ingested mass. Only the largest individuals, thus females, ingested frogs.

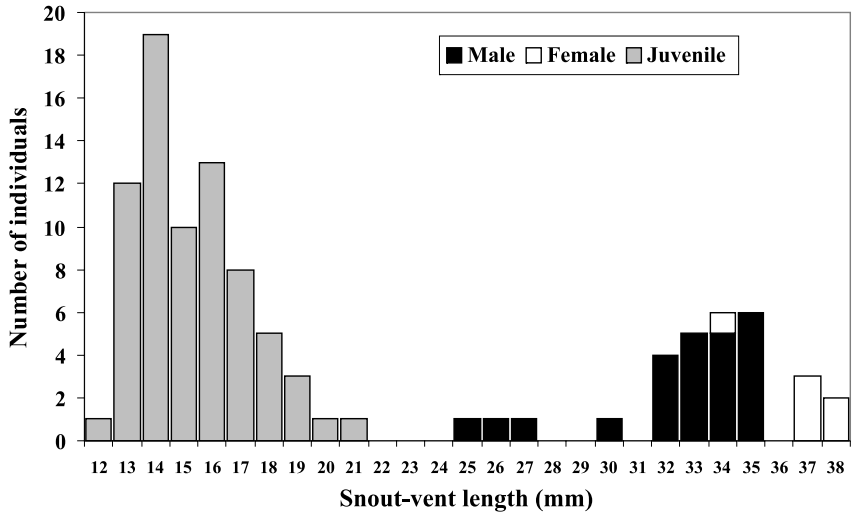

Figure 1. Snout-vent length distribution of *Leptodactylus natalensis* from Anchieta, state of Espírito Santo, southeastern Brazil.

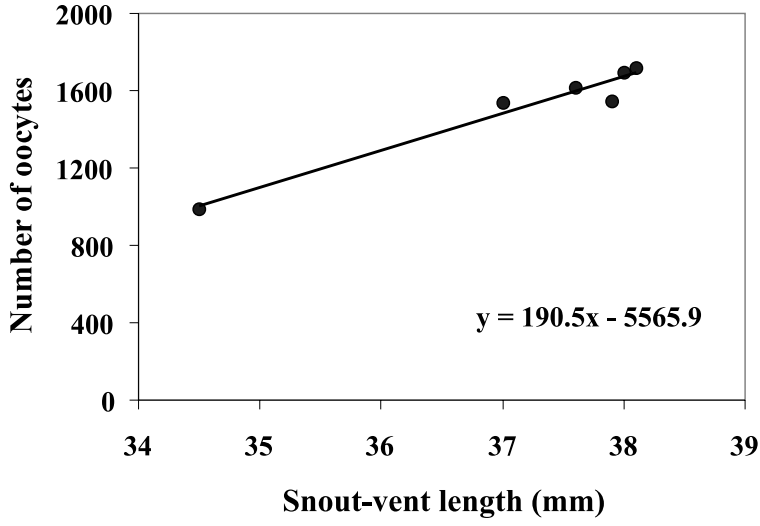

Figure 2. Regression analysis based on the number of oocytes against the snout-vent length of *Leptodactylus natalensis* from Anchieta, state of Espírito Santo, southeastern Brazil.

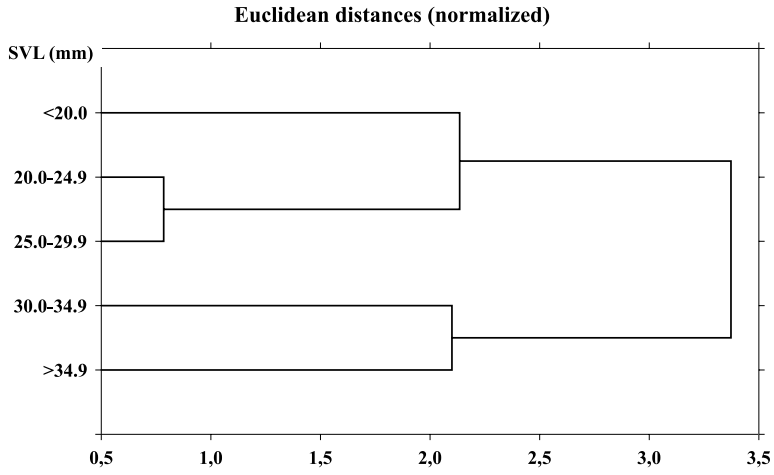

Figure 3. Cluster analysis showing trophic ontogeny in *Leptodactylus natalensis* from Anchieta, state of Espírito Santo, southeastern Brazil.

Table 1. Absolute values and percentages (in parenthesis) of each prey type found in stomach contents of *Leptodactylus natalensis* from Anchieta, Espírito Santo, southeastern Brazil.

