CADERNOS UniFOA

Edição 52 | Agosto de 2023

e-ISSN: 1982-1816

Data de submissão: 18/05/2022 Data de Aprovação: 25/05/2022

Tadpoles breeding method in a closed system with filtration

Método de criação de girinos em sistema fechado com filtragem

[1] Mauro Sérgio Cruz Souza Lima
[2] Bianca Leite Carnib de Sousa
[3] Jonas Pederassi
[4] Joara de Sousa Andrade
[5] Carlos Augusto Santos Sousa

1 Universidade Federal do Piauí, Campus Amílcar Ferreira Sobral, Laboratório de Herpetologia. Floriano, PI, Brasil.
2 Universidade Federal do Piauí, Campus Amílcar Ferreira Sobral, Laboratório de Herpetologia. Floriano, PI, Brasil.
3 Ong Bioma. Volta Redonda, RJ, Brasil.
4 Universidade Federal do Piauí, Campus Amílcar Ferreira Sobral, Laboratório de Herpetologia. Floriano, PI, Brasil.
5 Universidade Federal do Acre, Centro de Ciências Biológicas e da Natureza. Rio Branco, AC, Brasil.

ABSTRACT

Considering the need to differentiate tadpoles by species and stage of development, and to consolidate the preservation of the largest number of stages in herpetological collection, the collection of tadpoles and maintenance in aquarium until metamorphosis is common for final identification of its species. For this purpose, the present work aimed at creating a low-cost method for maintaining tadpoles in the laboratory that would minimize the space used and facilitate maintenance. Plastic organizers were used as aquariums, interconnected by closed water circuits with filtration. During a 21-day test, tadpoles of Leptodactylus macrosternum showed maximum mortality of 9% with this breeding method.

Keywords:

Amphibia. Anura. Tadpole identification. Metamorphosis.

RESUMO

Considerando a necessidade de diferenciar girinos por espécie, e por estágio de desenvolvimento, e consolidar a preservação do maior número de estágios em coleção herpetológica, a coleta de girinos e manutenção em aquário até metamorfose é comum para identificação final de sua espécie. Para tanto, o presente trabalho objetivou à criação de um método de baixo custo para a manutenção de girinos em laboratório que minimizasse o espaço utilizado e facilitasse a manutenção. Foram utilizados organizadores de plástico como aquário, interligados por circuitos fechados de água com filtragem. Durante teste por 21 dias, girinos de *Leptodactylus macrosternum* apresentaram mortalidade máxima de 9% com esse método de criação.

Palavras-chave:

Amphibia. Anura. Identificação de girinos. Metamorfose.

1 INTRODUCTION

Anurans (frogs, toads, and tree frogs) display two stages of development, that being the larval stage (tadpole), generally evolving in bodies of water present in still or flowing environments whereas, in the adult phase, they display greater ecological diversification (DUELLMAN; TRUEB, 1994). This condition for development in two environments requires knowing the larval stage in a way that relates it taxonomically to the adult stage in light of similarities between larvae of distinct species. In many instances, such differentiation is only possible following stage 37.

Tadpoles display 46 stages of development (GOSNER, 1960) and due to the dependence of bodies of water, the species, in general, reproduce during the rainy season (Lima & Pederassi, 2015). In this reproductive state, many tadpoles of varying species occupy the same environment, making it difficult to differentiate among the species.

The anuran larval phase is crucial for the description of new species and the greater the compilation of stages the better the evolution and natural history of the species is known (MCDIARMID; ALTIG, 2000).

Regarding the need to distinguish between tadpoles by species, developmental stages, and consolidating the preservation of the great number of stages in a herpetological collection, it is common to gather tadpoles in their natural habitat and attempt to breed subjects in a laboratory setting, so that they reach more advanced stages or even metamorphosis to identify them correctly. Nevertheless, there are many problems encountered upon maintaining the tadpoles, such as the illumination that interferes in the photoperiod and, consequently, delays development or leads the subject to death (BAMBOZZI *et al.*, 2004), as well as the temperature (KOFFLER *et al.*, 2011). Another preponderant factor for successful development is dissolved oxygen and pH that are extremely deleterious and cause mortality rates when outside of the tolerated limits (CASTRO; PINTO, 2000).

