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Captive breeding program for *Scinax alcatraz* (Anura: Hylidae): introducing amphibian *ex situ* conservation in Brazil

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***Abstract.—Scinax alcatraz* is endemic to a small island ("Ilha dos Alcatrazes"), and is threatened by restricted distribution and habitat loss. Here, we present present the establishment of a captive breeding program for *S. alcatraz* at São Paulo Zoo, and introduce *ex situ* conservation as a strategy for amphibians in Brazil. We recorded 125 breeding events with about 10,200 eggs laid. We also observed that *S. alcatraz* does not have a marked breeding season, laying eggs throughout the year, and that breeding events are positively correlated with relative humidity and negatively correlated with temperature. This program has shown great success in the maintenance and reproduction of *S. alcatraz* in captivity, and has great potential for conducting research relevant to amphibian conservation and for the development of educational materials to share information about the global amphibian crisis, using *S. alcatraz* as a flagship species.**

Keywords. Alcatrazes Island, artificial environments, threatened species, amphibian decline, conservation strategy

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Introduction

During the last few decades, populations and species of amphibians have been facing extinction and declines worldwide (Chanson et al. 2008; Halliday 2008; Mendelson 2011) due to many causes such as habitat destruction, overexploitation, and infectious diseases (Berger et al. 2004; Cushman 2006; La Marca et al. 2005; Wake and Vredenburg 2006). In 2005, these troubling circumstances led herpetologists and conservationists around the world to implement a global strategy to minimize or stabilize declines and extinctions, which resulted in the first version of the Amphibian Conservation Action Plan (ACAP) (Gascon et al. 2007), updated in 2015 (Wren et al. 2015). This plan incorporated actions to understand, reduce, and reverse declines and generated 11 thematic guidelines necessary for the conservation of amphibians (Gascon et al. 2007). Two of these (captive programs and reintroduction) deal with *ex situ* conservation, which has been considered essential for some threatened amphibian species, especially those suffering from threats that cannot be controlled or reversed *in situ* (McFadden et al. 2013; Pavajeau et al. 2008; Zippel et al. 2011).

Brazil is considered to be the most diverse country for amphibian species, being home to 1,136 species (Segalla et al. 2019). Similar to other countries, amphibian declines in Brazil began at the end of the 1980s in the Atlantic Forest, and initially, the suggested cause was climate change (Heyer et al. 1988; Weygoldt 1989). Recently, such declines (e.g., *Cycloramphus*, *Crossodactylus*, *Hylodes*, and *Phrynomedusa* species) and the extinction of *Thoropa petropolitana* and *T. lutzi* were associated with the infectious disease chytridiomycosis caused by the fungus *Batrachochytrium dendrobatidis* (Carvalho et al. 2017). Currently, the *Brazilian Red List of Threatened Amphibian Species* contains one extinct and 41 threatened species (ICMBio 2018).

Based on the national scenario, the Brazilian Amphibian Conservation Action Plan (BACAP) was created in 2012, and following the ACAP guidelines it is aimed at developing specific conservation strategies for Brazilian amphibians, including *ex situ* strategies (Verdade et al. 2012). Previously, a list of priority species to be included in *ex situ* conservation actions was

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Fig. 1. Adult male of *Scinax alcatraz*. *Photo by Cybele Lisboa.*

created during a Conservation Needs Assessment (CNA) workshop in 2009 (Amphibian Ark 2009).We know *Scinax alcatraz* was the first and only species where the *ex situ* recommendation from the CNA has been implemented until 2019, when a similar *ex situ* action was included for *Nyctimantis pomba* (Zoológico de São Paulo 2019).

Scinax alcatraz is listed as Critically Endangered both in the National (Ministério do Meio Ambiente 2014) and IUCN Red List (Rodrigues and Cruz 2004). This is due to the fact the species is endemic to a single small island (1.35 km^2, Ilha dos Alcatrazes) (Brasileiro 2008) and its area of occupancy is less than 10 km^2, with a continuing decrease of extent and quality of habitat. Until recently, the Brazilian Navy used the island as a target for heavy artillery practice, threatening the habitat of *S. alcatraz*. In 2004, as a result of this training activity, a fire destroyed a significant part of the island's vegetation (Bataus and Reis 2011), and because of this fire, the development of a captive breeding program for *S. alcatraz* was deemed urgent and necessary as the population was facing an imminent risk of extinction (Bataus and Reis 2011; Zippel and Mendelson 2008). This action was included as one of the goals of the *Plano de Ação Nacional para a Conservação da Herpetofauna Insular Ameaçada de Extinção* (National Conservation Plan for the Endangered Island's Herpetofauna, Bataus and Reis 2011), a national strategy managed by the governmental agency Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio). The captive breeding program for *S. alcatraz* was implemented in 2009 at the Fundação Parque Zoológico de São Paulo (São Paulo Zoo) and fortunately in 2013 the artillery practice at the island ended due to an agreement between the Navy, government, and environmental protectionists (ICMBio 2013).

