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Body size, reproduction and feeding ecology of *Pleurodema diplolister* (Amphibia: Anura: Leiuperidae) from Caatinga, Pernambuco state, Northeastern Brazil

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Abstract. We present data about body size, sexual dimorphism, reproductive traits and diet ecology of *Pleurodoma diplolister.* This species is sexually dimorphic with females larger than males, corroborating others Leiuperidae species. The number of eggs varied from 62 to 1241 and we found a positive relationship between SVL of females and number of mature ovarian eggs but there is no relationship between SVL and volume of eggs. The diet of *P. diplolister* was composed by 11 categories of which Formicidae, Coleoptera and Orthopterans were the most important items and showed generalist and oportunistic predator habits. Data presented here should be considered in the development of future conservation strategies of anurans from Caatinga biome and other semiarid/arid environments.

Keywords. Ecology, body size, reproduction, sexual dimorphism, diet, *Pleurodema diplolister.*

Frogs of the genus *Pleurodema* inhabit mainly dry forests and open areas (Andrade and Vaz-Silva, 2012). They are medium sized anurans that frequently show adaptations to arid environments, such as morphology, explosive reproduction strategies associated to rainfall, fossorial habits and fast larval development (Gould and Vrba, 1982; Hodl, 1992; Cardoso and Arzabe, 1993). *Pleurodema diplolister* (Peters, 1870) is the only known species of the genus inhabiting the Caatinga, which is an arid and exclusive biome of Brazil (Cardoso and Arzabe, 1993), but *P. diplolister* could also be found in Brazilian Cerrado and transition zones between Caatinga-Cerrado (Vitt et al., 2005; Valdujo et al., 2011; Andrade and Vaz-Silva, 2012; Roberto et al., 2013).

Data about ecology of *P. diplilister* are scarce and based on reproduction behavior (Hodl, 1992) and feeding ecology (Santos et al., 2003) of two diferent localities from Caatinga biome. Herein, we present data on body size, sexual dimorphism, reproduction and feeding ecology of the frog *P. diplolister* at a gallery forest, from Caatinga, Pernambuco state, Northeastern Brazil.

The study was conducted on February 2012 at the Angico Farm (08°07’S; 40°05’W), located at the rural zone of the municipality of Ouricuri, Pernambuco state, Brazil. The vegetation is characterized mainly by deciduous forest and hypoxerophytic Caatinga (Velloso et al., 2001). Local climate is hot and semiarid, with the rainy period ranging from October to April and the mean annual precipitation ranging from 500-800 mm (Velloso et al., 2001).

We collected *P. diplolister* specimens by pitfall traps with drift fences and by hand, in a gallery forest near a temporary river during and after one of the few rains occurred in that year. Four sets of pitfalls traps were placed, each set is composed by 4 buckets (20 l) organized in a “Y” form (Cechin and Martins, 2000) with the central bucket connected to three buckets at extremities by a drift fence about 8 m in lenght and 50 cm in height.

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The specimens were euthanatized with a lethal injection of lidocaine chlorohydrate 2%, fixed in 10% formalin and then preserved in 70% ethanol. Voucher specimens were deposited at Coleção Herpetológica da Universidade Regional do Cariri (URCA-H: 1068, 2852-2915, 2925-2926).

For each adult individual we measured with a digital caliper the following variables (± 0.01 mm): snout-vent length (SVL), head length (HL), head width (HW), interorbital distance (IOD), internarinal distance (IND), eye-nostril distance (END), eye diameter (EYD), tympanum diameter (TD), femur length (FML), tibia length (TL), tarsal length (TAL) and foot length (FOL).

Sex was assigned by dissection and direct examination of the gonads. To examine morphological variation due to sex, we made a multivariate discriminant function analysis with residuals of morphological variables to test if significant sexual differences exist in the body shape (using all morphometric variables) and among individual morphometric variables. The residuals from a linear regression between each variable and SVL were used to remove the effect of body size in the analysis of sexual dimorphism.

Clutch size was determined by counting a sample and then estimating the number of mature ovarian eggs. We recorded length and width (± 0.01 mm) of five eggs in females and testicles in males and estimated the volume for each using the ellipsoid formula:

$$V = \frac{4}{3}\pi\left(\frac{W}{2}\right)^2\left(\frac{L}{2}\right)$$

where W and L represent width and length, respectively.