Many other motives for tadpole maintenance in a laboratory exist, such as the utilization of these organisms in the indication of the biological state of the environment (Lima & Peixoto, 2007). There are many projects that demand the maintenance of tadpoles in a lab and many obstacles are faced during their upkeep (GRAEFF et al., 2001; MOURIÑO et al., 2006; SÃO PEDRO et al., 2008; MIAUD et al., 2011)

These cases reinforce the need for better insight of tadpole species and thus, laboratory structures that occupy little space, maintain controlled abiotic conditions, and enable daily monitoring of the 46 developmental stages. Moreover, it is estimated that in Brazil at least 40% of the anuran species haven't had their tadpoles described (DUBEUX *et al.*, 2020; PROVETE; SILVA, 2012; ROSSA-FERES *et al.*, 2015).

Considering the challenges as well as being privy to tadpole developmental stages of differing species, we proposed constructing a water tank of 12 aquariums, with possible expansion, with low-cost material, interconnected via a closed water circuit with filtration and minimized spatial usage.

2 MATERIALS AND METHODS

Technological

A proposal based on offering tadpole breeding technology in vertical shelves, with the purpose of reducing space for experimentation, to ensure that cleaning maintenance is reduced due to the filtration system in an enclosed circulation of water and to guarantee the availability of dissolved oxygen through the enclosed ventilation system.

Patented Tadpole Breeding Systems

We searched along with the bases of EPO patents, INPI, Google patents, USPTO patents related to the following keywords: aquarium and aquaculture, tadpoles, cultivation and farming. At the time, we associated words as follows: aquarium and tadpoles and aquaculture, tadpoles, cultivation, and farming tadpoles, cultivation, farming, and aquarium.

Biological

Testing the qualities of the aquarium drum while following the development of the species *Leptodactylus macrosternum* Miranda-Ribeiro, 1926 through the structured model of the investigated population (GOTELLI, 2007; LIMA *et al*., 2014).

Mathematical model

The mathematical model was proposed by Lima & Pederassi (2014), larval stage organized into columns regarding time units (x days); Lx fraction of survivors by larval phase, in each x interval; dX is the fraction of subjects that metamorphosed or died between the ages x e x+1 and was estimated by Lx-Lx=1. The value of X (average probability of survival among the larval stages in successive metamorphoses) was calculated by Lx-1/2(dx), the total number of days of remaining life that larval stage survivors had (x)l Tx was estimated by Σ Ex+(Ex+1)+Ew, where "w" is the maximum larval phase in weeks; the x life expectancy, was estimated by Tx/Lx. The mortality ratio (qx) by age range was estimated by 1000qx(dX/Lx).

Sample Population

We emphasized that the experiment aimed to verify if the aquariums bore suitable conditions for the development of tadpoles. Hence, no setbacks occurred with regard to the development of the tadpole and its natural history or ontogeny (License SISBIO 38966-9, CEUA – Process 23107.018096/2018-15 UFAC).

Two hundred and seventy *L. macrosternum* tadpoles were used in diverse stages that varied from Gosner stages 25 and 30 (1960), divided evenly in the aquarium tanks, without considering the state of stage development.

It was considered for qualitative effect, the stage evolution, 1/3 of the developed ones being withdrawn to be placed in The Natural History of the Federal University of Piaui's collection (CHNUFPI3196/pot 51).

Feeding

The animals were removed from temporary ponds and fed with the same sediment and suspension from the environment Vieira *et al*. (2016), with respect to the reduction of the water column (depth until the surface water) from 10 cm (maximum state) to 9 cm (minimum state) transpired with the reposition of the collection's water site. (7°30'22.1"N and 45°5'8.2"W).

