

PRELIMINARY MORPHOMETRIC DATA OF *PELOPHYLAX PEREZI* (AMPHIBIA: RANIDAE) IN SÃO JORGE ISLAND (AZORES)

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RESUMO

A rã verde ibérica, *Pelophylax perezi*, foi introduzida nos Açores e dispersou para todas as ilhas do arquipélago. Contudo, os únicos dados publicados sobre a espécie dizem respeito à sua presença e distribuição em grande escala. Este estudo pretende investigar a variabilidade morfológica de *P. perezi* em São Jorge, a partir de seis populações amostradas em diferentes locais da ilha. Os indivíduos foram identificados em função do género e medidos em relação ao peso corporal e comprimento. Neste artigo, apresentam-se dados preliminares sobre parâmetros morfológicos da espécie e estabelece-se a relação peso-comprimento. A análise de regressão mostra-se fortemente descritiva. Mais investigação será necessária para melhor entender o significado dos coeficientes da recta de regressão para a espécie, nos Açores.

PALAVRAS-CHAVE: Rã verde ibérica, *Pelophylax perezi*, morfometria, Açores.

ABSTRACT

The Iberian green frog, *Pelophylax perezi*, was introduced in the Azores and widespread in all the islands of the archipelago. However, the only published data about the species concerns its presence and large-scale distribution. This study aims to investigate the morphological variability of *P. perezi* in São Jorge, from six populations sampled at different sites of the island. Individuals were identified for gender and measured for body mass weight and snout-vent length. In this paper, preliminary data on morphological parameters of the species are presented and it is established the weight-SVL relationship. The regression analysis shows to be strongly descriptive. More research is needed to better understand the real mean of coefficients in the regression line for this species in the Azores.

KEYWORDS: Iberian green frog, *Pelophylax perezi*, morphometrics, Azores.

INTRODUCTION

Pelophylax perezi is a green frog species belonging to the Western Palearctic water frog complex (Frost, 2011). Only in 1973, Hemmer has clarified that the Iberian green frog (*P. perezi*) is a separate species and not a "subspecies" of *Pelophylax ridibundus* (Hemmer, 1973). The Iberian green frog is an endemic species of the Iberian Peninsula that spread into Southern France, being also introduced to the Balearic Islands and the Canary Islands (Spain), in two sites in the United Kingdom, and in Madeira and the Azores Islands (Portugal) (García-París, 1997).

The distribution of *P. perezii* in the Azores is poorly described, since the only information about the species concerns its presence and large-scale distribution, whereas its ecology and distributional features are less known in the region (Constância *et al.*, 2001), and its genetic status is lacking.

P. perezii is an ecological generalist which can inhabit a large variety of habitats in a wide range of environmental conditions but it is conditioned by the existence of water masses, including man-made water reservoirs in agricultural areas. The species can use both lotic and lentic waters and thus may resist to some degree of pollution and salinity (Llorente *et al.*, 2002).

In *P. perezii* males are smaller than females and have stronger forelimbs; they have grey vocal sacs which can be identified even when they are not in use. The dorsal coloration is very variable, usually green but also brown or greyish to dark grey. It usually shows a vertebral stripe, mostly yellow or green, and well-formed dorso lateral folds. In the breeding session, the green frogs inhabit water habitats.

In general amphibians are considered as one of the most sensitive vertebrates to environmental changes, due to an early aquatic-dependent development stage and a highly permeable skin (Duellman & Trueb, 1994). For these reasons, this kind of organism can represent a good bioindicator of aquatic contamination when compared to other aquatic vertebrates (Marques *et al.*, 2008). Moreover, the ecological plasticity of *P. perezii* seems to represent a reaction to global warming. In fact, worldwide, the species appears to be expanding its range to higher altitudes, possibly as a consequence of climate change (García-Paris, 1997).