Since *Scinax alcatraz* had never been kept in captivity previously, from 2009 to 2011 we conducted a pilot study using *S. perpusillus* as a surrogate (Lisboa and Vaz 2012). *Scinax perpusillus* is categorized as Least Concern by the IUCN, is the closest species phylogenetically to *S. alcatraz*, and has the same breeding behavior (Peixoto 1987). Both species belong to the *perpusillus* group and depend on certain types of vegetation, specifically bromeliads, to complete their life cycle (Peixoto 1987). After two years of learning and developing management and reproductive techniques with this surrogate species (see Lisboa and Vaz 2012), we were able to apply the knowledge acquired to the Critically Endangered *S. alcatraz*. Here, we present the establishment of the *ex situ* population of *S. alcatraz* at

São Paulo Zoo. We also present data obtained regarding the reproductive biology and the longevity of *Scinax alcatraz* in captivity.

Materials and Methods

Founder acquisition. Ilha dos Alcatrazes (24°05'25"S, 45°41'00"W) is the main island of the Alcatrazes archipelago located about 35 km from the coast of the State of São Paulo in southeastern Brazil and included in the Atlantic Forest domain (Bataus and Reis 2011). We visited the island twice to acquire founders of *Scinax alcatraz* for the captive colony (N = 22; Fig. 1). Our first visit took place October 2011 and we collected three females and eight males (referred to as "2011 founders"). Our second visit was in October 2013 and we collected two females, six males, and three juveniles ("2013 founders").

Captive management. Between October 2011 and December 2017, we maintained the founders and their progeny in 11 glass terrariums in an isolated laboratory at São Paulo Zoo. We followed biosecurity standards according to Pessier and Mendelson (2010) to avoid the introduction of potential diseases to the *ex situ* population. Staff sanitized their arms and hands with chlorhexidine and 70% alcohol, and used dedicated clothing in the laboratory. Powder-free latex gloves were used when handling animals, enclosures, or equipment. We disinfected all equipment that entered the laboratory with sodium hypochlorite.

Two different sizes of terrariums were used (30 x 30 x 45 cm and 45 x 45 x 60 cm) with no substrate (Fig. 2a) since this species is not associated with floor litter in nature. Water was provided *ad libitum* in flat pots and plastic cups with submerged plants for refuge (Fig. 2b). For adults, tadpoles, and eggs we used tap water filtered with activated carbon to reduce chlorine. The water quality presented pH levels of ~7.2, 80 ppm alkalinity, 0 mg/L of ammonia, and 0 mg/L of nitrite. The water temperature was not measured.

The population density of the founders in each terrarium varied during the study period from 2 to 10 frogs. We fed the animals with pinhead-sized crickets (*Gryllus* sp.) dusted with Repashy Superfoods Calcium Plus ICB® vitamins twice per week directly on the terrarium floor. Artificial heat or cooling sources were not provided to the laboratory since São Paulo Zoo facilities are located within an Atlantic Forest fragment, the same morphoclimatic domain of *S. alcatraz*. Average air temperature was 21.3 ± 2.6 °C (10.2–30.4 °C), and relative air humidity was 71.4 ± 4.8% (25–97%). As the exposure to radiation in nature is unknown for this species, we decided to be conservative, and provided animals with only six hours per week of UVB lighting with an Exo Terra® Repti Glo 2.0 compact fluorescent bulb (20 watts), since this sort of radiation has proven to be important in the synthesis of vitamin D_3 for amphibians (Michaels et al. 2014a). The natural light that came through the window dictated the laboratory's photoperiod.

To stimulate the 2011 founders to breed, and given that there is no information on the natural breeding trigger regime for *S. alcatraz*, we used the same strategy applied to the surrogate species *S. perpusillus*. The technique consisted of utilizing an ultra-sonic fogger on the terrariums to increase the night-time humidity (see Lisboa and Vaz 2012) every other night for 33 days between December 2011 and January 2012. This procedure was not applied to the 2013 founders since