2.1 DESCRIPTION OF THE AQUARIUM TANKS MODEL

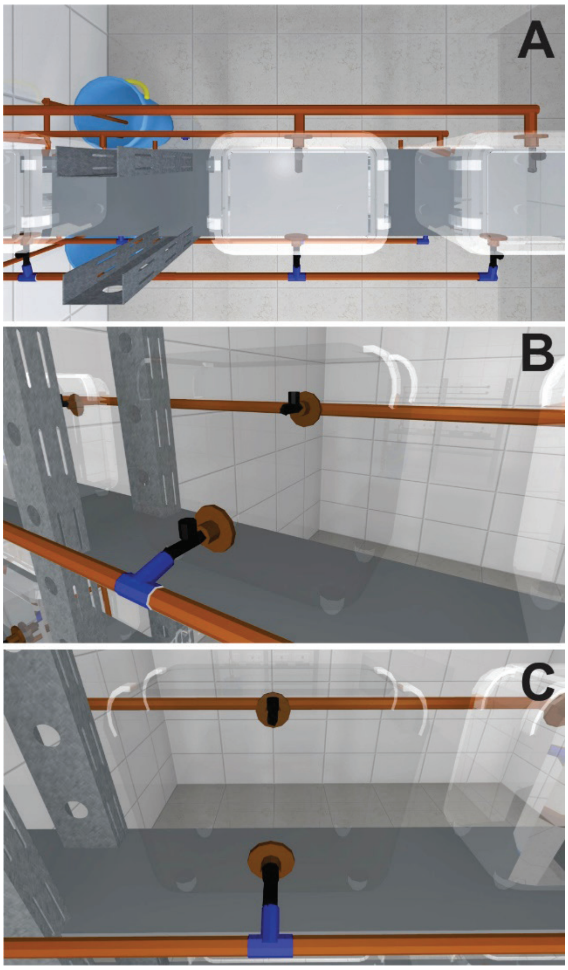
Description of the materials

Utilization of 20-liter plastic containers in an individualized aquatic state measuring 25 x 30 x 25 (cm), a ½" e ¼" plastic garden-like faucet, 20 mm PVC elbow, 20mm T adapter, 20mm connection to water tank, 20 mm sleeve, a six meter by 20mm pipe, 50 liter plastic bucket, 4000 (liters/hour) waterjet pump, 3-meter water column with dimensions of 16 x 100 x 140 (mm), NBR 14908:2004. standard filter

Build description

The plastic container relies on a 20 mm opening on one of its faces, the designated posterior side, withdrawn from the 8cm superior border, centered with respect to the outside edges connecting the water tank and ¼ plastic faucet facing inward to the entrance of the filtered water. On its other face, the designated front-side, with a similar opening and connection, withdrawn 18 cm from the edge and centered with respect to the sides with the ¼ faucet oriented outward. Coupled with the outflow of the water for filtration, this assembly was designated as the functional unit (Fig. 1 A-1C). Each working unit is interconnected with another through 20 mm PVC tubing with a continuous "t" connection.

