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Estimativa populacional de *Melanophryniscus montevidensis* (Philippi, 1902) (Anura, Bufonidae) através do método MARK-captura-recaptura no sul do Brasil

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Resumo

ROCHA, Alessandro Romano da. **Estimativa populacional de *Melanophryniscus montevidensis* (Philippi, 1902) (Anura, Bufonidae) através do método MARK-captura-recaptura no sul do Brasil** 44f. Dissertação (Mestrado em Biologia Animal) – Instituto de Biologia, Universidade Federal de Pelotas, Pelotas, 2020.

Estudar os parâmetros populacionais é uma etapa importante na avaliação do status de conservação das espécies, além de fornecer informações relevantes aos programas de manejo da fauna silvestre. *Melanophryniscus montevidensis* é classificado como uma espécie vulnerável de acordo com a IUCN, com distribuição restritas zonas costeiras do Uruguai e no extremo sul do Brasil. Embora exista alguma informação sobre sua dinâmica populacional uruguaia, não há dados disponíveis sobre suas populações brasileiras. O presente estudo teve como objetivo estimar o tamanho da população de *M. montevidensis* em uma região no sul do Brasil. Os dados foram coletados de junho de 2018 a junho de 2019 no município de Santa Vitória do Palmar, no Rio Grande do Sul. As taxas de sobrevivência foram altas, constantes e muito semelhantes em todos os modelos construídos. As probabilidades de captura foram influenciadas principalmente pelas chuvas. O modelo POPAN estimou um tamanho populacional de mais de 19.000 indivíduos na área de estudo. Esses dados serão úteis, uma vez que os habitats naturais dessa espécie no Brasil foram rapidamente substituídos por parques eólicos.

Palavras-chave: Bufonidae, *Melanophryniscus montevidensis*, Bioma Pampa, Tamanho populacional, Sul do Brasil.

Abstract

ROCHA, Alessandro Romano da. **Population size of the threatened Tandilean red-belly toad Melanophryniscus montevidensis: First estimate for southern Brazil** 44p. Dissertation (Master in Animal Biology) - Institute of Biology, Federal University of Pelotas, Pelotas, 2020.

Abstract. Studying population parameters is an important step in evaluating species conservation status as well as providing relevant information to wildlife management programs. *Melanophryniscus montevidensis* is classified as a vulnerable species according to IUCN and it is distributed in the coastal zones of Uruguay and extreme southern Brazil. Although there is some information regarding its Uruguayan population dynamics, no data is available on its Brazilian populations. The present study aimed to estimate the population size of M. montevidensis in a region in southern Brazil. Data were collected from June 2018 to June 2019 at Santa Vitoria do Palmar municipality in the state of Rio Grande do Sul. Survival rates were high, constant and very similar in all built models. Catch probabilities were mainly influenced by rainfall. The POPAN model estimated a population size of more than 19.000 individuals in the study area. These data will be useful since the natural habitats of this species in Brazil have been quickly replaced by wind farms.

Keywords: Bufonidae, *Melanophryniscus montevidensis*, Pampa Biome, Population size, Southern Brazil.

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1 Introdução

A América Latina abriga uma fauna altamente diversificada, representando metade da riqueza total de espécies do mundo (DUELLMAN, 1999). Neste contexto, em relação aos anfíbios, o Brasil tem o maior número de espécies conhecidas, atualmente com 1.228 espécies descritas (FROST, 2020). Nas últimas décadas vem ocorrendo diversas discussões sobre o declínio populacional de anfíbios em todo o mundo (YOUNG et al., 2001). No entanto, o principal problema para avaliar corretamente a extensão dos declínios dessas populações é a falta de informação básica, especialmente a necessária para separar as flutuações naturais dos declínios reais (HEYER et al., 1994). Embora haja evidências de que as populações de anfíbios estão diminuindo em todos os continentes, as contribuições para o estudo do problema vêm principalmente de cientistas dos Estados Unidos, da Europa e da Austrália (HOULAHAN et al., 2000). Um dos fatores apontados como responsáveis pelo declínio de populações de anfíbios são as alterações climáticas. Zank et al. (2014) demonstrou através de modelagens climáticas que algumas espécies de *Melanophryniscus* podem perder até 100% dos indivíduos e locais de ocorrência até 2080.