The objective of this study is to obtain preliminary morphometric data (body mass weight and total length) of *P. perezii* in São Jorge Island and to examine the structure and pattern of morphometric variability of its populations in the habitats under investigation. More knowledge on the morphometrics and the distribution of *P. perezii* in the archipelago will provide useful insights into the conservation status of its populations, and will detect sources of environmental stress over its habitats, thus allowing the identification of populations to use as biological indicators of environmental stress.

METHODS

Our study was based on a set of body length and body mass weight data collected in specimens of the Iberian green frog belonging to six different populations in São Jorge Island, from 25th to 31st of July 2011.

The sampling stations (Table 1) where *P. perezii* populations were investigated were located along a longitudinal E-W transect in the island. The altitudinal gradient of the island (highest altitude Pico da Esperança, 1053m) was taken into account, being the differences between the higher and the lower sites of 860 m. The major distance between opposite sampling stations (Engenho and S. Amaro) was of 37 km (Figura 1). Lentic and lotic habitats were included, in order to verify the distribution of the species in different habitats (Figura 2). Two stations (StSJ04 and StSJ05) were located into the area of a Site of Community Importance (PTJ0R0014-Costa NE e Ponta do Topo). Those stations and STSJ02 showed a higher naturalness degree. StSJ02 was an intermediate lotic-lentic habitat, because it was a stream with a low slope and a little barrage that provided a pond area. StSJ01 was located 590 m far from the Special Protected Area PTZPE0028 (Ilhéu do Topo e Costa adjacente), near a road and a little village. StSJ03 and StSJ06 consisted of water storage tanks for pasture.

Table 1: Site code, locality, UTM Coordinates, altitude, type of habitat and number of specimens for each sampling site.

Site Code	Locality	UTM Coordinates	Altitude (m A.S.L.)	Habitat	N (N tot= 54)
StSJ01	Engenho	26S 0431874 4267596	270	ponds river	12
StSJ02	S. Antão	26S 0429068 4267231	357	wetland area around river	10
StSJ03	Norte Pequeno	26S 0412162 4277937	439	water storage tank	6
StSJ04	Pico Esperanza	26S 0404233 4279749	864	pond	11
StSJ05	Fajã dos Cubres	26S 0415845 4277394	4	wetland area	5
StSJ06	S. Amaro	26S 0397772 4282218	385	water storage tank	10

Specimens were captured, measured and immediately released at the site of capture. Body mass weight (W) was measured to the nearest 0.3 % with a handling held spring balance. Sex was determined by the presence/absence of grey vocal sacs and nuptial pads. All individuals with a weight lower than 9 g were considered juveniles (sexually immature). Dorsolateral pictures of each specimen were taken. Snout-vent length (SVL) was recorded by the digital image analysis in laboratory, using ImageJ software (Abramoff *et al.*, 2004).

