

## Study on the population structure of the paradoxical frog, *Pseudis bolbodactyla* (Amphibia: Anura: Hylidae), using natural markings for individual identification

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**ABSTRACT.** The goal of this study was to assess the population structure of *Pseudis bolbodactyla* Lutz, 1925 using natural markings to identify individuals. Recruitment, survival, and population size estimations were obtained using the Jolly-Seber stochastic method. A total of 166 individuals were captured, and the striped, spotted, and dotted patterns that make their recognition possible were recorded. Of the specimens captured, 27 were recaptured, including some at pre and post-metamorphic stages. The estimate maximum population size was 52. The indices of survival and recruitment varied among samplings. Sexual dimorphism in size and in the operational sex ratio were detected. Despite the limited scope of our characterization of the *P. bolbodactyla* population, our data might be useful in the interpretation and elaboration of new hypotheses about ecological processes acting on anuran populations.

**KEY WORDS.** Amphibian; capture-recapture; Cerrado; photograph; estimate.

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Several methods have been used for marking and identifying anurans, for instance toe clipping, burning, tagging and banding (DONNELLY *et al.* 1994). These techniques, which import in various degrees of disfigurement, have been assessed in order to determine their impacts on the survival of the marked animals, their efficacy, and the ethical issues involving the use of animals in scientific research (GOLAY & DURRE 1994, MEASEY 2001, DAVIS & OVASKA 2001, MCCARTHY & PARRIS 2004, FUNK *et al.* 2005, FERNER 2007, CLEMAS *et al.* 2008). An alternative to these methods is identification through photographs, a relatively new technique that has been adopted by a number of researchers, but is still looked upon with some suspicion (REBELO & LECLAIR 2003, BRADFIELD 2004, MIRANDA *et al.* 2005, FERNER 2007, CLEMAS *et al.* 2008, KENYON *et al.* 2010). It involves the utilization of organic physical markings (scratches, mutilations, or defects) and color patterns (stripes, spots, etc.) to distinguish among individuals of the same species. The viability of this technique depends on the quality of the markings and the time it takes to compare images (FRISCH & HOBBS 2007).

Identification through photographs was recently used by MIRANDA *et al.* (2005) for the identification of *Pseudis cardosoi* Kwet, 2000 individuals. Their results showed that the variable

designs on the inner surface of the thighs of these frogs serve as a fingerprint that makes it possible to unequivocally identify them. Later, GARDA *et al.* (2010) suggested that these patterns of stripes on the thigh area vary between and within populations of different species of *Pseudis*, allowing population studies to be conducted.

Based on the patterns of the stripes on the ventral longitudinal section of the thigh, GALLARDO (1961) divided the *Pseudis paradoxa* (Linnaeus, 1758) species group into several sub-species, but this division was subsequently deemed unreliable (GARDA *et al.* 2010). The species that comprise *Pseudis* Wagler, 1830 are distributed in South America, east of the Andes, from Venezuela to eastern Argentina and Uruguay (DARST & CANNATELLA 2004, GARDA & CANNATELLA 2007, GARDA *et al.* 2010, FROST 2013).

Species of *Pseudis* are extremely dependent on water and display several morphologic, reproductive, and developmental adaptations to aquatic environments that distinguish them from other Hylidae (EMERSON 1988, GARDA & CANNATELLA 2007). The dispersion of individuals to different water bodies occurs mainly after heavy rains, although populations ordinarily tend to remain within their own watershed (GALLARDO 1961). With the exception of *Pseudis bolbodactyla* Lutz, 1925, *P. paradoxa*,

and *Lysapsus limellum* Cope, 1862, all paradoxical anuran species are restricted to one watershed. However, this group is still poorly sampled in most of the Central and North regions of South America (GARDA & CANNATELLA 2007).

*Pseudis bolbodactyla* has been found in four Brazilian states: Bahia, Minas Gerais, Goiás, and Mato Grosso (CARAMASCHI & CRUZ 1998, TEIXEIRA *et al.* 2004, VAZ-SILVA *et al.* 2007, GARDA *et al.* 2010, FROST 2013). It is medium sized when compared with other species of *Pseudis*, and is characterized by a vestigial carpal tubercle, head as wide as long, and dorsal skin sharply wrinkled (GALLARDO 1961, CARAMASCHI & CRUZ 1998, TEIXEIRA *et al.* 2004, GARDA *et al.* 2010). Very few studies have been conducted on the population ecology of *P. bolbodactyla*, and the existing ones are mainly focused on behavior, diet, and breeding (BRANDÃO *et al.* 2003, TEIXEIRA *et al.* 2004, VAZ-SILVA *et al.* 2007).