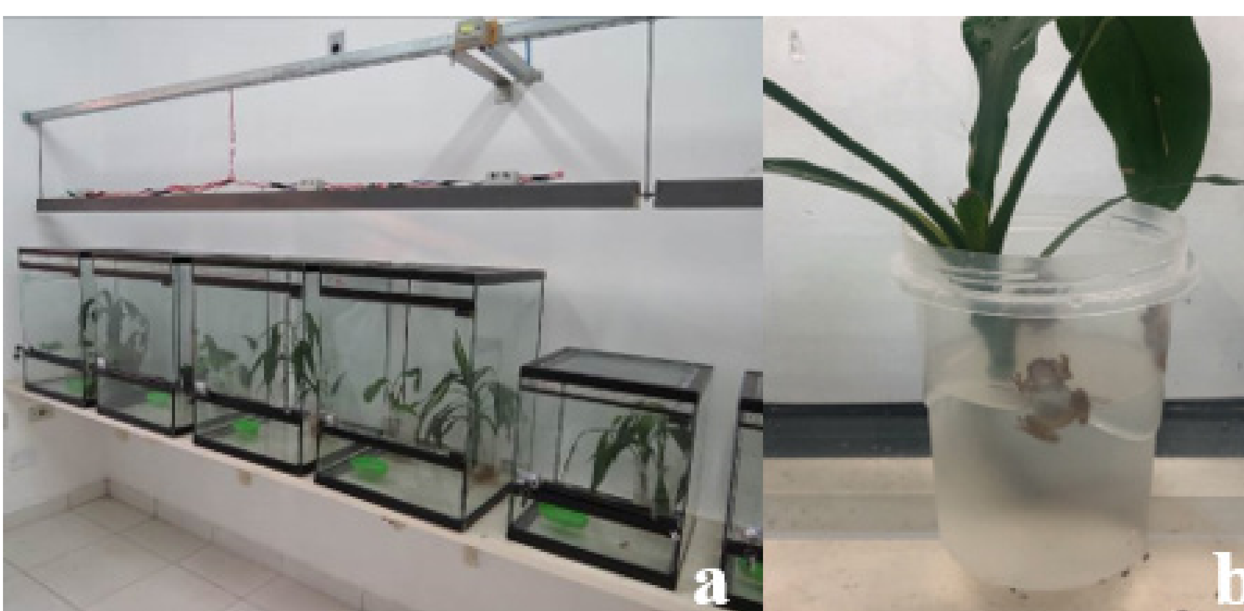

Fig. 2. Laboratory colony of *Scinax alcatraz* at Sao Paulo Zoo. a) Aquariums for maintanance of juveniles and adults. b) Plastic cups with filtered water and submerged plants for refuge. *Photos by Cybele Lisboa.*

they started reproducing spontaneously without artificial stimulus. Breeding information was collected from the captive-born group which started reproducing without artificial stimulus. The term "breeding events" was used for clutches found on the same day, in the same terrarium, due to the fact that some terrariums had more than one female and it was not possible to identify which one laid the eggs. We only kept 12 clutches from all the breeding events, which were enough to generate frogs to constitute a controlled *ex situ* population and for research and educational purposes.

When females deposited eggs in the water of the plastic cups we transferred the cups to an empty terrarium and monitored these eggs until they hatched. Subsequently, we transferred the tadpoles to plastic round containers (1.3 L; maximum of 30 tadpoles per container) with no substrate and containing 3–4 cm of filtered water (Fig. 3).

Tadpole feeding began the day after hatching and they were fed daily with Alcon® Spirulina Flakes. After metamorphosis, froglets were maintained in groups of four to seven individuals in plastic round containers (1.3 L), with a small pot of filtered water and no substrate, for 30 days. Subsequently, we transferred the groups of froglets to terrariums similar to those used for the adults (with 6 to 14 individuals per aquarium). Measurements of 10 eggs (diameter), nine tadpoles (body and tail length), and 97 froglets (snout-vent length) were taken using a digital caliper (0.01 mm).

Data analyses. The data were expressed as mean ± SD. Multiple Linear Regression based on Pearson's Correlation Coefficient (r) was performed to assess whether breeding events were correlated with environmental conditions (air temperature and relative humidity), based on data obtained from August 2013 (when the captive-born group began to reproduce) until December 2017 (53 months). The total of breeding events for the three groups (2011 founders, 2013 founders, and captive-born) occurring each month was considered and tested for correlations with monthly average air temperature and relative humidity. We also verified which ranges of relative humidity and temperature were optimal, comparing the number of breeding events that occurred between 60 and 69% relative humidity with those between 70 and 80%, and up to 22 °C with those above this temperature. Analyses were performed using the software Past 2.17 (Hammer et al. 2001) with significance set at the 0.05 confidence level.

Results

Captive breeding. The first breeding event of the 2011 founders occurred in January 2012, after three months in captivity and 33 days of the fogger stimulus. The second breeding event for this group occurred in July 2013. After that, the 2011 founders began to breed frequently (Fig. 4), without the fogger stimulus. The first breeding event of the 2013 founders occurred in July 2014, after nine months in captivity without fogger stimulus. In total, we recorded 88 breeding events with 7,743 eggs from the 2011 founders, and 37 events with 2,494 eggs from the 2013 founders (Table 1). From the 2011 and 2013 founders, we kept only the clutches from 12 breeding events (1.6% of total) through complete development. From these clutches, we obtained a total of 1,012 eggs, from which 327 (33%) tadpoles hatched and 184 (56%) metamorphosed. From the 327 tadpoles, 93 died at different stages immediately after water exchange on two different occasions.

Juveniles from the same clutch were kept in groups until adulthood when they started to breed. From the captive-born frogs, we recorded a total of 609 breeding events (37,976 eggs; Table 1), but we did not keep any of these clutches until 2018 when we separated siblings and paired new groups with animals from different parents. Some females laid unfertilized eggs without the presence of males in the same enclosure.

Scinax alcatraz laid eggs throughout the year in captivity with an average temperature of 20.6 °C ± 2.6 (13.1–29.5 °C) and relative humidity of 74.4% ± 6.4 (25–94%). Relative humidity and temperature were not correlated ($r = 0.08$; $p = 0.57$), but breeding events were correlated with both humidity and temperature ($r = 0.323$; $p < 0.001$). The number of breeding events occurring each month were positively related to relative humidity ($r = 0.467$; $p < 0.001$; Fig.5 a), and negatively related to air temperature ($r = -0.286$; $p = 0.04$; Fig.5 b). More breeding events occurred in the range of relative humidity between 70–80% (N = 611) than between 60–69% (N = 121), and at temperatures below 22 °C (N = 517) than above this temperature (N = 215; Fig.6).

In terms of life history and sexual maturity, the youngest female laying eggs was six months old after metamorphosis and the oldest female was 20 months old (mean = 12 ± 5 months, N = 5 females). Males were observed producing advertisement calls at ages as young as three months post-metamorphosis.