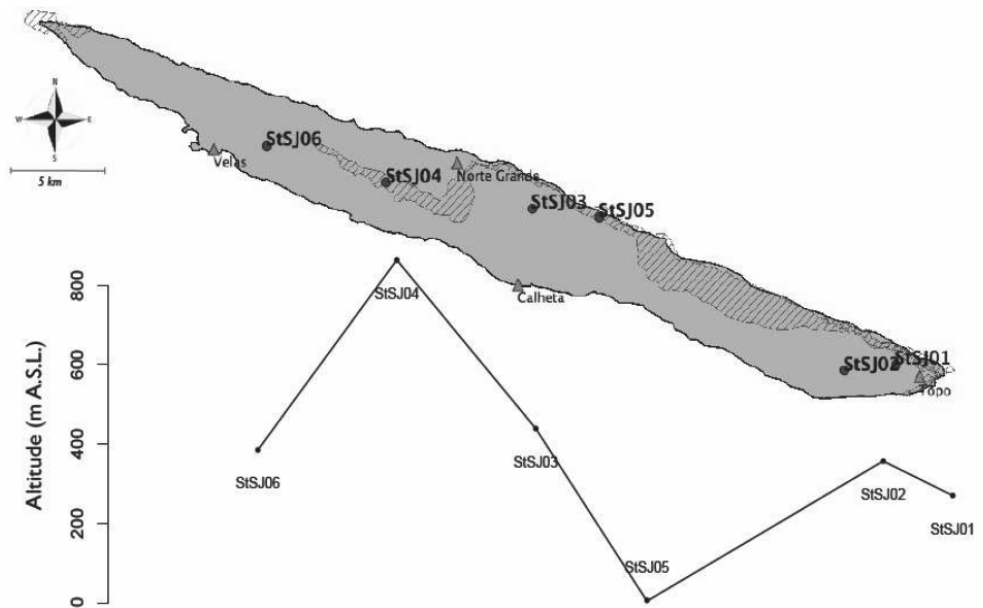


Figure 1: Map of the sampled localities of the São Jorge Island and corresponding altitude values. The stripe areas represent Sites of Community Importance of Natura 2000 ecological network.



Figure 2: Views of the sampling stations: StSJ01, Engenho; StSJ02, S. Antão; StSJ03, Norte Pequeno; StSJ04, Pico da Esperança; StSJ05 Fajã dos Cubres; StSJ06, S. Amaro.

The relationship between SLV and W was investigated by the equation $\log_{10}(W) = \log_{10}(a) + b \log_{10}(SVL)$, where W represents frog mass weight and SVL represents frog snout-vent length. The b value in the above formula represents the rate of increase (i.e., slope) of the weight against the length, whereas the a value represents the weight of an organism at a unit length (i.e., 1 mm).

In order to assess the variability of morphometric data, intra and inter populations analysis of variance were performed (ANOVA) followed by a Tukey's test.

RESULTS AND DISCUSSION

Fifty-four Iberian green frogs were collected in the sampling sites, 9 specimens in average per site (Table 2). Specimens have shown a high variability of the dorsal coloration, with the majority (96.3%) exhibiting a vertebral stripe, and all presenting dorsolateral folds (Figura 3).

Table 2: Mean and Standard Error (SE) of body mass weight and snout vent-length of *P. perezi* by sampling station.

Site code	N	Body mass (g)			SVL (mm)		
		max	Mean±SE	min	max	Mean±SE	min
StSJ01	12	40	19.29±3.28	6	70.41	51.99±3.26	36.15
StSJ02	10	31	14.8±2.70	7	70.49	48.39±3.53	37.12
StSJ03	6	29	20.83±2.41	13	77.21	59.36±4.86	44.5
StSJ04	11	57	24±4.60	9	71.26	52.56±3.49	39.05
StSJ05	5	26	20.2±2.28	14	56.19	51.59±1.70	46.99
StSJ06	10	79	39.8±6.75	7	85.87	65.09±3.99	39.5
<i>Life stage</i>	N						
Adult	45		28.16±3.27			58.81±2.34	
Juvenile	9		7.78±0.42			39.35±0.88	
<i>Gender</i>							
Female	15		30.1±5.85			58.06±3.85	
Male	30		24.87±1.66			57.52±1.58	



Figure 3: Dorsolateral views of two adult males sampled in São Jorge Island. The gray vocal sacs can be appreciated in both specimens. (a) A male with an evident vertebral green stripe; (b) A male without a vertebral green stripe, but whose dorsolateral folds are well shown.

Forty-five individuals were identified as adults (15 females and 30 males), and the remaining 9 were considered as juveniles (Figure 4). SVL values (average \pm SE) ranged from 36.15 mm (a juvenile specimen) to 85.87 mm (a female) (Figure 5) and the weight (average \pm SE) ranged from 6 g (a juvenile) to 79 g (a female) (Figure 6). The preliminary results obtained from the sampled populations are presented in Table 2.

