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Tese de Doutorado

Ecologia e Conservação do Sapinho-admirável-de-barriga-vermelha

Melanophryniscus admirabilis (Anura: Bufonidae)

Michelle Abadie de Vasconcellos

Porto Alegre, abril de 2021

Ecologia e Conservação do Sapinho-admirável-de-barriga-vermelha *Melanophryniscus admirabilis* (Anura:Bufonidae)

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Resumo

A maior parte dos anfíbios ameaçados no mundo têm distribuição inferior a 20.000 km². O que aconteceu na história recente de uma espécie para que ela seja encontrada em somente uma faixa restrita de distribuição? É uma espécie rara? É pouco abundante? Ou somente é pouco estudada? Esta tese de doutorado tem como objetivo contribuir com o conhecimento sobre história natural e abundância de um anuro microendêmico, ao mesmo tempo que reavalia seu risco de extinção e sugere ações de conservação. Além da introdução geral (em português), a tese é composta por três capítulos, cada um referente a um artigo científico (em inglês). No primeiro capítulo, abordamos "tudo que você gostaria de saber sobre *Melanophryniscus admirabilis*", o sapinho-admirável-de-barriga-vermelha. Descrevemos o girino, o canto de anúncio, o padrão temporal reprodutivo, as estratégias de reprodução e de defesa, tempo de eclosão do girino e de metamorfose, além do dimorfismo sexual, fidelidade de sítio e longevidade. No segundo capítulo, estimamos o tamanho da única população conhecida da espécie, e sua variação ao longo de oito anos, utilizando modelos de marcação e recaptura aliados à fotoidentificação. No último capítulo, descrevemos a distribuição microendêmica da espécie, além de identificar as ameaças e ordená-las por relevância. Utilizando essas informações e o tamanho populacional, nós reavaliamos o status de conservação da espécie sob os critérios B, C e D da Lista Vermelha da IUCN. Adicionalmente, discutimos ações prioritárias para a conservação do sapinho-admirável e como elas podem reduzir o risco de extinção da espécie. Demonstramos que, embora um grande esforço de ações protetivas precise ser feito, a distribuição de *M. admirabilis* é tão pequena que somente o encontro de uma nova população poderia alterar a sua condição de espécie ameaçada.

Palavras-chave: microendemismo, espécie ameaçada, *Melanophryniscus admirabilis*, história natural, tamanho populacional, distribuição geográfica

Abstract

Most threatened amphibians have a distribution smaller than 20,000 km^2. What happened in recent history for it to have such a restricted range? Is it a rare species? Is it not abundant? Or is it not just studied enough? This doctoral thesis aims to contribute to the body of knowledge about the natural history and abundance of a microendemic anuran while reassessing its risk of extinction and suggesting conservation actions. In addition to the general introduction (in Portuguese), the thesis consists of three chapters, each one referring to a scientific article (in English). In the first chapter, we address "everything you would like to know about *Melanophryniscus admirabilis*", the Admirable Redbelly Toad. We described the tadpole, the advertisement call, the temporal reproductive pattern, the reproductive and defensive strategies, the tadpole's hatching time and metamorphosis, in addition to sexual dimorphism, site fidelity and longevity. In the second chapter, we estimated the abundance for the only known population of the species, and its variation over eight years, using capture-recapture models with photoidentification. In the last chapter, we described the microendemic distribution of the species, besides identifying threats and ordering them by relevance. Using this information and population size, we reassessed the species' conservation status under IUCN Red List criteria B, C and D. Additionally, we discussed priority actions for the conservation of the toad and how they can reduce the extinction risk of the species. We demonstrated that, although a great effort is needed to protect this species, the distribution of *M. admirabilis* is so small that only finding a new population could remove the species from threatened categories.

Keywords: microendemism, threatened species, *Melanophryniscus admirabilis*, natural history, population size, geographic distribution

Sumário

Introdução Geral

“Raridade” é um termo amplamente utilizado na literatura científica, porém nem sempre é empregado no mesmo contexto (GASTON, 1994). Na maioria das vezes, esse termo está associado à baixa abundância ou à pequena área de distribuição de uma espécie e, em geral, abundância e distribuição não são independentes (GASTON, 1994). A relação positiva entre essas duas variáveis é um dos padrões empíricos mais gerais dentro da ecologia: espécies de distribuição restrita tendem a ser pouco abundantes (GASTON; BLACKBURN, 2007). Entretanto, uma espécie abundante na sua pequena distribuição pode ser considerada rara?

Algumas espécies tornam-se raras imediatamente antes de sua extinção, enquanto outras espécies mantêm essa raridade ao longo de toda sua existência. Embora o termo raridade ainda seja amplamente utilizado, Rabinowitz (1981) propôs um conceito mais preciso a partir de três componentes, onde uma espécie extremamente rara tem (1) pequena distribuição geográfica, (2) alta especificidade de habitat e (3) baixa abundância local. No sentido de endemismos restritos, espécies com alcance geográfico estreito e especificidade de habitat estreita são raridades clássicas e estão frequentemente ameaçadas (RABINOWITZ, 1981).

As características demográficas de espécies altamente endêmicas podem indicar um maior risco de extinção e devem ser consideradas nas iniciativas de conservação, uma vez que essas espécies são potencialmente mais sensíveis à estocasticidade ambiental e demográfica. Compreender a diferença entre uma população estável e uma população suscetível à extinção requer conhecimento sobre o tamanho da população e sua variação temporal (INCHAUSTI; HALLEY, 2003). As mudanças no número de indivíduos maduros de uma espécie, assim como a amplitude da distribuição geográfica, são critérios-chave para a avaliação do risco de extinção pela União Internacional para Conservação da Natureza (IUCN STANDARDS AND PETITIONS COMMITTEE, 2019), cuja abordagem tende a estar fortemente relacionada à estrutura proposta por Rabinowitz sobre raridade (TOLEDO *et al.*, 2014).

Esse conhecimento sobre os aspectos demográficos das espécies é especialmente importante para os anfíbios, que são particularmente propensos à extinção (BISHOP *et al.*, 2012; STUART *et al.*, 2004). Embora cerca de 40% das espécies de anfíbios estejam provavelmente ameaçadas (‘best estimate’; IUCN, 2021), parece ser o grupo de vertebrados menos conhecido: somente 12% das espécies têm informações disponíveis sobre aspectos de história de vida ou dados demográficos (CONDE *et al.*, 2019). Esse nível de informação cai para apenas 0,2% quando somente as ameaçadas são levadas em conta (CONDE *et al.*, 2019).

Na falta de informações sobre a abundância e sua tendência ao longo do tempo, a maior parte das classificações de anfíbios em alguma categoria de ameaça é baseada <u>exclusivamente</u> em critérios de distribuição geográfica (Critério B e D2; IUCN, 2021). Isso significa que, em escala global, cerca de 85% dos anfíbios ameaçados não dispõem de informações para serem avaliados por outros critérios (IUCN, 2021). Além disso, no mínimo 90% dos anfíbios ameaçados ou quase ameaçados (‘Near Threatened’ – NT) podem ser considerados endêmicos (*i.e.*, tem distribuição inferior a 20.000 km²; IUCN 2021). No Brasil, das 41 espécies de anfíbios listadas como ameaçadas, trinta e oito (93%) tiveram seu status de conservação definido com base nos critérios referentes à extensão de ocorrência (critério B1) ou à área de ocupação (critérios B2 ou D2; Portaria MMA Nº 444, 2014).

Nos Neotrópicos, a América do Sul se destaca nas taxas de endemismo de anuros (PIMM *et al.*, 2014; VILLALOBOS *et al.*, 2013). Entre esses destaca-se o gênero *Melanophryniscus* (Anura: Bufonidae), que possui uma notável riqueza de espécies endêmicas, além de ser um importante grupo para a conservação (ZANK *et al.*, 2014). Os sapinhos-de-barriga-vermelha, como são chamadas as espécies desse gênero, são representados atualmente por 29 espécies e distribuem-se exclusivamente nas regiões tropical e subtropical da América do Sul (FROST, 2021; ZANK *et al.*, 2014). Muitas das espécies desses pequenos anuros (comprimento rostro-cloacal inferior a 50 mm; GARRAFFO *et al.*, 2012) estão ameaçadas de extinção e apresentam uma distribuição muito restrita (ZANK *et al.*, 2014). Assim como para a maioria dos anfíbios anuros, os aspectos ecológicos e de história de vida dos sapinhos-de-barriga-vermelha ainda estão longe de serem conhecidos. As nove espécies de *Melanophryniscus* que se encontram listadas em alguma categoria de ameaça foram avaliadas com base apenas na sua restrita distribuição (CARREIRA; MANEYRO, 2015; IUCN, 2021; PORTARIA MMA Nº 444, 2014; VAIRA *et al.*, 2012). Entre as espécies do gênero que tive oportunidade de conhecer, uma delas me chamou a atenção. Considerando as chamadas '7 formas de raridade' (RABINOWITZ, 1981), *Melanophryniscus admirabilis*, o sapinho-admirável-de-barriga-vermelha ("Admirable Redbelly Toad", em inglês), se enquadra em dois dos três eixos de raridade: pequena amplitude de distribuição e alta especificidade de habitat. Entre os endemismos, ele é um dos mais extremos. Possivelmente, a vulnerabilidade de uma espécie tão restrita, tão particular e tão iminentemente ameaçada de desaparecer foram as motivações para meu envolvimento pessoal e acadêmico nessa história de conservação.

Melanophryniscus admirabilis foi descrito em 2006, para apenas uma localidade no extremo sul da Mata Atlântica brasileira (DI-BERNARDO; MANEYRO; GRILLO, 2006), cerca de três anos após a publicação das listas de espécies da fauna ameaçada no Brasil (INSTRUÇÃO NORMATIVA MMA Nº 3, 2003) e no Rio Grande do Sul (MARQUES *et al.*, 2002). Portanto, naquele momento, a espécie não estava legalmente protegida por qualquer política ou lei ambiental brasileira. Em 2010, quatro anos após a descrição da espécie, a Fundação Estadual do Meio Ambiente – FEPAM, instituição do estado do Rio Grande do Sul responsável pelo licenciamento ambiental do estado, concedeu a primeira de três licenças para a construção da Pequena Central Hidrelétrica (PCH) Perau de Janeiro. O projeto era localizado no rio Forqueta, a apenas 500 metros à montante do único local de ocorrência da espécie. Nosso trabalho na região foi inicialmente motivado pelo senso de urgência de conservação do sapinho-admirável-de-barriga-vermelha, já que nada se sabia a respeito da espécie que, naquele momento, corria o risco de ser extinta.

Graças a um esforço multi-institucional, a licença para a construção da PCH foi cancelada (FONTE *et al.*, 2014), e a situação serviu de gatilho para iniciarmos um projeto de monitoramento da espécie a longo prazo. Nesta tese de doutorado, apresento uma compilação (de parte) do trabalho que venho desenvolvendo com *M. admirabilis* ao longo dos últimos 10 anos, praticamente durante toda minha vida acadêmica.

Esta tese está estruturada em três capítulos, cada um correspondendo a um artigo. No primeiro capítulo, abordamos aspectos da história natural do sapinho-admirável-de-barriga-vermelha, passando pela descrição do girino, descrição do canto de anúncio, dimorfismo sexual, comportamento reprodutivo e longevidade. A abordagem utilizada vai desde observações oportunísticas de campo até marcação e recaptura aliada a técnica de fotoidentificação.

No segundo capítulo, estimamos o tamanho da única população da espécie e sua variação ao longo do tempo, abordando o uso de três estimadores: Modelo de População Fechada (OTIS *et al.*, 1978), Modelo de População Aberta – POPAN (SCHWARZ; ARNASON, 1996) e Desenho Robusto (POLLOCK, 1982). Para isso, construímos um banco de dados de marcação e recaptura ao longo de oito anos.

De maneira geral, quando se trata de espécies com distribuição restrita, espera-se que elas apresentem um risco de extinção mais elevado, pois são mais suscetíveis a efeitos estocásticos demográficos ou ambientais. Por outro lado, será possível que uma espécie microendêmica, como o sapinho-admirável-de-barriga-vermelha, não apresente um alto risco de extinção? No último capítulo, apresentamos a distribuição atual da espécie, fazemos a reavaliação do estado de conservação, ranqueamos ameaças e discutimos as ações prioritárias para tentar reduzir o grau de ameaça sobre a espécie.

Cada capítulo foi formatado conforme a norma da revista para a qual será submetido (informado no início de cada capítulo). Para facilitar a leitura, as tabelas e figuras foram inseridas no corpo de texto. Na última seção, após o terceiro capítulo, fiz uma compilação das principais conclusões de cada um dos artigos e como que eu espero que essa tese contribua para redução do risco de extinção do sapinho-admirável-de-barriga-vermelha e inspire outras(os) pesquisadoras(os) a trilhar o caminho da conservação.

Capítulo 1

Morphology, reproduction, longevity, and bioacoustics of the microendemic and threatened Admirable Redbelly Toad

Este manuscrito segue o formato da revista *Zoology*. A fim de facilitar a leitura, as figuras e tabelas foram inseridas no corpo do texto.

Morphology, reproduction, longevity, and bioacoustics of the microendemic and threatened Admirable Redbelly Toad

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Abstract

Although information about the natural history of a species is fundamental for conservation planning, little is known about threatened species, and this is more critical for amphibians. One of the most relevant aspects of amphibian natural history is its reproductive behaviour, particularly for explosive breeders that are often considered to be rare. *Melanophryniscus admirabilis* is a microendemic and Critically Endangered toad for which the lack of such information almost resulted in its extinction. In this study, we address aspects of morphology, reproduction, bioacoustics, and longevity of *M. admirabilis* and provide insights on the behaviour of *Melanophryniscus* species. From seven years of capture-recapture data and *in situ* observations, we found that the species is a seasonal explosive breeder and we recaptured individuals seven years from the first capture. The species has female-biased sexual size dimorphism, diurnal and nocturnal breeding activity and site fidelity. We also describe the tadpole and the advertisement call. Similar to other *Melanophryniscus* species, *M. admirabilis* characteristics seem to be adapted to ephemeral and particularly risky and noisy environments. Based on this study’s findings, it is possible to improve the identification of suitable habitats for the species, as well as facilitating in-situ protection.

Keywords

Melanophryniscus admirabilis, lifespan, capture-recapture, tadpole, advertisement call

1. Introduction

Knowing the natural history of a species is a fundamental step to effectively plan conservation actions. When there is limited knowledge about a species, it often becomes difficult to assess its conservation status. This can be of particular concern for rare or poorly known species, as the lack of information can lead to a Data Deficient (DD) categorization, when, in fact, it can be actually threatened (IUCN, 2021). The availability of information about a species, and its consequent categorization or not in a red list, can determine how it will be deemed by decision-makers.

Besides being the least known tetrapod group, with only 12% having any demographic information (Conde et al., 2019), amphibians are the most threatened vertebrate class globally (IUCN, 2021). When only threatened amphibians are taken into account, the level of demographic information drops to 0.2% (Conde et al., 2019). Out of the 7,212 species assessed by the IUCN (2021), 34% are currently listed in a threatened category (Critically Endangered (CR), Endangered (EN) or Vulnerable (VU)). While a considerable proportion of amphibian species is at risk of extinction, amphibians are also the group of tetrapods with the highest proportion of Data Deficient (DD) species, with 16% listed as such (IUCN, 2021). Estimates indicate that up to 50% of all amphibian species could be threatened (González-del-Pliego et al., 2019; IUCN, 2021). Alarmingly, other studies have indicated even higher estimates (Howard and Bickford, 2014).

Anurans are the most diverse group of amphibians, currently comprising more than 7,300 species (Frost, 2021). They present the greatest diversity of reproductive modes (Haddad & Prado, 2005) and their reproductive behaviour is one of the most relevant aspects of their life history. This is especially true for species with explosive reproduction. Explosive breeders (*sensu* Wells, 1977) usually spend much of their lives in hiding, only emerging during short periods of time to reproduce. For this reason, they are often considered to be rare. The breeding period can last from 2 days to a few weeks (Wells, 1977) and understanding the timing of these events may optimize research and management planning.

The South American Redbelly toads, genus *Melanophryniscus* (Anura: Bufonidae), exemplify the typical knowledge shortfalls for Neotropical anurans. They are small toads, explosive breeders with poor knowledge about their natural history and ecology, despite great interest in their conservation (Zank et al., 2014). Out of the 29 valid species, more than half (16 spp.) have been described in the last 20 years (Frost, 2021), suggesting that the genus' species richness is still underestimated. Fourteen species are endemic to the Atlantic Forest biome (Frost, 2021), a hotspot for conservation (Myers et al., 2000). Out of these, 4 are considered to be threatened (CR, EN, VU), 2 are listed as DD and 1 as Near Threatened (NT) in the Brazilian Red List (Portaria MMA Nº 444, 2014), with natural history and reproductive biology information available for only a few species (Baldo et al., 2014; Caorsi et al., 2014).

The microendemic and Critically Endangered Admirable Redbelly Toad (*Melanophryniscus admirabilis* Di-Bernardo, Maneyro, and Grillo, 2006) is an emblematic example. The lack of scientific knowledge about this species almost resulted in its extinction. It was described from a single site in the southern edge of the Brazilian Atlantic Forest, in 2006, four years after the last national and regional red list assessments, which were valid at that time (Instrução Normativa MMA nº 3, 2003; Marques et al., 2002). For this reason, its extinction risk was not assessed, and therefore it remained unprotected by any conservation policy or national environmental law. In 2010, the regional government granted a licence for the construction of a small hydroelectric power plant exactly in the type locality. Even though at the time, the only available information about the species was its original description, a multi-institutional effort led by the authors of this study allowed for the quick assessment of its conservation status (Fonte et al., 2014). Due to its extremely reduced area of occupancy (AOO = 0.035 km²) and the imminent threat imposed by the construction of the power plant, the species was further listed as CR at the global (IUCN SSC Amphibian Specialist Group, 2013), national (Portaria MMA Nº 444, 2014), and regional (Rio Grande do Sul, 2014) levels, remaining in this category at all levels since then (Haddad et al., 2018).

The licence for the construction of the hydroelectric power plant was cancelled (Fonte et al., 2014), and the situation triggered the beginning of a long-term monitoring project focused on the species. The main objective of this paper is to present the knowledge produced over seven years of population monitoring, from capture-recapture data and *in situ* observations. Here, we provide information about the population (longevity and sexual dimorphism), reproduction behaviour (site fidelity, reproductive strategies, temporal breeding pattern, oviposition and time to metamorphose), description of the tadpole and the advertisement call, and aspects of the defensive behaviour of *M. admirabilis*. Our results contribute to Neotropical anurans knowledge and provide insights into the behaviour of *Melanophryniscus* species. Moreover, we expect that the scientific knowledge presented here will contribute to implementing efficient conservation actions to protect the only known population of the Admirable Redbelly Toad.

2. **Materials and methods**

2.1 Study area

We performed the study at Perau de Janeiro (28° 51’ 25.3” S; 52° 18’ 12.3” W), the Admirable Redbelly Toad type and only locality, at the southernmost range of the

Atlantic Forest biome. Located on the slopes of the southern border of the Brazilian Southern Plateau, in the Brazilian state of Rio Grande do Sul, Perau de Janeiro is part of a ca.5 km^2 forest patch, one of the largest forest remnants in the region.

The climate of the region is classified as Subtropical Humid, without dry season and with hot summers (*Cfa*, Koeppen's climate classification; Alvares et al, 2013). Precipitation is evenly distributed throughout the year (1600 mm; Minella et al., 2009) and the temperature determines the seasonality of the region, with four well defined seasons (Supplementary Information Figure S1; Zepner et al., 2020). The *Cfa* climate type is characterised by average temperatures varying between -3 ºC and 18 ºC for the coldest month (July) and above 22 ºC for the hottest month (January). Negative temperatures are common during the winter, with an average of 19 frost days per year (Moreno, 1961).

Our study area is part of the Taquari-Antas River basin, at an elevation of around 550 m above sea level. It extends along 700 m of steep forested slopes of the Forqueta River. We sampled along ca. 400 m on the left margin, where most individuals concentrate to breed on pools formed on volcanic rock outcrops along the river bank. These flattened river bank outcrops are 1-14 m wide, from the river margin to the forest edge. Even though we concentrated our efforts on this site, we also made a few registers on the right margin of the river.

2.2 Data collection

Our final sampling effort totalled 122 days collecting capture-recapture data, starting in October 2010 and finishing in December 2017, at different intervals. Over the years, as we learned about the species, we adjusted our sampling design over this period. Fieldwork was carried out between October 2010 and October 2011 (monthly), July 2013 and August 2013 (monthly), and between August 2013 and October 2014 (bimonthly). In 2016 and 2017, a local resident and project partner visited the site once a week and sent us information about the Admirable Toad activity. From this information, we conducted surveys from July to December, whenever we detected breeding activity (see below for details). Fieldwork varied from two (2010-2011) to eight (2013-2014) continuous days in each survey. In 2012 and 2015 we did not survey the population.

We arbitrarily divided the flattened river bank outcrop into 27 sectors (15 metres in length). We used Visual Encounter Survey (Crump and Scott Jr., 1994) and Surveys at Breeding Sites (Scott Jr. and Woodward, 1994) to search for individuals during the day, starting about 9 am and stopping when new individuals were no longer detected, sometimes at night (i.e. surveys were not limited by time). Captured toads were sexed, measured (snout-vent-length – SVL; calliper to the nearest 0.01 mm), photographed and then released at the same place of capture. We classified individuals into three categories: juvenile, male (adult) or female (adult). We considered individuals as adults when sex recognition was possible, by the presence of brownish nuptial pads at the base of the male thumb (absent in females), by the calling behaviour of males, or by the presence of eggs in females. We considered as juvenile all individuals that did not have any secondary sex character (which in this study were those smaller than 28 mm).

We marked individuals using the Photographic Identification Method (PIM) following a semi-automatic procedure with the software Wild-ID (Bolger et al., 2012). We photographed the entire ventral surface of black pigmentation on each animal and used its unique colour pattern resulting from the yellowish-green glands for individual recognition (Caorsi et al., *in prep*). We took two to five pictures of each captured individual and selected the best one, which was cropped to the area of interest, between the throat and the cloaca, eliminating as much of the background as possible. The software Wild-ID returned the 20 closest matches in our photo catalogue. This list was subsequently examined by a researcher who attributed a new or existing individual identity to the captured toad. This approach was successfully tested and applied to this species (Abadie et al., 2021 - Chapter 2; Caorsi et al., *in prep*) and other South American Redbelly Toads (*e.g*. Bardier et al., 2019; Caorsi et al., 2012; Elgue et al., 2014). We used the capture-recapture method to address the longevity, site fidelity and dispersal of individuals.

To infer reproductive temporal pattern and reproductive strategies, we recorded the following evidence of reproductive activity: the presence of calling males, amplectant pairs and recently laid clutches (indicating current breeding activity), or presence of old clutches and tadpoles (indicating previous breeding activity). We considered as explosive reproductive events the aggregation of adults at the breeding site coupled with associated reproductive behaviour (Wells, 1977).

We recorded the number of eggs per clutch and time of hatching by monitoring *in situ* 17 recently laid clutches until hatching, in different pools at the breeding site in 2011 (n= 12) and 2013 (n=5). Furthermore, in October 2014, we collected clutches to conduct *ex situ* experiments for another study on the exposure to pesticides and the antioxidant capacity of the species (da Silva et al., 2021), allowing us to observe the development of tadpoles (control group) until metamorphosis. To perform this experiment, we collected clutches in different pools along the breeding site and, soon after hatching, tadpoles were randomly distributed in glass aquaria. The tadpoles were kept in the laboratory, with photoperiod set to 12 h light: 12 h dark, room temperature set to 23 ± 1 °C and fed once daily with commercial fish feed. For more details, see da Silva et al. (2021). The newly metamorphosed juveniles were fixed in 10% formalin.

Vocalisations were recorded in October 2014 using a Marantz PMD 670 and a Sennheiser ME 67 directional microphone at a distance of about 50-100 cm from the calling individual. To obtain the best signal-to-noise ratio and avoid distortion, the recording level was adjusted manually. Recording level, microphone distance and orientation were kept constant during each session. Sounds were recorded using a sampling rate of 48 kHz, 16-bit depth and uncompressed WAV as output format. After each recording, we measured the air and water temperature of the specific individual calling site using a digital thermometer (accuracy 0.1ºC). One recorded individual was collected (voucher) and fixed in 10% formalin.

To describe the tadpole, we randomly collected them in the field from different pools, in October 2011. Following the standard methodology to describe tadpoles, we used tadpoles between Gosner stages 32 - 37 (n= 20; Gosner, 1960). Collected tadpoles were fixed in 10% formalin.

Notes of reproductive and defensive behaviours were described based on opportunistic observations. All field procedures followed recommendations by the Chico Mendes Institute for Biodiversity Conservation (ICMBio) under licences number 40004-5 and 10341-1 issued by the Biodiversity Information and Authorization System (SISBIO), and Institutional Research and Ethics Committees on Animal Use at UFRGS (Projects 19541, 25528, 25526) to Márcio Borges-Martins. All collected individuals were deposited in the Coleção Herpetológica do Departamento de Zoologia da Universidade Federal do Rio Grande do Sul (UFRGS), in Brazil, under the numbers UFRGS XXXX (newly metamorphosed juveniles), UFRGS 7070 (vocalizing male voucher) and UFRGS 6358 (tadpoles).

2.3 Data analyses

To test for sexual size dimorphism, we used only the data collected in 2013 and 2014 since all individuals over that period were measured by the same researcher (TM), ensuring standardisation. We applied a Simple Linear Model to test whether the size is associated with the individuals' sex.

To infer lifespan (longevity), we analysed the time between captures, using the entire capture-recapture database (2010 – 2017). To infer site fidelity, we used the maximum distance (in sectors) between two captures of the same individual. We applied a binomial Generalised Linear Model to determine if movements were influenced by sex (male or female) or maturity (adult or juvenile). Analyses were performed using R version 4.0.2 and results were considered significant when $p < 0.05$.

To describe the annual reproductive activity pattern, we used the number of captured individuals (including the zeros) in a sampling day from two data subsets: 2010-2011 and 2013-2014. We assumed that all adult toads at the breeding site were reproductively active adults. First, we converted sampling dates into Julian days and then treated them as angles (0° to 360°). To identify if the distributions of sampling and frequency of captures were significantly different from a uniform distribution ($p < 0.05$), we performed Rayleigh's test using the "circular" R package (Agostinelli and Lund, 2017). All statistical analyses were performed using R version 4.0.2.

The advertisement calls were analysed using Raven Pro v. 1.4 software for Mac OS X (Bioacoustics Research Program 2011). We obtained temporal properties from oscillograms (temporal resolution = 5.33 ms) and spectral information using Fast Fourier Transformation (512 points Hann window; frequency resolution = 188 Hz). For call variables, the nomenclature follows Kohler et al. (2017) and Emmrich et al. (2020),, and for vocal repertoires and behaviours, Wells (2007). Since the advertisement call of the *Melanophryniscus* species is composed of two distinct segments (call part A and call part B), we analysed the following parameters: call duration (s), call part A duration (s), number of pulses per call part A, pulse duration of call part A (s), pulse interval of call part A (s), peak frequency of call part A (Hz), interval between call part A and call part B (s), call part B duration, number of pulses per call part B, pulse rate in call part B (pulses/sec), pulse duration of call part B (s), pulse interval of call part B (s), and peak frequency of call part B (Hz).