O gênero *Melanophryniscus* compreende espécies de sapos de porte pequeno (comprimento rostro-cloacal de 20-30 mm), que se caracterizam por, um padrão dorsal predominantemente negro ou escuro, geralmente com pontos contrastantes de coloração amarelo/laranja e com a presença de substâncias tóxicas (CARAMASCHI E CRUZ, 2002, CAORSI et al.,2014. Atualmente o gênero é composto por 29 espécies. Dentre elas, sete espécies estão listadas como ameaçadas de extinção a nível global (IUCN, 2020). Em um status maior de preocupação estão*M. peritus,*e*M. admirabilis* e *M. langonei*, classificados como criticamente em perigo (CR), as duas primeiras espécies endêmicas do Brasil e a última uma espécie do Uruguai. Classificados como vulneráveis (VU) estão *M. dorsalis*, *M. macrogranulosus*, *M. orejasmirandai* e *M. montevidensis* (LANGONE 2004). Na lista vermelha de animais ameaçados do Brasil quatro espécies do gênero consideradas ameaçadas de extinção segundo a última atualização feita em 2014, *M. admirabilis* (criticamente em perigo-CR), *M. dorsalis* (vulnerável-VU), *M. macrogranulosus* (em perigo-EN) e t *M. cambaraensis* (vulnerável-VU). Conhecido como Sapito-de-Darwin ou Sapinho-da-barriga-vermelha-uruguaio, *Melanophryniscus montevidensis* (PHILIPI, 1902) (Anura: Bufonidae) é restrito às costas uruguaias e ao extremo sul Brasil (BERNARDO-SILVA et al., 2012), sendo conhecido no Brasil apenas para o sul do Rio Grande do Sul, nos municípios de Santa Vitória do Palmar (MANEYRO E KWET, 2008) e Chuí (TEDROS et al., 2001).

No Brasil, inúmeros trabalhos tem sido feitos para o gênero *Melanophryniscus*, com múltiplas abordagens, desde descoberta de novos locais de ocorrência (ZANK, 2013; BORNSCHEIN et al., 2015), comportamento reprodutivo (CAORSI, 2011), extensão da distribuição (QUINTELA et al., 2007), status de conservação (CAORSI et al., 2014; COLOMBO, 2008; VASCONCELOS, 2015) e identificação de áreas prioritárias para conservação (BERNARDO-SILVA, 2012). No entanto, para a população brasileira de *M. montevidensis*, existem poucas informações detalhadas sobre abundância e distribuição claras que justifiquem o status de conservação para as populações já identificadas, apenas sobre sua história evolutiva (SILVA, 2012), variabilidade genética (SZYNWELSKI, 2016), locais de ocorrências (MANEYRO E KWET, 2008; TORANZA E MANEYRO, 2014) e áreas potenciais para a preservação da espécie (BERNARDO-SILVA, 2012). Por outro lado, para a população do Uruguai, onde a espécie era considerada endêmica até 2001 (TEDROS et al., 2001), inúmeros trabalhos tem sido publicados sobre a história de vida (PEREIRA E MANEYRO, 2016b; 2018), status de conservação (CARREIRA E MANEYRO, 2015). Várias populações locais dos departamentos de Montevidéu e Canelones foram extirpadas (LANGONE, 2003). Uma causa provável para este declínio é a urbanização e consequente fragmentação do habitat ao longo da costa uruguaia (LANGONE, 1995).

A foto-identificação é um método que consiste em registros fotográficos dos padrões de manchas e marcas naturais únicos para cada animal. (BRADFIELD, 2004). É considerada uma das técnicas mais eficazes, já que é rápida, pouco invasiva, barata e permite o reconhecimento individual a longo prazo (SACCHI et al., 2010). A foto-identificação foi validada em estudos anteriores para esta espécie (ELGUE et al., 2014) e outras espécies de *Melanophryniscus* na América do Sul (CAORSI et al, 2012; CAIRO et al., 2013).

Nosso principal objetivo é fornecer dados sobre uma população de M. montevidensis do extremo sul do Brasil para ajudar os tomadores de decisão na identificação de áreas prioritárias para esforços de conservação (ZANK et al., 2014). Também investigamos se as variáveis climáticas (ou seja, precipitação e temperatura) influenciam a abundância esperada desta espécie. Essa dissertação teve como objetivo principal auxiliar o aumento do conhecimento sobre uma parte da população de *Melanophryniscus montevidensis*.

2 Population size of the threatened Tandilean red-belly toad *Melanophryniscus montevidensis*: First estimate for southern Brazil

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Abstract

Population size of the threatened Tandilean red belly toad *Melanophryniscus montevidensis*: First estimate for southern Brazil

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Abstract. Studying population parameters is an important step in evaluating species conservation status as well as providing relevant information to wildlife management programs. *Melanophryniscus montevidensis* is classified as a vulnerable species according to Iucn and it is distributed in the coastal zones of Uruguay and extreme southern Brazil. Although there is some information regarding its Uruguayan population dynamics, no data is available on its Brazilian populations. The present study aimed to estimate the population size of *M. montevidensis* in a region in southern Brazil. Data were collected from June 2018 to June 2019 at Santa Vitoria do Palmar municipality in the state of Rio Grande do Sul. Survival rates were high, constant and very similar in all built models. Catch probabilities were mainly influenced by rainfall. The Popan model estimated a population size of more than 19.000 individuals in the study area. These data will be useful since the natural habitats of this species in Brazil have been quickly replaced by wind farms.

Keywords: Bufonidae, *Melanophryniscus montevidensis*, Pampa Biome, Population size, Southern Brazil.

Introduction

One of the great challenges in population studies is to understand how and why the abundance of wildlife populations fluctuates through time. In some cases, the main interest may be simply to detect trends in size or population density (Gerrodette 1987), but more complex analyses can reveal the causes of these changes (Bjornstad & Grenfell 2001). Time series data provide the information needed to detect fluctuations in population dynamics, reproductive rate and population density. They also reveal whether climate may be affecting these population parameters. (Boggs & Inouye 2012).