Eggs and tadpoles. Eggs were small, black, and enveloped in a gelatinous capsule, measuring 1.7 ± 0.06 mm in diameter (N = 10). The clutch size ranged from 11–142 eggs (mean = 54.6 ± 35 eggs; N = 18 clutches). Females deposited their clutches mainly in water in plastic cups with plants (Fig. 3b), but we also discovered some eggs scattered throughout the terrarium (walls, floor, leaves).

The development of the eggs lasted 6 ± 1 days (4–7 days; N = 12 clutches) before the tadpoles hatched. The body length of tadpoles (stages 25–26; Gosner 1960) was 2.1 ± 0.2 mm and tail length 4.5 ± 0.1 mm (N = 9). The development of tadpoles (Fig. 3c) until metamorphosis lasted an average of 84.7 ± 14.1 days (N = 97). The snout-vent length of post-metamorphic froglets (Fig. 3d) was 12.5 ± 0.7 mm (N = 107).

Fig. 3. Breeding of *Scinax alcatraz* at São Paulo Zoo. a) A pair in amplexus. b) Eggs deposited in the water. c) Maintanance of tadpoles in plastic pots with filtered water. d) Post-metamorph individuals (SVL $\bar{x}$=12.49 mm). *Photos by Cybele Lisboa.*

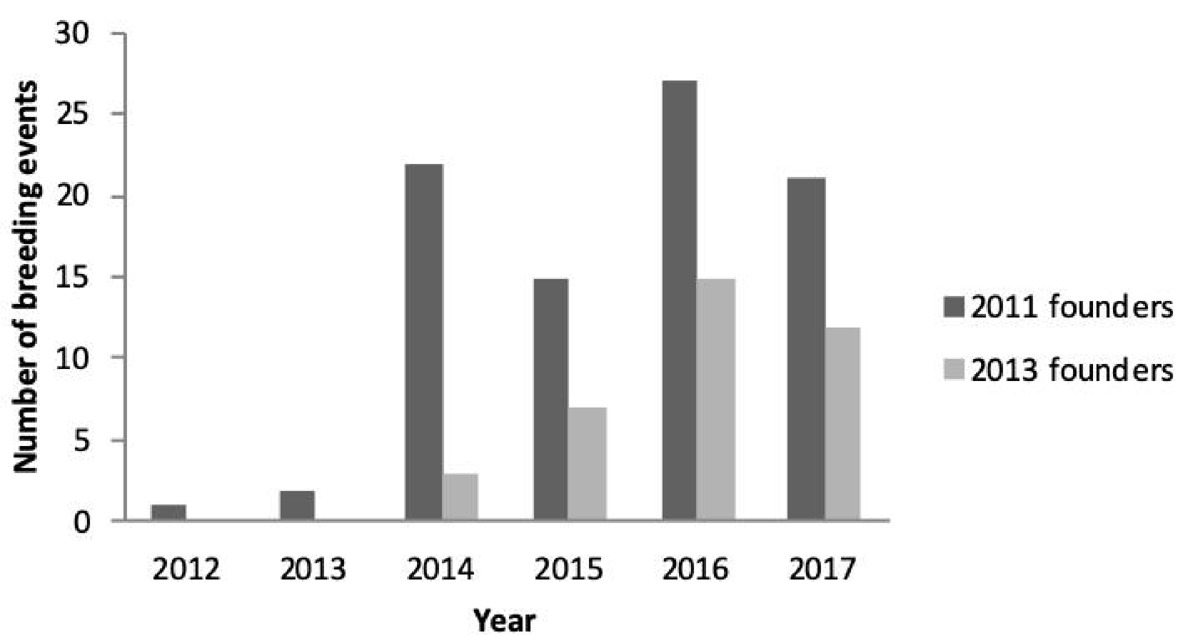

Fig. 4. Frequency of the founder *Scinax alcatraz* breeding events from 2012 to 2017.

Table 1. Breeding events of *Scinax alcatraz* from individuals collected in the wild (2011 and 2013 founders) and by captive-born individuals, which occurred at Sao Paulo Zoo from 2012 to 2017.

Groups	Number of breeding events	Total eggs
2011 Founders (N = 8)	88	7,743
2013 Founders (N = 9)	37	2,494
Captive-born (N = 148)	609	37,976
Total	**737**	**48,213**

Mortality. Of the 2011 founders, one female and two males died on the first day after collection. Two other males died a few years later (in July 2013 and May 2017). As of the current date (December 2021), two females and four males from the 2011 founders group are living. From the 2013 founders, two individuals died in June 2014, and one in July 2015.

From the 184 captive-born frogs, 58 died during the period of this study (ages ranging from one month to five years) and 15 were used for research purposes. In December 2017, the captive-born group comprised 111 adult frogs (19 males, 85 females, and seven sex-unknown).

Discussion

The maintenance and reproduction of *Scinax alcatraz* in captivity have proven to be very successful. Our results showed that within only three months in captivity, the founders started to breed. The skills acquired during the pilot study (Lisboa and Vaz 2012) using *S. perpusillus* as a surrogate species accelerated the results obtained for *S. alcatraz*. The use of a non-threatened surrogate to develop amphibian maintenance and breeding protocols is recommended in the absence of data on a target species (Michaels et al. 2014b). This is especially important for those species with specific ecological requirements (Crump and Grow 2007), as with *S. alcatraz*. Wild populations of threatened species are usually small and have limited geographic ranges (McGowan et al. 2017), and the surrogate approach is extremely helpful to develop adequate skills and experience before the acquisition of target founder specimens (Michaels et al. 2014b), which may prevent the loss of these specimens and reduce routine difficulties with captive maintenance.