For the description of the tadpole, we followed the anatomical nomenclature suggested by Altig and McDiarmid (1999). We took eight measurements (mm) with the aid of a stereoscope: total length (TL), body length (BL), tail length (TAL), tail musculature height (TMH), maximum tail height (MTH), internarial distance (IND, measured between the internal edges of narial apertures), interorbital distance (IOD, the distance between interior edges of eyes), and tail musculature width (TMW). To allow comparisons with other descriptions of *Melanophryniscus* spp., we took additional measurements suggested by Lavilla & Scrocchi (1986): body maximum width (BMW), body width at nostrils level (BWN), body width at eye level (BWE), body maximum height (BMH), rostro-spiracular distance (RSD, measured horizontally from tip of snout to posterior edge of the tube), frontonasal distance (FN, from the tip of snout to anterior edge of nostrils), eye diameter (E), oral disc width (OD, disc measured folded), dorsal gap length (DG) and ventral gap length (VG).

3. Results

3.1 Populational Data

Between 2010 and 2017, we obtained 4919 captures of 2069 individuals. Of these, males were more frequent (1298) than females (631) and juveniles (160). Twenty individuals were first captured as juveniles and recaptured as adults in the following years. The recapture rate was high, with 40% of the individuals (830 individuals) captured at least twice, while 332 (16%) were recaptured three times or more in different year intervals.

Females measured between 31.45 and 41.34 mm (36.26±2.06 mm, n = 302), while males measured between 29.00 and 39.19 mm (33.18±1.49 mm, n = 760). Although there is considerable overlap between measurements of both sexes, we observed sexual dimorphism in size (F-value = 730.54, p<0.01, df = 1060; Supplementary Figure S2).

Considering the maximum interval between the first and last captures of individuals, the maximum recorded lifespan was 7 years: 25 from 169 adult individuals which however could live longer, since all of them were recaptured in 2017, the last year of this monitoring (Figure 1). We do not have surveys from 2012 or 2015 and, consequently, certain combinations between first and last captures were not possible (e.g. 2010-2012, 2012-2014, 2012-2017, 2010-2015, 2013-2015, 2015-2017).

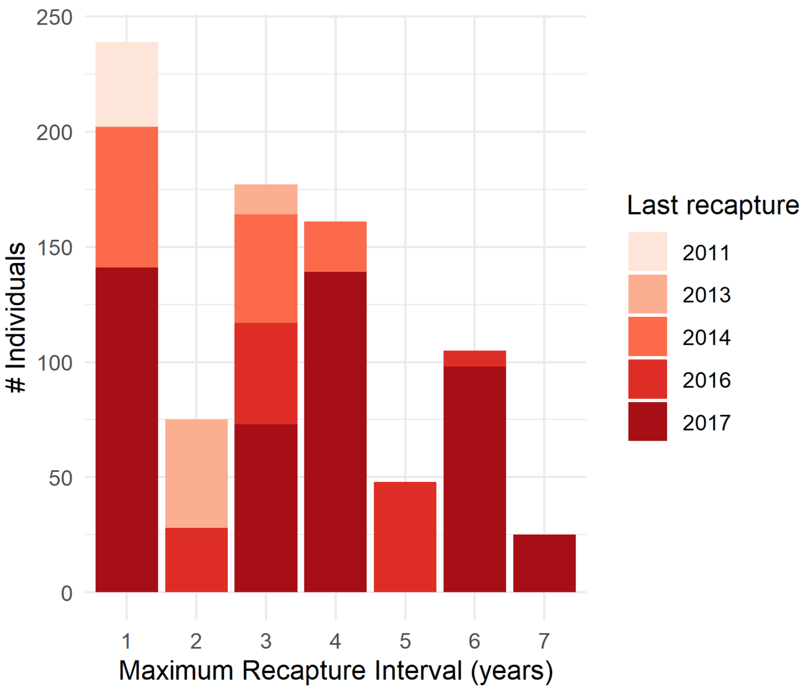

Figure 1. The maximum time interval between first and last captures of *Melanophryniscus admirabilis* over the study duration (2010 – 2017).

3.2 Reproductive activity

When toads were not breeding, they were hardly found on the riverbank, but some could be found in small hollows, leaf litter or other shelters in the adjacent forest (Supplementary Figure S3), but not far from the breeding site in the rocky riverbank. The maximum distance from the river we found toads, into the forest, was about 50 metres from the river bank (a walking male).

Site fidelity was high, with most recaptures occurring in the same (38%, n = 394) or the adjacent (28%, n = 295) riverbank sector from the first capture. However, a few recaptures (n = 2) correspond to individual movements of up to 300 m (Supplementary Figure S4). Site fidelity was not associated with sex, but with sexual maturity. Juveniles had a higher probability (62%; CI = 0.44 – 0.78) of changing sectors than adults (33%; CI = 0.30 – 0.36; $p<0.01$).

We recorded amplectant pairs not necessarily associated with explosive breeding events. We observed some evidence of reproductive activity (current or previous) in almost all months of the year, except for April, May and June (Figure 2). However, the number of captures at the breeding site varied widely among the months (2010-2011: $r = 0.7144$, $p<0.01$; 2013-2014: $r = 0.7757$, $p<0.01$), despite the uniform distribution of sampling effort over both periods considered in this analysis (2010-2011: $r = 0.1423$, $p=0.5266$; 2013-2014: $r = 0.0591$, $p=0.8023$; Figure 2). The explosive breeding events were concentrated between July and December.

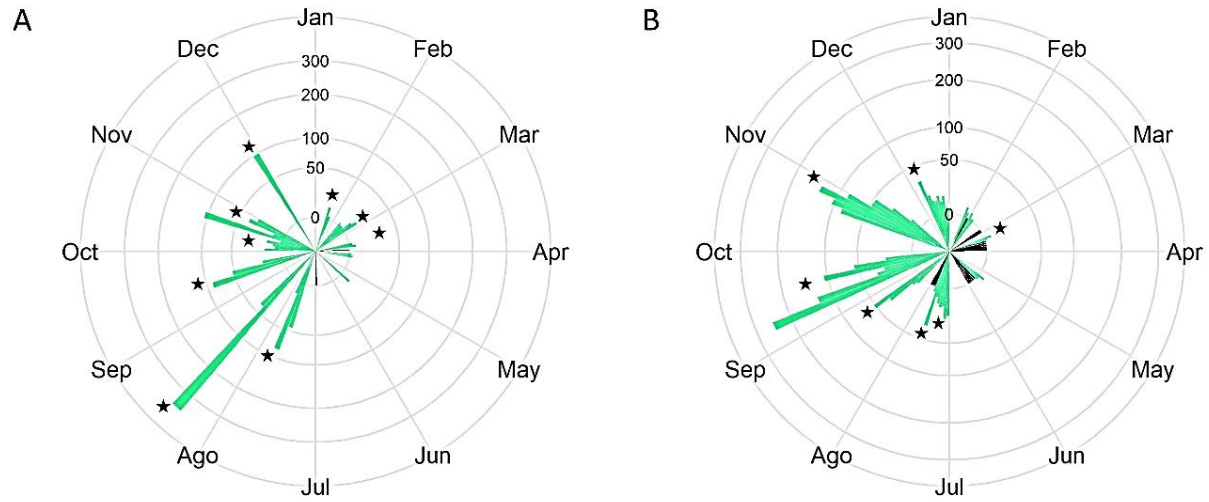

Figure 2. Seasonal pattern of reproductive activity of the Admirable Redbelly Toad from 2010 to 2011 (A) and from 2013 to 2014 (B), based on the number of adults captured at the breeding site. Field trips were conducted monthly in 2010-2011 and bimonthly in 2013-2014. Each bar represents the number of captures on a sampling day. The black bar indicates no capture on a sampling day. The black star represents any evidence of breeding activity, current or previous, based on calling males, amplectant pairs, clutches, and tadpoles.

In explosive breeding events, toads could be easily found, because hundreds of individuals aggregated on the rocky outcrop along the riverbank, using small pools as vocalisation and oviposition sites (Figure 3). In these events, it was more common to hear release calls than advertisement calls. In October 2013, individuals bred at least four consecutive days, during an explosive breeding event, and in September 2014 we recorded two events with two days between them (3 and 2 days of breeding activity, respectively). The maximum number of individuals recorded on the same day was 361 (August 2011). On rare occasions, some toads mated in small pools inside the forest, but always within a few meters of the river margin.

We observed diurnal and nocturnal reproductive activity, during explosive breeding events. Most males vocalised inside the water at or near small and shallow temporary pools (<1 m^2 of surface and <20 cm depth, Bordignon, 2019; Figure 3A-B). On occasion some males vocalised hidden under rocks or in rock crevices or underneath herbaceous vegetation (at the edge between the forest and the rocky outcrop), mainly during the day. The temporary pools are filled directly by rainwater and are maintained by the water that drips from the riparian forest growing on the steep slopes. After heavy rains, the river overflows and “washes” the rocky margins (Supplementary Figure S5), sometimes carrying egg clutches and tadpoles to the river. During the austral winter (June to August), the pools are filled with fallen leaves from the surrounding deciduous forest. In summer (December to February), at the end of the main reproductive period, we noted that the availability of water in pools is reduced, and there is an accumulation of fallen fruits from the abundant Myrtaceae trees. In this period, we recorded little reproductive activity restricted to pools free of rotting fruit.

In explosive breeding events, we recorded intense male-male combat and struggles including amplectant pairs and up to three additional dislocating males (Figure 3D). Besides, we noticed interspecific amplexus between a male and a juvenile of *Rhinella icterica* (Figure 3G), and males mating with dead individuals and inanimate objects, such as algae and a fish gut (Figure 3E-F). We recorded males often embracing other males. When this happens, the embraced male emits a release call, causing the clasping male to release him.

Figure 3. *Melanophryniscus admirabilis* breeding activity and behaviour at the breeding site: (A) Some individuals at a small temporary pool in the rocky river bank outcrop; (B) a male calling; (C) a pair in axillary amplexus in the water; (D) struggle between three males and an amplectant pair; (E) necrophiliac amplexus; (F) a male embracing an alga; (G) an interspecific amplexus with a juvenile of *Rhinella icterica*;

(H) a pair spawning in a pool with other clutches from other pairs; (I) a newly released spawning with about 19 eggs; (J) three clutches released at different times; (K) tadpoles almost hatching from clutch; (L) tadpoles at Gosner (1960) stage 27-29; (M) tadpoles at Gosner (1960) stages 30-31, showing evident golden iridophores. Photos: Rômulo Silveira (A), Valentina Caorsi (B), Simone Leonardi (H), Michelle Abadie (C–G, I–M).

We followed two amplexus until the female finished oviposition, both events during the day (January 2011 and October 2016). In the first one, we encountered the amplectant pair in a small shaded pool. While in amplexus, the female remained still with only her head sticking out of the water. Her feet and hands remained resting on the pool's surface. The male contracted his abdomen repeatedly (about one contraction per second). At a certain point, the female dived, taking the male into the water. The male then started rubbing his feet, collecting the eggs from the female's cloaca, and leading to his cloaca. Following this, the male left the eggs in submerged substrate (about 20 eggs). The female, still carrying the same male, left this pool and the process began again in another one. After they deposited the second egg clutch, we collected this amplectant pair in a plastic bag with water and they deposited two more clutches inside the bag, one with 31 eggs and another with 20 eggs. We recorded the second amplectant pair (Supplementary Video S6) when the female was spawning, and the male was collecting submerged leaves in the water with his hind feet and leading them to his cloaca to deposit the eggs. Even after laying the eggs, the female continued to do wavy abdominal movements until leaving the pool, carrying the male.

Melanophryniscus admirabilis' egg clutch is transparent, formed by gelatinous egg masses which are hatched inside the small, shallow pools on the volcanic flattened river bank outcrop, frequently adhered to vegetation, fallen leaves or just floating on the water. Immediately after oviposition, eggs were very close to each other (Figure 3I). A couple of hours later, they swelled to a point where they could be distinguished by eye.

We counted *in situ* five eggs as the minimum and 36 eggs as the maximum in each clutch (n = 17; mean = 15.4±6.6.). After about four or five days the tadpoles had begun to hatch from the eggs (Figure 3L). In the lab, we observed that on the 10th day from the hatching, the hind limbs had begun to appear (Gosner stage 26-30), and the forelimbs three days after. The tail resorption began on the 14th day from the hatching and was completed on the 15th day. On the 16th day, the metamorphosis was complete in all individuals, making a total of 20-21 days for the full development (egg-to-juvenil).

3.3 Advertisement Call

Call recordings were carried out along the breeding site under air temperatures ranging between 17.5 and 23.9 °C. The advertisement call of *M. admirabilis*, based on four individuals (total of nine calls), has a duration of 21±1.5 seconds and it is comprised of segments A and B (Figure 4; Supplementary Table S7). Part A has 2–19 single frequency modulated notes (0.1 seconds each), each one comprising one pulse separated by intervals of 2±2.4 seconds. Notes in part A present frequency modulation increasing from 1.92±0.13 kHz to 2.04±0.17 kHz, reaching the peak frequency on the second third of the note, then decreasing to 1.98±0.17 kHz. Call part B is a train of unmodulated pulses emitted at a rate of 50.8±2.0 pulses per second, with short time intervals (0.014±0.002), and lasting from 1 to 6.26 (4.1±1.7) seconds. The interval between call part A and B is on average 1.5±4.1 seconds. The peak frequency is on average 1.9±0.38 kHz in part A and 1.7±0.22 kHz in part B.

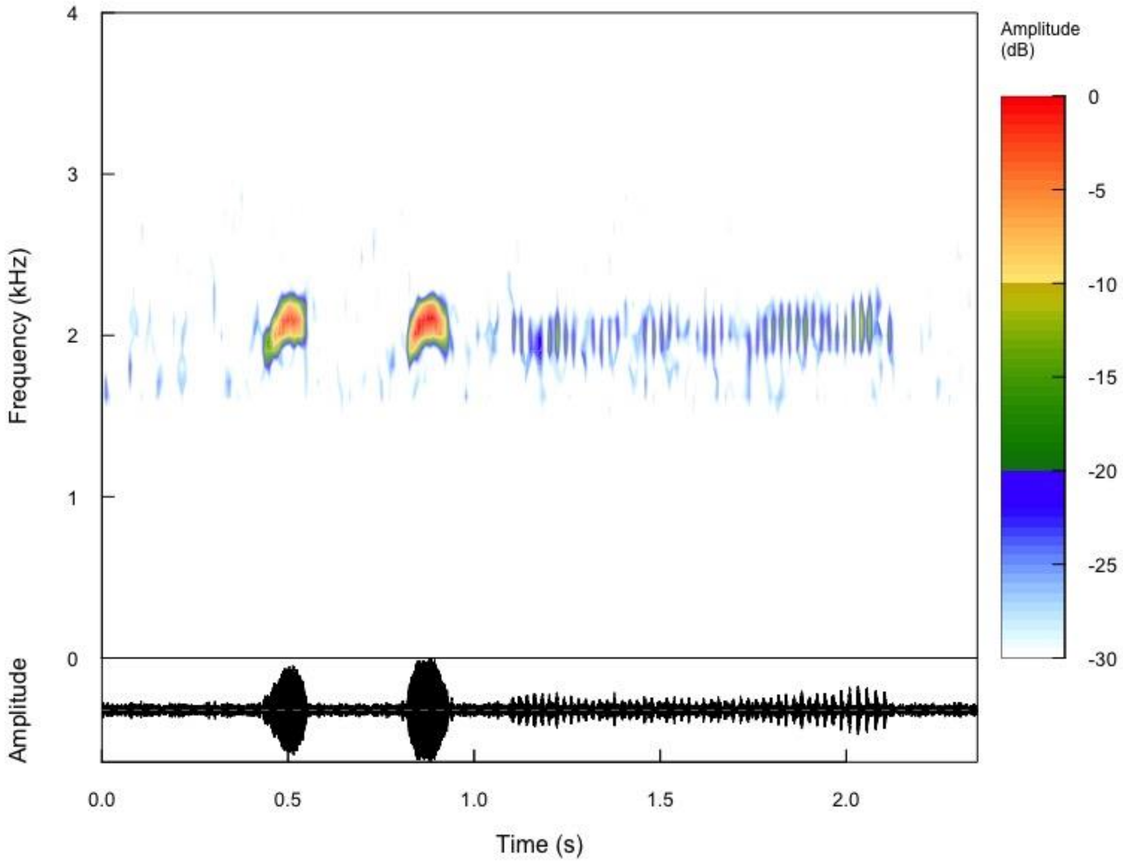

Figure 4. Advertisement call of *Melanophryniscus admirabilis*: spectrogram and oscillogram of two notes from the first segment (call part A) and the second segment (call part B).

3.4 Tadpoles

The total length of *M. admirabilis* tadpoles (Figure 5) at stages 32 – 37 was between 13.9 and 17.6 mm (Supplementary Table S8). Body shape slightly depressed (BMH/BMW= 0.85 ± 0.06 mm) and oval in dorsal view; body length approximately 40% of the total length, body width approximately 72% of the body length, and body height approximately 61% of the body length; the maximum body width was just behind the eyes. The snout was rounded in dorsal and lateral views, and the gular region was flat. Nostrils rounded, positioned dorsally with internarial distance about 34% of the body width, visible in frontal, dorsal and lateral views, opening anterolaterally, with a small projection on the marginal rim all around. Eyes were medium (E/BWE = 0.19 ± 0.02 mm) and placed dorsolaterally (interorbital distance approximately 49% of the body width). The pineal end-organ, present as an unpigmented spot between the anterior edges of the eyes, was almost imperceptible. The spiracle was sinistral, single, very short, opening at the end of the middle third of the body (RSD/BL = 0.63 ± 0.04 mm), at the lateral midline, and directed posterodorsally, with the inner fused to the body wall. The gut loop ("point de rebroussement", *sensu* Hourdry & Beaumont, 1985) is placed at the left of the abdomen. The vent tube was conical, short, running along its right side and associated with the ventral fin; the vent opening was dextral. The tail was straight, medium-sized (TAL/TL = 0.60 ± 0.02 mm) and slightly lower than the body (MTH/BMH = 0.82 ± 00.7 mm); myomeres were evident through the whole caudal musculature extension, narrowing gradually toward the end of the tail, not reaching the rounded tip of the tail; the dorsal fin was originated before the body-tail junction and reached its maximum height at the middle region, staying almost constant until converging with the ventral fin, at the end of the tail; the ventral fin was originated on the left side of the vent tube and its height was almost uniform until the tip.

The oral disc was anteroventral, not visible dorsally, with almost 50% of the body width and laterally emarginate (Figure 5, Supplementary Table S8). The marginal papillae were arranged in a single row, with wide rostral and medium-sized ventral gaps (66% and 50% of the oral disc, respectively). Labial tooth row formula (LTRF) was 2/3 in all analysed specimens (n=20). The upper jaw sheath was slightly curved, the lower sheath was smaller and V-shaped, both serrated.

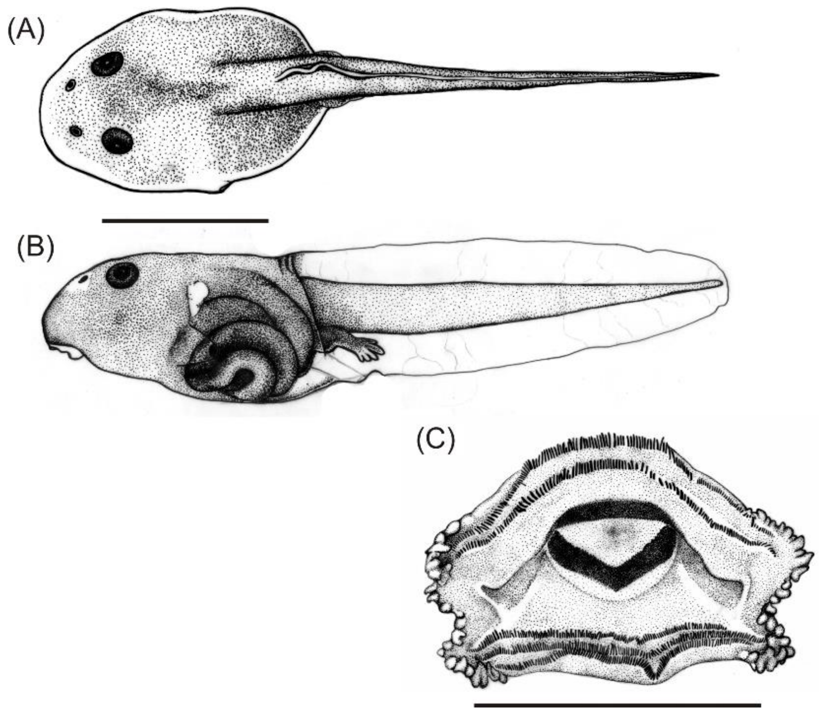

Figure 5. External morphology and oral disc illustration of the tadpole of M*elanophryniscus admirabilis*. Gosner (1960) Stage 37. (A) Dorsal view and (B) lateral view (scale bar = 5 mm). (C) Oral disc (scale bar = 1 mm). *Drawing by Marcelo Costa.*

The colouration of tadpoles in life was almost uniformly brown and opalescent immediately after hatching (Figure 3L). After stage 32, the dorsum colouration of the tadpoles was dark brown (melanophores, *sensu* Gosner 1960) with spreading small golden dots (iridophores, *sensu* Gosner 1960). The dorsal fin was translucent with scattered brown spots; the ventral fin was translucent and unpigmented. Caudal musculature with patches of brown and golden dots, especially in dorsal myosepts. The venter was translucent with scattered brown spots, except for the gular region which was almost entirely translucent. In preserved specimens, the golden dots disappeared, the skin was translucent, and the dorsal colouration was dark and opaque brown.

3.5 Juvenile and adult colouration and defensive behaviour

The colouration of the individuals' skin varies ontogenetically (Figure 6; Supplementary Figure S9). Newly metamorphosed individuals have a dark green (almost black) dorsum, translucent pale palmar and plantar surfaces and black, partially translucent venter (Figure 6B-C). Two to three days after metamorphosis, we observed the beginning of the development of dorsal glands and the emergence of green pigmentation (Figure 6D). We do not know when, in the life of an individual, the red colouration and the yellowish-green ventral spots (glands) emerge, but we do know this process is gradual (Figure 6C-F-I). The palmar, plantar and pelvic surfaces remain opaque (Figure 6F-I), varying from pale orange to dark red (but still translucent) until adulthood, when the red colouration is bright and conspicuous (Supplementary Figure S9). Even without presenting the brightness of the red aposematic colouration, young individuals can perform the *unken reflex* behaviour (Figure 6J).

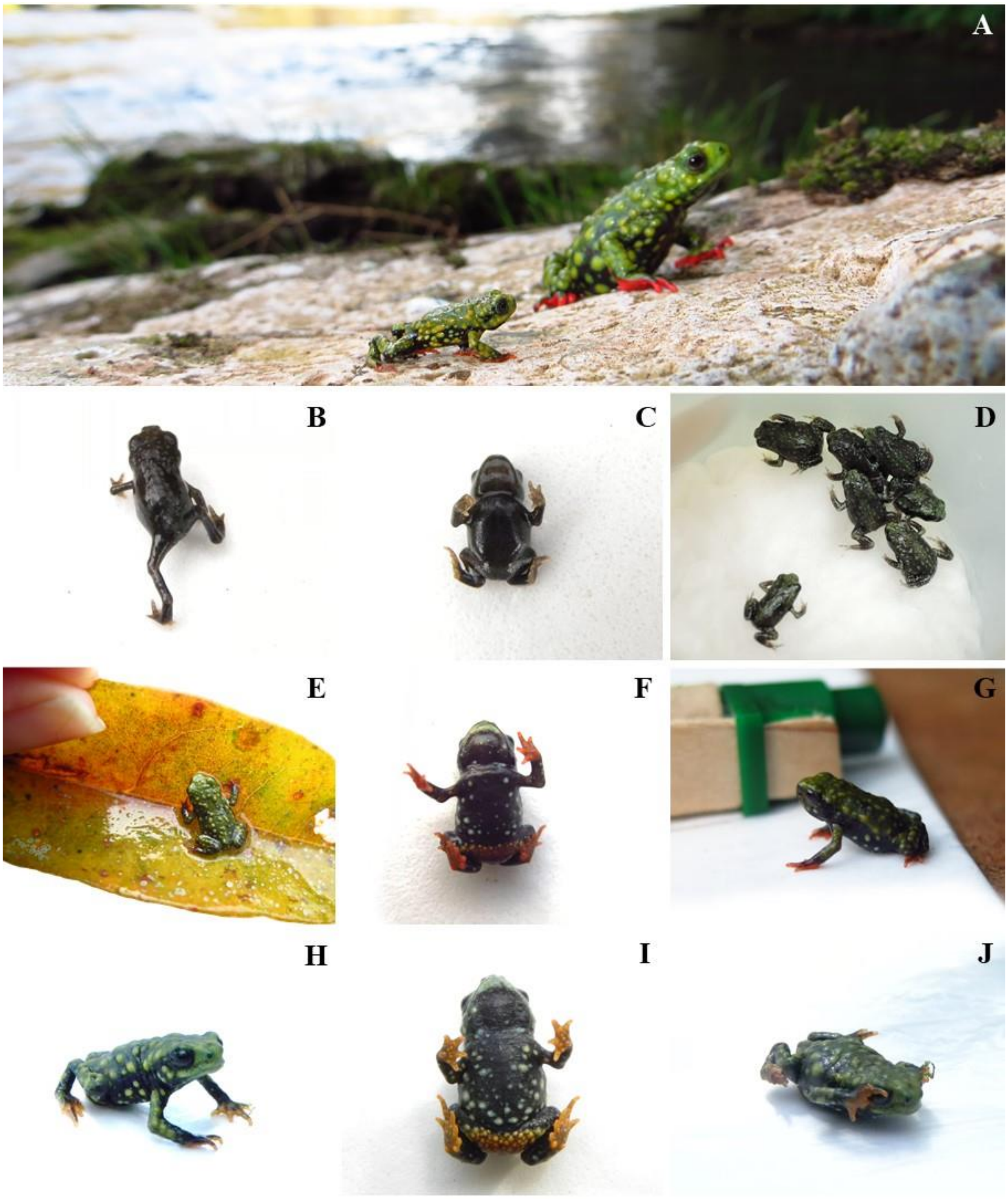

Figure 6. The colouration of juveniles of *Melanophryniscus admirabilis.* (A) A juvenile and an adult at the breeding site; (B–C) a newly metamorphosed individual without colouration, found at the breeding site; (D) a few individuals about four days after metamorphosis, presenting dorsal glands and some green pigmentation; (E–G) a juvenile individual, presenting conspicuous green colouration, yellowish-green dorsal glands, opaque red pigmentation on the palmar, plantar and pelvic surface and few ventral glands (green-bluish ventral dots); (H–J) a juvenile individual (11.04 mm and 0.2 g), presenting more ventral glands than the previous juvenile, still opaque and

slightly translucent red colouration and performing the *unken reflex* behaviour. Photos: Luis Fernando Marin da Fonte (A), Michelle Abadie (B–J).