Survival and recruitment rates are the main factors that influence fluctuations in the abundance and fertility of a population, and long-term sampling is one of the best tools to understand these dynamics (Tezanos-pinto et al. 2013). However, sampling is an expensive effort, logistically impracticable in most cases, and demands a lot of time (Bertulli et al. 2017). One of the most cost efficient methodologies is the use of individual identification (Tezanos-Pinto et al. 2013, Strandbr aten rødland & Bjorge 2015). These methods are well established and have been extensively published on in the past few decades (Willians et al. 2002). In this context, in relation to amphibians, Brazil has the largest number of known species, currently with 1.228 described species (Frost 2020). The majority of studies are based on data from marked animals and the created models allow inferring about population parameters, including demographic rates such as survival, population change, and population size or density (Royle et al. 2014). In Brazil, numerous works have been done for the genus *Melanophryniscus*, with multiple approaches, since the discovery of new places of occurrence (Zank 2013, Bornschein et al. 2015), reproductive behavior (Caorsi 2011), extension of distribution (Quintela et al. 2007), conservation status (Colombo 2008, Caorsi et al. 2014, Vasconcelos 2015) and identification of priority areas for conservation (Bernardo-silva 2012). However, few studies with population studies, mainly for *M. montevidensis*, as there is little detailed information on abundance and clear distribution that justify the conservation status for the populations already identified, only on their evolutionary history (Silva 2012).

The Tandilean red belly toad *Melanophryniscus montevidensis* (Philippi 1902) (Anura: Bufonidae) is restricted to the coastal zones of Uruguay and extreme southern Brazil (Bernardo-silva et al. 2012). The species is restricted to Santa Vitória do Palmar and Chuí in Brazil, the most austral municipalities in the country (Tedros et al. 2001, Maneyro & Kwet 2008). The species is associated to sandy soils of the coastal plain, being present in both coastal dunes and grassland fields. The species is categorized as “vulnerable” in the Iucn red list of threatened species (Langone 2004), and it is threatened at country and state levels in both countries where it occurs (Carreira & maneyro 2015).

Melanophryniscus montevidensis has periods of peak breeding (i.e. explosive reproduction), with reproductive events associated with heavy rainfalls along the year (Alonzo et al. 2002, Maneyro & Carreira 2012). It has a short period of activity in the post-rainfall phase with males vocalizing from temporary ponds for a maximum of three to four days. The main studies with the populations of the species show that they are decreasing in number due to the loss of habitat caused mainly by the increase of the urbanization of the coastal areas (Langone 2004).

Our main goal was to provide data on a *M. montevidensis* population from extreme southern Brazil to aid decision-makers in identifying priority areas for conservation efforts (Zank et al. 2014). We also investigate whether climatic variables (i.e. precipitation and temperature) influence the expected abundance of this species.

2.1 Materials and Methods

2.1.1 Study area and field methods

The study was conducted at Fazenda Charrua located at Barra do Chuí in the Santa Vitória do Palmar municipality at the extreme south of Rio Grande do Sul state, near the border between Brazil and Uruguay. The study site covers an area of 800ha (Figure 1), between the coordinates -33.716108° Lat./-53.408949° Long. (Wgs48 - Extreme north limit) and -33.741341° Lat./-53.402859° Long. (Wgs48 - Extreme south limit). The vegetation in the area is predominantly herbaceous, composed by grasses and patches of shrubs with a few introduced *Pinus elliottii* and *Eucalyptus spp.* individuals. The water bodies in the area are lentic and ephemeral, formed throughout the year in moments of heavy rainfall. In 2014, a wind farm was established inside the study area with the installation of 23 wind turbines.

The characteristics are of Subtropical climate with rains well distributed throughout the year. Summer is a hot season (with a maximum above 32 ° C). In winter, the average daily minimum is between 6 ° C and 12 ° C. Altitude and latitude facilitate a decrease in temperature. The South Region is homogeneous with respect to rainfall - The average height of annual precipitation: 1.250 mm to 2.000 mm. (https://en.weatherspark.com).

Located on the Coastal Plain, in the Laguna-Barreira depositional system, the region on the banks of Arroio Chuí is composed of sandy facies with a shallow marine and beach environment, composed of clear and fine quartz sand, with well developed stratifications. In several places ichnofossils of digging crustaceans and mollusk shell molds occur. The maximum height of occurrence of these fossils indicates that the transgression responsible for their formation reached about 7 meters above the current level (Buchmann et al. 2009).

2.1.2 Data collection

Sample collection was performed twice per month between June 2018 and June 2019 for a total of 26 field campaigns spaced by intervals ranging from 15 to 20 days. The methodology used to register individuals followed that of Heyer et al. (1994), consisting of a search for individuals in predefined transects. We covered nine transects of 500 meters each. Each transect was at least 500 meters apart. We captured all individuals found up to a maximum distance of 10 m perpendicular to the transects for a total sampled area of 10.000 m2 per transect (20 m wide x 500 m long). The area of transects is characterized as disturbed vegetation, in an open area with several bodies of water. The nine transects were covered in all campaigns, at the same time during the day, with the participation of only one person. Active searches were visual and acoustic and in the end the animals were released. Total sampling effort was 93 hours and a total of 3.051 photographs were taken.