We consider *S. perpusillus* as an appropriate surrogate species for *S. alcatraz*. However, other studies have shown limitations in using the surrogate species approach (Michaels et al. 2015, 2016), since even closely related species with similar husbandry have specific requirements for survival and reproduction (Michaels et al. 2015). Therefore, we highlight the importance of making the correct determination of whether the selected surrogate species is the most suitable one, considering existing knowledge, mainly about native climate and microhabitat selection (Michaels et al. 2016), for both surrogate and target species.

Captive breeding and reproductive biology. As observed with *S. perpusillus* (Lisboa and Vaz 2012), the high humidity produced by a fogger stimulated breeding activity in *S. alcatraz* and after a period in captivity this stimulus was no longer necessary. The captive population of *S. alcatraz* does not show a marked breeding season and lays eggs throughout the year. In contrast, wild reproductive activity of *S. alcatraz* seems to be more

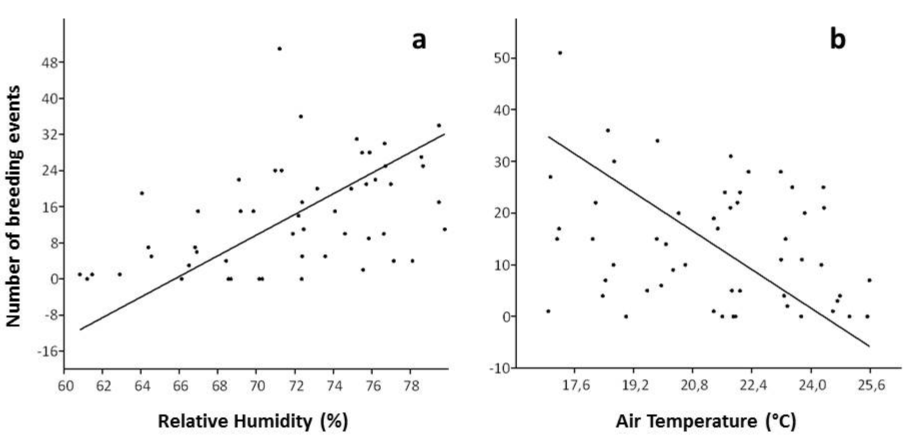

Fig. 5. Correlation between breeding events of *Scinax alcatraz* and environmental conditions (a) relative humidity and (b) air temperature from August 2013 to December 2017. Pearson product-moment Correlation Coefficient: $r = 0.323$; $p < 0.001$; N = 732.

concentrated between October and April, during the rainy season (Brasileiro 2008). In our findings, the correlation between relative humidity, air temperature, and number of breeding events suggests that high relative humidity (between 70–80%) associated with temperatures below 22 °C are indicators of suitable conditions for breeding in *S. alcatraz*.

Although *S. alcatraz* is a bromeligenous species (Brasileiro 2008), i.e., eggs and tadpoles develop in the water of bromeliads (reproductive mode 6, Haddad and Prado 2005), the presence of these plants were not essential for breeding in captivity. In fact, the most important factor for the reproduction of *S. alcatraz* is the availability of water, and we simply provided it in captivity with the supply of water pots, which replaced the bromeliads and ensured an ideal environment for the development of tadpoles.

The clutch size in captivity (up to 142 eggs) is a much larger number than recorded in the field, on average four to seven eggs (Brasileiro 2008). The difference may be due to the difficulty of making accurate counts in bromeliads under wild conditions, by the high predation rate of eggs in the wild, and/or by the size of the water pool available (water pots vs. bromeliad axil). Other species of the *S. perpusillus* group distribute eggs of one clutch among multiple bromeliad leaf-tanks by depositing a small number of eggs in each tank (Alves-Silva and Silva 2009), since there are insufficient resources in each leaf-tank to support a large number of tadpoles (Lehtinen 2004). Thus, *S. alcatraz* probably exhibits the same behavior, indicating that the clutch size recorded in nature may refer to only part of the entire clutch deposited among possibly many different leaves and bromeliads during one reproductive event. In captivity, clutches were found both shared among pots and aggregated in one portion, and it was common to find eggs scattered around the aquarium. As we offered only two or three pots per enclosure, less than the number of tanks found in a bromeliad, and with more water, this could explain the higher aggregation of eggs in each pot compared with wild conditions.

The high egg/embryo and tadpole mortality rates (67% and 47%, respectively) should be better investigated to promote improvements in husbandry of *S. alcatraz* in the future. One possible explanation may be the presence of unfertilized eggs, as they were included in the total count. However, this observation must be analyzed with caution because the exact numbers of fertilized and unfertilized eggs were not known. Another explanation could be the water conditions, since the two massive tadpole mortalities reported in 2014 occurred after water exchange, suggesting some possible toxicity in the water, including the observation of shuddering behavior of tadpoles before they died. The simple parameters like pH, chlorine, and ammonia were within expected standards, but we did not measure any other parameters that could also be toxic. Therefore, the cause of the high mortality of tadpoles remains uncertain. In conclusion, we consider it necessary to determine the parameters of water in which tadpoles develop in nature, to adjust the water in captivity and improve the survivorship rate (Poole and Grow 2012).