The dorsal colouration of adults does not vary widely, being bright green and, sometimes, presenting greyish-blue patches and/or yellow spots (more rarely red spots). The ventral surface of adults is a black background with well-defined yellowish-green spots (glands), sometimes with red or greyish-blue spots. There are no evident differences in the colour pattern of males and females.

The Admirable toads are hard to see in the green vegetation, especially against green mosses. Notably, it was common, even to trained collectors, to misidentify mosses and fallen green leaves as toads (Figure 7A - B). *Melanophryniscus admirabilis* exhibited the *unken reflex* when disturbed (Figure 7C), usually after trying to escape or freezing, often leaving the eyes uncovered.

Figure 7. Colouration and behaviour of *Melanophryniscus admirabilis*. (A) Two individuals on green moss at the breeding site; (B) an individual and a leaf with galls, often encountered at the breeding site; (C) an individual performing *unken reflex* behaviour, showing the red colouration on the palmar and plantar surfaces. Photos: Michelle Abadie.

4. Discussion

In this study, we gathered all unpublished information compiled from seven years and 122 days of *in situ* observation and monitoring of the only known population of *M. admirabilis*. We provide the most up-to-date and comprehensive synthesis of morphological, behavioural and other life-history information for the species. We expect this will lead to new research on the evolutionary origin and ecological relevance of the described traits for the species and stimulate comparative studies within the genus *Melanophryniscus*.

Melanophryniscus admirabilis exhibits a seasonal explosive breeding pattern and similar reproductive behaviour described for other *Melanophryniscus* species (Baldo et al., 2014), such as aggregation of hundreds of individuals, active searching and tactile identification (trial-and-error clasping behaviour), amplexus with inanimate objects or anything that moves, intense male combat, struggling including non-amplectant and amplectant males, diurnal and nocturnal breeding activity, breeding in temporary rain pools or other ephemeral habitats (Maneyro and Carreira, 2012; Cairo et al., 2013, 2008; Caldart et al., 2013; Caorsi et al., 2020, 2014; Pereira and Maneyro, 2018; Santos et al., 2010; Silva et al., 2009; Vaira, 2005). In this study, we recorded evidence of breeding activity in almost all months of the year. Nevertheless, the explosive breeding events, characterised by the high density of individuals at the breeding site and the typical associated behaviour, were not uniform throughout the year and were concentrated between mid-July and early December. Some geographically close species of *Melanophryniscus* (*M. cambaraensis*, Santos et al., 2010; *M. pachyrhynus*, Caldart et al., 2013; *M. macrogranulosus*, Caorsi et al., 2014) are aseasonal explosive breeders. Wells (1977) refers to the prolonged and explosive breeders as two ends of a continuum, and *M. admirabilis* is an example that appears to be somewhere in the middle.

Seasonality is reported for most *Melanophryniscus* species for which the reproductive cycle was described (*M. moreirae*, Van Sluys and Guido-Castro, 2011; *M.*

rubriventris, Vaira, 2005; *M. stelzneri*, Pereyra et al., 2011) and it is always associated with the seasonality of rainfall in those regions and the warm seasons in the austral hemisphere. In the Perau de Janeiro region, monthly rainfall is well distributed over the year (Alvares et al., 2013; Zepner et al., 2020) and the four seasons are well-defined, with hot summers and cold winters, and mild springs and autumns. In these latitudes, environmental seasonality is mostly determined by variation in temperature and photoperiod (Both et al., 2008). We encourage future studies to simultaneously monitor rainfall, temperature, photoperiod and reproductive activity to identify triggering factors of the breeding activity of *M. admirabilis*.

Admirable toads use small shallow temporary pools in riverbanks as oviposition sites, and they need to cope with a high risk of reproductive failure through desiccation or flooding of pools, risking both clutches and tadpoles. Species reproducing in these unpredictable and ephemeral environments have at least three not mutually exclusive strategies to increase offspring survival: (1) not release all eggs in a single clutch, but release them in a sequence of clutches, spreading several egg masses to different sites in the same pool and different pools at the breeding site; (2) rapid larval development; and (3) select more favourable pools. The first two strategies have already been reported for other *Melanoprhyniscus* species (*M. rubriventris*, Goldberg et al., 2006; *M. stelzneri*, Bustos Singer and Gutiérrez, 1997; *M. montevidensis*, Pereira and Maneyro, 2018; *Melanophryniscus sp.*, Cairo et al., 2008; *M. cambaraensis*, Caorsi et al., 2012). Spreading eggs in several sequential clutches has the potential to increase the probability that at least one spawning survives (Spieler and Linsenmair, 1997). Since temperature influences the larval growth rate and development (Hayes et al., 1993; Smith-Gill and Berven, 1979), direct or indirect sun warming of the water in pools may help to accelerate the development of tadpoles and, consequently, reduce the risks associated with water dependence. According to Goldberg et al. (2006), toads of *M. rubriventris* preferred warmer flooded pools. On the other hand, increasing temperature also increases the pool desiccation rate. Thus, we could expect that recruitment would be higher in pools that have the most favourable balance between development rate and mortality risk. All these aspects are open questions that should be explored in future studies. They can help explain why other available rocky outcrops along the river are not used for reproduction by *M. admirabilis*.

Even though it is the second-largest species of the genus, *M. admirabilis* does not have the largest tadpoles (BL= 6.33±0.46; see Table on Baldo et al., 2014). Like other *Melanophryniscus* species from the *stelzneri* group (Baldo et al., 2014), *M. admirabilis* exhibits one of the most basal reproductive modes found in anurans, which relies on lentic water bodies for spawning and tadpole development (Duellman and Trueb, 1994). Its tadpole is similar to other lentic tadpoles of the genus (*M. stelzneri* group; see Baldo et al., 2014), but the tail is slightly lower than body, the oral disc is wide and dorsal and ventral gaps are large, as reported for *M. rubriventris* (Baldo et al., 2014; Lavilla and Vaira, 1997). The tadpole of the Admirable Redbelly Toad has intermediate features among phenetic groups of the genus (*sensu* Caramaschi and Cruz, 2002), and an analysis of the body shape through geometric morphometrics (Haad et al., 2011) probably would put it closer to *M. rubriventris* (*M. stelzneri* group) or *M. devincenzii* (*M. tumifrons* group) (Baldo et al., 2014). As concluded by Baldo et al. (2014), *Melanophryniscus* tadpoles are very similar and the differences observed are mainly quantitative.

Similar to all *Melanophryniscus* species, the advertisement call of the Admirable Toad is structured in two parts: call A, comprised of notes of single pulses; and call B, a pulsed trill. The advertisement call is complex and has modulated frequency and may be assigned to Call Guild H (Emmrich et al., 2020). *Melanophryniscus admirabilis* presents one of the longest known advertisement calls among the species of the genus (see a comparative table in Caorsi et al., 2020), but while the long duration of the others is a result of the long call B, in *M. admirabilis* it is due to long silence intervals between notes of call A and, similar to species of the *M. stelzneri* group, it has a short call B. Call A appears to have subtantial variation in terms of the number of notes, the duration of each one and the length of the gap between them. During the performance of call A, we sometimes observed males alternating note emission with small movements, changing position on the same site, seemingly choosing the direction of the call. Moreover, we found some males calling inside burrows and rock crevices, especially during the day. This behaviour may be related to sound amplification, similar to that already described for other species that call in burrows (Penna, 2004; Penna and Hamilton-West, 2007). The torrent water of the Forqueta river introduces background noise that could mask certain frequency components of anuran calls and it could be expected that these toads presented higher dominant frequencies than other non-stream-dwelling *Melanophryniscus* spp. (Röhr et al., 2016 but see Vargas-Salinas and Amézquita, 2014), but Admirable toads appear to have other adaptations to this noisy environment. According to Goutte et al. (2018), torrent-dwelling species produced calls with more pronounced frequency modulations, being positively correlated with the ambient noise level. As far as we know, the notes of the call A we described here for *M. admirabilis* have the most modulated frequency for the genus, possibly being related to the very noisy environment that this species occurs. Besides, the call A of *M. admirabilis* presented on average one out of the longest notes (0.10 s) among *Melanophryniscus* species, similar to *M. atroluteus* (0.102 s; Baldo and Basso, 2004) and *M. stelzneri* (0.11 s; Barrio, 1964), while it is shorted in *M. dorsalis* (0.054 s and

0.042 s; Kwet et al, 2005) and *M. montevidensis* (0,0313 s; Kwet et al, 2005). In general, the advertisement call of *M. admirabilis* is more similar to calls of the *M. stelzneri* group. However, given the lack of phylogenies for this genus, any hypothesis about the relations and explanations of acoustic patterns for these species remain inconclusive.

Regarding sexual dimorphism, our results showed that males are smaller than females, as expected (female-biased sexual size dimorphism; Bidau et al., 2011). However, the body size alone is not an efficient parameter to identify the sex, since the ranges of size overlap at the extremes. Larger female body sizes are a pattern found in most anurans (Shine, 1979) and several explanations have already been proposed for this phenomenon, such as the capacity to produce bigger clutches or oocytes (Camargo et al., 2008; Kuramoto, 1978), mainly in small-bodied and explosive breeding species (Nali et al., 2014), or older age at first reproduction (Halliday and Verrell, 1988; Guimarães et al., 2011). Jeckel et al. (2015) found that females are larger and older than males in *M. moreirae*, suggesting that the different age structure of each sex could explain the sexual size dimorphism, as proposed by Monnet and Cherry (2002). However, in that study, females were still larger than males when the toads were separated by age cohorts, demonstrating that there is a sexual size dimorphism-related effect. Although both sexes can exhibit nuptial pads in some *Melanophryniscus* species (Jeckel et al., 2019), in our study we easily differentiated males from females using the presence of brownish nuptial pads at the base of the male thumb. We did not find any evidence of sexual colour dimorphism by eye, but a further interesting approach could be to investigate other parameters to identify gender.

The Admirable Redbelly Toad has changing ontogenetic colouration, and the bright green and red colours are conspicuous only in adulthood. A diet rich in carotenoids may result in a more saturated and brighter colour (Umbers et al., 2016). Carotenoids are largely responsible for the red, orange, and yellow colours in amphibians (Hoffman and Blouin, 2000) and must be acquired from the diet (Goodwin, 1984; Umbers et al., 2016). The red colouration in the genus *Melanophryniscus* comes from the accumulation of this pigment (Bonansea et al., 2017) and it is supposedly aposematic (Bonansea and Vaira, 2012, but see Bordignon et al., 2018). Together with lipophilic alkaloids obtained from an arthropod-rich diet (Hantak et al., 2013; Saporito et al., 2012, 2009), the aposematic colouration could be a result of the diurnal activity and could provide protection against visually oriented diurnal predators (Santos and Grant, 2010), especially when diurnal toads are breeding and are more exposed. Moreover, the bright green colouration acquired over a juvenile's lifetime may work as crypsis, reducing the animal's detection rate and protecting against visual predators as well (see Dallagnol Vargas et al., 2020). The ontogenetic modification of colour has already been observed in other *Melanophryniscus* species (*M. macrogranulosus*, Caorsi et al., 2014; *M. klappenbachi*, Bland, 2015; *M. montevidensis*, Bardier et al., 2017), but how and why this happens remains unclear.

We recorded the longest lifespan directly observed in the field for the genus *Melanophryniscus* to date. Our results reveal that Admirable Redbelly toads can live at least seven years in the wild, which represents our entire sampling window. We recaptured 25 individuals in 2017, seven years after the first capture (2010), when they were already adults. Additionally, in 2020 we occasionally recaptured three individuals captured for the first time in 2011, when they were already adults (Michelle Abadie, pers. obs.). Minimal lifespan has been estimated for another species of the genus, *M. moreirae*, using skeletochronology. Jeckel et al. (2015) reported adult female and male ages ranging from 3-6 and 2-6 years, respectively. In the same study, they recorded juvenile (all females) ages ranging from 1 to 5 years. These results suggest that *Melanophryniscus* individuals reach sexual maturity at least 1 year after metamorphosis, possibly more. Therefore, we may assume that *M. admirabilis* individuals can potentially live at least 10 years. The maximum lifespan of these two species suggests that *Melanophryniscus* species can be long-lived anurans, as observed for some other bufonids (e.g. La Marca, 1984; Lyapkov et al., 2020; see Carey and Judge, 2008). Chemical defences can play a role in this unexpected longevity for a small, subtropical and diurnal toad (Stark and Meire, 2018). Grant et al. (2012) and Jeckel et al. (2015) found that the larger (and older) the redbelly toad, the greater the diversity of alkaloids found in the skin and internal organs. Thus, we hypothesise that the longer they take to mature, the greater their protection from predators.

The high rate of recaptures in the same sector (within the breeding site) suggests site fidelity, with juveniles being less loyal than adults. Our results are in accordance with the long-standing assumption in the amphibian literature that juveniles are more likely to disperse than adults (Dole, 1971; Wells, 2010) although we cannot affirm that these movements configure dispersal. Dispersal in the juvenile stage may be a response to pressures such as mate availability, inbreeding avoidance, and conspecific competition for resources (Greenwood, 1980; Hamilton and May, 1977; Perrin and Mazalov, 2000; Dobson, 1982), as if they were looking for a breeding site to establish themselves. Other studies have already demonstrated site fidelity of *Melanophryniscus* species (Pereira and Maneyro, 2016, 2018). We observed fights between males while they were calling at the breeding site, which may be evidence of territoriality or site fidelity, but future studies are needed to confirm this. We found exceptions of adults who displaced up to 300 m and we hypothesise that they might be newly recruited individuals. In any case, it is the maximum distance ever recorded for a species of the genus and it seems to be substantial for such a small species.

5. Conclusions

The Admirable Redbelly toad is a seasonal explosive breeder that lives in noisy and risky conditions and seems to have adaptations to deal with this environment. In this study, we described the advertisement call of the species, which the notes of call part A have the most modulated frequency for the genus; the eggs are deposited in several clutches in different temporary pools; the tadpole has a rapid development and is one of the smallest of the genus, whereas the adults are one of the largest; the species has supposedly aposematic and cryptic colouration and observed longevity extended at least 9 years. Our study considerably increases knowledge about the Admirable Redbelly Toad, a microendemic and Critically Endangered anuran (IUCN SSC Amphibian Specialist Group, 2013). The information we present here can be used in the future to evaluate the risks to the persistence of the single-known population, guide the search for new populations, discuss management actions to protect the reproductive site and stimulate research on the species' reproductive patterns and the mechanisms that trigger them.

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Equity, Diversity, Inclusion – We highly value equity, diversity and inclusion in science. Our study includes researchers from different backgrounds, career stages and countries. Most authors, including the first one, are women.

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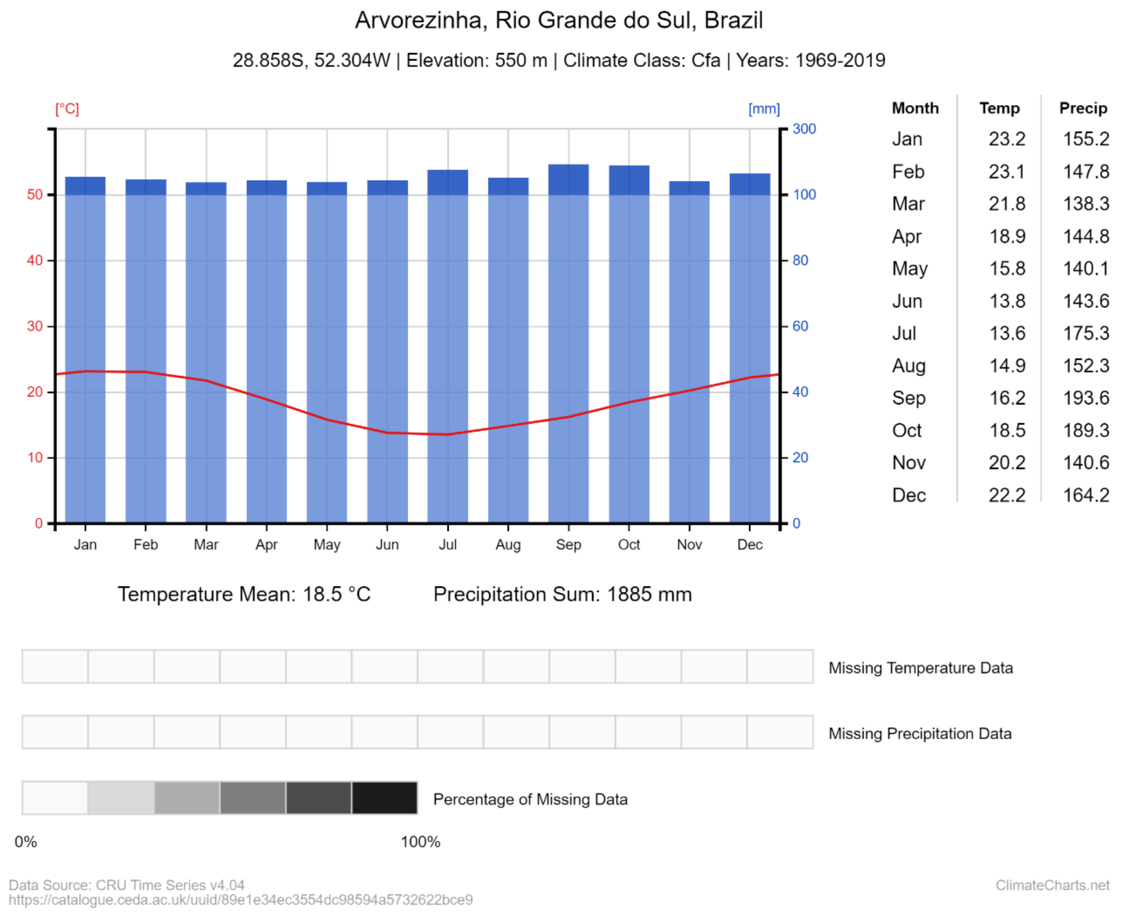
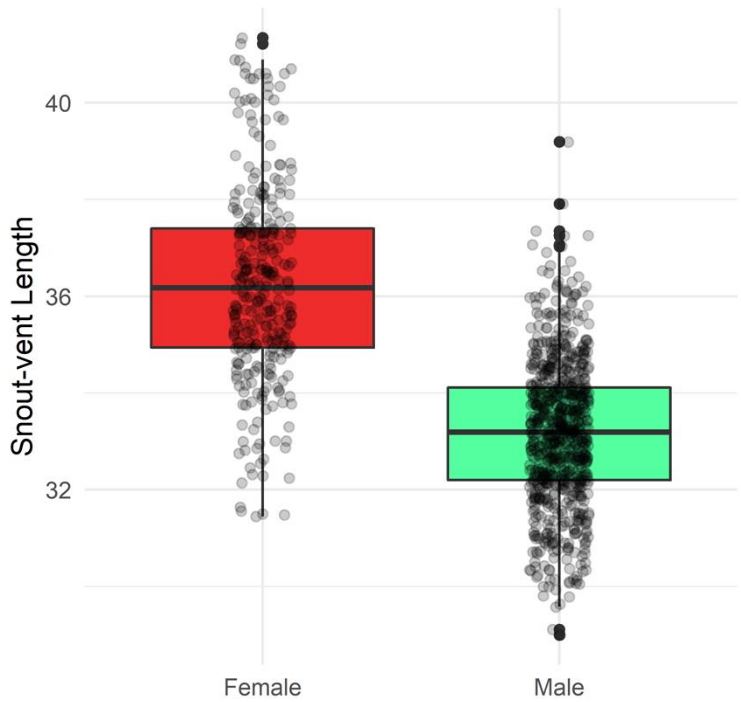
Appendix A - Supplementary Information

Morphology, reproduction, longevity, and bioacoustics of the microendemic and threatened Admirable Redbelly Toad

Michelle Abadie, Luis Fernando Marin da Fonte, Débora Bordignon, Thayná Mendes, Caroline Zank, Valentina Zaffaroni, Andreas Kindel, Raúl Maneyro, Márcio Borges-Martins.

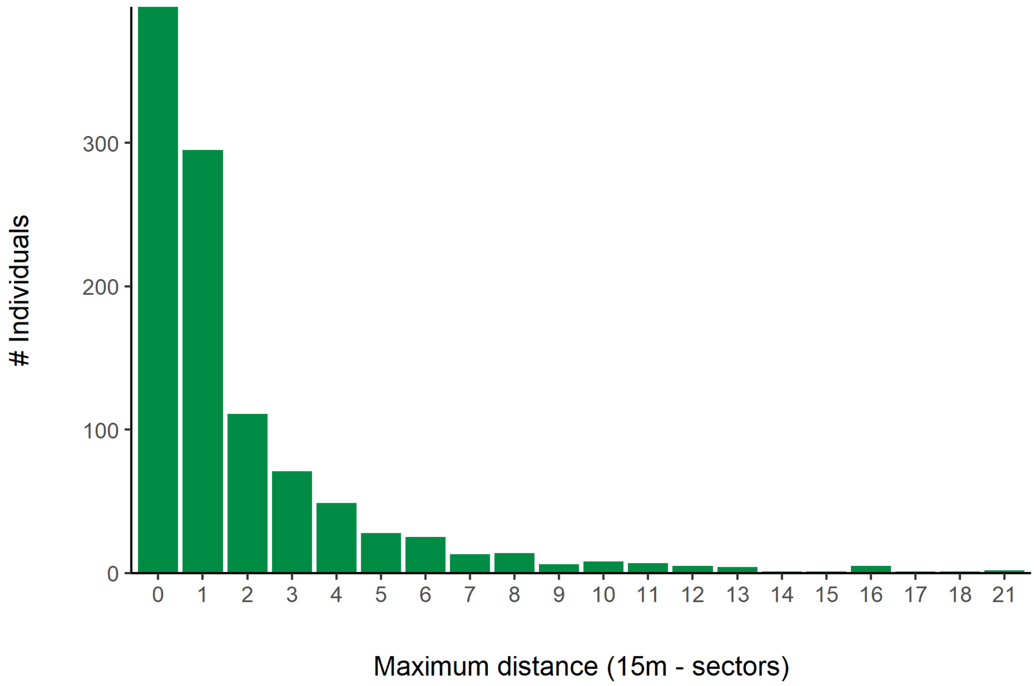


Supplementary Figure S1. Subtropical Humid Climate, variety *Cfa* (Koeppen's climate classification) in the region of Perau de Janeiro, Arvorezinha, Rio Grande do Sul state, Brazil. The type *Cfa* climate is characterised by precipitation evenly distributed throughout the year, with hot summers (average temperatures above 22 ºC for the hottest month) and cold winters (average temperatures varying between -3 and 18 ºC for the coldest month). Diagram generated in https://climatecharts.net/.

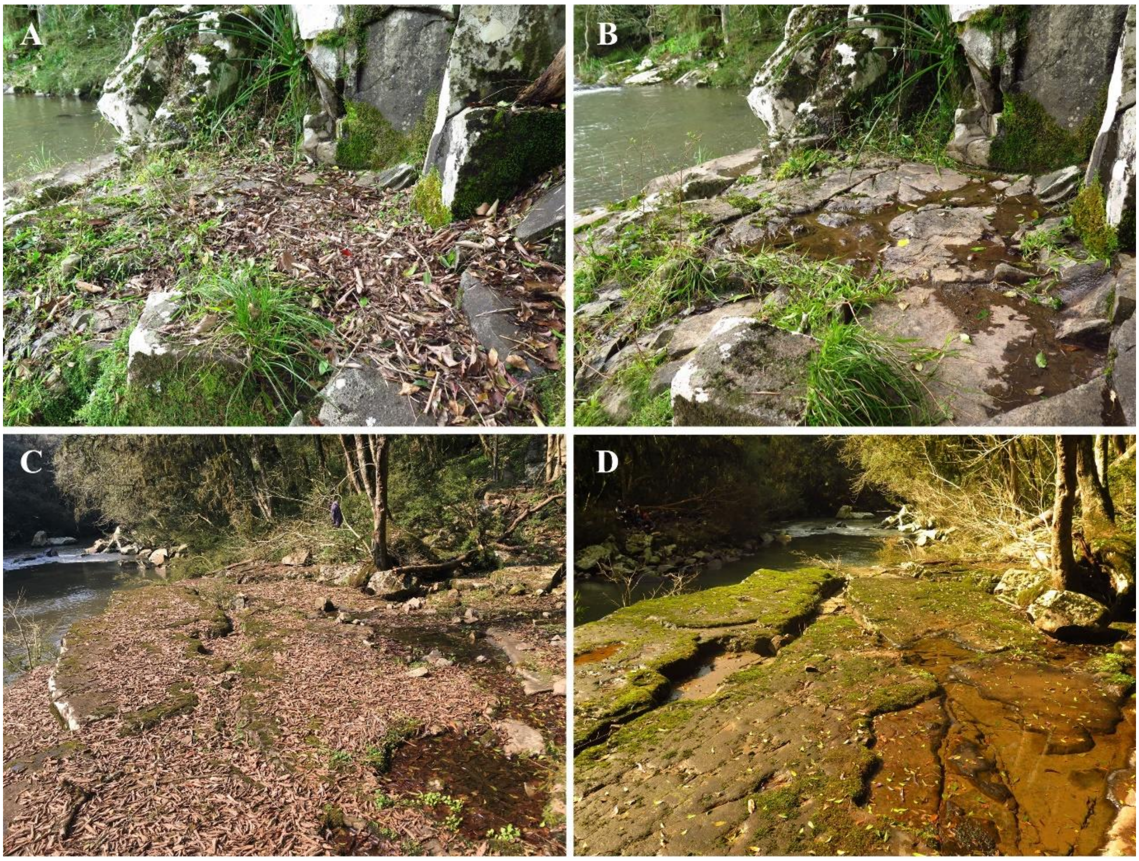


Supplementary Figure S2. Size sexual dimorphism on *Melanophryniscus admirabilis*. Boxplots showing the distribution of the snout-vent-length (mm) of 302 females and 760 males (point cloud). The rectangular boxes contain the central 50% of the values, the heavy line in the centre of each box is the median, the whiskers extend to 1.5 times the interquartile range above and below the box and outliers are indicated by black filled points.

Supplementary Figure S3. When *Melanophryniscus admirabilis* is not breeding. (A-B) two adult individuals in the adjacent forested steep slopes to the rocky river bank outcrop; (C-D) some individuals hiding in spaces under rocks; (E-F) some individuals hiding under vegetation and in a rocky slit, respectively, in moments when they are not breeding but they are at the breeding site. Photos: *(F) by Simone Leonardi*.



Supplementary Figure S4. Frequency of individuals for maximum distance between sectors registered. Each sector has 15 metres of length.



Supplementary Figure S5. Availability of water in the small pools at the breeding site of *Melanophryniscus admirabilis*, in the type locality. (A and C) Pools filled by dry leaves at the end of austral winter; (B and D) the same places after raining and "washing" the rocky river bank outcrop. Picture (B) was taken ten days after the picture (A).

Supplementary Video S6. Spawning behaviour of *Melanophryniscus admirabilis*. Video available clicking here.

Supplementary Table S7. Advertisement call characteristics of *Melanophryniscus admirabilis*, based on nine calls from four individuals in the type locality Perau de Janeiro, Arvorezinha, RS, Brasil. Call recordings were carried out under air temperatures ranging between 17.5 and 23.9 °C.