We consider males analyzed as reproductively mature (adult) because they all showed convoluted epididymides. We considered females as reproductive actively when they possessed mature ovarian eggs. We use residuals of morphometric variables to remove the effect of size and test for sexual dimorphism when showed ovarian eggs or convoluted oviducts.

We tested for relationships between body size and either egg number (females) or testicle volume (males) using the non-parametric Spearman correlation test. To verify if SVL influenced the volume of eggs in the ovarian, we use a simple linear regression.

Stomachs were removed from preserved specimens and examined under a stereomicroscope to identify prey items to the most inclusive taxonomic level possible (e.g., order). Prey items that were too fragmented to allow a reliable estimate of volume were excluded, and items that could not be identified due to advanced stage of digestion were included in the "other arthropods" category.

We recorded length and width (0.01 mm) of intact items with digital calipers and estimated prey volume (V) using the ellipsoid formula detailed above. Diet composition was determined based on number (N), volume (V) and frequency (F) of each prey type in the stomachs for individual frog and for pooled stomachs. To determine the relative contribution of each prey category, we calculated the relative importance index (I) using the formula (Powell et al., 1990):

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

where $F\%$, $N\%$ and $V\%$ are the percentages of frequency, number and volume of preys, respectively. Dietary niche breadth (B) was calculating using the inverse of Simpson's diversity index (Simpson, 1949):

$$B = 1 / \sum_{i=1}^{n} p_i^2$$

where p is the numeric proportion of prey category i, and n is the number of categories. Values of B vary from one (usage of a single category = specialized diet) to n (equal usage of all categories = generalist diet).

To evaluate the food niche overlap between the males and females, we use Pianka's overlap (Pianka, 1973) index in Ecosim 7.0 (Gotelli and Entsminger, 2004):

$$Ø_{jk} = \frac{\sum_{i=1}^{n} p_{ij}\, p_{ik}}{\sqrt{\sum_{i=1}^{n}\left(p_{ij}^2\right)\left(p_{ik}^2\right)}}$$

where i is the category of the resource, P is the proportion of resource category i, j and k represents compared sexes and n is the total number of categories. The overlap ranges from 0 (no overlap) to 1 (total overlap).

To evaluate the relationship between prey volume and SVL, HL and HW we performed Spearman correlations. Unless otherwise stated, we performed all statistical analysis using Statistica software version 10.0 (Statsoft, Inc 2011), presented means with ± standard deviation and a significance level of $\alpha = 0.05$.

We collected 67 specimens of *P. diplolister* (28 adult males, 37 adult females and two juveniles females). Females were significantly larger than males in the body shape (*Wilks* $\lambda = 0.6617$; $F_{12,51} = 2.1726$; $P = 0.027$) and individuals variables showed significantly difference in TL (Wilks $\lambda = 0.72$; $F_{1,51} = 0.91$; $P = 0.033$) and TAL (Wilks $\lambda = 0.76$; $F_{1,51} = 7.90$; $P = 0.007$) (Table 1).

Table 1. Morphological variables of *Pleurodema diplolister* from a Caatinga area, Pernambuco state, Northeasthern Brazil: discriminant function analyses results (DFUR). AGMV= all grouped morphometric variables, END = eye-nostril distance, EYD = eye diameter, FOL= foot length, HL= head length, HW = head width, IND= internarial distance, IOD = interorbital distance, TD = Tympanum diameter, FML= Femur length, SVL= snout-vent length, TL= tibia length, TAL= tarsal length. All values are given in mm.

	Females (n= 37) Mean (range)	Males (n = 28) Mean (range)	DFUR Wilks λ (p-value)
SVL	33.8 (25.3–38.0)	31.4 (23.6–36.4)	0.807 (0.002)
HL	14.3 (12.7–15.7)	13.6 (10.1–15.8)	0.662 (0.960)
HW	13.3 (11.5–15.0)	12.6 (9.1–14.1)	0.679 (0.254)
IOD	5.9 (5.2–6.8)	5.7 (4.7–6.6)	0.665 (0.638)
END	3.1 (2.3–3.5)	2.9 (1.2–3.6)	0.662 (0.913)
IND	3.2 (2.9–4.1)	3.2 (2.4–3.7)	0.665 (0.622)
EYD	4.5 (3.6–5.4)	4.5 (3.2–5.1)	0.665 (0.617)
TD	1.9 (1.2–2.4)	1.9 (1.9–2.6)	0.693 (0.128)
FML	13.5 (11.7–15.3)	12.9 (9.6–14.7)	0.670 (0.438)
TL	13.6 (12.0–14.7)	13.2 (10.0–15.0)	0.724 (0.033)
TAL	8.5 (7.0–10.3)	7.9 (5.9–9.5)	0.764 (0.007)
FOL	14.8 (13.4–16.4)	14.2 (11.2–16.5)	0.664 (0.707)
AGMV			0.6617 (0.027)