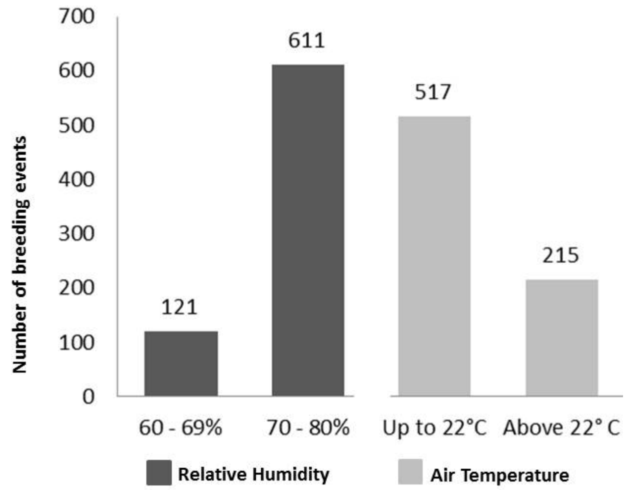

Fig. 6. Range of environmental conditions (relative humidity and air temperature) most favorable for reproduction of *Scinax alcatraz* in captivity.

Lifespan. Currently, six individuals of the 2011 founders group remain alive so they are at least ten years old. The mean longevity for anurans is 8.4 years and it is positively correlated with body size and negatively correlated with mean annual temperature (Stark and Meiri 2018). *Scinax alcatraz* shows that a small species (~23–28 mm; Brasileiro 2008) can potentially live more than one decade, indicating a relatively long lifespan for a small and tropical amphibian species. However, it is relevant to highlight that captive amphibians live, in general, 17% longer than their wild counterparts (Stark and Meiri 2018).

Fifteen of the captive-born individuals reached the age of eight years old in 2020. During this study, the mortality rate of specimens after metamorphosis was 31.5%, and they died individually, not in groups. The cause of death for most of them was inconclusive and their age varied from froglets to adults at the time of death.

The future of the program. During the development of the *ex situ* conservation program for *S. alcatraz*, *in situ* actions occurred in parallel by the Brazilian Government. Ilha dos Alcatrazes became a protected area in 2016 (Brasil 2016) and the bombing practice by the Navy was discontinued at the beginning of 2013. Nonetheless, the limited occurrence area of *S. alcatraz* on the island still demands the maintenance of a safe population in captivity (McGowan et al. 2017; Murray et al. 2017).

The wild population of *S. alcatraz* is abundant and stable (Brasileiro 2008), therefore the release of captive-bred individuals is not necessary at the present time. Due to this, we usually discard most new clutches to avoid overpopulation in captivity. As this species breeds easily in captivity and generates numerous offspring, we are conducting several scientific studies to improve our knowledge of the behavior, physiology, and disease resistance that can be used to conserve *S. alcatraz* in nature as well as other species, and thus avoid the need to collect additional specimens from the wild. The maintenance of a captive population allows long-term studies to be conducted, which is important because the access and logistics for working on Ilha dos Alcatrazes depend largely on appropriate sea conditions for navigation and landing, which hinders or even prevents long term studies in nature.

Another opportunity that has been provided by the program is the development of educational actions. The São Paulo Zoo receives about 1.5 million visitors per year, making it a great place to share information about the biology and conservation of amphibians, using *S. alcatraz* as a flagship species. Brazilian zoos and citizens are usually unaware of the importance of preserving amphibians, so an exclusive educational space has been created to exhibit some captive-born individuals of *S. alcatraz* (Rancura and Lisboa 2016) and educate the public.

Conclusions

At least to our knowledge, this was the first *ex situ* conservation initiative for an amphibian in Brazil, which has now guaranteed an assurance population of *Scinax alcatraz* at the São Paulo Zoo. From a practical perspective, numerous offspring can be produced if there is a need to reintroduce this species back to the wild. This captive population has shown great potential for amphibian conservation research since the species is very prolific. Finally, the use of a protocol developed with a surrogate species was very useful for the results achieved in the captive breeding program of *S. alcatraz*.

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Literature Cited

Alves-Silva R, Silva HR. 2009. Life in bromeliads: reproductive behavior and the monophyly of the *Scinax perpusillus* species group (Anura: Hylidae). *Journal of Natural History* 43(3): 205–217.

Amphibian Ark. 2009. Conservation Needs Assessments: Identifying priority species for conservation actions. Available: https://www.conservationneeds.org [Accessed: 11 April 2020].

Bataus YSL, Reis ML (Organizers). 2011. *Plano de Ação Nacional para a Conservação da Herpetofauna Insular Ameaçada de Extinção*. Series: *Espécies Ameaçadas*, Volume 21. Instituto Chico Mendes de Conservação da Biodiversidade, ICMBio. Brasília, DF, Brasil. 124 p.

Berger L, Speare R, Hines HB, Marantelli G, Hyatt AD, McDonald KR, Skerratt LF, Olsen V, Clarke JM, Gillespie G, et al. 2004. Effect of season and temperature on mortality in amphibians due to chytridiomycosis. *Australian Veterinay Journal* 82(7): 434–439.