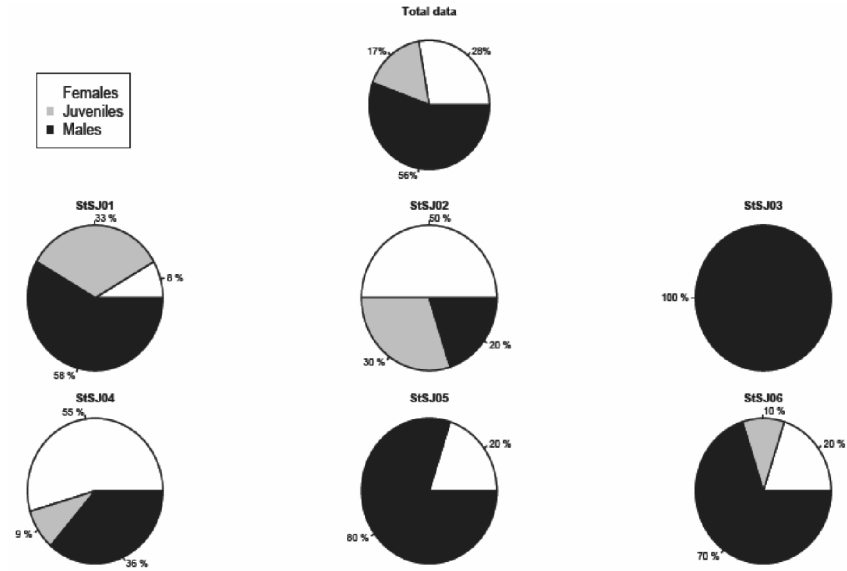


Figure 4: Distribution of *P. perezi* by gender, for all data and for each sampling site (juveniles are included).

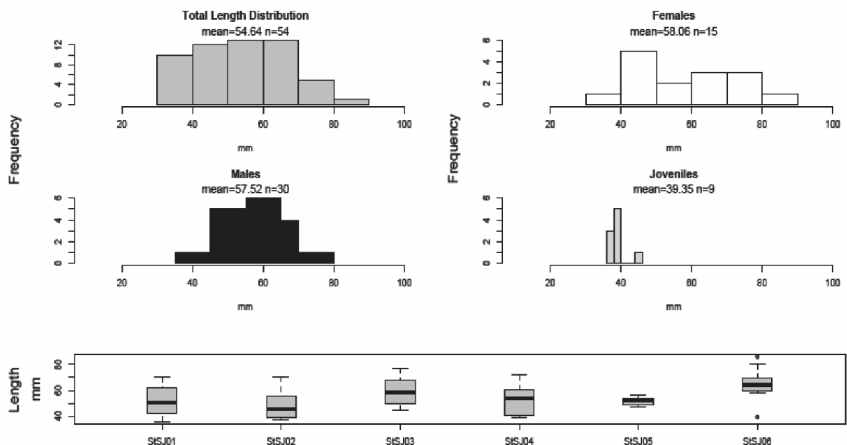


Figure 5: Distribution of *P. perezi* SVL by size class, for all data and for gender. The boxplot shows the SVL distribution in each site (the black bold horizontal line shows the median value, the box shows the lower and the upper quartile and the buffers indicate the maximum and minimum sample for each distribution).

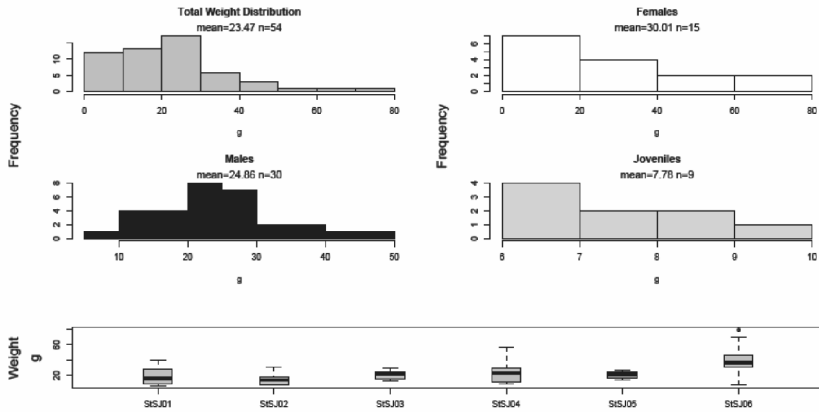


Figure 6: Distribution of *P. perezii* weight by size class, for all data and for gender. The boxplot shows the weight distribution in each site (the black bold horizontal line shows the median value, the box shows the lower and the upper quartile and the buffers indicate the maximum and minimum sample for each distribution).