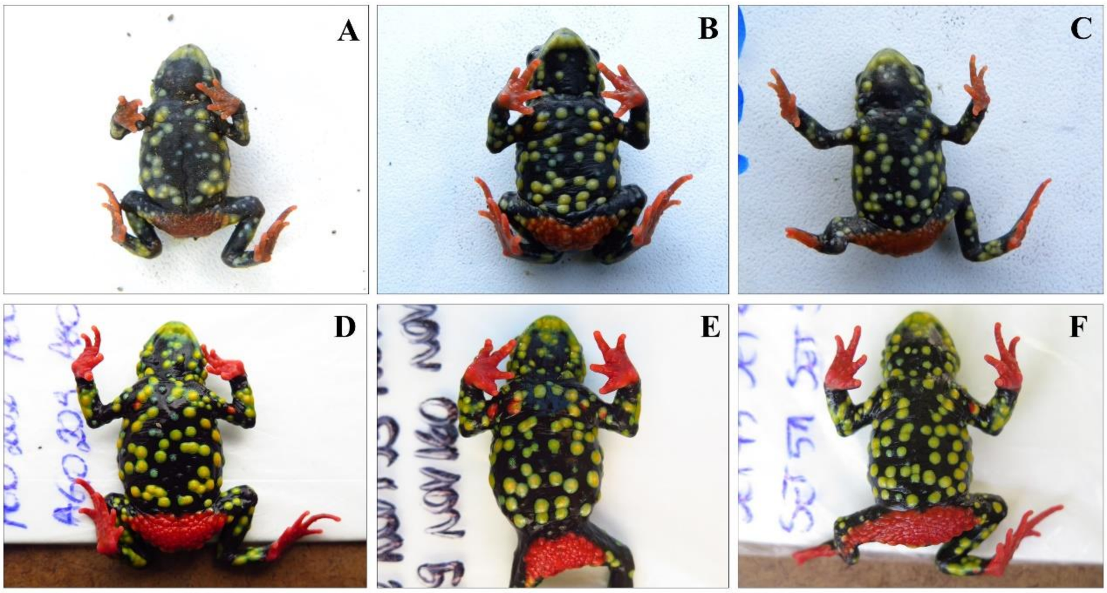
#	Acoustic parameter	Average ± SD [a] (range)
1	Call duration (s)	21.00 ± 1.50 (2.90 – 68.80)
2	Call part A duration (s)	15.40 ± 19.10 (0.32 – 64.30)
3	Number of notes Call part A	8.20 ± 4.90 (2.00 – 19.00)
4	Duration of notes Call part A (s)	0.10 ± 0.04 (0.01 – 0.17)
5	Interval among notes Call part A (s)	2.00 ± 2.40 (0.01 – 18.80)
6	Dominant frequency Call part A (Hz)	1939 ± 383 (1500 – 2800)
7	Interval between call parts A and B (s)	1.50 ± 4.10 (0.06 – 12.60)
8	Call part B duration (s)	4.10 ± 1.70 (1.00 – 6.26)
9	Number of pulses Call part B	206.30 ± 88.60 (53 – 324)
10	Pulses rate Call part B	50.80 ± 2.00 (49.00 – 54.30)
11	Duration of pulses Call part B (s)	0.006 ± 0.002 (0.002 – 0.020)
12	Interval among pulses Call part B (s)	0.014 ± 0.002 (0.002 – 0.030)
13	Dominant frequency Call part B (Hz)	1749 ± 219.50 (1500 – 1968)

[a] **SD** = standard deviation

1067 **Supplementary Table S8.** Body and oral disc measurements were taken from tadpoles
1068 of *M. admirabilis* (n=20) by development stage (*sensu* Gosner, 1960). Mean, standard
1069 deviation and range values are shown in millimetres.

Measurement	32 (n=5)	34 (n=2)	35 (n=2)	36 (n=5)	37 (n=6)
TL	14.68 ± 0.55 (13.9 – 15.3)	14.85 ± 0.92 (14.2 – 15.5)	15.55 ± 1.06 (14.8 – 16.3)	16.52 ± 0.94 (15.4 – 17.6)	16.22 ± 0.76 (15.2 – 17.1)
BL	5.82 ± 0.31 (5.3 – 6.1)	6.10 ± 0.57 (5.7 – 6.5)	6.35 ± 0.35 (6.1 – 6.6)	6.62 ± 0.41 (6.1 – 7.2)	6.57 ± 0.29 (6.1 – 6.9)
TAL	9.08 ± 0.51 (8.5 – 9.7)	8.65 ± 0.35 (8.4 – 8.9)	9.2 ± 0.71 (8.7 – 9.7)	9.9 ± 0.60 (9.3 – 10.5)	9.63 ± 0.49 (9.0 – 10.2)
BMW	4.26 ± 0.11 (4.1 – 4.4)	4.50 ± 0.42 (4.2 – 4.8)	4.70 ± 0.00 (4.7)	4.66 ± 0.36 (4.1 – 5.1)	4.70 ± 0.25 (4.3 – 4.9)
BWN	2.80 ± 0.16 (2.6 – 3.0)	2.95 ± 0.07 (2.9 – 3.0)	2.90 ± 0.14 (2.8 – 3.0)	3.04 ± 0.15 (2.8 – 3.2)	3.02 ± 0.16 (2.8 – 3.3)
BWE	3.70 ± 0.12 (3.5 – 3.8)	3.90 ± 0.28 (3.7 – 4.1)	3.90 ± 0.14 (3.8 – 4.0)	4.04 ± 0.18 (3.8 – 4.2)	4.03 ± 0.12 (3.9 – 4.2)
BMH	3.48 ± 0.16 (3.3 – 3.7)	4.20 ± 0.42 (3.9 – 4.5)	4.00 ± 0.00 (4.0)	3.90 ± 0.16 (3.7 – 4.1)	4.00 ± 0.18 (3.8 – 4.3)
MTH	2.98 ± 0.19 (2.7 – 3.2)	2.90 ± 0.14 (2.8 – 3.0)	3.15 ± 0.21 (3.0 – 3.3)	3.38 ± 0.22 (3.2 – 3.7)	3.27 ± 0.26 (2.8 – 3.5)
TMH	1.14 ± 0.05 (1.1 – 1.2)	1.15 ± 0.07 (1.1 – 1.2)	1.20 ± 0.00 (1.2)	1.30 ± 0.10 (1.2 – 1.4)	1.32 ± 0.04 (1.3 – 1.4)
TMW	1.04 ± 0.11 (0.9 – 1.2)	1.15 ± 0.07 (1.1 – 1.2)	1.20± 0.00 (1.2)	1.28 ± 0.08 (1.2 – 1.4)	1.28 ± 0.13 (1.1 – 1.4)
RSD	3.72 ± 0.22 (3.4 – 4.0)	3.80 ± 0.0 (3.8)	4.10 ± 0.28 (3.9 – 4.3)	4.12 ± 0.23 (3.9 – 4.4)	4.05 ± 0.16 (3.8 – 4.3)
FN	0.76 ± 0.09 (0.7 – 0.9)	0.85 ± 0.07 (0.8 – 0.9)	0.90 ± 0.14 (0.8 – 1.0)	0.94 ± 0.13 (0.8 – 1.1)	0.83 ± 0.08 (0.7 – 0.9)
IND	0.95 ± 0.05 (0.9 – 1.0)	1.00 ± 0.0 (1.0)	1.05 ± 0.07 (1.0 – 1.1)	1.04 ± 0.05 (1.0 – 1.1)	1.05 ± 0.05 (1.0 – 1.1)
E	0.66 ± 0.07 (0.6 – 0.7)	0.67 ± 0.04 (0.7 – 0.7)	0.75 ± 0.07 (0.7 – 0.8)	0.80 ± 0.07 (0.7 – 0.9)	0.83 ± 0.10 (0.7 – 1.0)
IOD	1.75 ± 0.05 (1.7 – 1.8)	1.85 ± 0.07 (1.8 – 1.9)	1.90 ± 0.00 (1.9)	2.00 ± 0.10 (1.9 – 2.1)	2.00 ± 0.13 (1.8 – 2.1)
OD	2.02 ± 0.04 (2.0 – 2.1)	2.20 ± 0.28 (2.0 – 2.4)	2.35 ± 0.07 (2.3 – 2.4)	2.28 ± 0.28 (2.0 – 2.7)	2.27 ± 0.14 (2.1 – 2.4)
DG	1.30 ± 0.11 (2.3 – 2.8)	1.38 ± 0.04 (1.4 – 1.4)	1.60 ± 0.14 (1.5 – 1.7)	1.52 ± 0.15 (1.3 – 1.7)	1.53 ± 0.10 (1.4 – 1.7)
VG	0.98 ± 0.09 (1.7 – 2.2)	1.00 ± 0.21 (0.9 – 1.15)	1.05 ± 0.07 (1.0 – 1.1)	1.21 ± 0.19 (1.1 – 1.5)	1.14 ± 0.09 (1.0 – 1.3)

TL= total length; BL= body length; TAL= tail length; BMW= body maximum width; BWN= body width at nostrils; BWE= body width at eyes; BMH= body maximum height; MTH= maximum tail height; TMH= tail musculature height; TMW= tail musculature width; RSD= rostro-spiracular distance; FN= fronto-nasal distance; IND= internarial distance; E= eye diameter; IOD= interorbital distance.



Supplementary Figure S9. Ontogenetic variation of the ventral colour pattern of three individuals of *M. admirabilis*. Figures A-C are juveniles and D-F are their respective adults. (A) Female juvenile (SVL= 15.52 mm) captured on October 2013 at sector #30 and (D) recaptured as adult (SVL = 35.04 mm) on August 2017 at sector #28. (B) Male juvenile (SVL= 21.82 mm) captured on October 2013 at sector #22 and (E) recaptured as adult (SVL = 32.86 mm) on November 2017 at sector #15. (C) Male juvenile (SVL= 15.88 mm) captured on October 2013 at sector #22 and (F) recaptured as adult (SVL = 31.23 mm) on September 2016 at sector #18.

Capítulo 2

The abundance of the microendemic Admirable Redbelly Toad: a comparison of population size estimates.

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The abundance of the microendemic Admirable Redbelly Toad: a comparison of population size estimates.

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Abstract

1. Limited range and habitat specificity make microendemic species particularly vulnerable to environmental change. Their persistence may be a result of random luck or of demographic processes that confer resistance to change. Population size and variability are key elements for understanding the demography of small populations as well as their chances of persistence. Among Neotropical anurans, the genus *Melanophryniscus* stands out as having a large number of species with small, microendemic populations that may be threatened.

2. The focus of this study is the Admirable Redbelly Toad (*Melanophryniscus admirabilis*), a Critically Endangered and microendemic anuran. We aim to estimate the size of the species population, as it varies through time in an eight-year window of observation.

3. We employ capture-recapture models to estimate population size, using individual recognition by photo-identification in a data set with more than seventeen hundred capture histories. We used three analytical approaches, the Closed population

(Closed) and the Jolly-Seber POPAN approaches which model data one year at a time and Pollock's robust design (PRD), which simultaneously models data from the whole observation period and provides annual estimates of abundance and apparent survival probability.

4. Our estimates of population size varied more between methods in the same year than between years, ranging from 561 ± 39 (estimate ± standard error) to 1,734 ± 361 individuals over the whole study period. There is a substantial decrease in the estimated number of individuals in the middle of the study period. POPAN and PRD abundance estimates were closer to each other than either was to the Closed model estimates.

5. We conclude that the only known population has fewer than 2000 reproductive individuals. Movements, within or between seasons, are likely the most important factor behind temporal variation in the number of reproductive Admirable Toads. When multi-year analyses are not possible, our results favor analyses that account for movement in and out of the study area throughout one breeding season. Otherwise, the PRD offers the most efficient approach for extracting information from the multi-year capture histories.

Key-words: Capture-mark-recapture, Closed Population model, Jolly-Seber POPAN, Pollock's Robust Design, *Melanophryniscus admirabilis*, microendemic, survival estimates, threatened species.

Introduction

If extinction cannot happen without population decline, every population must be rare at least once throughout its existence. Whether rarity is demographically informative, however, is mostly a matter of time. Some populations may be rare for only a moment before a cataclysmic event brings them to an end, while others may remain rare—and observable—for extended periods. Rarity is a slippery concept with multiple definitions (Rabinowitz 1981), but it always involves a small (or relatively small) population size. The number of individuals in the population and the variability of this number through time are key for interpreting the demography of rare populations as well as their chances of persistence (Inchausti & Halley, 2003; Pertoldi et al., 2008).

Among rare species, persistent microendemics are the most intriguing, because their limited range and often high habitat specificity make them vulnerable to environmental change. Yet, their observed persistence may be a result of random luck or of demographic processes that make them particularly resistant to change. Neotropical anurans have particularly high endemism rates (Pimm et al., 2014; Villalobos et al., 2013), and among them *Melanophryniscus* stands out as one of the genera with more endemic species of strong conservation concern (Zank et al., 2014). The focus of this study is the Admirable Redbelly Toad (*Melanophryniscus admirabilis*, Anura: Bufonidae; Figure S1), a Critically Endangered and microendemic anuran (IUCN SSC Amphibian Specialist Group, 2013) known from only one population, which occurs over a stretch of a few hundred meters along the margins of one stream in the southern end of the Brazilian Atlantic Forest (Di-Bernardo et al., 2006; Fonte et al., 2014). Admirable Toads are small (about 35 mm in length) and occur in a very specific environment consisting of forested river margins with flattened rocky outcrops along the water’s edge. They are difficult to find when they are not breeding, but occasionally become conspicuous when hundreds of individuals aggregate for a few days at a time on rocky outcrop pools where adults mate, clutches are laid, and eggs develop. Such explosive breeding events (*sensu* Wells, 1977) take place during the second half of the year (July – December: breeding season), coinciding with late winter, spring, and early summer in the southern hemisphere (Abadie et al., 2021 – Chapter 1). This paper aims to estimate the abundance of Admirable Redbelly Toad, a species whose individuals are rarely and irregularly available for observation.

As we accumulated observations of individually recognizable toads over eight years of study, we built more than seventeen hundred capture histories that we analyze with a combination of three different statistical approaches that make different assumptions about the data and Admirable Toad biology. We aim to inform herpetologists about the advantages and disadvantages of each analytical approach and, above all, to offer an assessment of *M. admirabilis* abundance, its variation through time, and the demographic processes operating behind such variation.

Materials and Methods

Study area

We conducted our study at Perau de Janeiro, the Admirable Redbelly Toad type locality, at the extreme south of the Atlantic Forest biome (Figure 1). Located in the Brazilian state of Rio Grande do Sul, Arvorezinha municipality, Perau de Janeiro is part of an approximately 5 km^2 patch of forest, which is one of the largest and few forest fragments of the region. Regional forest cover is under pressure from the expansion of eucalyptus, tobacco, soybean and livestock farming. Our study area extends ca. 400 m along the margins of Forqueta River, which cuts through the forest patch at an elevation of approximately 550 m above sea level and is part of the Taquari-Antas River basin. The local humid subtropical climate has hot muggy summers and mild to cold winters, with precipitation evenly distributed throughout the year (Alvares et al., 2013; Zepner et al., 2020). The site is more humid than the surrounding area due to its forest cover and steep slopes on both sides of the river (Figure S2). Our sampling area encompassed a flattened rocky outcrop along the left bank of the river (Figure 1; Figure S3), where most of the known Admirable Redbelly Toad population concentrates when breeding. Admirable Toads find shelter in the forest and often spawn in hundreds of small ephemeral pools on the rocky margins (Figure S3). Successful reproduction potentially depends on the duration of the pools, which can be shortened by river flooding or by high temperatures that lead to evaporation (Abadie et al., 2021 – Chapter 1).

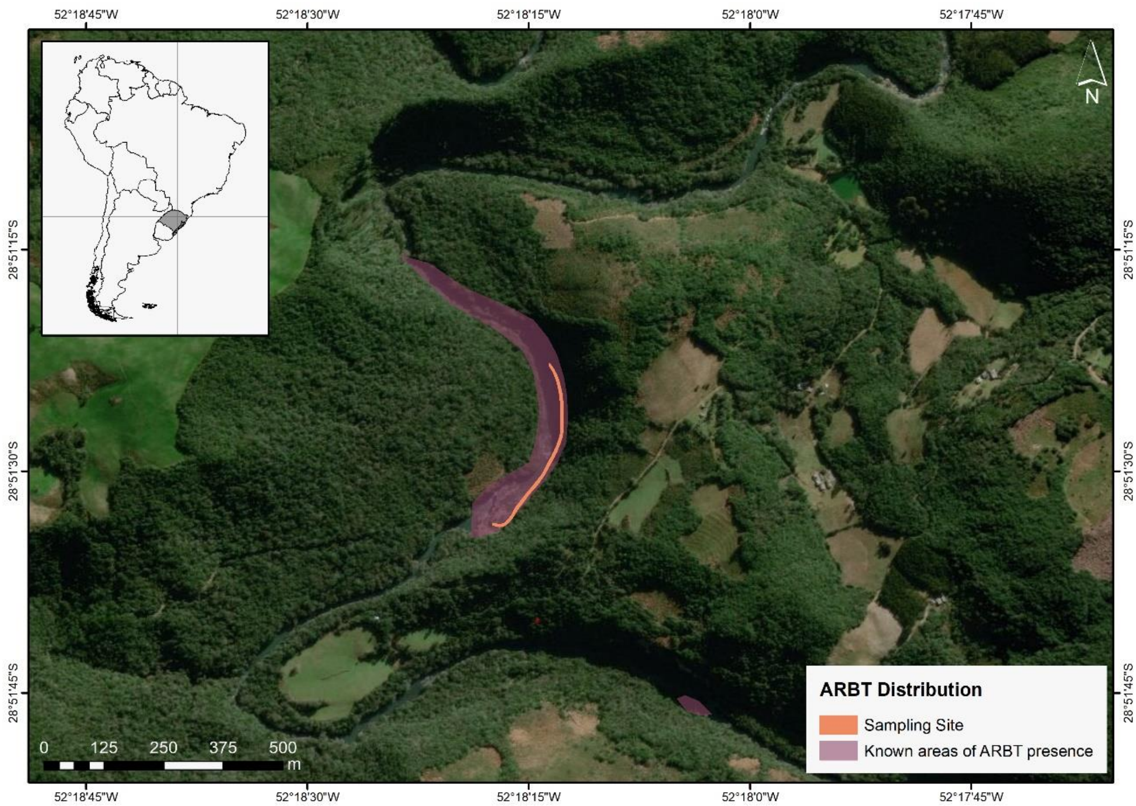

Figure1. Known geographic distribution of Admirable Redbelly Toad at Perau de Janeiro, municipality of Arvorezinha, Rio Grande do Sul state, Brazil. The sampling site for this study is at the main breeding site of Admirable Redbelly Toad (ARBT), on the flattened rocky outcrop along the left bank of the Forqueta River.

Data collection

We divided the sampling area in 27, 15-m-long sectors, and captured toads in fifteen sectors per day of fieldwork. Nine of these fifteen sectors were fixed, because they consistently held more animals than the others, while the remaining six were randomly selected each day. We visited the sectors while moving in opposite directions along the margin on consecutive days, to avoid always going through the same sector at the same time of the day. That is, if we visited fifteen sectors moving downstream in one day, we would visit the next set of fifteen sectors while moving upstream on the subsequent day. Surveys took place from 2010 to 2017, with gaps in 2012 and 2015 due to a lack of resources (Table 1). Up to and including 2014, we visited the site every 30 to 60 days, regardless of species activity, and stayed there from one to eight days, depending on breeding activity. From 2016 onward, we hired a local resident to visit the site once a week and send us information about Admirable Toad activity. Intending to increase capture success, we only visited the site if there was evidence of a breeding event. On each visit of this second stage, we stayed at the site for three days or until reproductive activity ceased, whichever came first. We limited visit duration to three days and the frequency of visits to once per month to minimize disturbance to the population.

Table 1. Surveys dates, number of individuals, and the number of captures of the Admirable Redbelly Toad throughout the study period (2010 – 2017). Detected breeding events are identified by their abbreviated month and followed by the corresponding number of trapping days in brackets. 'Individuals' refers to the number of individuals captured at least once in each year. 'Captures (days)' and 'Captures (events)' are, respectively, the number of captures summed over all fieldwork days and the number of captures summed over all events. The latter number excludes recaptures from the same event. Letters 'F' and 'M' stand for 'female' and 'male', respectively.

Year	Reproductive events	Individuals		Captures (days)		Captures (events)	
		F	M	F	M	F	M
2010	Oct(1); Nov(1)	24	106	24	110	24	110

2011	Jul(2); Aug(2); Sep(2); Oct(1)	111	394	123	496	116	482
2013	Aug(2); Oct(6); Dec(1)	126	363	145	457	130	399
2014	Jul(1); Sep(5)	90	359	98	469	92	371
2016	Sep(3); Oct(2); Nov(2); Dec(1)	177	477	207	767	190	687
2017	Aug(3); Sep(2); Oct(2); Nov(2)	202	513	264	1085	240	931
Total	19 events (41 survey days)	556	1211	861	3384	792	2980

In the field, we visually scanned and hand-captured toads throughout the day beginning at 9 am and stopping, sometimes at night, when new individuals were no longer detected in the selected sectors. All captured toads were sexed and photographed. Sex was determined based on a brownish nuptial pad at the base of the male thumb, the calling behavior of males, or the position of pairs in amplexus. To minimize disturbance and handling time, we did not clip toes or use any form of physical marking. Instead, we photographed the ventral patch of black pigmentation on each animal and used its unique outline for individual recognition (Figure S4). An alphanumeric code was assigned to each capture, and a photographic catalogue was assembled and updated after each sampling visit. We released all photographed toads at the same site of capture.

The individual identification of ventral patch photographs followed a semi-automatic procedure. The best photograph from each individual's capture was cropped to the area of interest, between the throat and the cloaca, and presented to the software Wild-ID (Bolger et al., 2012), which returned the 20 closest matches in our photo catalogue. This list was subsequently manually examined by a researcher who attributed a new or existing individual identity to the captured toad. This approach was successfully tested and applied to other species of South American Redbelly toads by Caorsi et al. (2012) and by Bardier et al. (2019). The mapping of individual identities over fieldwork days produced a database of capture histories spanning the entire duration of our sampling period and included all captured toads. Field procedures followed recommendations by the Chico Mendes Institute for Biodiversity Conservation (ICMBio) under licenses number 40004-5 and 10341-1 issued by the Information and Authorization System in Biodiversity (SISBIO) to MBM.

Data analysis overview

We organized captures through time in a hierarchical data structure where the entire study period is divided into years, which are further divided into breeding events. Each breeding event can also be divided into capture days, with the possibility for some individuals to be recaptured on different days of the same event. For simplicity, however, our analyses used the breeding event as the shortest time unit, treating multiple captures of the same individual within one event as a single capture. Some capture days, before 2016, did not coincide with a breeding event and were excluded from our analysis based on criteria specified in the results section. In our data set, a sample capture history could be given by:

$$X = 00\ 0110\ 011\ 00\ 1100\ 1011,$$

where year i represents the survey years in the study period ($i = 1 - 6$), and the breeding event j varies from 1 to l_i, the maximum number of breeding events in a year i. Table 1 shows that l_i takes the values {2, 4, 3, 2, 4, 4} as i varies from 1 to 6. A capture history element X_{ij} equals 1 when the individual is captured at least once during the breeding event j of the year i and equals 0 otherwise.

Our statistical analyses aimed to estimate the population size in each year and understand the variation between years. To achieve this goal, we modeled the set of all capture histories under three different approaches. Each approach has its own model structure, but they all share the recognition that individuals may be alive and present in the study site while not captured. First, we model capture histories within each year using a closed-population capture-recapture model (henceforth called 'Closed'; Otis et al., 1978). As a second approach, which considers the population open but still models each year separately from the others, we employed the POPAN formulation of the Jolly-Seber model (POPAN; Schwarz & Arnason, 2019; Schwarz & Arnason, 1996). Our third approach, Pollock's Robust Design (PRD; Kendall, 2019; Kendall et al., 1997; Pollock, 1982), considers the population closed within each year but open between years. The PRD model combines information from all years in the same analysis. All three approaches estimate yearly numbers of reproductively active males and females present at the sampling site during the reproductive season. All models were implemented in the RMark package (Laake & Rexstad, 2014), an R interface to build, run and rank capture-recapture models in a likelihood framework using Program MARK (White & Burnham, 1999). We ranked models within each approach using the Akaike Information Criterion (AIC) adjusted for small sample sizes (AICc; Burnham & Anderson, 2002) and drew inference about abundance from model-averaged estimates. The following sections detail assumptions and model structure for each approach.

Closed Population Capture-recapture

The Closed model uses marked toads in successive breeding events of the same year to estimate capture (p) and recapture (c) probabilities and the number of individuals not captured (f_0) to obtain the abundance estimate. The approach assumes geographic and demographic closure, i.e. no migration, recruitment, or mortality within each breeding season. This model also assumes that marks are permanent. Our use of natural marks for individual identification supports this assumption since the Admirable Toad ventral patches do not change with age to the point of precluding individual identification (Caorsi et al., *in prep*; Elgue et al., 2014). At the same time, the noninvasiveness of our identification technique is unlikely to cause a trap response. Finally, the Closed model assumes that all animals are available to be detected during the annual survey period. We have relatively less evidence to support this assumption and address the possibility of its failure with the POPAN and PRD models.

We implemented our Closed analysis using the 'Full likelihood *p* and *c*' data type (Lukacs, 2016), which directly estimates *p* (the probability of the first capture), *c* (the probability of recapture) and f_0 (the number, or *frequency*, of individuals that were observed *0* times, i.e., the estimated number of individuals in the population that were never captured). Estimated population size ($\widehat{N}$) under the Closed model is a derived parameter, obtained with the equation:

$$\widehat{N} = \hat{f}_0 + M_{t+1},$$

where M_{t+1} is the total number of marked animals. Thus, since M_{t+1} is a constant number, uncertainty about $\widehat{N}$ equals uncertainty about f_0. We estimate f_0 separately for males and females and use the logit link to model *p* and *c* as functions of covariates. We let *p* vary with sex, amount of rain accumulated during the seven days before the onset of surveys (rain), and with the median temperature of the first survey day in the event (temperature). The parameter *c* is modeled as a function of sex and time, taking one value for each event. We note that, even in the absence of trap aversion, it is reasonable to consider the possibility of $p \neq c$, as well as their variation with sex and environmental factors, because males and females may remain at the breeding site with different probabilities, which in turn will be affected by the availability of pools. We built twelve models that represent twelve hypothetical combinations of covariate effects and fit them separately to data from each year with more than two reproductive events (Table S1). The model set includes the possibility of constant *p* and *c*, represented by model '*p*(.)

c(.)' in Table S1. The year 2013 has only seven models because, in this year, we could not obtain convergence for models with an effect of temperature on *p*.