Table 2. Diet composition of *Pleurodema diplolister* from a Caatinga area, Pernambuco state, Northeasthern Brazil. F = frequency; n = abundance; V = volume; I = relative importance index.

Prey item	F	F %	n	n%	V	V%	I
Araneae	4	10.81	4	6.78	33.79	6.61	8.07
Coleoptera	7	18.92	12	20.34	151.07	29.56	22.94
Diptera	1	2.70	1	1.70	1.22	0.24	1.55
Hemiptera	1	2.70	1	1.70	1.12	0.22	1.54
Hymenoptera							
Formicidae	11	29.73	27	45.76	98.09	19.19	31.56
Others	2	5.41	3	5.09	53.32	10.43	6.97
Lepidoptera	2	5.41	2	3.39	0.82	0.16	2.99
Neuroptera	1	2.70	1	1.70	0.98	0.19	1.53
Orthoptera	3	8.11	3	5.09	148.31	29.02	14.07
Others arthropods	4	10.81	4	6.78	10.95	2.14	6.58
Seeds	1	2.70	1	1.70	11.4	2.23	2.21
TOTAL	37		59		511.07		
Niche breadth			3.74		4.45		

Thirty-three females presented ovarian egg, ranging from 62 to 1241 (mean = 573.18 ± 289.06). Mature ovarian eggs (n = 18915) averaged 1.08 ± 0.13 mm in length, 0.93 ± 0.11 mm in width and 0.68 ± 0.23 mm^3 in volume. The smallest reproductive female was 25.33 mm SVL. We found no relation between female SVL and volume of eggs ($r^2 = 0.7853$; $F_{1,31} = 2.6421$; $P = 0.11$), however, there was positive significant correlation between female SVL and number of eggs ($r_s = 0.595$; $P < 0.05$). The smallest reproductive male was 23.65 mm SVL and there was no correlation between males SVL and testicles volume ($r_s = 0.395$; $P > 0.05$).

Only twenty-two specimens (32.8% of the total, being 21 adult individuals, 11 males, 10 adult females, and one juvenile female) had a total of 59 prey items in their stomachs. Mean diversity of preys by stomach was 1.75 ± 1.22, with females having more diversified diet (2.22 ± 1.39), than males (1.18 ± 0.6). Females juvenile ingested four prey types.

Eleven categories of prey items were identified in diet of *P. diplolister*: hymenopetarns of the family Formicidae (45.76%) and the order Coleoptera (20.34%) were more important numerically in diet composition, whereas Coleoptera (29.56%), Orthoptera (29.02%) and Formicidae (19.19%) were more important volumetrically. Regarding the relative importance of each prey item, Formicidae (31.56%), Coleoptera (22.94%), Orthoptera (14.07%) were the main contributors in the diet composition (Table 2).

Niche breadth of *P. diplolister* was 3.74 in number and 4.45 in volume. Food niche overlap between the males and females was 0.7071. There was no correlation between prey volume and: SVL ($r_s = 0.1968$; $P > 0.05$), HL (0.1111; $P > 0.05$), or HW (0.2415; $P > 0.05$).

Sexual differences in body size are common among anurans, with approximately 90% of the species having females larger than males, but males could be equal size or larger than females in those species which males engage physical combat (Shine, 1979). That pattern was also found in *P. diplolister*, where females tend to be larger and more robust than males. Many leiuperids follow this trend at many places of South American (Table 3). Additionaly, different populations of *Pleurodema thaul* (Lesson, 1827) could (or not) present sexual dimorphism, as was demonstrated by Iturra-Cid et al. (2010) from Chile at different latitudes/longitudes.

In the present study, the average egg volume remains constant and independent of female SVL. However, larger females produce more eggs, resulting in the large difference in the amount of eggs found in ovaries. This pattern can most likely increase the reproductive success rate, once larger females producing more eggs will result in a greater amount of individuals that will hatch and reach maturity. The number of ovarian mature eggs found here was similar to that reported by Hodl (1992) in a population of *P. diplolister* from Paraiba state, with 528-742 eggs reported in foam nests.