Complementary data on each individual collected was organized in spreadsheets containing the following information: date, transect number, geographical coordinate, rostro cloacal length (mm), weight (g), and the sex of each captured individual. The sexes were separated by the presence of a vocal sac in the males, which have a black colored gular region. (Pereira & Maneyro 2016a). The distinction between adult and young individuals was made according to Bardier et al. (2017) who determined a minimum size for adult individuals of 13 mm (millimeters).

We monitored the individuals distinguishing each by their ventral markings according to Elgue et al. (2014) and Pereira & Maneyro (2018). The methodology used was photo-identification with each individual having its ventral portion photographed. All images were taken with a 16 megapixel digital camera (® Sony - Resolution: 72dpi, Dimensions: 4128x2322 pixels, maximum aperture: 1.85, Focal length: 4mm). We take three to four photos per individual. To capture the image, the individual was manually arrested by the hind limbs, so that his ventral portion was exposed for the photo. The images were analyzed by direct observation and using the I3S Pattern + identification software (version 4.1). All climatic data used here were extracted from the Inmet Santa Vitória do Palmar / Barra do Chuí automatic station (-33.742297 ° Lat / -53.372218 ° Long.) Located about 2 km from the study area (available at www.inmet.gov.br)

2.1.3 Statistical analysis of the data

We employed an open population Jolly Seber model (Schwarz & Arnason 1996) to estimate population size using the Popan module in RMark (Laake 2013). This model enabled us to estimate four parameters: apparent survival (Phi), capture probability (p), the probability of entry into the population (pent), and the population size (N) on each sampling occasion, which in the end will result in a final "N" with the number of all animals present in the population during the study period. We opted for this model because 1) the Popan model allows estimating the total population size, 2) the study area covers ca. nine percent of the known distribution of this species in Brazilian territory (see www.icmbio.gov.br), and 3) the time scale between field trips was such that it allowed the assessment of immigration, birth or emigration and death.

We assume that p varies over time (t), as the species has explosive reproduction. Therefore, we model p according to the environmental variables "average monthly temperature" and "accumulated monthly precipitation". We also model the apparent survival (Phi) and the probability of capture (p) as sex functions, since females tend to withdraw from the reproductive site before males. The probability of entry (pent) varied only with time. We created 12 candidate models(Table 1) to be included in the analysis for the parameter estimates (Popan). All based on additive effects of covariates (temporal and group) and without temporal or covariable effects (constant parameters). Correlation between climatic variables temperature and precipitation was low (r = -0.24; p = 0.44), as such, the interaction term between them was excluded from the analysis to avoid multicollinearity issues.

We developed a historical data series of captures for each individual with each month of sampling constituting an occasion, that is, we did not counted frogs marked and then recaptured in the same month as the capture. The number "1" indicated that an individual was present in our area during a month of sampling, while a "0" denoted its absence.We used the corrected Akaike information criterion to compare models for small samples (AICc). We calculated the AICc for each one of the models in the candidate set, and classified the models in descending order based on their AICc or AICc weight (W) (that is, the most parsimonious model is the one with the lowest AICc value). Finally, we used Akaike's corrected information criterion to compare models for small samples (AICc). We calculate the AICc for each of the models in the candidate set and classify the models in descending order based on their AICc (that is, the most parsimonious model is the one with the lowest AICc value). Finally, we calculate means and standard deviations (SE) of the monthly estimates of the models accepted by the value of the AICc to present the values of the real parameters (White & Burnham 1999, Burnham & Anderson 2002). All tests were performed in the R software environment for statistical computing (R core team, 2018).

We performed Pearson's correlation test to check whether there is influence of climatic variables (air temperature and precipitation) on parameters apparent survival (PHI), capture probability(p), probability of entry(Pent) and population size(N).

2.2 Results

A total of 650 captures were recorded with 18 recaptures (2.9%), from which 14 individuals were recaptured once and two individuals twice. Thus, the number of individuals captured was 634. We photo identified 584 adult males (92%) and 50 adult females (8%) of *M. montevidensis*, resulting in a sex ratio proportion of 11:1 (Table 2). There was no capture of juveniles

Catches occurred in all sampling months with August and September 2018 being the months with the highest count of individuals captured. In contrast, only one individual was captured in February 2019(Figure 2). No specific model provided an appropriate fit, but three models were acceptable according to the AICc analysis (Table 3). They differed in covariate effects, on the probability of survival, and on the probability of capture. The latter responded to non-variation in time (W = 0.65, Table 3), to variation in time (W = 0.31 Table 3), and to the additive effect of rain (W = 0.02, Table 3). Survival did not vary over time, but capture probability and probability of entry did (W = 0.64, Table 3) in the best model selected during the Popan analysis.