Brasil. 2016. Decreto s/n, de 2 de Agosto de 2016. Estabelece a criação do Refúgio de Vida Silvestre do Arquipélago de Alcatrazes, no litoral norte do Estado de São Paulo, Município de São Sebastião. Diário Oficial da União (3 August 2016). Available: http://pesquisa.in.gov.br/imprensa/jsp/visualiza/index.jsp?data=03/08/2016&jornal=1&pagina=3&totalArquivos=104 [Accessed: 9 April 2020].

Brasileiro CA. 2008. *Scinax alcatraz*. Pp. 305–306 In: *Livro Vermelho da Fauna Brasileira Ameaçada de Extinção.* Editors, Machado ABM, Drummond GM, Paglia AP. MMA, Brasília, DF. Fundação Biodiversitas, Belo Horizonte, Minas Gerais, Brazil. 645 p.

Carvalho T, Becker CG, Toledo LF. 2017. Historical amphibian declines and extinctions in Brazil linked to chytridiomycosis. *Proceedings of the Royal Society B* 284: 20162254.

Chanson J, Hoffmann M, Cox N, Stuart S. 2008. The state of the world's amphibians. Pp. 33–52 In: *Threatened Amphibians of the World.* Editors, Stuart SN, Hoffmann M, Chanson JS, Cox NA, Berridge RJ, Ramani P, Young BE. Lynx Editions, Barcelona, Spain. 758 p.

Crump P, Grow S. (Organizers). 2007. Action plan for *ex situ* amphibian conservation in the AZA community. Association of Zoos & Aquariums, Amphibian Taxon Advisory Group. 46 p.

Cushman SA. 2006. Effects of habitat loss and fragmentation on amphibians: a review and prospectus. *Biological Conservation* 128(2): 231–240.

Gascon C, Collins JP, Moore RD, Church DR, McKay JE, Mendelson J. (Editors). 2007. *Amphibian Conservation Action Plan*. IUCN/SSC Amphibian Specialist Group. Gland, Switzerland and Cambridge, United Kingdom. 64 p.

Gosner KL. 1960. A simplified table for staging anuran embryos and larvae with notes on identification. *Herpetologica* 16(3): 183–190.

Haddad CFB, Prado CPA. 2005. Reproductive modes in frogs and their unexpected diversity in the Atlantic Forest of Brazil. *BioScience* 55(3): 207–217.

Halliday TR. 2008. Why amphibians are important. *International Zoo Yearbook* 42: 7–14.

Hammer Ø, Harper DAT, Ryan PD. 2001. PAST: Paleontological Statistics Software Package for Education and Data Analysis. Version 2.17. *Palaeontologia Electronica* 4(1): 1–9.

Heyer WR, Rand AS, Cruz CAG, Peixoto OL. 1988. Decimations, extinctions, and colonizations of frog populations in Southeast Brazil and their evolutionary implications. *Biotropica* 20: 230–235.

ICMBio (Instituto Chico Mendes de Conservação da Biodiversidade). 2013. MMA defende criação de parque em Alcatrazes. Available: http://www.icmbio.gov.br/portal/index.php?option=com_content&view=article&id=4062&Itemid=999 [Accessed: 9 April 2020].

ICMBio (Instituto Chico Mendes de Conservação da Biodiversidade). 2018. Livro Vermelho da Fauna Brasileira Ameaçada de Extinção: Volume V - Anfíbios. In: *Livro Vermelho da Fauna Brasileira Ameaçada de Extinção.* ICMBio, Brasília, DF, Brasil. 128 p.

La Marca E, Lips KR, Lotters S, Puschendorf R, Ibáñez R, Rueda-Almonacid JV, Schulte R, Marty C, Castro F, Manzanilla-Puppo J, Young BE, et al. 2005. Catastrophic population declines and extinctions in Neotropical Harlequin Frogs (Bufonidae: *Atelopus*). *Biotropica* 37(2): 190–201.

Lehtinen RM. 2004. Tests or competition: cannibalism, and priority effects in two phytotelm-dwelling tadpoles from Madagascar. *Herpetologica* 60(1): 1–13.

Lisboa CS, Vaz RI. 2012. Captive breeding and husbandry of *Scinax perpusillus* at São Paulo Zoo: preliminary action for *ex situ* conservation of *Scinax alcatraz* (Anura: Hylidae). *Herpetological Review* 43(3): 435–437.

McGowan PJK, Traylor-Holzer K, Leus K. 2017. IUCN guidelines for determining when and how *ex situ* management should be used in species conservation. *Conservation Letters* 10(3): 361–366.

McFadden M, Hobbs R, Marantelli G, Harlow P, Banks C, Hunter D. 2013. Captive management and breeding of the Critically Endangered Southern Corroboree Frog (*Pseudophryne corroboree*) (Moore 1953) at Taronga and Melbourne Zoos. *Amphibian & Reptile Conservation* 5(3): 70–87.

Mendelson III JR. 2011. Shifted baselines, forensic taxonomy, and Rabbs' Fringe-limbed Treefrog: the changing role of biologists in an era of amphibian declines and extinctions. *Herpetological Review* 42(1): 21–25.

Michaels CJ, Antwis RE, Preziosi RF. 2014a. Impacts of UVB provision and dietary calcium content on serum vitamin D_3, growth rates, skeletal structure, and coloration in captive Oriental Fire-bellied Toads (*Bombina orientalis*). *Journal of Animal Physiology and Animal Nutrition* 99(2): 1–13.

