Body condition of *Pseudis minuta* Günther, 1858 (Anura: Hylidae) inhabiting an agroecosystem from south Santa Fe Province, Argentina

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Abstract. We present the first data on the body condition of *P. minuta* adults in a pond associated with an agroecosystem of the south Santa Fe Province, Argentina. Fieldwork was conducted from November 2012 to December 2013. Females and males did not differ in body condition, weight, nor length. However, males from December 2013 were in better condition than males from November 2012. Similarly, females from January 2013 were in better condition than those registered in November 2012. Our report provides for the first time an observation of the species in a landscape dominated by intensive agriculture in southern Santa Fe Province, corresponding to the Pampean region. Further studies should investigate habitat conditions and resources that allow the growth and development of this population of *P. minuta* in order to ensure its long-term permanence in the region.

Keywords. Lesser swimming frog, croplands, residual index, Humid Pampas

Introduction

Body condition is an important determinant of the health and fitness of an individual, a proxy of energy reserves, a nondestructive index, useful and necessary tool in ecology (Schulte-Hostedde et al., 2005; Peig and Green, 2010). Several authors have addressed the relationship between body condition of anurans and their food intake, fecundity, survival, anti-predator response, proportion of life stages, and call structure (e.g., Reading, 2007; McCracken and Stebbings, 2012; Middleton, 2012; Bennett and Murray, 2014; Ziegler et al., 2016). Body condition is an important proxy of environmental stress (Băncilă et al., 2010; Brodeur et

al., 2011), habitat quality (Scheele et al., 2014), and population size (Unglaub et al., 2018). In this sense, in Argentina, a reducing condition factor has been clearly demonstrated in frogs from agricultural lands (Brodeur et al., 2011).

Body condition indices, such as the residual index (Schulte-Hostedde et al., 2005), have been previously used in several studies for determining body condition differences amongst amphibian populations (Denoël et al., 2002; Băncilă et al., 2010; Scheele et al., 2014; Unglaub et al., 2018). Using this approach, frogs from agricultural lands were demonstrated to present a reduced body condition in the Pampa Region of Argentina (Brodeur et al., 2011). Pseudis minuta (Günther, 1858) is a small aquatic anuran species in which females are slightly bigger than males; both genders rarely exceed 48 and 40 mm in snout-vent length, respectively (Ghirardi and López, 2017). Pseudis minuta has long hind limbs, well-developed interdigital membranes extending to the base of the terminal discs, and a broad head with prominent eyes of dorsal position (Ghirardi and López, 2017). Males have a double lateral vocal sac (Gallardo, 1987). In Argentina, P. minuta breeds from July to April (Manzano et al., 2004), attaching its eggs to aquatic vegetation (Noguer, 2000). Tadpoles are much larger than adults, and develop in lentic environments

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(Manzano et al., 2004). It is a generalist-opportunistic predator with a broad feeding niche and it usually feeds on aquatic preys with large individual biomasses (Huckembeck et al., 2014). The conservation status of *P. minuta* is classified as "Least Concern" (Kwet et al., 2004) or "Not Threatened" in Argentina (Vaira et al., 2012).

The distribution of *P. minuta* encompasses Uruguay, extreme southern Brazil, and northeastern Argentina (Frost, 2018). In terms of ecoregions, P. minuta occurs in the Paraná-Paraba Rainforest, Araucaria Rainforest, Chaco Savanna, Humid Chaco, Espinal, Humid Pampa, Mesopotamian Savanna and Flood Savanna of Paraná (Lavilla, 2005). In Argentina, P. minuta has been mainly associated with large rivers, protected areas, lands used for cattle breeding and in anthropized, rural, or semi-urban environments (Noguer, 2000; Manzano et al., 2004; Guzmán and Raffo, 2011; Agostini, 2013; Ghirardi and López, 2017). Within Santa Fe Province, the known distribution of *P. minuta* encompasses the North Salado River basin, the Paraná River basin (Ghirardi and López, 2017), and the southwestern section of the province, which corresponds to the headwater of the South Salado River (Vera-Candioti et al., 2018).

We provide here the first data on the body condition of *Pseudis minuta* in an agroecosystem from south Santa Fe Province.