Prey item	Frequency	Number	Wet mass (mg)
INSECTA			
Blattodea	2 (2.2)	2 (1.3)	26.8 (2.3)
Coleoptera (adults)	4 (4.3)	4 (2.6)	30.7 (2.7)
Coleoptera (larvae)	9 (9.7)	11 (7.2)	42.3 (3.7)
Collembola	1 (1.1)	1 (0.7)	0.3 (0)
Diptera	2 (2.2)	2 (1.3)	3.3 (3.3)
Lepidoptera (larvae)	5 (5.4)	5 (3.3)	53.9 (4.7)
Homoptera	2 (2.2)	2 (1.3)	1.6 (0.1)
Hymenoptera (Formicidae)	38 (40.9)	71 (46.4)	39.5 (3.4)
Isoptera	29 (31.2)	32 (20.9)	24.2 (2.1)
Orthoptera	2 (2.2)	2 (1.3)	15.5 (1.3)
ARACHNIDA			
Araneae	10 (10.8)	10 (6.5)	7.1 (0.6)
Opilionidae	1 (1.1)	1 (0.7)	0.9 (0.1)
Pseudoescorpionidae	3 (3.2)	3 (2.0)	2.3 (0.2)
CRUSTACEA			
Isopoda	3 (3.2)	3 (2.0)	3.2 (0.3)
AMPHIBIA			
Hyla aff. *decipiens*	1 (1.1)	1 (0.7)	141.3 (12.3)
Physalaemus sp.	1 (1.1)	1 (0.7)	302.5 (26.3)
Scinax argyreornatus	2 (2.2)	2 (1.3)	456.4 (39.6)
Total	-	153 (100)	1151.8 (100)

Cluster analysis shows trophic ontogeny in *L. natalensis* (Figure 3), in which individuals smaller than 30.0 mm SVL fed mainly on Formicidae, Isoptera, and Coleoptera (adults and larvae). There is a trend in individuals larger than 30.0 mm SVL to prey more upon small anuran species. *Leptodactylus natalensis* displays a strong tendency to eat terrestrial colonial insects (67.3%), followed by terrestrial hidden insects (22.2%), terrestrial active insects (6.5%), and flying insects (3.9%). According to these results, we infer that *L. natalensis* forages actively, searching, and sitting near trails.

Discussion

The population of *L. natalensis* appears to be represented by a few individuals, although the flooded area around Maimbá lagoon is very large, and has several microhabitats, especially during the rainy season. Our results suggest that reproduction of *L. natalensis* is limited to the summer, a period of continuous rainfall. Most anuran species use rainy season for their reproductive activities, but unexpected rainfall during the dry season may lead to unusual breeding activity (Prado *et al*., 2005), and these sporadic activity patterns may lead to misinterpretation in short term studies. Based on the relatively high fecundity found in *L. natalensis*, natural mortality may be high in tadpoles and juveniles.

In most organisms, equality in sex ratio is expected (West *et al*., 2002). In *L. natalensis* from Maimbá Lagoon, sex ratio was skewed toward males, suggesting a strong competition among males for acquiring mates. Females of *L. natalensis* are larger than males, as usual in frogs (Duellman & Trueb, 1994). Effect size, widely used in the behavioral sciences and in meta-analysis studies in biology, proves to be the most useful single metric to evaluate whether statistically significant results are biologically meaningful (Hayek & Heyer, 2005). Larger females are able to produce more eggs. Differences between egg production from the smallest to the largest female *L. natalensis* examined here were around 800. In other words, the largest female examined (38.1 mm SVL) produces almost twice the number of eggs the smallest one (34.5 mm SVL) produces. Sexual size dimorphism is common in both plants and animals, and current evidence suggests that it reflects the adaptation of males and females to their different reproductive roles (Fairbairn, 1997).

Specimens of *L. natalensis* were in positive energetic balance *sensu*

Huey *et al*. (2001), since most individuals examined had at least one prey item in their stomachs. *Leptodactylus natalensis* appears to have a broad spectrum of food types, varying from small arthropods to small frogs. Our result corroborates other studies focusing on the diet of leptodactylid frogs (Cardoso & Sazima, 1977; Strussmann *et al*., 1984; Woolbright & Stewart, 1987; Lajmanovich, 1994, 1996; Teixeira & Vrcibradic, 2003; Maneyro *et al*., 2004; Santos *et al*., 2004). Our fieldwork suggests that *L. natalensis* is an opportunistic and sedentary predator, favoring terrestrial, colonial insects, which are abundant throughout the humid tropics. But males and females may display different behaviors in exploring the available habitat. All males collected were immobile under the leaf-litter, while the few females collected were moving toward breeding areas. Only the largest specimens, thus females, consumed small anurans, which are apparently the most mobile prey item found in stomach contents.

The effect size appears to play an important role in determining the use of food resources among anurans. Larger anurans usually show more diverse prey spectrum (Woolbright & Stewart, 1987; Duellman & Lizana, 1994; Lajmanovich, 1994, 1996; Teixeira & Vrcibradic, 2003; Maneyro *et al*., 2004; Santos *et al*., 2004). A large mouth width allows them to explore greater variety of prey items (Emerson, 1985), and trophic ontogeny is common within species (Woolbright & Stewart, 1987). When juveniles and adults share the same area, trophic ontogeny is an important strategy of prey division, increasing chances of coexistence when there is limited food supply. Resource partitioning is important especially in diversity hotspots, such as the Atlantic Rainforest, where several anuran species share food and reproductive resources (such as egg releasing sites), and the degree of specialization becomes higher and higher in order to avoid competition. Conservation strategies for such highly endangered areas will benefit from understanding the interactions within the anuran community about sharing the same habitat, and the best way to start is at the species level.

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