Jolly-Seber POPAN

As in the Closed analysis, our implementation of POPAN models use each year's data independently of the others. In contrast with Closed, however, POPAN accounts for the possibility of entry and departure of individuals between breeding events of the same year (Schwarz & Arnason, 2019). Specifically, the model includes probabilities of entry (*b*) and survival (φ) between breeding events within a year. From these two parameters, POPAN derives an estimate of the superpopulation size (*N*), which is the total number of individuals entering the breeding site in one year. The parameter *b* is the probability of each individual of the superpopulation entering the study site before a given breeding event. Using indices to designate event-specific parameter values, the expected number of individuals present during each breeding event is given by:

$$E[N_1] = Nb_0$$

$$E[N_{t+1}] = E[N_t]\varphi_t + Nb_t,$$

where b_0 is the probability of each individual of the superpopulation (*N*) to be available in the first breeding event. To account for imperfect detection, POPAN also estimates a probability of detection per capture occasion (*p*). Parameters *p*, *b*, and φ can be held constant or modeled as functions of covariates.

To keep the number of models down to an interpretable level, we followed a sequential procedure of first fitting a set of capture probability functions while holding *b* and φ at the most parameterized structure, which was φ as a function of sex and *b* as an additive function of sex and breeding event. Our set of seven capture probability functions consisted of all the additive combinations of the effects of sex, rain (accumulated in the seven days before the sampling event), and temperature, including a *p*(.) option of constant capture probability (Table S2). Combining the presence or absence of an effect of sex on φ with the four possible additive combinations of the effects of sex and time ('event') on *b*, we obtain eight possible joint models of φ and *b*. In the second stage of our sequential procedure, we fit combinations of the eight joint models of φ and *b* with all the models of *p* from the first stage that have $w > 0.1$ in each year (Tables S3, S4, S5, and S6). The POPAN superpopulation estimate (*N*) for each year is given for both males and females.

Pollock's Robust Design

The PRD approach combines the advantages of both open and closed population models, providing estimates of abundance, apparent survival, and temporary emigration (Kendall, 1999). This is the only one out of our three approaches that combines information from all years in one single analysis. The PRD data structure aggregates data on two temporal scales: the primary periods and, within each of these, multiple secondary occasions. There should be sufficient time between consecutive primary periods for the population to change via recruitment, mortality, and/or migration. Secondary occasions, on the other hand, should be close enough in time to allow for closure within each primary period. In our analysis, each year's reproductive season is a primary period, subdivided in breeding events, which constitute secondary occasions. According to the PRD model, each individual that is alive on primary period *t* survives or does not permanently emigrate to primary period *t*+1 with probability φ, termed apparent survival. Those individuals that survive or do not permanently emigrate may be unavailable for detection during primary period *t*+1 with probability γ, termed temporary emigration. Those that are available, with probability (1- γ), will be captured according to probabilities of first capture (*p*) and recapture (*c*), as in the Closed model. Also as in the Closed model, the PRD model derives yearly estimates of abundance from the sum of the number of individuals captured in that year (or primary period) with the estimated number of individuals that were alive and available at the site in that year but were never captured (f_0). Thus, our estimates of abundance from this analysis in year *t* refer to that part of the superpopulation, which was available to be captured at the site (i.e. breeding site) in year *t*. In our application of the PRD, temporary emigration may be random or Markovian (Kendall & Nichols, 1995; Kendall et al., 1997). Markovian emigration draws a distinction between the probability γ'' that an individual is unavailable in year *t*+1 given that it was available in year *t*, and the probability γ' that an individual was unavailable in year *t*+1, given that it was unavailable in *t*. Under the random emigration model, individuals become unavailable in year *t*+1 independently of their state in year *t*.

As with the POPAN analysis, we use a sequential procedure to fit the sampling and biological components of the PRD model. The most parameterized form of the biological component represents φ, γ'', and γ' as different additive functions of sex and year. While holding the biological component of the model in its most parameterized form, we fit 25 candidate models where *c* is either constant or a function of sex, and *p* appears as various functions of effort, rain, temperature (linear and quadratic effects), and sex (Table S7). The covariate effort equals the number of sampling days in a breeding event (Table 1). The covariate rain is the amount of rainfall accumulated during the seven days before the onset of sampling. After selecting the lowest AIC structure for *p* and *c*, we subsequently fit and ranked 24 models of apparent survival and temporary emigration (Table S8). These consist of Markovian and random temporary emigration versions of twelve models of φ and γ. Apparent survival (φ) appears in four structures: constant, as a function of sex, as a function of year, and as an additive function of sex and year. Temporary emigration appears in the same structures except as a constant parameter, because we didn't find that option biologically informative. We considered the irregular time intervals between the primary periods to obtain annual apparent survival and temporary emigration.

We compared estimates of abundance under the three analytical approaches; nonetheless, we know that PRD extracts information from our multi-year dataset. Therefore, we derive sex ratio and life expectancy statistics from the PRD results alone. We obtain variances of derived statistics based on the Delta method (Powell, 2007) implemented in the R package 'emdbook' (Bolker, 2019). The sex ratio values result from the division of the estimated number of males by the estimated number of females. We used the average annual apparent survival probability ($\bar{\varphi}$) to derive life expectancy for the age of reproductive maturity e_m using Seber's (1973): $e_m = 0.5 + 1 / (1 - \bar{\varphi})$, assuming that all adult individuals found in the breeding site are mature adults

Results

Overview of data and abundance estimates

We obtained 4,620 captures of 1,862 adult Admirable Toad individuals during 88 days of fieldwork between October 2010 and November 2017. Capture effort and capture success were unevenly distributed between the periods before and after 2016. There was less effort in the latter period (18 days in 8 events), but an almost fourfold increase in the number of captures per unit effort, from an average of 33 to 130 captures per day. To focus on breeding events, we only analyze days with more than 30 captures, thus working with a dataset of 41 capture days, all with evidence of reproductive activity (*e.g.* recent clutches, pairs in amplexus, calling males). These contain 4,245 captures of 1,767 adults (Table 1), which is more than 90% of both captures and individuals in the complete dataset. The number of breeding events detected per year varied between two and four (Table 1). We did not fit POPAN and Closed Population models to data from 2010 and 2014 because these years had only two breeding events each. Fifty-one percent of all the individuals captured were recaptured at least once in a different breeding event, while 16% were recaptured at least three times. The number of males captured per breeding event was always between two and five times larger than the number of females.

Estimates of the number of reproductive adults in the population varied more between methods in the same year than between years. Abundance estimates (± standard error) ranged from 561 ± 39 individuals (95% C.I. 484–638), for the Closed Population model in 2011, to 1,734 ± 361 individuals (95% C.I. 1,026–2,442) for the POPAN model, also in 2011 (Figure 2). POPAN results showed the least evidence of temporal variability, while, at the same time, producing the least precise estimates. Abundances estimated by the Closed Population model were consistently lower and more precise than those obtained with POPAN or PRD. POPAN and PRD estimates were closer to each other than either was to the estimates of the Closed Population model. In all but one instance (POPAN 2011), the estimated number of males was higher than that of females (Figure 2). Capture probability was also consistently higher for males than for females, except for the closed population model results from 2011 (Table S9). Likewise, recapture probabilities estimated under the Closed and PRD Models were higher for males than for females (Table S10). The apparent survival estimated under POPAN is particularly low for females in 2016 (Table S11).

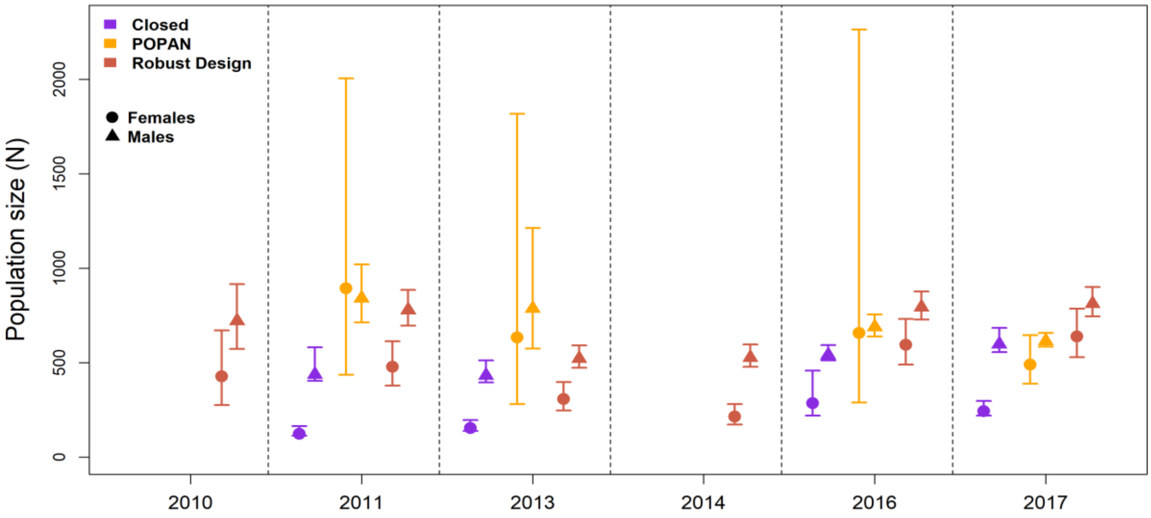

Figure 2. Estimates of adult female and male Admirable Redbelly Toad abundances per year, following the three approaches explored in this study: Closed Population, POPAN Jolly-Seber, and Pollock's Robust Design.

Robust Design Results

The best-ranking model in our comparison of 25 PRD detection models with the same biological component has an AIC weight of 99% (Table S7). We thus model recapture probability (*c*) as a function of sex and capture probability (*p*) as a function of sampling effort, cumulative rainfall, a quadratic effect of temperature, and sex. The big difference in AIC between models #12 and #13 in Table S7 reveals the strong impact of sampling effort on *p*. Models with an effect of rainfall on *p* consistently rank better than similar models without rainfall. The same applies to models with a quadratic effect of temperature, which consistently rank better than similar models with only a linear effect of temperature. Sex explains a large part of the variation in detection probabilities (*p* and *c*), as expressed, for *p*, by the consistently higher ranking of models with an effect of sex, and for *c* by the more than 200 unit difference in AIC between models #23 and #25. Males presented higher capture and recapture probabilities than females (Table 2). Rainfall had a positive effect on *p* (Figure 3A), as expressed by a $\Delta AIC > 8$ for model

#2 in Table S7. The relationship between temperature and *p* shows a maximum capture probability near 20ºC (Figure 3B). The quadratic nature of such relationship is supported by the considerable difference in AIC between models #3 and #1 (>10), as well as between models #10 and #8 (>5) in Table S7. The effort covariate explains most of the variation in *p*, as shown in Fig. 3C and in the more than 450 unit increase in AIC between model #12, the lowest-ranking model with effort, and model #13, the highest-ranking model without effort (Table S7).

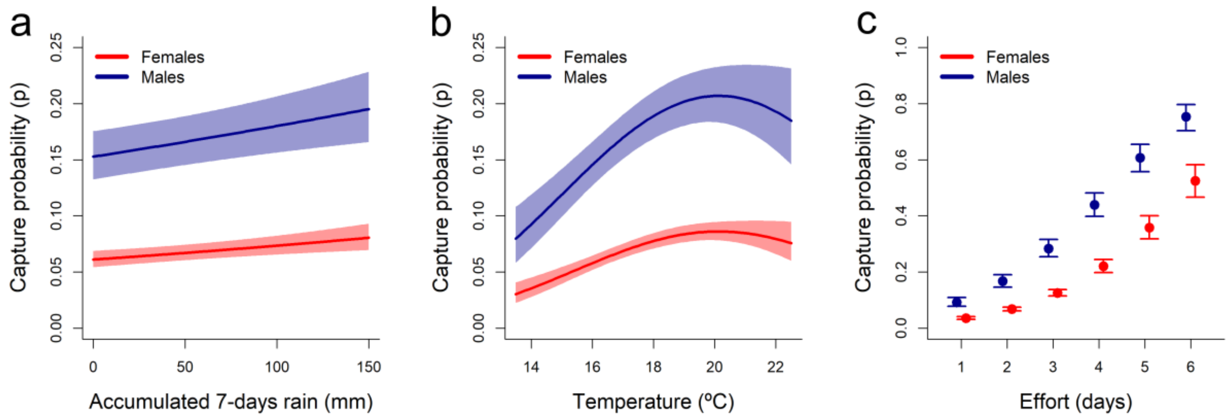

Figure 3. Relationship of capture probability (*p*) of adult female and male Admirable Redbelly Toad with accumulated rainfall (A), temperature (B), and sampling effort (C), under the top-ranking Pollock Robust Design model. Each plot's prediction holds the other two covariates at their average value. The structure of the top-ranking model is given by: φ(year) γ(sex + year) *p* (sex + rain + temp2 + effort) *c* (sex) f_0(sex*year).

Model-averaged PRD parameter estimates show a decrease in abundance for the years 2013 and 2014 (Figure 2, Table 2). Such decrease coincides with relatively high temporary emigration in 2011 and 2013 when compared with 2010; also in agreement, survival probability for 2011 and 2012, before the period of decreased abundance, is estimated at its lowest value throughout the study period (Table 2). The lowest abundance estimate, for the year 2014, coincides with the most biased sex ratio, of nearly two males per female in the population. When abundance was highest, in 2017, the sex ratio was statistically indistinguishable from 1. There is no evidence of differential survival between males and females throughout the period, but females do show consistently higher values of temporary emigration than males. In agreement with the model selection results of the previous paragraph, we see no evidence of difference between model-averaged γ'' and γ'. The model-averaged point estimate of life expectancy for females (8.46±1.65 years) is slightly higher than that of males (7.50±0.77 years) but the uncertainty about those estimates is too large to establish a statistical difference between the sexes.

Table 2. Yearly abundance, sex ratio, annual apparent survival, and annual temporary emigration of adult female and male Admirable Redbelly Toad obtained under the top-ranking Pollock Robust Design model. Sex ratios are expressed as the estimated number of males (M) divided by females (F). Temporary Emigration” is the probability that a toad present in the current year leaves the site, whereas Temporary Emigration’ is the probability that a toad absent in the current year remains out of the breeding site. Values of temporary emigration and survival are not estimable for the last year of the study, and temporary emigration’, likewise, cannot be estimated for the first year. Asterisks indicate uninterpretable estimates, with confidence intervals between zero and one. Survival and temporary emigration estimates for the years 2011 and 2014 are derived under the simplifying assumption of constant survival and temporary emigration during the two-year intervals 2011-2012 and 2014-2015, respectively.

Year	Abundance	Sex Ratio	Survival		Temporary Emigration”		Temporary Emigration’	
			F	M	F	M	F	M
2010	1148±141	1.68±0.14	0.99±0.01*	0.99±0.01*	0.20±0.20	0.09±0.09	-	-
2011	1256±87	1.63±0.14	0.80±0.04	0.78±0.01	0.60±0.08	0.32±0.06	0.57±0.16	0.30±0.09
2013	828±59	1.70±0.11	0.93±0.04	0.92±0.02	0.65±0.07	0.37±0.05	0.66±0.08	0.36±0.06
2014	747±49	2.45±0.31	0.82±0.03	0.80±0.04	0.001±0.012*	0.000±0.004*	0.000±0.015*	0.000±0.012*
2016	1388±82	1.33±0.13	0.94±0.05	0.93±0.05	0.002±0.052*	0.001±0.017*	0.001±0.033*	0.001±0.033*
2017	1451±87	1.27±0.22	-	-	-	-	-	-

Discussion

Knowledge about population size is key for effective wildlife management and for the assessment of species' conservation status (IUCN, 2012). If we may consider the Pollock Robust Design as the most adequate model for this biological system, our results reveal a breeding population of Admirable Toad varying between 650 and 1,650 individuals (PRD model, lower confidence limit – higher confidence limit) over eight years, while spending more than half of the time above 1,000 individuals. Accounting for temporary emigration, in those years for which we could estimate it, the total population of breeding and non-breeding toads would fall between 1,500 and 2,500. Is this the global Admirable Redbelly Toad population size? Since 2010, we have searched for other populations near our study site, along the Forqueta River, and throughout the Taquari-Antas basin. Despite our search over more than seven kilometers of forested river margins (Fonte et al., 2014), all we found was a group of fewer than 20 individuals, in 2017, breeding on a small rocky outcrop 1,500 m downstream from our site (Abadie et al., 2021 – Chapter 3). Across the Forqueta River from our sampling area, there is another small (~200 m^2) rocky outcrop with confirmed *M. admirabilis* breeding activity. We cannot confirm that breeding on the right bank of the river occurs as frequently as on the left. Nonetheless, even if it does, and if it includes approximately 15% of the individuals estimated for the left bank, the total breeding population size will be lower than 2000 individuals.

Our methodological choice of estimating abundance with three different approaches aims at comparing results under a variety of assumptions. The variation between approaches spans hundreds of individuals, an arguably small difference but still revealing informative patterns. The Closed population model consistently produced the lowest abundance estimates, especially for females, in all years, regardless of effort or number of captures. The Closed model assumes closure throughout the sampling period and applies to data from only one year at a time. Therefore, any individual that is not available for capture because it did not breed in our sampling area in one year will be treated as non-existent. Understandably, such a model should produce relatively low estimates, even if it is one of the most reasonable analytical options with a single year of data. One should look for alternative approaches when data from different years reveal information about temporary unavailability. Also, when there are intervals of several weeks between breeding events—as in our case—one should be cautious about assuming closure within a whole year, or breeding season.

Pellet et al. (2011) suggested that POPAN is a useful approach for amphibians with reproductive strategies that are less amenable to closure assumptions, as in the case of prolonged (i.e. not explosive) reproduction. Using a simulation study, they estimated abundances under scenarios of high and low survival and capture probabilities and found that lower values of these probabilities led to higher inaccuracy in abundance estimates. In our study, POPAN results are the least precise among the three approaches, particularly for female estimates, likely due to low capture probabilities (Table S9). The POPAN estimates of apparent survival refer to intervals between breeding events and are thus partly interpretable as emigration from the breeding site. This estimated apparent survival is particularly low for females in 2016, in agreement with the low number of 2016 female recaptures, evident in Table 1. When working with data from only one year, one may face the choice between Closed and POPAN approaches. We believe that this choice should not be guided by the low precision of the POPAN estimates, as it is better to be uncertain about a reasonable result than to be certain about a biased estimate. POPAN is an interesting way of accounting for inevitable changes in the availability of individuals between breeding events. Although we agree with Pellet et al.'s (2011) suggestion that POPAN is appropriate for prolonged breeders, since it models the superpopulation of toads using the breeding site throughout one reproductive season, we believe that it applies equally well to seasonal explosive breeders. The choice between POPAN and Closed, however, may also be informed by comparison with results from a PRD analysis, which simultaneously models data from the whole study period.

The PRD analysis assumes closure within each year, just like the Closed model does; however, individuals that are unavailable in any given year are treated by the PRD model as temporarily emigrated. A probability of temporary emigration is estimated, and both survival and capture parameter estimates will reflect the inference on emigration. One key benefit of PRD models is this ability to quantify temporary emigration from the information of individuals that are not captured in one year but reappear in future years, being unequivocally part of the superpopulation (Kendall, 1999). The difference between Closed and PRD abundance estimates, however, goes beyond the estimation of temporary emigration. Our PRD estimates of capture probability (p) were always lower than those obtained under the Closed analysis, for both males and females (Table S9). This consistent difference in p explains why the PRD abundances tend to be higher than their Closed counterparts, even after accounting for temporary emigration. In the end, despite the lack of precision in the POPAN estimates for 2011, 2013, and 2016, we would recommend the use of POPAN to anyone who had only one year of data obtained under circumstances similar to those of our study. Figure 2 and the inevitable lack of within-year closure lead us to believe that Closed models are underestimating abundance, and especially so for females.

The analysis of capture histories that span multiple breeding seasons, afforded by the PRD, also improves our ability to examine temporal changes in population size.

The estimated number of breeding individuals is lowest in 2013 and 2014 (Table 2). Such a decrease coincides with the highest estimated temporary emigration probabilities, for both sexes, which occurred in 2013 (Table 2). It also follows the lowest estimated apparent survival for males and females, obtained in 2011. Since temporary emigration varies more than survival, we are inclined to attribute most of the variation in abundance to temporary emigration, but some of the decrease in abundance could also result from reduced recruitment in 2012. The (austral) summer of 2012 saw the most severe drought of the previous 15 years in Southern Brazil (Zepner et al., 2020; Estado do Rio Grande do Sul, 2012). If Admirable Toads take one or two years to reach maturity, which appears likely in the similar-sized *Melanophryniscus moreirae* (Jeckel, Saporito, & Grant, 2015), unfavorable conditions to reproduce (and consequently fewer couples breeding), clutch desiccation and/or high juvenile mortality in 2012 could impact breeding population size in 2013 and 2014.

Admirable Redbelly Toad breeding events often concentrate several individuals in one pond with multiple males trying to mate with one female at the same time. Understandably, females may find such episodes stressful and possibly evolve mechanisms for successful breeding while minimizing exposure to large numbers of sexually active males. Besides anecdotal evidence of behavioral avoidance, there at least two lines of population-level evidence that are compatible with exposure minimization: first, females have higher temporary emigration (Table 2); and second, among individuals that are available for capture, females have lower capture (Table S9) and recapture (Table S10) probabilities than males. After accounting for differences in capture probability between sexes, we estimate sex ratios between 1.27 and 2.45 males per female. These estimates are lower than the average 3.3 ratio of captured males to females in Table 1, but they are statistically different from 1 in all but the last year.

Male-biased sex ratios have been reported for other bufonid species based on capture counts (Lampo et al., 2012), and for other species of *Melanophryniscus* based on capture counts (Cairo et al., 2013; Pereira & Maneyro, 2018; Vaira, 2005) and abundance estimates (Bardier et al., 2019). According to Wells (1977), anuran males usually outnumber females at explosive mating aggregations as well as throughout the breeding season, a pattern that should result in competition for mates (Lodé et al., 2005). Zug & Zug (1979) compiled evidence of intraspecific variation in the sex ratio of anuran populations and suggested that extensive variation among *Rhinella marina* populations was more due to the local environment than to phylogenetic history. Some anuran females become reproductively active at an older age than males. This seems to apply to *Melanophryniscus moreirae* as suggested by Jeckel et al (2015) and is common in sexually dimorphic species where females are larger than males (Monnet & Cherry, 2002). Considering we found evidence of similar survival for both sexes (or slightly higher survival for females), and assuming balanced sex ratios at birth, we could expect to find more males than females in the breeding population as a possible role of age maturity in the determination of sex ratios.

Demographic information about amphibians is scarce and patchy (Conde et al., 2019), but we found our estimates of Admirable Toad annual survival probability are generally higher than those published for another redbelly toad (*M. montevidensis*, Bardier et al., 2019) or other bufonids (Lampo et al., 2012; Vasconcellos & Colli, 2009). If *M. admirabilis* survival is indeed high, we hypothesize that it may be due to chemical defense against predators. Among Bufonidae anurans, *Melanophryniscus* is the only genus that has lipophilic alkaloids for chemical defense (Daly et al., 1984; Hantak et al., 2013). Species of the genus accumulate abundant and diverse alkaloids throughout their lives, from the ingestion of arthropods rich in these toxic substances

(Jeckel et al., 2015). The older the toad, the greater the diversity of alkaloids found in the skin and internal organs (Grant et al., 2012; Jeckel et al., 2015). Although not yet clear, alkaloid sequestration may reduce the range of predators that can feed on sequestering species (Savitzky et al., 2012; Stynoski et al., 2014; Toledo & Jared, 1995), as well as minimize the chance of parasite infection (Grant et al., 2012; Mina et al., 2015). In our case, annual survival estimates resulted in a life expectancy of about eight years for an adult toad. Such life expectancies are surprisingly high for animals as small as Admirable Redbelly Toad, but they are well supported by our data, which includes live recaptures seven years after the first capture for both sexes (see Abadie et al, 2021 – Chapter 1).

Based on this study, we are confident that the only known population of *M. admirabilis* has fewer than 2000 reproductive individuals. Our comparison of analytical approaches highlights the usefulness of carrying out multi-year monitoring efforts that account for population openness due to recruitment, mortality, and movement of live individuals. When multi-year analyses are not possible, our results suggest it is best to employ analysis that accounts for movement in and out of the study area throughout one breeding season. Such movements, within or between seasons, may be the most important factor behind the substantial temporal variation in the number of reproductive Admirable Toads, even though we see reasons to suspect that breeding failure during an extremely dry year may have contributed as well. Eight years of observation do not inform a proper assessment of extinction risk (Fieberg & Ellner, 2000) but we find it striking that such a small population could decrease to almost one half of its initial size and subsequently rebound during such a short time. If high survival probability is a characteristic trait of the Admirable Redbelly Toad, we wonder how much does it contribute to the persistence of the species at a low population size.

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Online Supporting Information

Abundance of the microendemic Admirable Redbelly Toad: a comparison of population size estimates.

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Figure S1. An individual of Admirable Redbelly Toad, *Melanoprhyniscus admirabilis* (snout-vent length about 3.5 cm). Photo: Documentation of Threatened Species Project (DoTS).

Figure S2. Forest cover and steep slopes on both sides of the Forqueta River at the Perau de Janeiro region. Photo: Documentation of Threatened Species Project (DoTS).

Figure S3. Breeding site of Admirable Redbelly Toad, at Perau de Janeiro, Arvorezinha/RS, Brazil. (a) flattened rocky outcrop along the left bank of the Forqueta River; (b) a small ephemeral pool on the rocky margins; (c) male individual of ARBT calling on a small pool at rocky outcrop; and (d) a pair in amplexus surrounded by egg clutches.

Figure S4. Individual variation in ventral patch of black pigmentation used as marking for individual recognition of the Admirable Redbelly Toad.

Table S1. Model-selection results for year-specific Closed population model analyses of adult female and male Admirable Redbelly Toad. Letters in front of parentheses stand for first capture (*p*) and recapture (*c*) probabilities. Words in parentheses indicate covariates for each parameter, where 'sex' denotes differences between males and females, 'rain' for rainfall accumulated in seven days before sampling, 'temp' for the median temperature of the first sampling day, and 'effort' for the number of days in each sampling event. For simplicity, we omit model structure specification for the number of individuals that were never caught, which was fixed as *f0* (sex).