In this study, we present information on the biology and ecology of a population of *P. bolbodactyla* in an artificial pond located on the boundary of the Serra de Caldas Novas State Park, in the municipality of Caldas Novas, state of Goiás, Brazil. The natural markings of individuals were recorded with the aid of photographs. Based on our data, we endeavored to: 1) verify the existence of biometric variations among males, females, and juveniles; 2) determine whether the variability in length and weight of males and females is indicative of sexual dimorphism; 3) verify whether there is a tendency for operational sex ratio in the species; 4) estimate population size during the breeding season; and 5) evaluate whether photographic identification methods using natural markings can be used in population studies of *P. bolbodactyla*.

## MATERIAL AND METHODS

This study was conducted at Lajeado farm (17°52'10.1"S/48°41'26.5"W, 663 m a.s.l.; WGS84 datum), situated in the buffer zone of Serra de Caldas Novas State Park, Goiás, Brazil (Fig. 1). Five excursions, lasting two days each, were conducted to the research site between April and May, 2009, and January and May, 2010. The samples were collected from an artificial pond measuring about 280 m<sup>2</sup>, beginning at 6:00 p.m. and lasting for a variable number of hours.

The anurans were captured manually, kept in buckets of water, and taken to the laboratory for biometrics and photographic registration. The snout-vent length (SVL) was measured with a caliper (0.1 mm precision) and their body mass with a Diamond model 500 digital scale (0.1 g precision). After these procedures, the specimens were released in the same place where they had been collected.

The photographic registration method used for individual recognition consisted in photographing the inner thighs of each individual specimen where the spotted designs are used for identification (BRADFIELD 2004), according to the method implemented by MIRANDA *et al.* (2005). Photographs were taken with a Sony DSC Hx1 digital camera mounted on a tripod, 15 cm from the

subject. Voucher specimens were deposited in the Herpetological Collection of the Pontifícia Universidade Católica de Goiás (CEPB-NUROG; male: CN18 and female: UF08).

We assumed that the population studied varied due to mortality, birth rates, emigration, and immigration. Therefore, the population size was estimated using the Stochastic Method of Jolly-Seber. This method was also used to estimate the probability of survival ( $\Phi_{i,i+1}$ ) and recruitment ( $B_{i,i+1}$ ) rates of the population between sampling sessions (LEBRETON *et al.* 1993, FERNANDEZ 1995).

Based on the observations of VAZ-SILVA *et al.* (2007), individuals with yellowish throat regions were identified as adult males and those with whitish throat regions were considered adult females. Sexual dimorphism was verified using SVL and body mass measurements used as reference. In order to compare the SVL and body mass measurements between males and females, a Student t-test was applied. Sexual Size Dimorphism (SSD) was analyzed by dividing the average SVL of females by the average SVL of males, where  $SSD > 0$  = females larger than males, and  $SSD < 0$  = males larger than females (SHINE 1979, MONNET & CHERRY 2002).

The operational sex ratio (OSR) was obtained by dividing the number of males by the number of females present at the breeding sites (assuming that males and females breed every year after reaching maturity) each night and by the grand total recorded in the samplings (EMLEN & ORING 1977, SILVA & ROSSA-FERES 2010). The qui-square ( $\chi^2$ ) (ZAR 1984) was used to evaluate possible differences in sex ratio.

The Pearson correlation coefficient ( $r$ ) was used to verify the relationship between abundance and climatic variables, the abundance of males and females, the abundance and operational sex ratio, and the weight/length relation of the individuals. All statistical analyses were conducted with a significance level of 0.05.

## RESULTS

A total of 169 *P. bolbodactyla* specimens (88 males, 40 females, and 41 juveniles) were collected and photographed. Individuals were considered juveniles in the absence of secondary sexual characteristics. There was a positive correlation between the number of individuals and the following climatic variables: precipitation ( $r = 0.92$ ,  $gl = 4$ ) and relative humidity ( $r = 0.89$ ,  $gl = 4$ ). Individuals were more abundant in April and May, 2009.

All individuals presented striped, spotted, and dot patterns on the ventral surface of their thighs, making their identification possible. Of the 169 individuals captured, 27 represent recaptures (21 males, five females and one undefined), totaling 142 individual identifications. One specimen captured during the pre-metamorphic phase (Fig. 2) was recaptured in the post-metamorphic phase without any alteration of the thigh imprint (Fig. 3).