The estimated rates of survival probability for anurans when parameterizing the best model with weighted averages were 0.95 ± 0.12 (mean ± SE) for both males and females (Figure 3A), which means a 95% of survival probability from between study periods. The survival probabilities were correlated was not significant and positively with the accumulated monthly rainfall (r = 0.39, p = 0.18), but they were moderately and negatively correlated with temperature (r = -0.22, p = 0.46). The mean capture probability was 0.07 ± 0.04 (mean ± SE) for males and females (Figure 3B). Capture probability varied considerably across months according to the models (Figure 3), and was weakly associated to monthly accumulated rainfall (W = 0.02, Table 3). The correlation between temperature and precipitation with (r = -0.37, p = 0.21) was not significant and had little influence on the probability of capture. (Table 3).

The apparent survival into the population (pent) fluctuated widely across time (Figure 3C) with an average of 8% (0.08 ± 0.04 SE). It not was correlated with rain (r = 0.43, p = 0.08) and temperature (r = - 0.43, p = 0.14). The estimated abundance ranged from 771 to 8,737 males and from 67 to 766 females (Figure 4) between each sampling period. There was also no significant correlation with the accumulated monthly precipitation (r = 0.68, p = 0.01) and temperature (r = 0.60, p = 0.03) for both sexes.

We found a total of 17,492 ± 6,805 males and 1,760 ± 634 females when considering the mean abundances of all valid models (Figure 2D). We observed that the model “Phi (*), p (t), pent (t)”, was the most appropriate representation of the abundance of *M. montevidensis* in the area. The Akaike weights (“W” - Table 2) allow us to state that the best model is twice as much supported as the next best model ”Phi (t) p (t) pent (t) N”, since (0, 65 / 0.31) = 2.09.

2.3 Discussion

We obtained an estimated 17,492 number of adult males and 1,760 sexually mature females. Bardier (2018) estimated the size of a *M. montevidensis* population over 72 months to be 581 ± 753 males, 182 ± 141 females, and 20 ± 38 young using statistical models of closed populations in a time-frame that is comparable to the present study. The results of Bardier (2018) differ from our study with regard to the number of individuals captured, as the size of the study area in this study was larger and in different environments. The observed population size fluctuated considerably during the study period, in line with what was observed previously for most anuran species (Vasconcellos & Colli 2009). These fluctuations may be associated with variations in reproductive activity associated with variables that have not been tested (for example, precipitation 7 days before sampling, temperature on sampling days, relative humidity, etc.).

Survival probabilities (Phi) were high in our study (about 95%). High survival rates are expected for larger species with large displacement capacities such as large bufonid toads like *Rhinella diptycha* and *R. rubescens* (see Vasconcellos & Colli 2009). However, some studies suggest that some small species may have high survival rates. For example, Guimarães et al. (2014) encountered a survival rate of 0.98 for *Hylodes asper*, a small frog species typically from streams in the Atlantic Rainforest. These high survival probabilities may be associated with investments in chemical defense against predators and minimizing the chance of parasitic infections (Hantak et al. 2013), as reported by other bufonids (Vasconcelos 2015). For *M. montevidensis*, survival was dependent on sex, being higher for females than for males (Bardier 2018).

Adults of *M. montevidensis* were recaptured almost a year after the first capture, suggesting that individuals of this species may have a longer life expectancy than our studied temporal scale. A recent study in osteochronology determined that the lifespan of adults may varies between five to seven years in females, and between four to eight years in males of the bufonid toad species *Melanophryniscus moreirae* (Jeckel et al. 2015).

The low average probabilities of capture (about 7%) among valid models can be explained by the small body size of these frogs that reduces their detectability (Guimarães et al. 2011). A higher probability of capture in periods with greater precipitation may be related to the reproductive and foraging activity of many species of anurans (for example Hatano et al. 2002, Almeida-Gomes et al. 2007, Machado et al. 2016). Unlike Bardier (2018), in our best models, it included the covariable precipitation, despite the weak relationship found between these two variables, differently from what we expected for the species. Precipitation and humidity are important variables for the survival and reproduction of amphibians (Rollins-Smith 2017), consequently also in their capture, mainly for *M. montevidensis*, whose characteristic is explosive reproduction. There was no difference between men and women in the probability of capture in our study. It cannot be said that this is a real effect or just an effect of sampling.

Higher numbers of individuals occurred during August, September, November and April according to the capture/recapture data, which is in accordance with that found in previous studies (Pereira & Maneyro 2016a, Pereira & Maneyro 2018, Bardier 2018), except April that occurred only in our study. It was not possible to correlate the volume of rain with the number of monthly catches, although one of the best classified models included precipitation as a covariate. However the species was in reproductive activity in the months of greatest capture. These data are corroborated by observations in previous studies on some aspects of the species life cycle. The reproduction of

M. montevidensis is associated with large amounts of rainfall (Maneyro & Carreira 2012) which is necessary to form ephemeral ponds. Our results are in accordance with Maneyro & Carrera (2012), who consider the reproduction of species to be non-seasonal, that is, it can occur several times over a given year. More evidence is needed to corroborate this information, for example, collections over a longer period of time and over a larger area. It is only possible to state that the largest number of catches occurred in autumn and winter. Bardier's (2018) results, on the other hand, detect a seasonal pattern, with individuals more active in the spring and less in the fall. The reproduction of this species is also characterized by a short active period after heavy rains, from hours to a few days, resulting in mass migration to reproductive sites (Pereira & Maneyro 2016b), which would justify a large amount of catch during these periods. We expected a rain effect on some or all of the four parameters, but we did not find a strong and clear effect. We speculate on three broad possibilities - real biological effect, sampling artifact or modeling.