Materials and Methods

The study area is located along National Route N° 33, 25 km southwest of Venado Tuerto City, General López Department, Santa Fe Province, Argentina (33.8752°S, 62.2211°W).

Field samplings were conducted in November 2012, January and December 2013, as part of a long-term study of anuran from agroecosystems in south Santa Fe, Argentina. We captured specimens of P. minuta on a permanent pond with aquatic vegetation of 8.4 ha of total area and 48 cm of maximum depth. Native grassland separated the pond from a corn crop on one side (70 m of distance), and a soybean crop on the other side (100 m of distance). We captured frogs by hand, measured them on site, and immediately released specimens at the same site of capture. We measured snout-vent length (SVL) with an electronic caliper (± 1 mm), and body weight (W) with a Sartorius portable electronic balance (± 0.001 g). We determined sex by the presence (in males), or absence (in females), of subgular pigmentation (Ghirardi and López, 2017). We excluded juveniles from the analyses because of the small sample size (see Results). We considered juveniles those individuals without subgular pigmentation and an SVL < 30 mm (Melchiors et al., 2004). All procedures involving live frogs were conducted according to the guidelines for research with laboratory, farm, and wild animals from the National Scientific and Technical Research Council of Argentina (CONICET, 2005).

We estimated body condition based on the residual index according to Băncilă et al. (2010). We calculated this index using log10-transformed data to insure the linearity of the relationship between W and SVL. The theoretical body weight value of each frog, which was obtained by introducing the length of the animal into the equation of the regression line, was subtracted from the measured body weights in order to obtain the value of the "residual", a representation of body condition (Denoël et al., 2002; Băncilă et al., 2010; Scheele et al., 2014).

We compared body size, body weight and, residuals describing body condition between sexes and within the same sex, between different sampling dates, using a one-way ANOVA, followed by Fisher Least Significant Difference for multiple ranges comparisons (Fisher's LSD). We verified normality and equal variance of the data before conducting all ANOVAs and multiple comparisons. We used Kruskal-Wallis one-way analysis of variance on ranks (KW) when normality or homogeneity of variance could not be obtained, even after transformation. The criterion for significance was set at P < 0.05, in all cases. All analyses and graphs were performed using Statgraphics Centurion XV statistical software (StatPoint, Inc., Virginia, USA) and GraphPad Prism Version 5.00 (GraphPad Software, Inc., USA).

Results

We present here the first data on body condition of P. minuta adults in a pond associated with an agroecosystem area of the South Santa Fe Province, Argentina. We registered 60 individuals (2 juveniles, 39 adult males, and 19 adult females) (Table 1). The larger number of captures occurred in November 2012 with 29 frogs sampled. The relationship between SVL and W was described by the following linear regression equation: $\log W = -9.31518 + 3.026 \times \log SVL$ (Fig. 1). Body condition of males and females did not differ (ANOVA, P = 0.85; F value: 0.036; df: 1); neither W (Kruskal-Wallis, P = 0.57; Q value: 0.327; df: 1); nor SVL (ANOVA, P = 0.31; F value: 1.039; df: 1). On the other hand, residual index indicated that males exhibited a better condition in December 2013 than in

	n	Mean body mass (g) ± SE (min-max)	Mean snout-vent length (mm) ± SE (min-max)	
Life stage				
Adult	58	$5.05 \pm 0.20 \ (3.13 - 10.56)$	$37.06 \pm 0.56 \ (31.08 - 58.00)$	
Juvenile	2	$2.41 \pm 0.21 \ (2.2 - 2.62)$	$28.32 \pm 0.75 \ (27.57 - 29.06)$	
Gender				
Male	39	$4.72 \pm 0.12 (3.13 - 6.21)$	$36.66 \pm 0.63 \ (31.19 - 58.0)$	
Female	19	$5.72 \pm 0.55 (3.21 - 10.56)$	$37.88 \pm 1.14 (31.08 - 47.69)$	

Table 1. Mean, SE (Standard Error), and range (min-max) of body mass and snout-vent length of *Pseudis minuta* in an agroecosystem from south Santa Fe Province.

November 2012 (ANOVA, P < 0.05; F value: 6.539; df: 1); whereas females demonstrated better condition in January 2013 than in November 2012 (ANOVA, P < 0.01; F value: 14.499; df: 1) (Fig. 2).