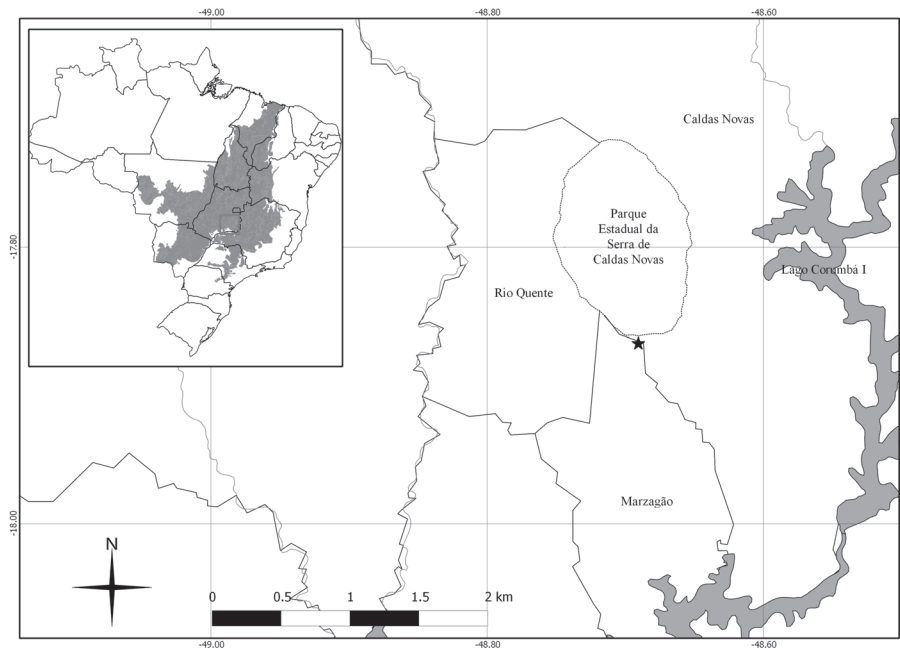


Figure 1. Geographic localization of the artificial pond used as breeding sites by *Pseudis bolbodactyla*, Municipality of Caldas Novas, Goiás, Brazil.



Figures 2-3. *Pseudis bolbodactyla* specimen captured during metamorphic phase (2) and recaptured in its post-metamorphic phase (3).

The low recapture rate and the proximity to a second pond suggest that this is an open population. The estimates revealed a population apex ( $N_3 = 52$ ) in the third sampling performed in May, 2009. The survival index between samplings varied between 0.02 and 0.05, with greatest value ( $\Phi = 0.45$ ) in the interval between the first and second samplings. Recruiting rates were 0.94 between the first to second samplings, and 0.36 from third to fourth samplings. The lowest estimates were observed in the fourth sampling due to the low survival and recruiting rates in that period (Table I).

Table I. Numbers of captured individuals ( $n_i$ ), released ( $R_i$ ), and recaptured ( $r_i$ ) during the samplings (I) of *Pseudis bolbodactyla* and corresponding estimates of survival ( $\phi_i, i+1$ ), recruitment ( $B_i, i+1$ ), and population size ( $N_i$ ).

| I | $n_i$ | $M_i$ | $R_i$ | $r_i$ | $\phi_i, i+1$ | $B_i, i+1$ | $N_i$ |
|---|-------|-------|-------|-------|---------------|------------|-------|
| 1 | 61    | 0     | 59    | 25    | 0.45          | –          | –     |
| 2 | 29    | 17    | 29    | 6     | 0.21          | 0,94       | 45.21 |
| 3 | 52    | 8     | 52    | 1     | 0.02          | 0,36       | 52.00 |
| 4 | 20    | 1     | 20    | 0     | –             | –          | 20.00 |
| 5 | 7     | 1     | 7     | –     | –             | –          | –     |

Males and females had similar color patterns, with sexual dimorphism in the coloration of the throat region, which is yellow in males and white in females. The SVL varied as follows: males, 35.0-48.8 mm (average =  $39.89 \pm 2.76$ ,  $n = 67$ ), females, 36.7-56.1 mm (average =  $45.06 \pm 4.50$ ,  $n = 30$ ); and juveniles, 21.2 and 37.8 mm (average =  $32.45 \pm 2.82$ ,  $n = 36$ ). The body mass of males ranged between 6-17 g (average =  $8.61 \pm 2.18$ ,  $n = 67$ ), of females between 6-26 g (average =  $11.95 \pm 4.49$ ,  $n = 30$ ), and of juveniles between 5-26 g (average =  $8.19 \pm 3.47$ ,  $n = 36$ ). Males and females differed in size (t test = 5.82,  $gl = 96$ ) and body mass (t test = 3.88,  $gl = 96$ ): females were larger and heavier. The SSD index for the species was 1.12, also suggesting sexual dimorphism in size.