Year	#	Model name	AICc[a]	ΔAICc[b]	*w*[c]	*k*[d]	Deviance[e]
2011	1	*p*(rain + temp) *c*(sex + event)	-2878.4	0	0.73	9	54.3900
	2	*p*(sex + rain + temp) *c*(sex + event)	-2876.4	2.00	0.27	10	54.3714
	3	*p*(temp) *c*(sex + event)	-2866.0	12.39	0.00	8	68.8033
	4	*p*(sex + temp) *c*(sex + event)	-2864.4	14.00	0.00	9	68.3904
	5	*p*(sex + rain + temp) *c*(sex)	-2859.5	18.91	0.00	8	75.3155
	6	*p*(rain + temp) *c*(event)	-2855.5	22.84	0.00	8	79.2484
	7	*p*(sex + temp) *c*(sex)	-2847.5	30.91	0.00	7	89.3345
	8	*p*(rain) *c*(sex + event)	-2828.9	49.53	0.00	8	105.9376
	9	*p*(sex + rain) *c*(sex + event)	-2826.9	51.54	0.00	9	105.9285
	10	*p*(sex) *c*(sex + event)	-2805.4	73.02	0.00	8	129.4241
	11	*p*(sex) *c*(sex)	-2788.5	89.93	0.00	6	150.3682
	12	*p*(.) *c*(.)	-2770.6	107.79	0.00	4	172.2563
2013	1	*p*(rain) *c*(sex + event)	-3708.0	0	0.70	7	14.8753
	2	*p*(sex + rain) *c*(sex + event)	-3706.1	1.84	0.28	8	14.6955
	3	*p*(sex + rain) *c*(event)	-3701.3	6.67	0.02	7	21.5471
	4	*p*(sex + rain) *c*(sex)	-3648.1	59.90	0.00	7	74.7794
	5	*p*(sex) *c*(sex + event)	-3220.7	487.31	0.00	7	502.1853
	6	*p*(sex) *c*(sex)	-3162.6	545.37	0.00	6	562.2692
	7	*p*(.) *c*(.)	-3159.8	548.15	0.00	4	569.0736
2016	1	*p*(sex + rain + temp) *c*(sex + event)	-3883.4	0	0.98	10	70.2127
	2	*p*(rain + temp) *c*(sex + event)	-3875.0	8.41	0.01	9	80.6358
	3	*p*(sex + rain) *c*(sex + event)	-3867.5	15.95	0.00	9	88.1785
	4	*p*(rain) *c*(sex + event)	-3857.8	25.58	0.00	8	99.8269
	5	*p*(sex + temp) *c*(sex + event)	-3818.6	64.85	0.00	9	137.0755
	6	*p*(rain + temp) *c*(event)	-3811.0	72.45	0.00	8	146.6957
	7	*p*(temp) *c*(sex + event)	-3809.8	73.60	0.00	8	147.8461
	8	*p*(sex) *c*(sex + event)	-3778.2	105.19	0.00	8	179.4330
	9	*p*(sex + rain + temp) *c*(sex)	-3761.7	121.77	0.00	8	196.0085
	10	*p*(sex + temp) *c*(sex)	-3696.8	186.61	0.00	7	262.8713
	11	*p*(sex) *c*(sex)	-3656.5	226.96	0.00	6	305.2288
	12	*p*(.) *c*(.)	-3585.1	298.29	0.00	4	380.5720

[a] **AICc** =Akaike's Information Criteria adjusted for small sample sizes
[b] **ΔAICc** = difference in the AICc values between top model and the current model
[c] ***w*** = AIC weight
[d] ***k*** = number of estimated parameters
[e] **Deviance** = difference between the saturated model and the current model

Table S1. (Cont.)

Year	#	Model name	AICc[a]	ΔAICc[b]	*w*[c]	*k*[d]	Deviance[e]
2017	1	*p*(rain + temp) *c*(sex + event)	-3611.0	0	0.29	9	52.5343
	2	p(temp) *c*(sex + event)	-3610.3	0.75	0.20	8	55.2941
	3	*p*(rain) *c*(sex + event)	-3609.6	1.45	0.14	8	55.9965
	4	*p*(sex + rain + temp) *c*(sex + event)	-3609.5	1.47	0.14	10	51.9948
	5	*p*(sex + temp) *c*(sex + event)	-3608.7	2.30	0.09	9	54.8364
	6	*p*(sex + rain) *c*(sex + event)	-3608.0	3.01	0.06	9	55.5500
	7	*p*(sex) *c*(sex + event)	-3607.3	3.71	0.04	8	58.2585
	8	*p*(sex + rain + temp) *c*(sex)	-3605.8	5.25	0.02	8	59.7956
	9	*p*(sex + temp) *c*(sex)	-3604.9	6.08	0.01	7	62.6372
	10	*p*(sex) *c*(sex)	-3603.5	7.49	0.01	6	66.0593
	11	*p*(rain + temp) *c*(time)	-3484.0	126.98	0.00	8	181.5340
	12	*p*(.) *c*(.)	-3479.8	131.18	0.00	4	193.7670

[a] **AICc** =Akaike's Information Criteria adjusted for small sample sizes
[b] **ΔAICc** = difference in the AICc values between top model and the current model
[c] ***w*** = AIC weight
[d] ***k*** = number of estimated parameters
[e] **Deviance** = difference between the saturated model and the current model

Table S2. Year-specific model-selection results for capture probability structures using the POPAN model of adult female and male Admirable Redbelly Toad. The letter *p*, in front of parentheses, stands for detection probability, with words in parentheses indicating detection covariates. 'sex' denotes differences between males and females, 'rain' for rainfall accumulated in seven days before sampling, and 'temp' for the median temperature of the first sampling day. All models contain the structures *φ*(sex) *b*(sex + event), as explained in the methods section.

Year	#	Model name	AICc[a]	ΔAICc[b]	*w*[c]	*k*[d]	Deviance[e]
2011	1	*p*(sex + temp)	631.0	0	0.43	11	-1205.44
	2	*p*(sex + rain + temp)	631.6	0.53	0.33	12	-1206.98
	3	*p*(sex + rain)	632.2	1.17	0.24	11	-1204.26
	4	*p*(sex)	639.2	8.20	0.00	10	-1195.16
	5	*p*(.)	643.3	12.30	0.00	9	-1188.99
	6	*p*(rain + temp)	644.2	13.14	0.00	11	-1192.30
	7	*p*(temp)	645.1	14.09	0.00	10	-1189.31
	8	*p*(rain)	645.3	14.24	0.00	10	-1189.12
2013	1	*p*(sex + temp)	272.0	0	0.49	10	-541.75
	2	*p*(temp)	273.7	1.64	0.21	9	-538.04
	3	*p*(sex + rain + temp)	274.1	2.05	0.17	11	-541.79
	4	*p*(rain + temp)	275.6	3.57	0.08	10	-538.18
	5	*p*(sex + rain)	277.4	5.31	0.03	10	-536.44
	6	*p*(rain)	280.1	8.08	0.00	9	-531.59
	7	*p*(.)	585.2	313.15	0.00	8	-224.45
	8	*p*(sex)	601.8	329.75	0.00	9	-209.93
2016	1	*p*(rain + temp)	1102.5	0	0.72	11	-1363.43
	2	*p*(sex + rain + temp)	1104.4	1.91	0.28	12	-1363.58
	3	*p*(sex + temp)	1146.1	43.64	0.00	11	-1319.78
	4	*p*(temp)	1153.5	51.01	0.00	10	-1310.36
	5	*p*(sex)	1202.2	99.75	0.00	10	-1261.63
	6	*p*(rain)	1229.6	127.14	0.00	10	-1234.23
	7	*p*(sex + rain)	1230.7	128.20	0.00	11	-1235.23
	8	*p*(.)	1236.6	134.13	0.00	9	-1225.19
2017	1	*p*(sex)	1697.3	0	0.27	10	-1769.86
	2	*p*(sex + rain + temp)	1697.5	0.19	0.24	12	-1773.74
	3	*p*(sex + temp)	1697.7	0.43	0.22	11	-1771.46
	4	*p*(sex + rain)	1697.9	0.55	0.20	11	-1771.34
	5	*p*(rain + temp)	1701.5	4.15	0.03	11	-1767.74
	6	*p*(temp)	1702.8	5.54	0.02	10	-1764.32
	7	*p*(rain)	1704.4	7.05	0.00	10	-1762.80
	8	*p*(.)	1705.9	8.58	0.00	9	-1759.24

[a] **AICc** =Akaike's Information Criteria adjusted for small sample sizes
[b] **ΔAICc** = difference in the AICc values between top model and the current model
[c] ***w*** = AIC weight
[d] ***k*** = number of estimated parameters
[e] **Deviance** = difference between the saturated model and the current model

Table S3. Biological process model-selection results for the POPAN analysis 2011. Model name letters in front of parentheses stand for apparent survival (φ), detection probability (p), and the probability of entrance (b). Words in parentheses indicate covariates for each parameter: 'sex' denotes differences between males and females, 'rain' for accumulated rainfall for the last seven days before the sampling day, 'temp' for the median temperature of the first sampling day, and 'event' for temporal variation among sampling events. We omit specification of the parameter N, derived from φ and b for males and females.

#	Model name	AICc[a]	ΔAICc[b]	*w*[c]	*k*[d]	Deviance[e]
1	φ(sex) *b*(sex + event) *p*(sex + temp)	631.0	0	0.30	11	-1205.4
2	φ(sex) *b*(sex + event) *p*(sex + rain + temp)	631.6	0.53	0.23	12	-1207.0
3	φ(sex) *b*(sex + event) *p*(sex + rain)	632.2	1.17	0.17	11	-1204.3
4	φ(sex) *b*(event) *p*(sex + rain + temp)	634.4	3.34	0.06	11	-1202.1
5	φ(.) *b*(sex + event) *p*(sex + rain)	634.6	3.58	0.05	10	-1199.8
6	φ(.) *b*(sex + event) *p*(sex + temp)	634.9	3.94	0.04	10	-1199.4
7	φ(.) *b*(event) *p*(sex + rain + temp)	635.0	3.99	0.04	10	-1199.4
8	φ(sex) *b*(event) *p*(sex + rain)	635.1	4.08	0.04	10	-1199.3
9	φ(.) *b*(event) *p*(sex + rain)	635.4	4.39	0.03	9	-1196.9
10	φ(.) *b*(sex + event) *p*(sex + rain + temp)	636.7	5.62	0.02	11	-1199.8
11	φ(.) *b*(event) *p*(sex + temp)	640.7	9.63	0.00	9	-1191.7
12	φ(sex) *b*(event) *p*(sex + temp)	642.7	11.62	0.00	10	-1191.7
13	φ(sex) *b*(sex + event) *p*(rain + temp)	644.2	13.14	0.00	11	-1192.3
14	φ(.) *b*(.) *p*(sex + rain + temp)	644.7	13.64	0.00	8	-1185.6
15	φ(.) *b*(sex) *p*(sex + rain + temp)	646.0	14.97	0.00	9	-1186.3
16	φ(sex) *b*(sex) *p*(sex + rain + temp)	649.2	18.20	0.00	10	-1185.2
17	φ(sex) *b*(.) *p*(sex + rain + temp)	652.4	21.36	0.00	9	-1179.9
18	φ(.) *b*(.) *p*(sex + temp)	652.7	21.71	0.00	7	-1175.5
19	φ(.) *b*(sex) *p*(sex + temp)	653.2	22.18	0.00	8	-1177.0
20	φ(sex) *b*(.) *p*(sex + temp)	653.6	22.56	0.00	8	-1176.7
21	φ(sex) *b*(sex) *p*(sex + temp)	654.9	23.91	0.00	9	-1177.4
22	φ(.) *b*(.) *p*(sex + rain)	656.5	25.50	0.00	7	-1171.7
23	φ(sex) *b*(.) *p*(sex + rain)	656.8	25.76	0.00	8	-1173.5
24	φ(.) *b*(sex) *p*(sex + rain)	658.5	27.49	0.00	8	-1171.7

[a] **AICc** =Akaike's Information Criteria adjusted for small sample sizes
[b] **ΔAICc** = difference in the AICc values between top model and the current model
[c] ***w*** = AIC weight
[d] ***k*** = number of estimated parameters
[e] **Deviance** = difference between the saturated model and the current model

Table S4. Biological process model-selection results for the POPAN analysis 2013. Model name letters in front of parentheses stand for apparent survival (*φ*), detection probability (*p*), and the probability of entrance (*b*). Words in parentheses indicate covariates for each parameter: 'sex' denotes differences between males and females, 'rain' for accumulated rainfall for the last seven days before the sampling day, 'temp' for the median temperature of the first sampling day, and 'event' for temporal variation among sampling events. We omit the specification of the parameter *N*, which is derived from *φ* and *b*.

#	Model name	AICc[a]	ΔAICc[b]	*w*[c]	*k*[d]	Deviance[e]
1	*φ*(.) *b*(event) *p*(sex + temp)	268.4	0.00	0.18	8	-541.2
2	*φ*(.) *b*(.) *p*(sex + rain + temp)	268.9	0.50	0.14	8	-540.7
3	*φ*(.) *b*(.) *p*(sex + temp)	270.2	1.74	0.08	7	-537.4
4	*φ*(.) *b*(event) *p*(sex + rain + temp)	270.3	1.84	0.07	9	-541.5
5	*φ*(.) *b*(sex + event) *p*(sex + temp)	270.3	1.87	0.07	9	-541.4
6	*φ*(sex) *b*(event) *p*(sex + temp)	270.5	2.07	0.06	9	-541.2
7	*φ*(sex) *b*(.) *p*(sex + rain + temp)	270.7	2.30	0.06	9	-541.0
8	*φ*(.) *b*(sex) *p*(sex + rain + temp)	270.7	2.33	0.06	9	-541.0
9	*φ*(.) *b*(sex) *p*(sex + temp)	271.4	3.02	0.04	8	-538.2
10	*φ*(sex) *b*(event) *p*(sex + rain + temp)	272.0	3.62	0.03	10	-541.8
11	*φ*(sex) *b*(sex + event) *p*(sex + temp)	272.1	3.64	0.03	10	-541.8
12	*φ*(sex) *b*(.) *p*(sex + temp)	272.1	3.72	0.03	8	-537.5
13	*φ*(.) *b*(sex + event) *p*(sex + rain + temp)	272.3	3.88	0.03	10	-541.5
14	*φ*(sex) *b*(sex) *p*(sex + rain + temp)	272.8	4.34	0.02	10	-541.1
15	*φ*(sex) *b*(event) *p*(temp)	273.0	4.56	0.02	8	-536.7
16	*φ*(sex) *b*(sex) *p*(sex + temp)	273.1	4.73	0.02	9	-538.6
17	*φ*(.) *b*(event) *p*(temp)	273.2	4.81	0.02	7	-534.4
18	*φ*(sex) *b*(sex + event) *p*(temp)	273.7	5.28	0.01	9	-538.0
19	*φ*(sex) *b*(sex + event) *p*(sex + rain + temp)	274.1	5.68	0.01	11	-541.8
20	*φ*(sex) *b*(.) *p*(temp)	274.4	5.94	0.01	7	-533.2
21	*φ*(.) *b*(.) *p*(temp)	274.9	6.53	0.01	6	-530.6
22	*φ*(sex) *b*(sex) *p*(temp)	275.6	7.16	0.01	8	-534.1

[a] **AICc** =Akaike's Information Criteria adjusted for small sample sizes
[b] **ΔAICc** = difference in the AICc values between top model and the current model
[c] ***w*** = AIC weight
[d] ***k*** = number of estimated parameters
[e] **Deviance** = difference between the saturated model and the current model

Table S5. Biological process model-selection results for the POPAN analysis 2016. Model name letters in front of parentheses stand for apparent survival (*φ*), detection probability (*p*), and the probability of entrance (*b*). Words in parentheses indicate covariates for each parameter: 'sex' denotes differences between males and females, 'rain' for accumulated rainfall for the last seven days before the sampling day, 'temp' for the median temperature of the first sampling day, and 'event' for temporal variation among sampling events. We omit the specification of the parameter *N*, which is derived from *φ* and *b*.

#	Model name	AICc[a]	ΔAICc[b]	*w*[c]	*k*[d]	Deviance[e]
1	*φ*(sex) *b*(sex + event) *p*(rain + temp)	1102.5	0.00	0.60	11	-1363.4
2	*φ*(sex) *b*(sex + event) *p*(sex + rain + temp)	1104.4	1.91	0.23	12	-1363.6
3	*φ*(sex) *b*(sex) *p*(rain + temp)	1105.6	3.17	0.12	9	-1356.2
4	*φ*(sex) *b*(sex) *p*(sex + rain + temp)	1107.6	5.17	0.05	10	-1356.2
5	*φ*(.) *b*(.) *p*(sex + rain + temp)	1117.6	15.12	0.00	8	-1342.2
6	*φ*(.) *b*(sex) *p*(sex + rain + temp)	1118.5	16.04	0.00	9	-1343.3
7	*φ*(sex) *b*(.) *p*(sex + rain + temp)	1119.6	17.13	0.00	9	-1342.2
8	*φ*(.) *b*(event) *p*(sex + rain + temp)	1120.6	18.16	0.00	10	-1343.2
9	*φ*(.) *b*(sex + event) *p*(sex + rain + temp)	1121.3	18.86	0.00	11	-1344.6
10	*φ*(sex) *b*(event) *p*(rain + temp)	1147.3	44.84	0.00	10	-1316.5
11	*φ*(sex) *b*(event) *p*(sex + rain + temp)	1148.5	46.03	0.00	11	-1317.4
12	*φ*(sex) *b*(.) *p*(rain + temp)	1153.9	51.39	0.00	8	-1305.9
13	*φ*(.) *b*(sex + event) *p*(rain + temp)	1166.2	63.72	0.00	10	-1297.7
14	*φ*(.) *b*(sex) *p*(rain + temp)	1170.7	68.24	0.00	8	-1289.1
15	*φ*(.) *b*(event) *p*(rain + temp)	1184.4	81.93	0.00	9	-1277.4
16	*φ*(.) *b*(.) *p*(rain + temp)	1186.9	84.47	0.00	7	-1270.8

[a] **AICc** =Akaike's Information Criteria adjusted for small sample sizes
[b] **ΔAICc** = difference in the AICc values between top model and the current model
[c] ***w*** = AIC weight
[d] ***k*** = number of estimated parameters
[e] **Deviance** = difference between the saturated model and the current model

Table S6. Biological process model-selection results for the POPAN analysis 2017. Model name letters in front of parentheses stand for apparent survival (φ), detection probability (*p*), and the probability of entrance (*b*). Words in parentheses indicate covariates for each parameter: 'sex' denotes differences between males and females, 'rain' for accumulated rainfall for the last seven days before the sampling day, 'temp' for the median temperature of the first sampling day, and 'event' for temporal variation among sampling events. We omit the specification of the derived parameter *N*, which is a function of sex.

#	Model name	AICc[a]	ΔAICc[b]	*w*[c]	*k*[d]	Deviance[e]
1	φ(sex) *b*(sex) *p*(sex + rain + temp)	1696.8	0.00	0.09	10	-1770.4
2	φ(sex) *b*(event) *p*(sex)	1697.0	0.28	0.08	9	-1768.1
3	φ(sex) *b*(sex + event) *p*(sex)	1697.3	0.55	0.07	10	-1769.9
4	φ(sex) *b*(.) *p*(sex + rain + temp)	1697.4	0.61	0.07	9	-1767.8
5	φ(.) *b*(sex + event) *p*(sex + temp)	1697.4	0.61	0.07	10	-1769.8
6	φ(.) *b*(sex + event) *p*(sex + rain)	1697.4	0.67	0.07	10	-1769.7
7	φ(sex) *b*(sex + event) *p*(sex + rain + temp)	1697.5	0.74	0.06	12	-1773.7
8	φ(sex) *b*(event) *p*(sex + rain + temp)	1697.6	0.81	0.06	11	-1771.6
9	φ(.) *b*(sex + event) *p*(sex)	1697.7	0.89	0.06	9	-1767.5
10	φ(sex) *b*(sex + event) *p*(sex + temp)	1697.7	0.98	0.06	11	-1771.5
11	φ(sex) *b*(sex + event) *p*(sex + rain)	1697.9	1.10	0.05	11	-1771.3
12	φ(sex) *b*(event) *p*(sex + temp)	1698.0	1.19	0.05	10	-1769.2
13	φ(.) *b*(event) *p*(sex + rain + temp)	1698.0	1.23	0.05	10	-1769.2
14	φ(sex) *b*(event) *p*(sex + rain)	1698.3	1.49	0.04	10	-1768.9
15	φ(.) *b*(sex + event) *p*(sex + rain + temp)	1699.4	2.65	0.03	11	-1769.8
16	φ(sex) *b*(sex) *p*(sex + temp)	1699.5	2.72	0.02	9	-1765.6
17	φ(sex) *b*(.) *p*(sex + temp)	1699.7	2.90	0.02	8	-1763.4
18	φ(sex) *b*(.) *p*(sex + rain)	1701.0	4.25	0.01	8	-1762.1
19	φ(sex) *b*(sex) *p*(sex + rain)	1701.1	4.38	0.01	9	-1764.0
20	φ(sex) *b*(.) *p*(sex)	1703.4	6.64	0.00	7	-1757.7
21	φ(sex) *b*(sex) *p*(sex)	1704.6	7.83	0.00	8	-1758.5
22	φ(.) *b*(event) *p*(sex + temp)	1706.8	10.04	0.00	9	-1758.3
23	φ(.) *b*(event) *p*(sex + rain)	1709.5	12.74	0.00	9	-1755.6
24	φ(.) *b*(sex) *p*(sex + temp)	1710.9	14.12	0.00	8	-1752.2
25	φ(.) *b*(sex) *p*(sex)	1711.2	14.47	0.00	7	-1749.8
26	φ(.) *b*(sex) *p*(sex + rain)	1711.4	14.59	0.00	8	-1751.7
27	φ(.) *b*(sex) *p*(sex + rain + temp)	1711.6	14.84	0.00	9	-1753.5
28	φ(.) *b*(event) *p*(sex)	1712.6	15.85	0.00	8	-1750.5
29	φ(.) *b*(.) *p*(sex + temp)	1718.6	21.81	0.00	7	-1742.5
30	φ(.) *b*(.) *p*(sex + rain)	1719.1	22.30	0.00	7	-1742.0
31	φ(.) *b*(.) *p*(sex)	1719.2	22.43	0.00	6	-1739.9
32	φ(.) *b*(.) *p*(sex + rain + temp)	1719.4	22.63	0.00	8	-1743.7

[a] **AICc** =Akaike's Information Criteria adjusted for small sample sizes
[b] **ΔAICc** = difference in the AICc values between top model and the current model
[c] ***w*** = AIC weight
[d] ***k*** = number of estimated parameters
[e] **Deviance** = difference between the saturated model and the current model

Table S7. Sampling process model-selection results for the Pollock Robust Design analysis. Model name letters in front of parentheses stand for first capture (*p*) and recapture probability (*c*). Words in parentheses indicate covariates for each parameter, with ‘sex’ standing for the sex and ‘rain’ for rainfall accumulated in seven days before sampling. The words ‘temp’ and ‘temp2’ stand, respectively, for linear and quadratic effects of the median temperature of the first sampling day. The word ‘effort’ is the number of trapping days in the sampling event. For simplicity, we omit specification of the biological component of the model, which was fixed as *φ*(sex + year) *γ’’*(sex + year) *γ’*(sex + year). We also omit the model structure for parameter *f*0, fixed as *f0*(sex*year), as explained in the methods section.

#	Model name	AICc[a]	ΔAICc[b]	*w*[c]	*k*[d]	Deviance[e]
1	*p*(effort + rain + temp2 + sex) *c*(sex)	-13427.4	0	0.99	37	-2398.27
2	*p*(effort + temp2 + sex) *c*(sex)	-13418.4	8.98	0.01	36	-2387.24
3	*p*(effort + rain + temp + sex) *c*(sex)	-13415.3	12.15	0.00	36	-2384.08
4	*p*(effort + rain + temp2) *c*(sex)	-13414.4	13.07	0.00	36	-2383.16
5	*p*(effort + rain * temp + sex) *c*(sex)	-13413.3	14.14	0.00	37	-2384.12
6	*p*(effort + temp + sex) *c*(sex)	-13.411.1	16.36	0.00	35	-2377.83
7	*p*(effort + rain + temp) *c*(sex)	-13405.2	22.21	0.00	35	-2371.98
8	*p*(effort + temp2) *c*(sex)	-13403.8	23.65	0.00	35	-2370.54
9	*p*(effort + rain * temp) *c*(sex)	-13403.3	24.11	0.00	36	-2372.12
10	*p*(effort + temp) *c*(sex)	-13399.3	28.11	0.00	34	-2364.04
11	*p*(effort + rain + sex) *c*(sex)	-13362.4	65.08	0.00	35	-2329.11
12	*p*(effort + rain) *c*(sex)	-13340.0	87.45	0.00	34	-2304.70
13	*p*(rain + temp2) *c*(sex)	-12850.0	577.41	0.00	35	-1816.78
14	*p*(rain + temp2 + sex) *c*(sex)	-12849.1	578.37	0.00	36	-1817.86
15	*p*(rain * temp + sex) *c*(sex)	-12803.2	624.27	0.00	36	-1771.95
16	*p*(rain * temp) *c*(sex)	-12802.8	624.60	0.00	35	-1769.59
17	*p*(rain + temp) *c*(sex)	-12782.4	645.08	0.00	34	-1747.07
18	*p*(rain + temp + sex) *c*(sex)	-12781.8	645.65	0.00	35	-1748.54
19	*p*(rain + sex) *c*(sex)	-12776.7	650.76	0.00	34	-1741.39
20	*p*(temp2 + sex) *c*(sex)	-12758.6	668.81	0.00	35	-1725.38
21	*p*(temp + sex) *c*(sex)	-12452.6	974.82	0.00	34	-1417.33
22	*p*(sex) *c*(sex)	-12409.9	1017.5	0.00	33	-1372.57
23	*p*(.) *c*(sex)	-12409.4	1018.0	0.00	32	-1370.00
24	*p*(sex) *c*(.)	-12207.3	1220.1	0.00	32	-1167.93
25	*p*(.) *c*(.)	-12206.8	1220.7	0.00	31	-1165.37

[a] **AICc** =Akaike’s Information Criteria adjusted for small sample sizes
[b] **ΔAICc** = difference in the AICc values between top model and the current model
[c] ***w*** = AIC weight
[d] ***k*** = number of estimated parameters
[e] **Deviance** = difference between the saturated model and the current model

Table S8. Biological process model-selection results for the Pollock Robust Design analysis. Model name letters in front of parentheses stand for survival probability (*φ*) and the probability of temporary emigration (*γ*). Models where *γ* has no prime(s) treat temporary emigration as a random process independent from the migration state of individuals in the previous year. The remaining models portray temporary emigration as a Markov process where the state of an individual at time *t* depends on its state at time *t*–1, as given by the probability *γ'* that an individual stays out of the study area in primary occasion *t*, given that it was already out in *t*–1; and the probability *γ"* that an individual leaves the study area in primary occasion *t*, given that it was present there in *t*–1. Words in parentheses indicate covariates for each parameter: 'sex' stands for the sex, and 'year' for temporal variation among years. For simplicity, we omit specification of the detection component of the model, which was fixed as *p* (sex + rain + temp2 + effort) *c* (sex) *f0*(sex*year), as explained in the methods section.