Michaels CJ, Gini BF, Preziosi RF. 2014b. The importance of natural history and species-specific approaches in amphibian *ex-situ* conservation. *Herpetological Journal* 24: 135–145.

Michaels CJ, Tapley B, Harding L, Bryant Z, Grant S, Sunter G, Gill I, Nyingchia O, Doherty-Bone T. 2015. Breeding and rearing the Critically Endangered Lake Oku Clawed Frog (*Xenopus longipes* Loumont and Kobel 1991). *Amphibian & Reptile Conservation* 9 [General Section]: 100–110.

Michaels CJ, Fahrbach M, Harding L, Bryant Z, Capon-Doyle JS, Grant S, Gill I, Tapley B. 2016. Relating natural climate and phenology to captive husbandry in two Midwife Toads (*Alytes obstetricans* and *A. cisternasii*) from different climatic zones. *Alytes* 33: 2–11.

Ministério do Meio Ambiente. 2014. Portaria nº. 444, de 17 de dezembro de 2014. Lista Nacional Oficial de Espécies da Fauna Ameaçadas de Extinção. Diário Oficial da União (17 December 2014). Available: http://pesquisa.in.gov.br/imprensa/jsp/visualiza/index.jsp?jornal=1&pagina=121&data=18/12/2014 [Accessed: 9 April 2020].

Murray MJ, Keith DA, Bland LM, Nicholson E, Reagan TJ, Rodríguez JP, Bedward M. 2017. The use of range size to assess risks to biodiversity from stochastic threats. *Diversity Distribution* 23: 474–483.

Pavajeau L, Zippel KC, Gibson R, Johnson K. 2008. Amphibian Ark and the 2008 Year of the Frog Campaign. *International Zoo Yearbook* 42: 24–29.

Peixoto OL. 1987. Caracterização do grupo "perpusilla" e revalidação da posição taxonômica de *Ololygon perpusilla perpusilla* e *Ololygon perpusilla v-signata* (Amphibia, Anura, Hylidae). *Arquivos da Universidade Federal Rural do Rio de Janeiro* 10: 37–49.

Pessier AP, Mendelson III Jr. (Editors). 2010. *A Manual for Control of Infectious Diseases in Amphibian Survival Assurance Colonies and Reintroduction Programs*. IUCN/SSC Conservation Breeding Specialist Group, Apple Valley, Minnesota, USA. 229 p.

Poole VA, Grow S. (Editors). 2012. *Amphibian Husbandry Resource Guide, Edition 2.0.* Association of Zoos and Aquariums, Silver Spring, Maryland, USA. 238 p.

Rancura K, Lisboa CS. 2016. Educational exhibit as a conservation tool for the Alcatraz Snouted Tree Frog. *AArk Newsletter* (September 2016). Available: http://www.amphibianark.org/Newsletters/AArk-newsletter-36.pdf [Accessed: 8 April 2020].

Rodrigues MT, Cruz CAG. 2004. *Scinax alcatraz*. The IUCN Red List of Threatened Species 2004. Available: https://www.iucnredlist.org/species/55924/11393985 [Accessed: 8 April 2020].

Segalla MV, Caramaschi U, Cruz CAG, Garcia PCA, Grant T, Haddad CFB, Santana DJ, Toledo LF, Langone JA. 2019. Brazilian amphibians: list of species. *Herpetologia Brasileira* 8(1): 65–96.

Stark G, Meiri S. 2018. Cold and dark captivity: drivers of amphibian longevity. *Global Ecology and Biogeography* 27: 1,384–1,397.

Verdade VK, Valdujo PH, Carnaval AC, Schiesari L, Toledo LF, Mott T, Andrade GV, Eterovick PC, Menin M, Silvano DL, et al. 2012. A leap further: the Brazilian Amphibian Conservation Action Plan. *Alytes* 29(1–4): 27–42.

Wake DB, Vredenburg VT. 2008. Are we in the midst of the sixth mass extinction? A view from the world of amphibians. *Proceedings of the National Academy of Sciences of the United States of America* 105: 11,466–11,473.

Weygoldt P. 1989. Changes in the composition of mountain stream frog communities in the Atlantic mountains of Brazil: frogs as indicators of environmental deteriorations? *Studies on Neotropical Fauna and Environment* 24(4): 249–255.

Wren S, Angulo A, Meredith H, Kielgast J, Santos M, Bishop P. (Editors). 2015. Amphibian Conservation Action Plan. IUCN SSC Amphibian Specialist Group. Available: https://www.iucn-amphibians.org/resources/acap/ [Accessed: 8 April 2020].

Zippel KC, Mendelson III JR. 2008. The amphibian extinction crisis: a call to action. *Herpetological Review* 39: 23–29.

Zippel K, Johnson K, Gagliardo R, Gibson R, McFadden M, Browne R, Martinez C, Townsend E. 2011. The Amphibian Ark: a global community for *ex situ* conservation of amphibians. *Herpetological Conservation and Biology* 6(3): 340–352.

Zoológico de São Paulo. 2019. Available: http://www.zoologico.com.br/noticias/programa-de-conservacao-da-perereca-pintada/ [Accessed: 11 April 2020].

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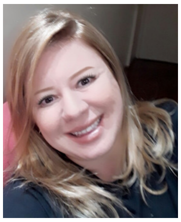

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