There was a positive correlation between the SVL and body mass of males ( $r = 0.66$ ,  $gl = 66$ ) and females ( $r = 0.85$ ,  $gl = 29$ ). There was no correlation between the SVL and body mass ( $r = -0.13$ ;  $gl = 35$ ) of toadlets and juveniles, granted that it is not possible to determine their gender, possibly the result of atrophy of the tail. In toadlets, the length of the tail varied between 7.1 and 89.4 mm (average =  $47.77 \pm 27.07$ ,  $n = 32$ ) equivalent to three times the SVL in some examples.

The number of males and females correlated positively during the samplings ( $r = 0.98$ ,  $gl = 4$ ). The females, although present in all samples, were often in fewer numbers, which resulted in an operational sex ratio of 2.2:1 males/female (average =  $2.6 \pm 0.61$ ,  $n = 5$ ) and a greater than expected Chi-squared value ( $\chi^2 = 63.64$ ,  $gl = 4$ ). It is a fact that the numbers of males and females differed from the expected proportion of 1:1. During the sampling performed in May, 2010, which coincided with the end of the rainy season and the pond area is smaller, a reduction in the number of males reflected in an operational sex ratio of 1:1.

## DISCUSSION

Photographs have been used in some studies involving anurans, including species of *Pseudis* (MIRANDA *et al.* 2005), in the place of marking methods, which are considered invasive (REBELO & LECLAIR 2003, BRADFIELD 2004, MIRANDA *et al.* 2005, FERNER 2007, CLEMAS *et al.* 2008, KENYON *et al.* 2010). Photographs are probably more effective than "toe clipping" to identify *P. cardosoi* individuals, since individuals easily lose the phalanx of their fingers, quickly regenerating them (MIRANDA *et al.* 2005). The species is ideal for photo identification because individuals can be easily identified by the unique pattern of spots, stripes, and dots located on their inner thighs, allowing the recapture of 19% of the photographed individuals. In this study, the natural patterns used for individual identification remained unchanged over time, indicating that the photographic identification method using natural markings can be effectively applied to this species. However, we suggest the use of a second control method in order to reduce uncertainties.

Population studies using capture-mark-recapture methods to estimate the size of open populations must meet certain

criteria (JOLLY 1965, SEBER 1965, 1982). Due to the non-standardization of sampling intervals in this study, the assumption of equal probability of survival cannot be met, which means that the survival and recruiting estimates obtained for the intervals are not comparable (FERNANDEZ 1995). The low survival rate verified in the interval between the third and fourth samplings ( $\Phi = 0,02$ ) reflects the assumption that survival is a constant over the interval between  $i$  and  $i+1$ , with the proportion of surviving animals decreasing over time, following a negative exponential function (FERNANDEZ 1995). Therefore, the population structure obtained for this period cannot be considered constant. Beyond time, factors such as competition, predation, and density can cause fluctuations in amphibian populations (THOMÉ & BRASILEIRO 2007, ERISMIS 2011).

The color pattern and sexual dimorphism of *P. bolbodactyla* individuals in our data are consistent with previous descriptions (CARAMASCHI & CRUZ 1998, VAZ-SILVA *et al.* 2007, GARDA *et al.* 2010). Furthermore, we have identified sexual dimorphism in this population: females are larger and heavier. Larger females are found in 90% of all anuran species (SHINE 1979). Due to undetermined growth (HALLIDAY & VERREL 1988) associated with fast growth rates and late maturity, females of most species become larger than males. Larger females produce larger eggs, or spawns, when compared with smaller females, and have a higher probability of producing more than one offspring per breeding season (MONNET & CHERRY 2002, SILVA & ROSA-FERES 2010). In species where males are larger than females, size is associated with territoriality, combat, and mating success (MONNET & CHERRY 2002, WOGEL & POMBAL 2007, SILVA & ROSA-FERES 2010).