#	Model name	AICc[a]	ΔAICc[b]	*w*[c]	*k*[d]	Deviance[e]
1	*φ*(year) γ(sex + year)	-13435.7	0.00	0.66	31	-2394.34
2	*φ*(sex + year) γ(sex + year)	-13434.3	1.43	0.32	32	-2394.95
3	*φ*(year) γ"(sex + year) γ'(sex + year)	-13428.7	6.99	0.02	36	-2397.54
4	*φ*(sex + year) γ"(sex + year) γ'(sex + year)	-13427.4	8.30	0.01	37	-2398.27
5	*φ*(year) γ(year)	-13424.4	11.34	0.00	30	-2380.97
6	*φ*(.) γ(sex + year)	-13423.6	12.11	0.00	27	-2374.11
7	*φ*(sex + year) γ(year)	-13423.5	12.19	0.00	31	-2382.16
8	*φ*(sex) γ(sex + year)	-13421.9	13.82	0.00	28	-2374.43
9	*φ*(.) γ"(sex + year) γ'(sex + year)	-13420.4	15.29	0.00	32	-2381.09
10	*φ*(sex) γ"(sex + year) γ'(sex + year)	13418.8	16.89	0.00	33	-2381.52
11	*φ*(year) γ"(year) γ'(year)	-13417.3	18.38	0.00	34	-2382.07
12	*φ*(sex + year) γ"(year) γ'(year)	-13416.7	19.08	0.00	35	-2383.41
13	*φ*(.) γ(year)	-13411.4	24.37	0.00	26	-2359.82
14	*φ*(sex) γ(year)	-13410.3	25.42	0.00	27	-2360.80
15	*φ*(.) γ"(year) γ'(year)	-13406.7	29.07	0.00	30	-2363.24
16	*φ*(sex) γ"(year) γ'(year)	-13405.4	30.29	0.00	31	-2364.05
17	*φ*(year) γ(sex)	-13392.7	43.03	0.00	27	-2343.19
18	*φ*(sex + year) γ(sex)	-13392.1	43.65	0.00	28	-2344.60
19	*φ*(year) γ"(sex) γ'(sex)	-13391.3	44.45	0.00	29	-2345.83
20	*φ*(sex + year) γ"(sex) γ'(sex)	-13389.5	46.28	0.00	30	-2346.04
21	*φ*(sex) γ(sex)	-13349.2	86.57	0.00	24	-2293.57
22	*φ*(.) γ(sex)	-13348.2	87.55	0.00	23	-2290.56
23	*φ*(.) γ"(sex) γ'(sex)	-13346.3	89.45	0.00	25	-2292.72
24	*φ*(sex) γ"(sex) γ'(sex)	-13345.5	90.25	0.00	26	-2293.94

[a] **AICc** =Akaike's Information Criteria adjusted for small sample sizes
[b] **ΔAICc** = difference in the AICc values between top model and the current model
[c] ***w*** = AIC weight
[d] ***k*** = number of estimated parameters
[e] **Deviance** = difference between the saturated model and the current model

Table S9. Model-averaged estimates ± SE of capture probability for Closed, POPAN, and PRD models. Each row corresponds to one breeding event identified by month and year. The letter 'F' indicates females and 'M' males.

Month/ Year	Closed		POPAN		Robust Design	
	F	M	F	M	F	M
Oct/2010	-	-	-	-	0.02±0.003	0.05±0.007
Nov/2010	-	-	-	-	0.04±0.004	0.10±0.007
Jul/2011	0.20±0.024	0.20±0.020	0.08±0.080	0.37±0.164	0.08±0.008	0.19±0.016
Aug/2011	0.50±0.046	0.50±0.041	0.06±0.028	0.36±0.061	0.09±0.009	0.22±0.016
Sep/2011	0.46±0.078	0.46±0.076	0.03±0.018	0.20±0.042	0.05±0.005	0.14±0.009
Oct/2011	0.58±0.081	0.53±0.079	0.03±0.018	0.21±0.056	0.03±0.003	0.09±0.006
Aug/2013	0.13±0.026	0.14±0.020	0.12±0.222	0.24±0.219	0.06±0.005	0.15±0.009
Oct/2013	0.77±0.067	0.78±0.058	0.22±0.136	0.48±0.135	0.35±0.039	0.60±0.038
Dec/2013	0.13±0.026	0.14±0.020	0.01±0.008	0.04±0.012	0.04±0.006	0.10±0.013
Jul/2014	-	-	-	-	0.02±0.003	0.07±0.006
Sep/2014	-	-	-	-	0.40±0.040	0.66±0.034
Sep/2016	0.18±0.051	0.35±0.029	0.25±0.097	0.27±0.018	0.14±0.013	0.32±0.017
Oct/2016	0.37±0.087	0.60±0.040	0.44±0.141	0.47±0.029	0.08±0.007	0.20±0.011
Nov/2016	0.21±0.057	0.40±0.030	0.30±0.110	0.32±0.020	0.08±0.007	0.19±0.011
Dec/2016	0.09±0.040	0.20±0.050	0.07±0.033	0.08±0.014	0.03±0.003	0.09±0.007
Aug/2017	0.32±0.032	0.33±0.023	0.22±0.090	0.51±0.131	0.10±0.009	0.24±0.014
Sep/2017	0.38±0.055	0.39±0.049	0.21±0.073	0.50±0.037	0.09±0.008	0.22±0.013
Oct/2017	0.39±0.054	0.40±0.047	0.20±0.083	0.49±0.032	0.09±0.011	0.22±0.021
Nov/2017	0.39±0.075	0.40±0.070	0.21±0.081	0.50±0.041	0.08±0.007	0.20±0.012

Table S10. Model-averaged estimates ± SE of recapture probabilities for Closed and PRD models. Each row corresponds to one breeding event identified by month and year. The letter 'F' indicates females and 'M' males. Estimates of recapture probability under our PRD model were considered as constant.

<table>
<tr><th rowspan="2">Month/Year</th><th colspan="2">Closed</th><th colspan="2">Robust Design</th></tr>
<tr><th>F</th><th>M</th><th>F</th><th>M</th></tr>
<tr><td>Nov/2010</td><td>-</td><td>-</td><td rowspan="13">0.06±0.007</td><td rowspan="13">0.24±0.007</td></tr>
<tr><td>Aug/2011</td><td>0.05±0.025</td><td>0.27±0.047</td></tr>
<tr><td>Sep/2011</td><td>0.02±0.012</td><td>0.14±0.021</td></tr>
<tr><td>Oct/2011</td><td>0.01±0.007</td><td>0.08±0.014</td></tr>
<tr><td>Oct/2013</td><td>0.15±0.072</td><td>0.38±0.061</td></tr>
<tr><td>Dec/2013</td><td>0.01±0.006</td><td>0.04±0.010</td></tr>
<tr><td>Sep/2014</td><td>-</td><td>-</td></tr>
<tr><td>Oct/2016</td><td>0.08±0.023</td><td>0.38±0.035</td></tr>
<tr><td>Nov/2016</td><td>0.05±0.014</td><td>0.27±0.022</td></tr>
<tr><td>Dec/2016</td><td>0.01±0.003</td><td>0.06±0.011</td></tr>
<tr><td>Sep/2017</td><td>0.14±0.024</td><td>0.48±0.036</td></tr>
<tr><td>Oct/2017</td><td>0.11±0.018</td><td>0.42±0.024</td></tr>
<tr><td>Nov/2017</td><td>0.10±0.016</td><td>0.37±0.023</td></tr>
</table>

Table S11. Model-averaged estimates ± SE of apparent survival and entrance probabilities for POPAN models. Each row corresponds to one interval of the breeding event identified by month and year. The letter 'F' indicates females and 'M' males. Estimates of apparent survival probability do not vary with time. Asterisks indicate uninterpretable estimates, with confidence intervals between zero and one.

Year	Apparent Survival		Probability of Entrance	
	F	M	F	M
Jul/2011			0.16±0.224	0.41±0.118
Aug/2011	0.89±0.143	0.63±0.105	0.07±0.128	0.26±0.093
Sep/2011			*	*
Aug/2013	0.90±0.147	0.92±0.114	0.054±0.243	0.55±0.233
Oct/2013			0.09±0.183	0.10±0.190
Sep/2016			0.19±0.068	*
Oct/2016	0.16±0.107	0.83±0.041	0.13±0.045	*
Nov/2016			0.34±0.107	*
Aug/2017			0.15±0.161	0.024±0.131
Sep/2017	0.71±0.151	0.89±0.032	0.06±0.056	0.09±0.045
Oct/2017			0.04±0.044	0.04±0.035

Capítulo 3

Assessment of threats, extinction risk and conservation priority actions for the microendemic Admirable Redbelly Toad

Este manuscrito segue o formato da revista *Perpectives in Ecology and Conservation*. A fim de facilitar a leitura, as figuras e tabelas foram inseridas no corpo do texto.

Assessment of threats, extinction risk and conservation priority actions for the microendemic Admirable Redbelly Toad

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Abstract

Red Lists are essential conservation tools, which influence conservation outputs, such as scientific knowledge gain and public awareness. Carefully identifying all known and potential threats to a species is important not only to assess the extinction risk of a species but also to plan priority actions for its conservation. In this study, we used geographical distribution metrics and global population size, combined with an objective approach to rank the threats, to reassess the conservation status of the microendemic *Melanophryniscus admirabilis* (Anura: Bufonidae), the Admirable Redbelly Toad, under three IUCN criteria. We found a known distribution of 0.049 km² and an area of occupancy (AOO) of 4 km² as a maximum distribution for *M. admirabilis*. The most relevant threats to the species are Bd and agrochemicals from tobacco and soy plantations, and the most concerning one in terms of intensity and extent is the hydropower plant construction, even though it is under control. Finally, we discussed priority actions for the conservation of the Admirable toad in order to reduce the species’ extinction risk. Although a great effort is needed to protect the toad, the distribution of *M. admirabilis* is so small that only finding a new population could remove the species from threatened categories.

Keywords: *Melanophryniscus admirabilis*, Critically Endangered, area of occupancy, extent of occurrence, IUCN Red List criteria, ranking of threats

1. Introduction

A quantitative framework widely used to assess the conservation status of a taxon is the IUCN Red List Categories and Criteria, which highlights those species that are most likely to become extinct in a not-so-distant future (IUCN Standards and Petitions Committee, 2019). This standardized approach has been periodically updated and improved and is now widely applied to regional, national, and local species assessments worldwide, even though it was designed for global assessments (Mace et al, 2008). Red Lists are essential conservation tools, influencing conservation outputs such as scientific knowledge gain and public awareness, although measuring their impact on positive changes for species persistence is still a challenge (Betts et al., 2020).

The IUCN Red List approach is based on five criteria, which refer to currently available knowledge about population size, geographic distribution, trends in population and distribution, and recent, current, or projected threats acting on the species (IUCN Standards and Petitions Committee, 2019). Data for assessing the complete set of criteria are not available for the majority of species, but the approach is flexible enough to be applicable to most of the known taxa. For example, in the absence of information on abundance and population trends, about 85% of threatened amphibians have their assessments based exclusively on the criteria of geographic distribution (criteria B and D2; IUCN, 2021). For assessing a species' extinction risk adequate data are required for at least one criterion; otherwise the species is assessed as Data Deficient (DD).

Carefully identifying threats is important not only to assess the extinction risk of a species but also to plan priority actions for its conservation. Sound planning on how to mitigate threats includes monitoring and reviewing the plan, to subsequently improve its implementation (CMP, 2020). Conservation Action Plans are usually focused on promoting threat reduction to conservation targets (TNC, 2007). At the global level, in response to the global amphibian crisis (Young et al., 2001; Stuart et al., 2004), the Amphibian Conservation Action Plan - ACAP (Gascon et al., 2007; Wren et al., 2015) addressed the most important threats and the priority action steps for amphibian conservation (Bishop et al, 2012). At the national level in Brazil, there are tools designed for prioritizing conservation actions through threat suppression or mitigation, such as the National Action Plans for Conservation of Species Threatened with Extinction (PAN; Instrução Normativa MMA nº 21, 2018; e.g. Baptista et al., 2019).

Melanophryniscus admirabilis (Admirable Redbelly Toad) is an example of a priority species for conservation (Portaria MMA Nº 350, 2019). It is a microendemic and Critically Endangered anuran that faced a high risk of extinction due to the imminent construction of a hydroelectric power plant just 500 meters upstream of the species' breeding site (Fonte et al., 2014). At that time, the species was not legally protected by any environmental law in Brazil, and it had been categorized as Near Threatened (NT) in The IUCN Red List of Threatened Species (Maneyro, 2008). This assessment was questioned by local researchers, and its conservation status was reassessed with the scarce information that was produced in a year (Fonte et al., 2014). It was reassessed as Critically Endangered based on criteria B1ab(iii,v)+2ab(iii,v) (IUCN SSC Amphibian Specialist Group, 2013). Simultaneously, a team of researchers and conservationists began a long-term project to produce as much information as possible about its population, habitat and threats.

The main objective of this study is to present all the acquired information of the Admirabilis Project in the past ten years and offer a reassessment of the conservation status of the Admirable Redbelly Toad. Specifically, we addressed: (1) distribution of the species in terms of area of occupancy (AOO) and extent of occurrence (EOO); (2) population size of the species; (3) identification, description and ranking of threats; and (4) conservation status. Finally, we discuss some possible strategies to protect the population of the Admirable Redbelly Toad.

2. Study species and area

Melanophryniscus admirabilis is a colorful (bright green back, black and bright red belly), small (< 42 mm), poisonous (lipophilic alkaloids from diet) and explosive breeding bufonid. The species occurs in a very specific environment, breeding on a flattened river bank outcrop, which is surrounded by forested steep slopes in a river valley (Figure 1A). Small and shallow temporary pools on the rocky outcrop are used for calling, spawning, and development of tadpoles (Figure 1B-D). The species can live at least 9 years and seems to have high site fidelity, particularly among adults (Abadie et al, 2021 – Chapter 1).

Figure 1. Admirable Redbelly Toad (*Melanophryniscus admirabilis*) and its only known locality. (A) Forested steep slopes in a river valley, in Southernmost Atlantic Forest; (B) colorful Admirable toads breeding in a small pool; (C) a calling male; and (D) amplexus pair spawning in a small pool. Photos: Pedro Peloso - DoTS Project, Michelle Abadie, Valentina Caorsi, and Simone Leonardi, respectively.

The Admirable Redbelly Toad is known from a single locality on the margins of the Forqueta River, in Perau de Janeiro, on the border between the municipalities of Arvorezinha and Soledade, Rio Grande do Sul, Brazil (28° 51’ 25.3” S; 52° 18’ 12.3”

W). This area is located in the Southernmost Atlantic Forest biome, about 550 m asl, on the southern border slopes of the Brazilian Southern Plateau. The climate of the region is classified as Subtropical Humid, without a dry season and with hot summers (*Cfa*, Koeppen's climate classification; Alvares et al, 2013). The species' type locality is part of the largest remaining forest fragments in the region (about 500 ha), which is under pressure mainly due to the expansion of soy, tobacco and eucalyptus monocultures and livestock production. The only known population of the species is found in an unprotected and easily accessible area. A nearby tourist facility and upstream pesticide usage expose the site to direct and indirect human threats.

3. Distribution

The original description of *Melanophryniscus admirabilis* informed its occurrence on 200 m along the Forqueta River margins (Di-Bernardo et al., 2006). To improve our understanding of the distribution of *M. admirabilis* and to verify whether the species represented a true microendemism or whether its range was underestimated due to lack of sampling, we conducted three different approaches: active search by the project team, review of environmental impact assessment studies of hydropower projects in the region, and a BioBlitz.

3.1 Project Team Search

We searched for new occurrences of the species along the Forqueta River and other rivers in the same basin, and areas where the local people reported that the species could occur (Figure 2B-C). We adjusted our methodology and sampling effort, both spatially and temporally, as we improved our knowledge about the Admirable Toad behavior and reproduction (see Abadie et al., 2021– Chapter 1).

Since the region is characterized mostly by steep and inaccessible slopes, from October 2010 to March 2011, we started by searching accessible areas of the river margins. Before searches, we always checked if there was reproductive activity at the known breeding site. In 2017, when we knew more about the species behavior, we used satellite images to select and prioritize potential searching sites as riverbanks associated with steep slopes of forest (slope > 45º) and rocky outcrops and concentrated surveys between August and December. In the remaining years (2013 to 2016), we worked to refine knowledge about the local distribution at Perau de Janeiro, the regular breeding site of the species.

From 2010 to 2017, we conducted visual and audio surveys (Visual Encounter Survey, Crump and Scott Jr., 1994; Surveys at Breeding Sites, Scott Jr. and Woodward, 1994) looking for adult individuals or any other evidence of the species presence, as clutches of eggs or tadpoles. We mainly focused on environmental conditions similar to the known reproductive site of the species (rocky river bank outcrops with nearby forest remnants), as a clue for potential new areas of occurrence. We visited at least twice all potential rocky river bank outcrops to reduce false-negative observations from unsuccessful survey events.

3.2 Review of environmental impact assessment studies of hydropower plant projects

Simultaneously to the beginning of fieldwork, we analyzed databases from pre-permitting biodiversity inventories and follow-up studies conducted between 2005 and 2010 in other rivers in the Taquari-Antas river basin (Figure 2A; Biolaw Consultancy Company, *unpublished data*). This information and 2010-2011 project team surveys were used to assess the global conservation status of the species in 2013 (IUCN SSC

Amphibian Specialist Group, 2013) and national and regional status in 2014 (Portaria MMA Nº 444, 2014; Rio Grande do Sul, 2014).

3.3 BioBlitz

In 2017, we also conducted a five-day modified BioBlitz approach with the involvement of taxonomic experts, a socio-environmental NGO and undergraduate students. The goals of this BioBlitz were to record as many vertebrates as possible in the Perau de Janeiro region and to generate data to design and propose a protected area for the Admirable Redbelly Toad (Instituto Curicaca, *unpublished data*). Since the Admirable toad was the target species, we also searched for adult individuals, tadpoles or eggs through transects in riparian forests and along the riverbanks in the surroundings of the breeding site (Figure 2D).

3.4 EOO, AOO and range description and calculation

We used the coordinates of the searched sites and transects to produce a sampling effort map. Only confirmed records were used to calculate the extent of occurrence (EOO) and the area of occupancy (AOO). The EOO was measured by a minimum convex polygon (MCP), and the AOO by counting the occupied cells in a 2 x 2 km cell grid (IUCN Standards and Petitions Committee 2019). We also delimited the range of the species, defined by “current known limits of distribution, accounting for all known, inferred or projected sites of occurrence”, that is, the occupied sites and the surrounding river margin forests (~50 m to each margin). Besides AOO and EOO being essential to assess the conservation status under criterion B of IUCN standards (IUCN Standards and Petitions Committee. 2019), distribution metrics, including the species range presented here, are also useful for monitoring habitat loss, mapping threats and planning and locating priority conservation actions.

3.5 Admirable Toad range

We searched for the species over 40 sampling days and covered 65 km along the riverbanks of the Forqueta River sub-basins (Figure 2). The areas sampled by herpetologist consultants (for environmental impact assessment studies) covered 16 km in areas along the sub-basins of Carreiro and Turvo Rivers. We found three potential new population sites, which seemed appropriate for the Admirable toad's reproduction, as temporary pools in rocky outcrops. Although we have returned several times to these sites, we have never found any evidence of the species. These sites are located in areas lacking either adjacent forests or steep slopes. Probably as a consequence, they also lack herbaceous vegetation, are exposed to the sunlight and are drier than the sites where the species is usually found. This lower humidity possibly hinders the persistence of the pools and reduces the minimum necessary period for tadpole development.

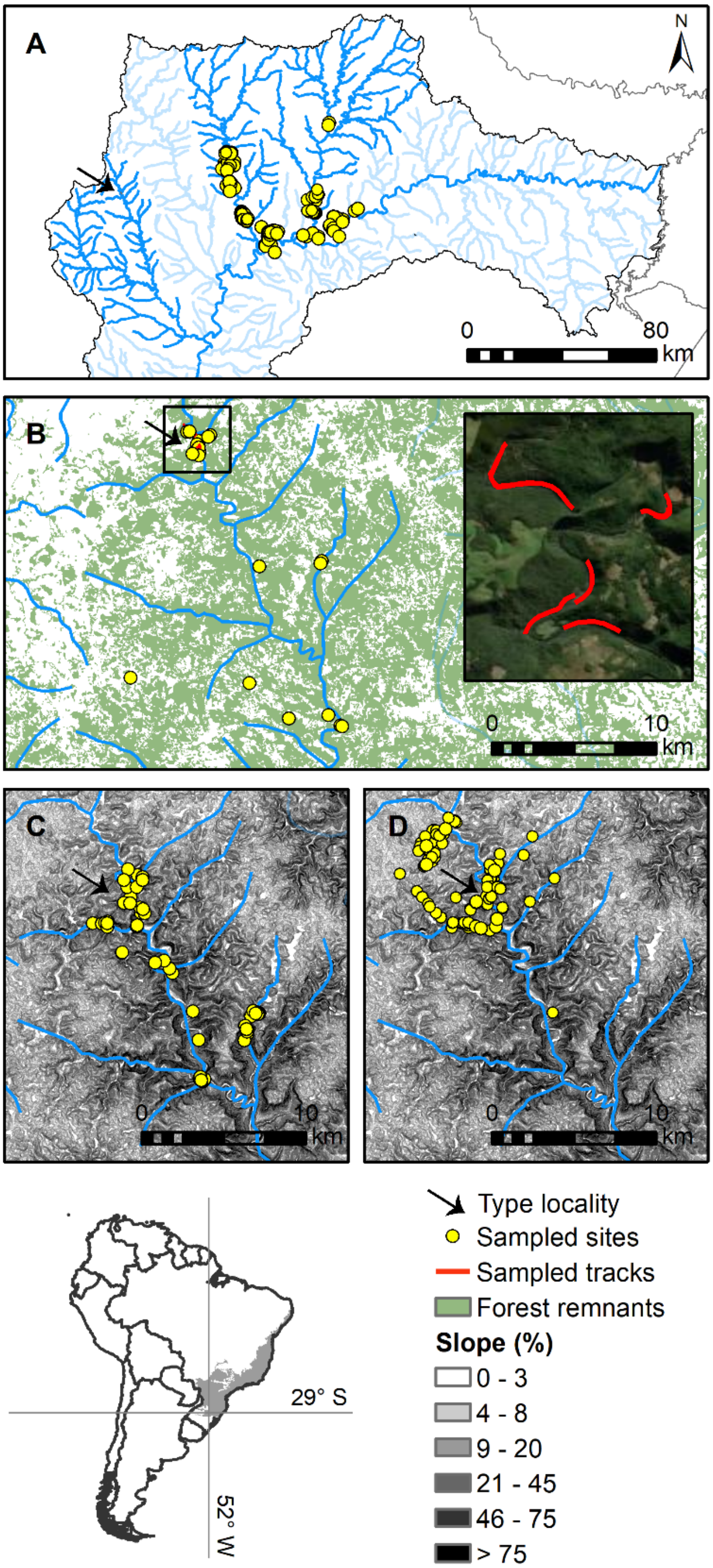

Figure 2. Areas searched for *Melanophryniscus admirabilis*. (A) sites from the review of environmental impact assessment studies of hydropower projects in Taquari-Antas Basin; (B)

sites from active searches by the project team in 2010-2011 and (C) 2017 ; and (D) sites from the Bioblitz.

We found *M. admirabilis* in a unique new site, expanding its range by only 1.43 km downstream and 492 m in Euclidean distance from the previously known site. Species presence was detected after heavy rains in September of 2017 based on auditory encounters (male calling) and confirmed by the visual encounter and capture of some individuals. The new site is a rocky river bank outcrop with less than 100 m^2, few pools, and surrounding steep slope forests. This new record increases the known EOO (measured by a minimum convex polygon – MCP) from 0.035 to 0.253 km^2. The new AOO is a 4 km^2 square encompassing the area where the toad lives, following the novel approaches applied by IUCN (Figure 3). As AOO should not exceed EOO (IUCN Standards and Petitions Committee, 2019), we adjusted EOO to 4 km^2. The range was estimated to be 0.418 km^2 (Figure 3). We estimated the known species occurrence to be 0.049 km^2 along the river, including both river margins and 50 m of adjacent forests (see Abadie et al, 2021 – Chapter 1).

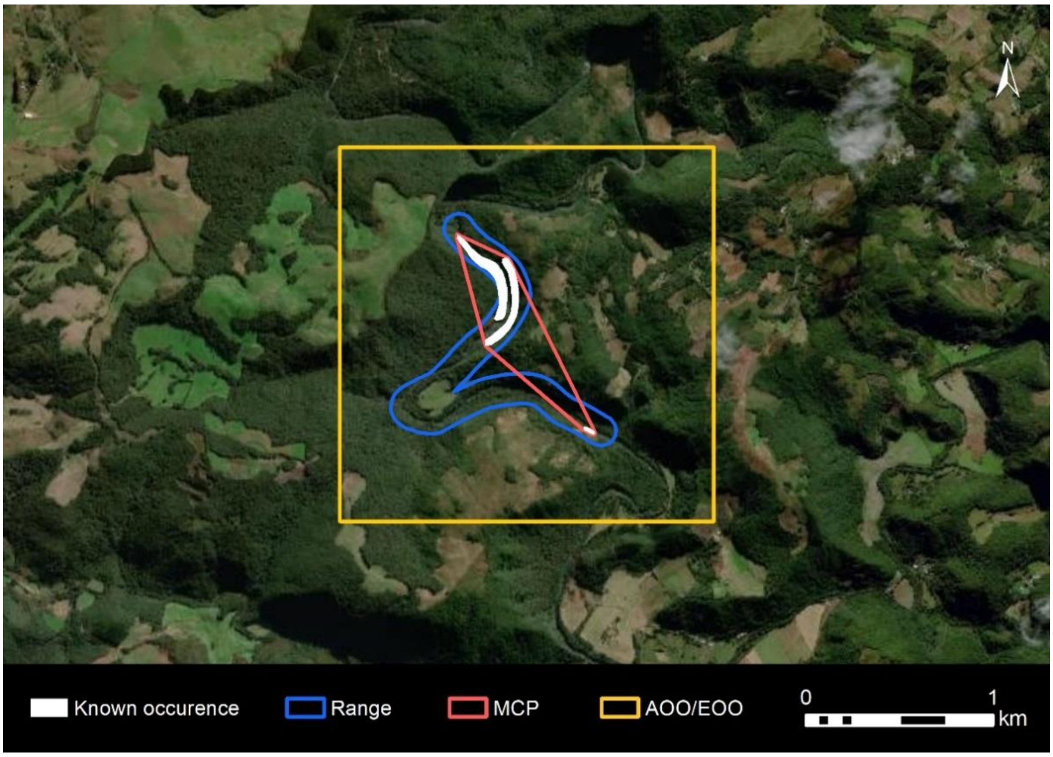

Figure 3. Distribution of *Melanophryniscus admirabilis* under different metrics. Range is the current known limits of distribution; MCP is the minimum convex polygon; AOO is the area of occupancy and EOO is the adjusted extent of occurrence.

The new record considerably expands the species distribution (almost 10 x the previous EOO; IUCN SSC Amphibian Specialist Group, 2013); however, the range is still very restricted, and we kept considering it as a single population and locality.

4. Population Size

For assessment purposes, we followed the IUCN Red List Categories and Criteria v. 3.1 (2012) nomenclature which defines ‘population’ as the total number of individuals of the taxon, and ‘population size’ as the number of mature individuals of the population. Since most of the mature individuals of *M. admirabilis* reproduce at the same moment in explosive breeding events, we considered the breeding adults (i.e., participating in the reproductive events at the breeding site) as ‘mature individuals’.

Abadie et al. (2021 – Chapter 2) estimated the abundance of Admirable Redbelly Toad breeding adults over an eight-year window (2010-2017), using individual photo-identification and capture-recapture models (Pollock Robust Design; Pollock, 1982). The abundance estimate was obtained from the site where most of the population concentrates for breeding. This breeding site is about 400 m long and one to 14 m wide (Abadie et al., 2021 – Chapter 1). The authors provided annual estimates of abundance for males and females, which varied over time from 742 (CI= 645-838) individuals in 2014 to 1451 (CI= 1280-1622) individuals in 2017 (Table 1). They also presented a male-biased sex ratio for the species, even after accounting for imperfect detection (from 1.27:1, in 2017, to 2.45:1, in 2014).