Apparently, body size has an influence on egg number of leiuperid species, since larger species presented

Table 3. Data of eleven leiuperines from South America, with snout vent–length of males and females (SVL M and F), average number of mature ovarian eggs (EGGS), egg diameter (ED), sexual size dimorphism (SSD), breeding activity patterns (BAP) and source.

Specie	SVL		EGGS	ED	SSD	BAP	Reference
	M	F					
Physalaemus albonotatus	29.6	30.2	1474	0.98	Absent	Continuous	Rodrigues et al. (2004)
Physalaemus centralis	36.3	34.5	1842	1.3	Absent	Explosive	Brasileiro and Martins (2006)
Physalaemus cuvieri	—	—	473.5	1.3	Present	—	Barreto and Andrade (1995)
Physalaemus fernandezae	23.2	26.6	—	—	Present	—	Marangoni et al. (2011)
Physalaemus kroyeri	31.1	30.2	1332		Absent	Continuous	Gally and Zina (2013)
Physalaemus maculiventris	23.3	20.5	190	—	—	—	Heyer et al. (1990)
Physalaemus nattereri	47.3	51.3	3765	1.2	Present	Explosive	Rodrigues et al. (2004)
Physalaemus signiffer	25	27.4	273.14	—	Present	Continuous	Wogel et al. (2002)
Pleurodema diplolister	31.44	33.79	573.18	1.08	Present	Explosive	Present study
Pleurodema diplolister	30.7	34.3	678	—	—	Explosive	Hodl (1992)
Pleurodema guayapae	—	41.7	1137	1.44	—	Explosive	Valetti et al. (2013)

more eggs both in ovarian and in foam nests; on the other hand, breeding activity patterns (explosive or continuous) does not affect egg number in this anuran family (see Table 3). Additional studies with another populations of *P. diplolister* are necessary to confirm this patterns.

Amphibians of arid environments usually are active only two-three months in a year (during rainy season), and then start aestivation process (Carvalho et al., 2010). In Caatinga, *P. diplolister* stay on aestivate ten or eleven months, emerging from the burrow at first rains, and start to reproduce explosively in temporary water bodies (Carvalho et al., 2010). Feeding in *P. diplolister* starts just after reproduction (Carvalho et al., 2010), which may explain the large number of empty stomachs observed in this study.

We found no correlation between SVL and prey size. Based on the numeric and volumetric niche breadth, we consider *P. diplolister* as a generalist and opportunistic predator. The food niche overlap between males and females was relatively high, which was already expected since males and females use similar microhabitats to forage and reproduce in the sampled area (Sousa pers. obs.).

The diet of *Pleurodema* species has been reported from some countries of South America: in Brazil, Santos et al. (2003) found Chilopoda, Isoptera and Coleoptera as the most important prey item for *P. diplolister*, whereas Formicidae, Coleoptera and Orthoptera were the most important prey categories found in the present study; in Chile, ants and insect larvae were the most important item for *Pleurodema bufoninum* Bell, 1843 (Pincheira-Donoso, 2002) and Diptera and arachnids for *Pleurodema thaul* (Schneider, 1799) (Díaz-Páez and Ortiz, 2003); in Argentina, dipterans (Stratiomyidae), spiders (Araneidae) and beetles (Coleoptera) to *Pleurodema cinereum* Cope, 1878 (Hulse, 1979) and Hemiptera and Hymenoptera to *Pleurodema nebulosum* (Burmeister, 1861) (Sanabria et al., 2007). Considering that *P. diplolister* is a generalist and opportunist predator, these differences in diet composition are probably due to variation in abundance and prey diversity of each studied area.

The population of *P. diplolister* studied showed generalist and opportunistic predator habits, and males and females showed high food niche overlap, which is probably due the similarity in microhabitat use. *Pleurodema diplolister* also presented sexual dimorphism (females larger than males), whereas probably as result of sexual selection, larger females (and probably older) had greater amount of mature ovarian eggs than smaller females. Other studies with Leiuperines are required to confirm the reproductive pattern here discussed. Data presented here should be considered in the development of future conservation strategies of anurans from Caatinga biome and other semiarid/arid environments.

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