Body size is a fundamental morphological feature, important in a physiological, ecological, and social contexts (SCHÄUBLE 2004). Several factors can be responsible for geographic variations in morphology (KUPFER 2007), including predation (SCHNEIDER *et al.* 2000), climatic changes, or other environmental parameters (CASTELLANO *et al.* 2000) such as sexual selection, sexual dimorphism (STORZ *et al.* 2001), genetic drift, founder effect (DEMETRIUS 2000), and distance from the Equator (BLACKBURN *et al.* 1999). However, the causes and maintenance of these variations are complex and are not always understood (AVISE 2000). The amplitude in body size found in this population of *P. bolbodactyla* is within the parameters for the genus (BRANDÃO *et al.* 2003), and for the species (GALLARDO 1961, TEIXEIRA *et al.* 2004, VAZ-SILVA *et al.* 2007, GARDA *et al.* 2010). However, the individuals studied by us were larger than individuals in other populations in different locations of Minas Gerais (CARAMASCHI & CRUZ 1998) and in the municipality of Pirenópolis, Goiás (BRANDÃO *et al.* 2003). The variation in size and maturity may be due to the size of the tadpoles in metamorphosis, post-metamorphic growth, or an interaction between these factors (ALFORD & HARRIS 1988, GARDA *et al.* 2010). According to ROCEK *et al.* (2006), ecological factors of specific locations allow species of *Pseudis* to grow in gigantic proportions, especially in large temporary lakes, and in the absence

of predators. GARDA *et al.* (2010) confirmed the descriptions of ROCEK *et al.* (2006) for several populations of *Pseudis*, where the largest specimens of *P. bolbodactyla* were found in large flooded areas under a bridge that crosses the São Francisco River, between the cities of Pirapora and Buritizeiro, Minas Gerais. The smallest individuals were found in small temporary ponds on sandy soils in the Municipality of Iaciara, Goiás. The fact that this study has been limited to the reproductive period of the species does not explain the larger size of the individuals, as in the case of the study of SILVA & ROSSA-FERES (2010) on another species, because in *Pseudis*, individuals practically do not grow after metamorphosis (DOWNIE *et al.* 2009, FABREZI *et al.* 2009).

The operational sexual ratio (OSR) is the proportion of males and females that are ready to mate in a population within a determined timeframe (EMLEN & ORING 1977), and may be an important determinant regarding competition for mating and the intensity of sexual selection (PROHL 2002, KUPFER 2007). This is the case for several species of amphibians (EMLEN & ORING 1977, BASTOS & HADDAD 1999, PROHL & HÖDL 1999, PROHL 2002, WOGEL *et al.* 2002, SILVA & ROSSA-FERES 2010). The operational sex ratio found in this population of *P. bolbodactyla* was biased towards a larger number of males, with an average of 2.6:1 males/female. This large male-to-female ratio had been previously recorded for a population of *P. bolbodactyla* (2.3:1 males/female; TEIXEIRA *et al.* 2004). However, this information must be treated with caution when the estimate is based on the number of captures, because collectors may be more tuned to calling males, creating a bias.

While this data affords only a limited interpretation, our results suggest that the studied area, located in the buffer zone of Serra de Caldas Novas State Park, represents an appropriate environment for populations of *P. bolbodactyla*. However, for a better evaluation of the population structure with estimation, recruiting, and survival rates taken into consideration, longer studies with equivalent intervals between samplings must be implemented. We must consider that the differences in size found between the individuals of this and other populations of *P. bolbodactyla* may be the result of biological and ecological factors, such as reproductive periods, lake size, and the absence of predation. Lastly, this data can be considered useful in the interpretation and elaboration of new hypotheses on the ecological processes acting on amphibian populations. It can also help establish conservation measures for the species, in addition to demonstrating that population structure and sex ratio data provide valuable information on animal populations.

#### ACKNOWLEDGEMENTS

We thank the owners of the farm Lajeado (Rui Gilberto and Paula Cristina) for permission to access the study area; Adrian A. Garda, Tatiana Miranda, and Hélio R. da Silva for the valuable suggestions during the preparation of this manuscript; Walter Boeger and the anonymous reviewer for very

useful critiques on the manuscript; the CNPq by the scholarships given to Rafael Filgueira Jorge and Werther Pereira Ramalho. Specimens were collected under SEMARH license no. 5601207252008-5.

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Submitted: 24.I.2013; Accepted: 01.IX.2013.

Editorial responsibility: Walter A.P. Boeger