We propose herein a first estimate of *M. montevidensis* population size in Brazil using mark/recapture modeling. This method is especially useful due to its low cost and non-invasive nature. We propose the use of the Popan method as an alternative to estimate population size in the absence of additional data. We recommend that future studies investigate other areas of this species' distribution for longer periods of time to validate results presented here as well as provide additional data that would assist in conservation efforts.

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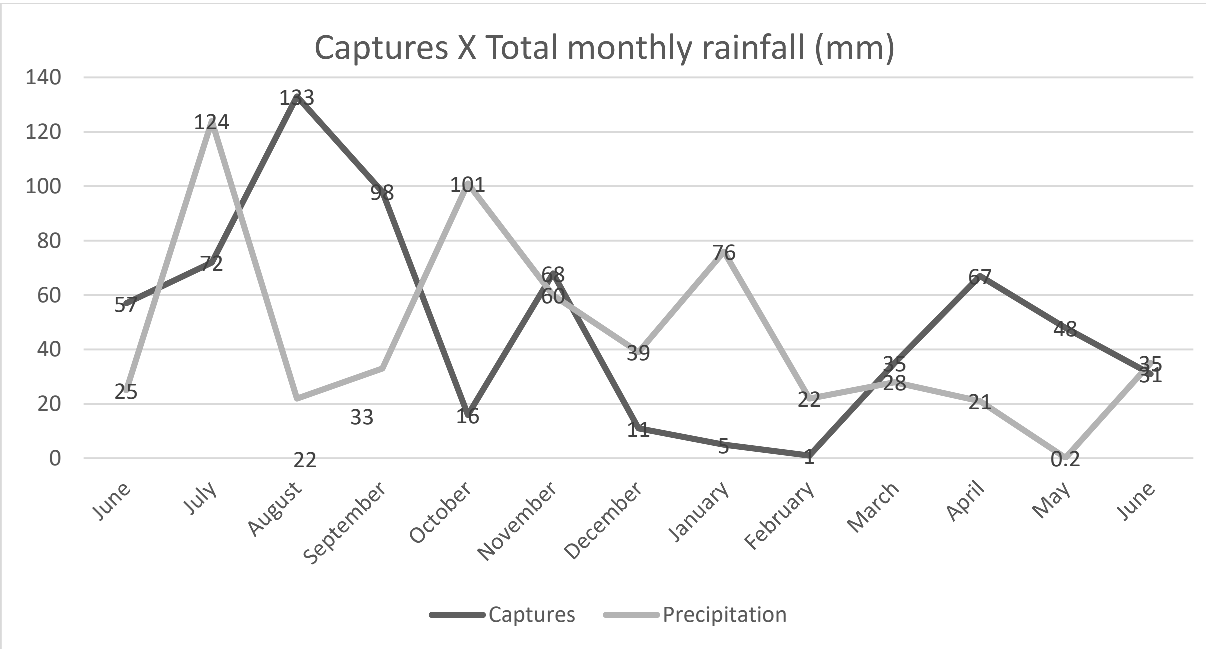
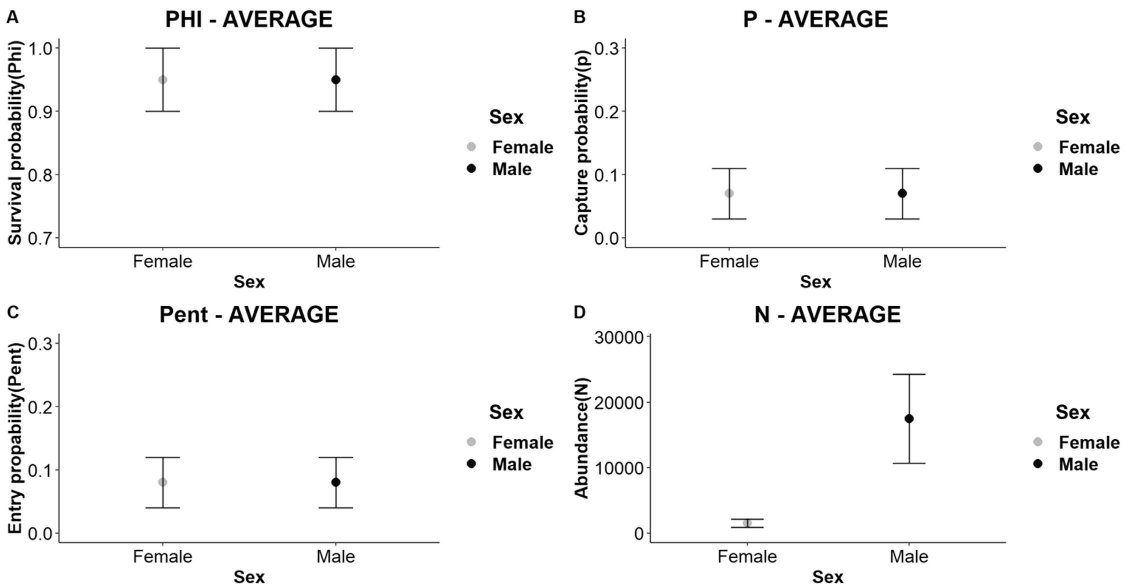
Supplementary material

5 Supplementary Figures and 3 Supplementary Tables.

Supplementary Figure S1. Map showing the location of the study area (red rectangle) in the extreme south of Rio Grande do Sul, close to the border with Uruguay.