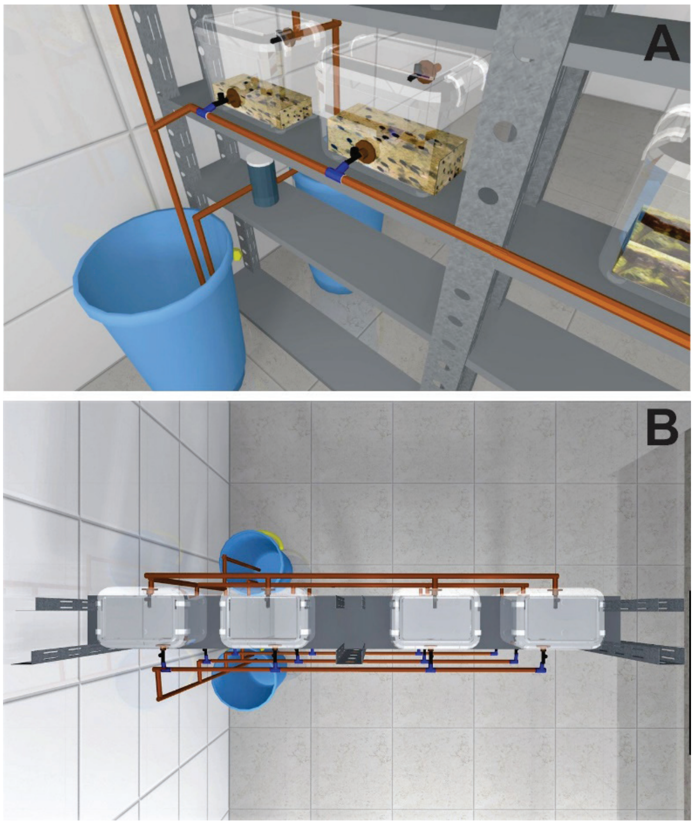
Fig. 1 - (**A**) overhead/superior view, (**B**) side view, (**C**) front view: a functional unit with the ¼ faucets oriented into the inside of the accumulator and ½ faucet turned towards the outside of the accumulator.



Source: pictures of Mauro Sérgio.

From the 1st 18-liter bucket, the pipe sends the flow of filtered water across the connected elbow tube and tube that runs along the entire working unit line, every functional unit being connected to the ½" faucet with an inlet flow. On the other face with a similar interconnection with a substituted ½" PVC faucet and radiating outflow of the container with a return to bucket 2 (Fig. 2 A and B).

Fig. 2 - (**A**) a front view of the 2 interconnected operating units, (**B**) aerial view of four interconnected operating units.

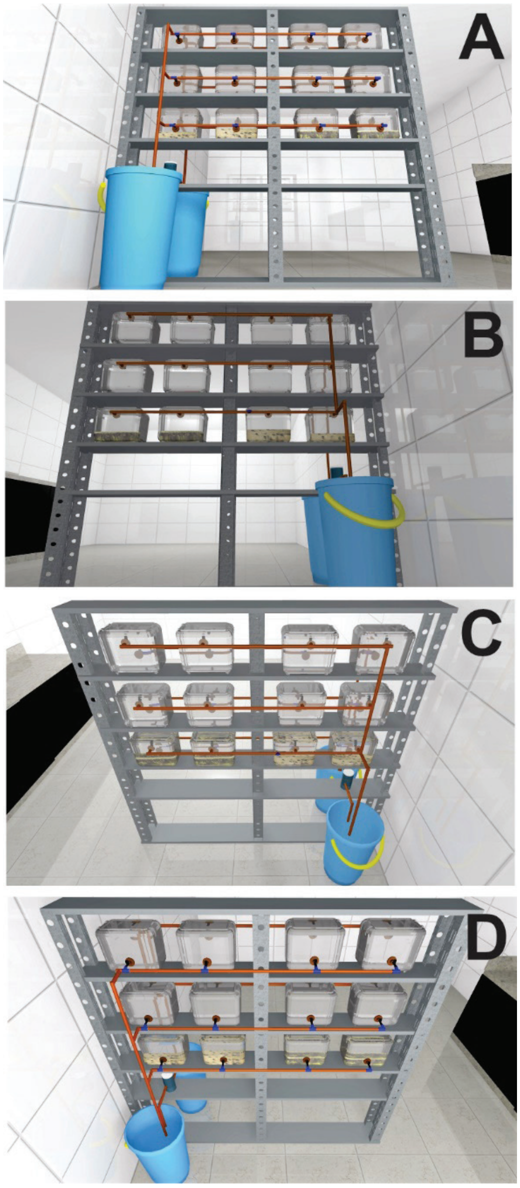


Source: pictures of Mauro Sérgio.