Table 1. Estimated annual population size for males and females of *Melanophryniscus admirabilis* and respective sex ratio, from 2010 to 2017 using capture-recapture data**.** Adapted from Abadie et al., 2021 – Chapter 2.

Year	Population Size (–95% CI – +95% CI)		Sex Ratio
	F	M	
2010	428 (276-670)	721 (573-916)	1.68±0.14
2011	477 (378-612)	779 (695-885)	1.63±0.14
2013	307 (247-396)	521 (473-591)	1.70±0.11
2014	215 (172-280)	527 (478-596)	2.45±0.31
2016	594 (491-732)	794 (728-876)	1.33±0.13
2017	638 (528-785)	813 (745-900)	1.27±0.22

According to IUCN Standards and Petitions Committee (2019), when the population size fluctuates or has a biased sex ratio, we should use the lowest estimate. Although *M. admirabilis* population has a male-biased sex ratio, females attend to more than one breeding event per year (Abadie et al, 2021 – Chapter 1). Thus, to assess the species conservation status we used the estimate from 2014 (females and males = 742 individuals, CI= 645-838; Abadie et al., 2021 – Chapter 2).

In addition to the main breeding site where the capture-recapture study was conducted, there are three other small breeding sites, which represent less than 10% of the surface of the main one. Even if we extrapolate a density estimate to these sites, the total population size would remain below 1,000 individuals.

5. Threats

The list of threats presented here is the result of 10 years of the Admirabilis Project and was elaborated based on regular visits to the locality and regular communication and knowledge shared with local people. The final selection is a consensus list that was gathered through a series of specific meetings with the project team to enumerate, describe and rank all current and potential direct threats.

First, we sorted all direct threats that we considered relevant for *M. admirabilis* from the CMP Direct Threats Classification v 2.0 (Salafsky et al., 2008; CMP, 2016). We then ranked all threats using three criteria based on the Threat Reduction Assessment (TRA) approach (Salafsky and Margoluis, 1999): 1) Extent: portion of the area of occupancy (AOO) that is already or will be affected by the threat; 2) Intensity: degree of damage or disturbance severity caused by the threat (i.e. will the threat change the overall supporting system, such as environmental conditions, potentially affecting the entire population or will it affect some individuals' survival?); 3) Urgency: the immediacy of the threat (i.e. is the threat occurring now or may the impact arise only in future years?). We adapted the TRA criteria as a tool for ordering and prioritizing the species threats and do not conduct a threat reduction assessment. The number of ranking levels for the three criteria varied from one to the total number of threats, and the same number could not be repeated within the same criterion. Since the species is microendemic, the differences between threats of the same criterion are very small in absolute terms, making it sometimes difficult to assign rankings. Thus, it was essential to weigh the differences and discuss the ordering until a consensus was reached. We then added up the rankings for all three criteria for each threat to obtain the total ranking. Finally, we calculated the Threat Relevance Index (TRI), which is the proportion of a given threat to the total sum of the three criteria:

$$TRI_i = {(E_i + I_i + U_i)}\Big/{(\sum_{i=1}^{n} E_i + \sum_{i=1}^{n} I_i + \sum_{i=1}^{n} U_i)},$$

where TRI is the Threat Relevance Index for each threat i $\{1, \ldots, n = 10\}$, E is extent, I is intensity, and U is urgency.

We kept the threats related to Climate Change, such as large temperature variations or changes in the precipitation regime, out of our classification and ranking not because these are not potential threats to the species, but because we have no direct influence on them.

We identified a total of ten direct threats to the Admirable Redbelly Toad and its habitat (Table 2; Figure 4). Amphibian fungal disease (Chytridiomycosis) followed by agricultural pesticides were classified as the greatest general concern. Both livestock and American bullfrog (*Lithobates catesbeianus*) invasion were equally ranked as of least general concern. Although cattle-raising is an expected threat to Atlantic Forest (Fundación Vida Silvestre Argentina and WWF, 2017), particularly by virtue of use of forests as shelter by cattle, with risk of trampling and changes in litter humidity, the slope in the adjacent areas and the site itself do not favor this activity. Thus, cattle presence is sporadic. The risk of a hydropower plant construction has the highest rank on extension and intensity criteria. But it was considered as the lowest urgency because this threat is currently controlled due to the prohibition for new HPP projects on the Upper Forqueta River Basin (Becker et al., 2017). Agricultural pesticide was the most urgent threat because soy production is quickly expanding and dominating the landscape matrix. Although there is a general demand for poison and colorful frogs for the pet trade (CITES, 2020), we do not have any record of *M. admirabilis* individuals found in trade.

Figure 4. Identified direct threats to *Melanophryniscus admirabilis*. (A) Pesticides used in tobacco plantations; (B) soy and eucalyptus plantations; (C) cattle breeding; (D) tourists at the breeding site of the species; and (E) sign indicating the construction of a hydropower plant. Photos: Talita M. Ribeiro (A), Pedro Peloso - DoTS Project (B), Michelle Abadie (C-E).

Table 2. *Melanophryniscus admirabilis* threats ranking according to CMP Direct Threats Classification v 2.0 levels. The ranking is in descending order within each criterion (E, I, U), where “10” is the highest level for the threats. E: Extent - portion of the area of occupancy (AOO) that is already or will be affected by the threat; I: Intensity – degree of damage or disturbance severity caused by the threat; U: Urgency - the immediacy of the threat; TRI: Threat Relevance Index of each threat (not in order).

CMP Direct Threats Classification v 2.0 levels								
General type	**Definition**	**Example for *M. admirabilis***	**Definition/Justification**	**E**	**I**	**U**	**TR**	**TRI (%)**
2. Agriculture & Aquaculture								
2.1 Annual & Perennial Non-Timber Crops	Crops planted for food, fodder, fiber, fuel, or other uses	Tobacco and soy plantations	Native vegetation areas have been converted into tobacco and soy plantations. It threatens the population of *M. admirabilis* due to the use of pesticides in the plantations (see item 9.3) and the loss of local humidity from the native forest. Together these mechanisms lead to the loss of local habitat quality	6	4	6	16	9.70
2.2 Wood & Pulp Plantations	Stands of trees planted for timber or fiber outside of natural forests, often with non-native species	Eucalyptus plantations	Native forest areas have been replaced by eucalyptus plantations (slower than conversion into tobacco and soy plantations). It threatens the population of *M. admirabilis* due to the use of pesticides in the plantations (see item 9.3) and the loss of local humidity from the native forest. Together both mechanisms lead to the loss of local habitat quality	5	3	2	10	6.06
2.3 Livestock Farming & Ranching	Domestic terrestrial animals raised in one location on farmed or non-local resources (farming); also domestic or semi-domesticated animals allowed to roam in the wild and supported by natural habitats (ranching)	Cattle breeding	Small-scale cattle breeding. It affects the population of *M. admirabilis* due to trampling on seedlings of riparian native flora, hindering the regeneration of the local vegetation and resulting in forest floor drying	3	1	5	9	5.45
5. Biological Resource Use								
5.1 Hunting & Collecting Terrestrial Animals	Killing or trapping terrestrial wild animals or animal products for commercial, recreation, subsistence, research or cultural purposes, or for control/persecution reasons; includes accidental mortality/bycatch	Collection of specimens for the pet trade	Sporadic capture of specimens from the wild. Potential use of the species in the pet trade due to its colorful pattern and easy access to the locality. It affects the population of *M. admirabilis* due to the removal of mature individuals from the population	1	8	4	13	7.88

Table 2. (continued)

General type	Definition	Example for *M. admirabilis*	Definition/Justification	A	I	U	TR	TRI (%)
5.3 Logging & Wood Harvesting	Harvesting trees and other woody vegetation for timber, fiber, or fuel, including site preparation and other forestry management practices	Harvesting trees	Tree harvesting for firewood, which is used in the tobacco drying process. It threatens the population of *M. admirabilis* due to the loss of local humidity from the native forest, leading to the loss of local habitat quality	7	6	8	21	12.73
6. Human Intrusions & Disturbance								
6.1 Recreational Activities	People spending time in nature or traveling in vehicles outside of established transport corridors, usually for recreational reasons	Tourism	Local people and tourists walking across the reproductive site of the species. It affects the population of *M. admirabilis* due to the trampling of individuals and pools used for reproduction	2	7	7	16	9.70
7. Natural System Modifications								
7.2 Dams & Water Management / Use	Changing water flow patterns from their natural range of variation either deliberately or as a result of other activities	Risk of dam construction	Possible construction of hydropower plants for energy production. Dam construction would affect the *M. admirabilis* population due to changes in the hydrologic regime and quality	10	10	1	21	12.73
8. Invasive & Problematic Species, Pathogens & Genes								
8.1 Invasive Non-Native / Alien Plants & Animals	Harmful plants and animals not originally found within the ecosystem(s) in question and directly or indirectly introduced and spread into it by human activities	American Bullfrog (*Lithobates catesbeianus*)	Presence of the American Bullfrog (*Lithobates catesbeianus*). It can affect the *M. admirabilis* population due to its potential as a vector of diseases	4	2	3	9	5.45
8.4 Pathogens & Microbes	Harmful native and non-native agents that cause disease or illness to a host, including bacteria, viruses, prions, fungi, and other microorganisms	Chytridio-mycosis	Presence of the fungus *Batrachochytrium dendrobatidis* (Bd) in specimens and/or in the environment. It can affect the population of *M.admirabilis* due to the development of the chytridiomycosis disease	8	9	9	26	15.76
9. Pollution								
9.3 Agricultural & Forestry Effluents	Water-borne pollutants from agricultural, silvicultural and aquaculture systems that include nutrients, toxic chemicals and/or sediments including the effects of these pollutants on the site where they are applied	Agricultural pesticides	Use of pesticides in tobacco, soy and eucalyptus plantations on the upper slope of the Forqueta River and upstream. It affects the population of *M.admirabilis* due to the flow of residues from the plantations to the reproductive site of the species, which can cause malformations or even the direct death of individuals	9	5	10	24	14.55
			TOTAL	**55**	**55**	**55**	**165**	**100**

6. Conservation Status Reassessment

According to the Guidelines for Using the IUCN Red List Categories and Criteria (2019), it is important to use the best available data to assess taxa status against all five criteria (IUCN Standards and Petitions Committee, 2019). Based on the information gathered in this study, we could assess the extinction risk of *M. admirabilis* under criterion B (geographic range metrics, severe fragmentation, number of locations, decline or fluctuations), criterion C (small and declining population size and fragmentation, fluctuations, or few subpopulations), and criterion D (very small population or very restricted distribution). We were not able to assess under criteria A (population size reduction) and E (quantitative analysis of extinction risk) because we do not have ten years (or three generations) of population monitoring, and there is not enough available information for a population viability analysis.

6.1. Criterion B: (1) The species has a measured EOO of 0.253 km^2, adjusted to 4 km^2, which is within the threshold for Critically Endangered (100 km^2) under criterion B1; (2) The species has an estimated AOO of 4 km^2, which is within the threshold for Critically Endangered (10 km^2) under criterion B2; (3) With the observed presence of the fungus *Batrachochytrium dendrobatidis* (Bd) in individuals of *M. admirabilis* (M.R. Pontes, *personal communication*), if a Bd outbreak occurs, it could cause a fast decline in the entire population (number of locations=1; subcriterion a); (4) There is an observed ongoing decline of the species' terrestrial and aquatic habitat quality, as a result of heavy and continued usage of agrochemicals and loss of forest to agriculture and harvesting of trees for fuel (subcriterion b, item iii); (5) the locality is a target area for hydroelectric power plants. Under this criterion, *M. admirabilis* would remain categorized as Critically Endangered, based on criteria B1ab(iii) + 2ab(iii).

6.2. Criterion C: (1) The species has less than 2,500 mature individuals, which is within the threshold for Endangered; (2) There is no observed, estimated, projected, or inferred continued decline in the number of mature individuals. Therefore, under this criterion, *M. admirabilis* could not be classified in any of the threat categories.

6.3. Criterion D: (1) The species population size was estimated at less than one thousand individuals, which is within the threshold for Vulnerable (< 1,000 mature individuals) under criterion D1; (2) The species occurs in a very restricted area of occupancy (AOO < 20 km^2) and has less than five locations, which is within the threshold for Vulnerable under criterion D2. Also, the species may be prone to "future threats that could drive the taxon to CR or EX in a very short time", such as the HPP construction, Bd infection or forest loss. Under this criterion, *M. admirabilis* must be categorized as Vulnerable, based on criteria D1 + 2.

Given the precautionary principle adopted by IUCN guidelines, which recommend that the taxon should be listed under the most threatened category, we propose *M. admirabilis* to remain categorized as Critically Endangered (criterion B).

7. Conclusions and Future Directions

For Red Lists to be useful for conservation planning and effectiveness monitoring, the regular reassessment of the species conservation status is fundamental. New threats can emerge and affect the priority of conservation actions, particularly for species with restricted distributions. In this study, we conducted a detailed conservation status reassessment of *M. admirabilis*, a microendemic species from the Southern Atlantic Forest, addressing three out of five criteria from IUCN Red List Categories and Criteria. We compiled information about the species distribution and population size and adapted a methodology used to monitor threat reduction to rank threats by extent, intensity and urgency.

The most concerning threats, Bd infection and agrochemicals, are of complex control because they have a diffuse origin and chronic action, that is, even if these threats are introduced in some local quite far upstream catchment, they could affect the species and/or the quality of the species' habitat in a difficult way to control. There is evidence that *M. admirabilis* has a potential xenobiotic degradation pathway in the oral microbiota (Mann et al., 2021), which may be an indicator of contact with contaminants, although the tadpole is quite resistant to pesticides (da Silva et al, 2021). Synergistic effects among pesticides, skin microbiome and chytridiomycosis are still poorly understood for amphibians (Jani and Briggs, 2018), but environmental contaminants could play an important role in altering the amphibian skin microbiome and therefore disease susceptibility (McCoy and Peralta, 2018). The quantification and monitoring of both agrochemical concentration and Bd incidence in *M. admirabilis* range are imperative to anticipate crashes in population size. Promoting best practices and/or alternatives to intensive agrochemical usage among landowners in the entire river basin is also urgent.

An important conservation action would be the creation of a protected area for the Admirable Redbelly Toad, and this was formally recognized in the National Action Plan for the Conservation of Amphibians and Reptiles in Southern Brazil (Portaria MMA Nº 350, 2019). Here, it is important to note that the area is already considered a global priority for conservation, being recognized as a Key Biodiversity Area and an Alliance for Zero Extinction site (Key Biodiversity Areas Partnership, 2020; AZE, 2020). This strategic action could protect the population from most of the prioritized threats but is scale-dependent. Tree harvesting, livestock disturbance, tobacco, eucalyptus and soy plantations, tourist activities and illegal collection of specimens at or near the main reproductive site could be eradicated by a small-scale protected area. However, agrochemical contamination effects could only be reduced with broader-scale economic incentives. Tobacco plantations are monocultures dependent on neurotoxic pesticides (Krawczyk et al., 2014), which besides harming flora, fauna and funga, directly affect farmers (Arcury and Quandt, 2006). Also, soy plantations and their inputs are expanding within the species' range. Another conservation action would be to subsidize the substitution of tobacco and soy monocultures for yerba mate (cultural South American tea) in agroforestry and organic systems, availing on the region's vocation known as the "land of yerba mate". Linking yerba mate culture to consumption of organic produce and the conservation of an endemic species in the region might be a solution that benefits the entire production chain involved. Moreover, promoting mitigation strategies to reduce pesticide inputs in the entire basin should be a priority for government programs, since the deliberate abuse of pesticides (often used above legal levels) directly impacts water quality and produces different health impacts for humans and wildlife (Pimentel et al., 1992). Although difficult to put in place, the replacement of monocultures by yerba mate agroforestry and the creation of a protected area have the potential to reduce the threats listed above. These priority actions could genuinely change the extinction risk degree of *M. admirabilis* from Critically Endangered B1ab (iii) + 2ab (iii) to Vulnerable D1 + 2.

We considered the hydropower plant (HPP) construction as the most extensive and intensive (but least urgent) threat to *M. admirabilis* (see Fonte et al., 2014). Currently, the species is protected by a legal instrument that prohibits the HPP construction in its habitat (Resolução Consema, Nº 388, 2018). If for any reason the validity of this regulation ceases, the species may be susceptible to loss of habitat quality and a potential reduction in the number of mature individuals. A recent study found that this kind of regulation is the main reason for HPP cancellations (Macedo, 2021). Therefore, the permanent avoidance of this potential threat would only be ensured by either the maintenance of the referred legal instrument throughout the next governments or the creation of a protected area encompassing all the potential HPP sites upstream. With this perspective and if the number of mature individuals increases above the one thousand individuals threshold, the species could even be categorized as Near Threatened (NT). Nonetheless, this would only happen if other populations were found in other areas, expanding the AOO, and if the likelihood of a plausible future threat that could quickly drive the species to Critically Endangered or Extinct is removed. With the current distribution, even if those conditions were achieved, the species would remain categorized as Vulnerable because of criterion D2 (AOO < 20 km^2).

Some research actions also deserve further and continuous efforts due to their repercussion for conservation planning and species persistence. First, it is necessary to keep monitoring the population size and habitat quality to track for any positive or negative trends. Second, the search for new populations should be reinforced since their finding and protection would increase the likelihood of the species' persistence. Since *M. admirabilis* is a quite elusive explosive breeder, indirect detection techniques, such as environmental DNA and passive acoustic recorders, could improve the chance to detect the species in new areas (e.g. Lopes et al., 2020).

Microendemic species like the Admirable toad are a great challenge for conservation. There are many examples of fast decline and disappearance in the amphibian literature (see Bishop et al., 2012). The restricted range alone is an underlying condition for increased extinction risk. However, it also gives us a great opportunity to control most of the main mechanisms or factors that could affect the species persistence since the landscape to be targeted for action is relatively small. Time will be the limiting dimension for planning and implementing conservation actions since any significant shift in the extent or intensity of the main threats could drive important population losses. This requires that proper indicators of threats and the population need to be regularly and continuously monitored, with obvious pressures on sustainable project fundraising. This challenge could only be overcome with increasingly close cooperation between researchers, locals, NGOs, environmental agencies and other government structures and funders. In the recent history of the Admirable toad conservation program, this kind of coordinated partnership was fundamental for the toad's conservation (Fonte et al., 2014; and the present study).

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Considerações Finais

Nesta tese, apresento informações inéditas sobre ecologia e conservação do sapinho-admirável-de-barriga-vermelha *Melanophryniscus admirabilis*, uma espécie microendêmica e ameaçada. No primeiro capítulo, nós demonstramos que a espécie apresenta reprodução sazonal e explosiva e que parece ter desenvolvido adaptações para lidar com o ambiente barulhento e arriscado em que vive. Uma dessas possíveis adaptações é a modulação de frequência do canto A da espécie (GOUTTE *et al.*, 2018), primeira vez registrada para o gênero *Melanophryniscus*. Nós registramos que os ovos são depositados em pequenas porções (média de 15,4 ovos por desova; n= 17), sendo cada porção em uma poça temporária diferente. Juntamente com o rápido desenvolvimento dos girinos (em torno de 21 dias desde a oviposição até a metamorfose completa), essas podem ser adaptações ao ambiente efêmero das pequenas poças em beira de rio, que rapidamente seca (devido à exposição ao sol) e frequentemente alaga (tanto pelas cheias do rio, como diretamente pela água da chuva que contribui para que as poças transbordem). A energia depositada em uma rápida metamorfose pode contribuir para que o girino de *M. admirabilis* seja um dos menores já descritos para o gênero, apesar dos adultos serem um dos maiores. Nós também registramos a maior longevidade diretamente observada em *Melanophryniscus*: 7 anos (25 indivíduos) e 9 anos (3 indivíduos). Esperamos que as espécies do gênero sejam anfíbios de vida longa, uma vez que (1) o registro de nove anos de longevidade foi ocasional (não analisamos todo o banco de dados), que (2) os indivíduos recapturados sete anos depois já eram adultos na primeira captura, que (3) nossa janela temporal de amostragem sistemática também tem sete anos, e que (4) Jeckel et al. (2015) registraram por osteocronologia uma variação de 1 a 5 anos para uma fêmea de *M. moreirae* se tornar adulta. Discutimos que as defesas químicas

(GRANT *et al.*, 2012) e a suposta coloração aposemática (BONANSEA; VAIRA, 2012; mas veja BORDIGNON *et al.*, 2018), observada no gênero possam desempenhar um papel importante na determinação dessa inesperada longevidade para um anuro pequeno, subtropical e diurno (STARK; MEIRI, 2018). Nós também registramos dimorfismo sexual de tamanho, sendo fêmeas maiores que machos, e alta fidelidade de sítio, sendo os jovens mais propensos a se deslocar ao longo do sítio reprodutivo. A partir do conhecimento produzido neste capítulo da tese, diversas outras perguntas ecológicas e evolutivas surgiram e fica evidente o quão importante e interessante são os estudos sobre a história natural das espécies.

No segundo capítulo, estimamos o tamanho populacional anual da única população conhecida da espécie e a probabilidade de sobrevivência aparente. Nossas estimativas de abundância variaram mais entre os métodos no mesmo ano do que entre anos. As estimativas de POPAN e do Desenho Robusto ficaram mais próximas entre si do que as estimadas pelo Modelo Fechado. Porém, a baixa recaptura de fêmeas influenciou diretamente na precisão das estimativas, principalmente no método POPAN. Consideramos o Desenho Robusto mais adequado para estimar abundância ao longo dos anos, já que ele considera toda a janela temporal em uma mesma análise. As estimativas para esse método variaram de 747±49, em 2014, a 1541±87 indivíduos adultos, em 2017. Discutimos que essa importante flutuação do tamanho populacional no meio do período (2013 e 2014) pode estar relacionada à estiagem de 2012. Nesses intervalos, a sobrevivência dos indivíduos adultos também foi menor do que em outros anos. Com base nessas estimativas, levantamos a hipótese de que a estiagem pode ter contribuído principalmente por ter sido um ano de pouca atividade reprodutiva e, provavelmente, muita mortalidade de ovos e girinos por dessecamento das poças. Consequentemente isso contribuiria para o menor recrutamento de indivíduos maduros nos anos imediatamente posteriores, já que esperamos que os indivíduos levem ao menos um ano desde a metamorfose para se tornarem adultos reprodutivos (JECKEL; SAPORITO; GRANT, 2015). Por fim, concluímos que, considerando os outros pequenos sítios reprodutivos não amostrados neste trabalho, a população global da espécie não passaria de 2 mil indivíduos adultos reprodutivos nos anos das maiores estimativas.

No terceiro e último capítulo, nós usamos as informações sobre abundância, produzida no capítulo II, a distribuição geográfica e o levantamento das ameaças para reavaliar o estado de conservação de *M. admirabilis*. Usamos o conhecimento adquirido no primeiro capítulo da tese para identificar as ameaças e como elas se relacionam com a biologia da espécie. A partir dessa lista de ameaças, usamos uma metodologia objetiva para ranquear desde as de menor importância até as mais relevantes, usando três parâmetros: abrangência, intensidade e urgência da ameaça. Com base nesse método, identificamos que a presença do fungo quitrídio na população e os agrotóxicos utilizados nas plantações de fumo e soja são as ameaças mais preocupantes atualmente. A ameaça inferida causada pela construção da hidrelétrica foi unanimemente classificada como a que causaria danos mais severos e teria a maior abrangência. No entanto, atualmente é uma ameaça controlada, já que existe uma diretriz ambiental que proíbe a construção de barragens hidrelétricas nas cabeceiras do rio (Resolução Consema, Nº 388, 2018). Além disso, nesse capítulo, nós mostramos que mesmo após o novo registro de ocorrência da espécie, a área de ocupação (AOO) não passa dos 4 km². O risco de extinção da espécie pôde ser avaliado com base nos critérios B, C e D da IUCN (IUCN STANDARDS AND PETITIONS COMMITTEE, 2019), podendo ser categorizada como Criticamente em Perigo (CR), Em Perigo (EN) e Vulnerável (VU), respectivamente. No entanto, pelo princípio da precaução adotado pela IUCN, a espécie permaneceria categorizada como CR B1ab(iii) + 2ab(iii). A partir disso, discutimos que as ações prioritárias para conservação seriam (1) a proteção legal da área e (2) a mudança no uso da terra, incentivando e subsidiando a substituição das monoculturas de fumo, soja e eucalipto, por agroflorestas de erva-mate nativa. Ainda assim, a distribuição da espécie é tão pequena que, embora essas ações citadas sejam fundamentais para a redução do risco de extinção, *M. admirabilis* somente poderia ser removido das listas de espécies ameaçadas caso uma nova população fosse encontrada. Então, sugerimos que é tão importante continuar buscando a espécie em outros locais, usando outras metodologias como DNA ambiental ou gravadores passivos, quanto é necessário que seja mantido o monitoramento na área, a fim de acompanhar as tendências populacionais e de qualidade do hábitat.

Gostaria de aproveitar o espaço, nas considerações finais desta tese, para expor minhas conclusões sobre os cinco anos de doutorado no Programa de Pós-Graduação em Ecologia da Universidade Federal do Rio Grande do Sul. O aprendizado ao longo desse período vai muito além do que está previsto nas seções deste documento. Além dos artigos aqui apresentados (e dos que estão por vir), considero que alcancei objetivos importantes para a minha formação como pesquisadora e conservacionista. O trabalho de conservação que venho desenvolvendo com o sapinho-admirável-de-barriga-vermelha foi reconhecido nacional e mundialmente. Em 2018, durante o I Simpósio Brasileiro de Conservação de Anfíbios – ANFoCO, recebi o Prêmio Jovem Conservacionista, conferido pelo Grupo de Especialistas em Anfíbios do Brasil (ASG Brasil - *Amphibian Specialist Group Brazil*) e Sociedade Brasileira de Herpetologia (SBH). Dois anos depois, recebi bolsa parcial para apresentar no Simpósio de Pesquisa em Conservação de Anfíbios (ACRS - *Amphibian Conservation Research Symposium*), promovido pela Aliança para a Sobrevivência dos Anfíbios (ASA - *Amphibian Survival Alliance*) durante o Congresso Mundial de Herpetologia (Nova Zelândia, 2020), o trabalho de conservação que desenvolvemos com a espécie: '*Victory against all odds: the successful conservation* *story of the Admirable Red-Belly Toad in Brazil'* ("Vitória contra todas as probabilidades: a bem-sucedida história de conservação do sapinho-admirável-de-barriga-vermelha"). Lá também recebi o prêmio de Futura Líder em Conservação de Anfíbios (*Future Leader of Amphibian Conservation*), conferido pela ASA. Além disso, durante o doutorado, coorientei alunos de graduação (e.g. RIBEIRO, 2017), participei de outros estudos acadêmicos (BORDIGNON, 2019; BORDIGNON *et al.*, 2018; CAORSI *et al*., *in prep*), dei palestras, fui banca examinadora de Trabalho de Conclusão de Curso, revisei artigos em periódicos, participei da organização de eventos, participei (participo) como articuladora de ação do PAN Herpetofauna do Sul, fui representante discente durante dois anos e, no último ano de doutorado, iniciei meu trabalho no Centro de Pesquisa e Conservação de Répteis e Anfíbios do Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio/RAN). Todas essas experiências extracurriculares, com certeza, fortalecem e diferenciam a minha formação.

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