Supplementary Figure S2. Figure showing the distribution of Captures X Total monthly rainfall in the 13-month study period.



Supplementary Figure S3. Average probabilities of Phi (A), P (B), Pent (C) and N (D) for males and females of *Melanophryniscus montevidensis* from south Brazil.

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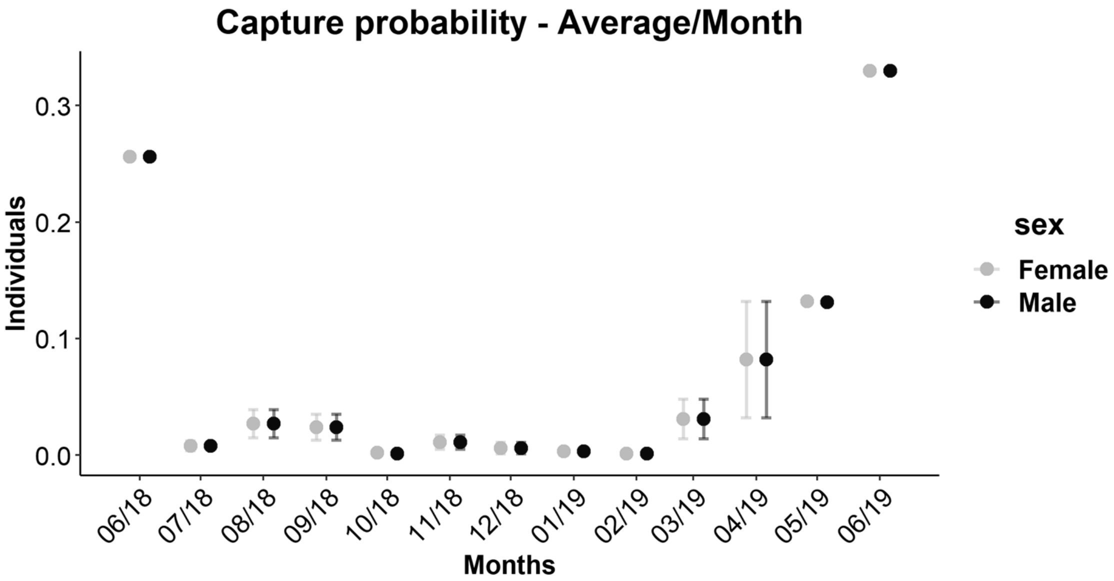
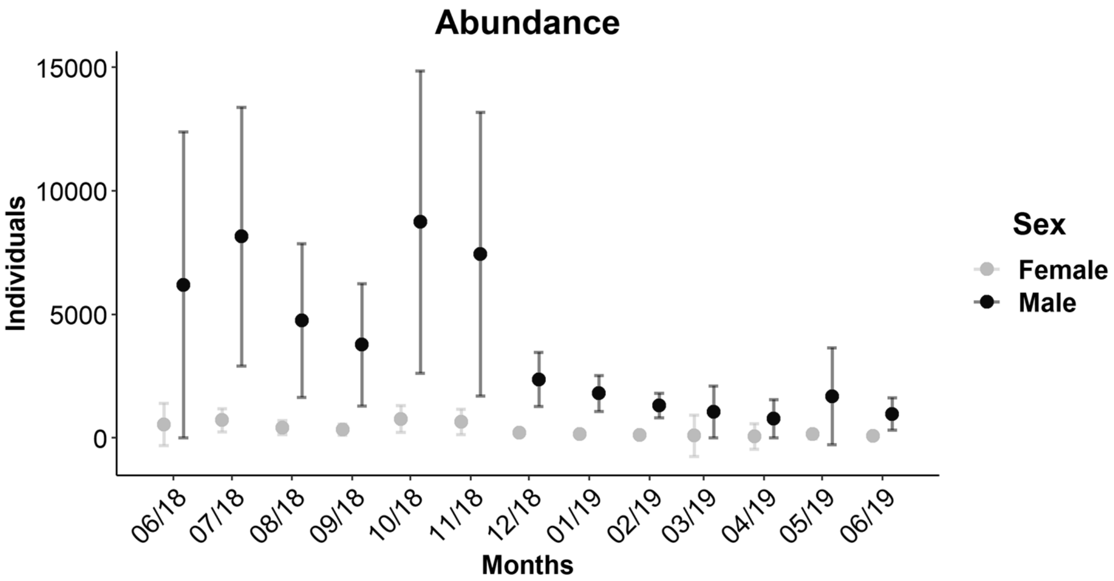


Supplementary Figure S4. Estimates of probability of capture (p) for the population of *Melanophryniscus montevidensis*, generated from the average of the models. 95% confidence interval represented by vertical lines



Supplementary Figure S5. Average estimates of the population size (N) of males and females at each sampling occasion during the 13 months (mean + - SE).