Discussion

As the aim of body condition indices is to determine the mass of the animal associated with energy reserves, an individual with a greater body condition is assumed to have more energy reserves than an animal with a low condition (Denoël et al., 2002). Consequently, the lower body condition observed in frogs on November 2012 with respect to January and December 2013, indicates that animals from this first sampling had reduced fitness

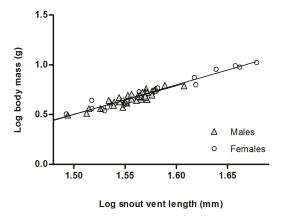


Figure 1. Relationship between body mass and snout-vent length in males and females of *Pseudis minuta* from an agroecosystem of south Santa Fe Province, Argentina (n = 56).

and suggests that ecologically relevant parameters such as reproductive output and survival were perhaps affected (Brodeur et al., 2011).

Body condition is influenced by habitat quality (e.g., resource availability, food intake, humid micro-climatic conditions, and water availability) (Brodeur et al., 2011; Scheele et al., 2014), and population size (Unglaub et al., 2018). The highly variable size at maturity observed in *Pseudis* may be due to tadpole size at metamorphosis (Alford and Harris, 1988). Specific local ecological factors allow larvae to grow to gigantic proportions, most notably the presence of very large temporary ponds with low densities of predators (Roček et al., 2006). Post-metamorphic growth in *Pseudis* is believed to be nil or very limited (Fabrezi et al., 2009).

In November 2012, we registered individuals with the lowest body condition in coincidence with the larger capture rate (n = 29). Few studies has been demonstrated that body size variation and body condition of some amphibian species are density-dependent or have a negative association with population size (Burton et al., 2009; Middleton, 2012; Unglaub et al., 2018).

In a long-term survey of amphibians in agricultural landscapes from northwestern Buenos Aires Province, within the Pampas region of Argentina, *P. minuta* was only mentioned in view of specimens held at herpetological collections (Agostini et al., 2016). After 10 years of fieldwork without any new records of the species, authors suspected that the species could not have maintained permanent populations in northwestern Buenos Aires Province (Agostini et al., 2016). In the present study, we found the lesser swimming frog within an agroecosystem in the southwest Santa Fe Province corresponding to the Pampean region, from 2012 until at least 2016 (Vera-Candioti et al., 2018).

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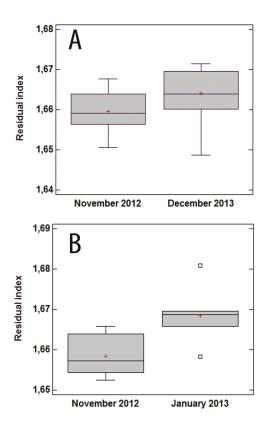


Figure 2. Residual index of males (A) and females (B) of *Pseudis minuta* for different sampling times from an agroecosystem of south Santa Fe Province, Argentina.

We not only observed juveniles, males and females, but we also registered vocalizations and amplexus, in an environment characterized by a reduced number of habitats suitable for amphibian life (Peltzer et al., 2006), and the ecoregion with the lowest amphibian species richness of Santa Fe Province (Ghirardi and López, 2017). *Pseudis minuta* shows highly plastic patterns of habitat and microhabitat occupancy and presents a generalist-opportunist predator condition (Huckembeck et al., 2012, 2014). In terms of feeding, generalist species should be less vulnerable to disturbances than species that forage more specialized food (López et al., 2015). Thus, this generalist-opportunistic condition of *P. minuta* might be contributing to its survival in agroecosystems.

Further studies should investigate habitat conditions and resources that allow the growth and development of this population of *P. minuta* in order to ensure its long-

term permanence in agricultural fields of the Humid Pampas of Santa Fe, Argentina.

Acknowledgements. Financial support was provided by Agencia Nacional de Promoción Científica (PICT2010-0390). Captures were conducted under the authorization of Dirección General de Recursos Naturales y Ecología del Ministerio de Medio Ambiente de la Provincia de Santa Fe (Expedient number 02101-0017838-7). We thank the landowner for giving us access to the sampling location.

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