The SVL data sampled during this study are consistent with those present in literature, where snout-vent length for *P. perezii* is indicated from 40 to 70 mm, but sometimes is longer than 10 cm (Docampo & Milagrosa-Vega, 1988).

Females were always larger than males both for SVL and body mass. Average SVL in females was 58.06 ± 3.85 mm, compared to 52.57 ± 1.57 mm in males; females weighed on the average 30.10 ± 5.85 g and males 24.86 ± 1.66 g. However, there was no significant differences between males and females, concerning weight ($F = 1.22$; $P = 0.27$) and SVL ($F = 0.02$; $P = 0.87$).

The comparison of the mean SVL values of each population, revealed significant differences within the mean SVL values ($F=2.82$; $p < 0.05$). The Tukey's test revealed a significant difference between the mean SVL value of StSJ02 (48.39 mm) and StSJ06 (65.09 mm) (Table 2). This result is significant at $p = 0.05$ level.

The equation describing the pooled W-SVL relationship is (Figure 7):
 $\log_{10}(W) = -3.32 + 2.67 \log_{10}(SVL) \quad r^2 = 0.89, df = 52, P < 0.001$

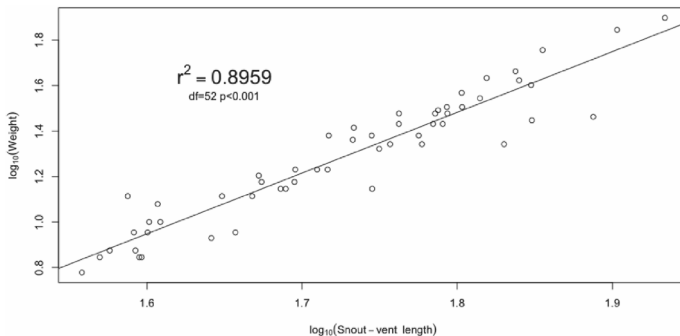


Figure 7: Relationship between \log_{10} transformed body mass and snout-vent length in *P. perezii* ($N = 54$).

A data pool size of 54 is small for describing weight-length relationships. Because 89% of the variation in weight of the sampled frogs was explained by the length, it is possible to believe that equation provides a reasonable description of the relationship between the weight and the length for the range of sampled lengths. Moreover, the ANOVA F-test shows that the regression relationship provides a significant statistical model ($F=447.64$; $P<0.001$). In reviewing the literature, no data was found on the relationship between length and weight for *P. perezii*. However, the b value found is consistent with literature data about this parameter for other amphibian species with a length comparable to *P. perezii* (Takahara *et al.*, 2008).

CONCLUSIONS

The capture effort in most of the sampled stations was low, regarding the spread degree of specimens over the habitats. Contrarily, as populations StSJ03 and StSJ06 were confined to tanks, it is possible to think that they were extensively sampled.

Reports on the relationship of body length to body mass in *P. perezii* are absent, but estimates of biomass are essential for studies modelling its structure, biomass growth, and energy production and flow in the ecosystems.

Our data suggest that there are statistically significant differences in morphometric data among populations of *P. perezii* in São Jorge Island. An increase in morphological and environmental studies could allow a better understanding of the origin and the rate of these differences.

It could be interesting to analyse mark-recapture frogs data from this geographical area to estimate annual growth rates and summertime body conditions for the different life stages (juveniles, males, and females). From our studies, more information will be provided detecting relationships between morphometric variations and climate parameters.

ACKNOWLEDGEMENTS

We thank Dinarte Teixeira and Susana Sargo Silva for their encouragement and assistance during the fieldwork. The authors would also like to thank the Department of Biology and Mrs Regina Tristão da Cunha and Dr. Fernando Tristão da Cunha for their logistical support during the expedition.

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