Supplementary Table S1. All 12 candidate models to be included in the analysis for the parameter estimates (Popan). All models are based on additive effects of covariates (temporal, group and climatical) and without temporal or covariable effects (constant parameters).

Models (Popan)
Phi(*) p(t) pent(t) N
Phi(t) p(t) pent(t) N
Phi(t) p(rain) pent(t) N
Phi(t) p(sex) pent(t)N
Phi(sex) p(rain) pent(t)N
Phi(*) p(rain) pent(t) N
Phi(*) p(sex) pent(t) N
Phi(t) p(temperature) pent(t) N
Phi(*) p(temperature) pent(t) N
Phi(sex) p(sex) pent(t) N
Phi(sex) p(temperature) pent(t) N
Phi(sex) p(t) pent(t) N

Supplementary Table S2. General description of the *M. montevidensis* capture and recapture data set obtained from the sampling periods (months). “a” animals captured only once, “b” animals captured and recaptured once and “c” animals recaptured twice.

	MALE	FEMALE	TOTALS
Captured a	584	50	594
Recaptured b	13	1	14
Re-recaptured c	2	0	2
Total	599	51	650

Supplementary Table S3. Comparison of Popan models for capture probability (p), survival probability (Phi), entry probability (Pent) and abundance (N) for *M. montevidensis*. Npar = Number of parameters for each model, AICc = Akaike information criterion (lower values mean better adjustment of the models); DeltaAICc = difference between the first model and the current model, W = adjustment weight for each model and Deviance = difference between the current model and the saturated model.

Models	Npar	AICc	DeltaAICc	W	Deviance
Phi(*) **p**(t) **pent**(t) **N**	28	381.98	0	0.65	-2632.12
Phi(t) **p**(t) **pent**(t) **N**	34	383.44	1.45	0.31	-2643.91
Phi(t) **p**(rain) **pent**(t) **N**	28	388.58	6.60	0.02	-2625.51
Phi(t) **p**(sex) **pent**(t)**N**	28	394.06	12.08	<0.01	-2620.03
Phi(sex) **p**(rain) **pent**(t)**N**	18	417.27	35.29	<0.01	-2575.29
Phi(*) **p**(rain) **pent**(t) **N**	17	417.46	35.48	<0.01	-2572.99
Phi(*) **p**(sex) **pent**(t) **N**	17	421.23	39.24	<0.01	-2569.22
Phi(t) **p**(temperature) **pent**(t) **N**	17	422.46	40.48	<0.01	-2567.98
Phi(*) **p**(temperature) **pent**(t) **N**	17	422.46	40.48	<0.01	-2567.98
Phi(sex) **p**(sex) **pent**(t) **N**	18	422.58	40.60	<0.01	-2569.98
Phi(sex) **p**(temperature) **pent**(t) **N**	18	423.25	41.26	<0.01	-2569.32
Phi(sex) **p**(t) **pent**(t) **N**	29	449.98	68.00	<0.01	-2566.31

3 Considerações Finais

Essa dissertação teve como principal objetivo auxiliar o aumento do conhecimento sobre uma parte da população de *Melanophryniscus montevidensis* em território brasileiro. Obtivemos um número estimado de 17.492 machos adultos e 1.760 fêmeas. O tamanho da população observada flutuou consideravelmente ao longo do período do estudo. Reconhecemos que nossa análise pode não necessariamente cobrir toda a faixa geográfica da população, por isso restringimos nossas conclusões a indivíduos nas proximidades de nossa área de estudo durante o ano.

A maioria das espécies do gênero *Melanophryniscus* ocorre em áreas muito pequenas, são endêmicas, ameaçadas ou deficientes de dados (ZANK et al. 2014; IUCN 2015). A inclusão das espécies em listas de espécies ameaçadas é o primeiro passo para sua conservação. Apesar de Unidades de Conservação constituírem em muitos casos a melhor alternativa de conservação de áreas e espécies, o país ainda sofre com a falta de informação para o manejo adequado dessas unidades. Nesse contexto, o conhecimento gerado por pesquisas científicas são importantes ferramentas para a conservação.

Propomos aqui uma primeira estimativa do tamanho da população de *M. montevidensis* em território brasileiro usando o modelo de marcação / recaptura. Este método é especialmente útil devido ao seu baixo custo e natureza não invasiva. Propomos o uso do método POPAN como uma alternativa para estimar o tamanho da população na ausência de dados adicionais. Esperamos que conhecimento gerado por este trabalho seja capaz acrescentar mais subsídios para o planejamento de programas de conservação, monitoramento em longo prazo. Recomendamos que estudos futuros investiguem outras áreas da distribuição dessa espécie por períodos mais longos para validar ou não os resultados aqui apresentados, além de fornecer dados adicionais que ajudem nos esforços de conservação.