A 20 mm PVC register can be placed between two operating units for unit isolation or between pairs of units. In our model, we didn't utilize the register. Thus, we used two lined pairs forming four repeated operating units in two others making a total of 12 operating units (Fig. 3 A and B).

Two 50-liter plastic buckets were placed on the front and rear portion of the 12 units, since a 4000 (liters/hour) waterjet pump was fitted inside of each bucket, a 3-meter water column with dimensions of 216 x 100 x 140 mm. These two buckets were interlinked by standard filter model NBR 14908:2004 (Fig. 3 C and D) so that the descending water that reached the bucket was pumped to the interconnected filter and the stream, upon reaching the bucket in the posterior region and it was sent to a new stream across the pipe.

Fig. 3 - (**A**) front view, (**B**) rear view of the pairs of lines formed by the 4 operating units totaling 12 interconnected units, **(C)** front view with descending stream of water and (**D**) posterior view with rising stream of water.



Source: pictures of Mauro Sérgio.

The regulation of inflow and outflow, with the desired standard water column, must be obtained with the management of the faucets and register of each pipe with regards to the rising water column so that the flowing circuit of water remains closed and continuous.

3 RESULTS

No patent was found with the aim of tadpole breading in closed ventilation, filtration, and water flow system, whether it was for scientific purposes or zootechnical purposes. The patents found for these keywords (Table 1) are related to devices for liquid transferal among containers, devices for fluorescence-based mediation, methodologies for vegetative and zootic culture, creation of natural-based ornaments, substances for aquariums, and for mosquito and parasite control, material engineering procedures and equipment for concrete production.

Table 1 - Search on platforms for approved patents and respective words for patent search. Keyword + Search Engine.

Keywords	**Plataforma de Busca**			
	EPO	**INPI**	**USPTO**	**Google patents**
Aquarium and aquaculture	3	0	1	6209
Tadpoles	0	1	0	4168
cultivation and farming	4	0	20	123943
Aquarium and Tadpoles	0	0	0	88
Tadpoles and cultivation and farming	0	0	0	429
Tadpoles and cultivation and farming and Aquarium	0	0	0	22

Source: table made by Mauro Sérgio.

The development of *Leptodactylus macrosternum* tadpoles was monitored for 21 days, of which the highest mortality rate was 9% and the lowest 0.5% as provided by the structured tracking tablet of the population of development in stages.

Table 2 – Description of the tadpole development across the structured population model (GO-TELLI,200715, LIMA et.al. 201416).

X	Lx	dx	Ex	Tx	ex	1000qX
1	270	10	265.0	2486.0	9.21	2.00
2	260	20	250.0	2221.0	8.54	4.15
3	240	12	234.0	1971.0	8.21	2.70
4	228	16	220.0	1737.0	7.62	3.79
5	212	21	201.5	1517.0	7.16	5.35
6	191	30	176.0	1315.5	6.89	8.48
7	161	12	155.0	1139.5	7.08	4.02
8	149	8	145.0	984.5	6.61	2.90
9	141	10	136.0	839.5	5.95	3.83
10	131	15	123.5	703.5	5.37	6.18
11	116	18	107.0	580.0	5.00	8.38
12	98	16	90.0	473.0	4.83	8.82
13	82	2	81.0	383.0	4.67	1.32
14	80	11	74.5	302.0	3.78	7.43
15	69	3	67.5	227.5	3.30	2.35
16	66	2	65.0	160.0	2.42	1.64
17	64	30	49.0	95.0	1.48	25.31
18	34	12	28.0	46.0	1.35	19.06
19	22	16	14.0	18.0	0.82	39.27
20	6	5	3.5	4.0	0.67	45.00
21	1	5	0.5	0.5	0.50	270.00

Source: table made by Mauro Sérgio.

4 DISCUSSION

The results found in the development of tadpoles in a cleaning filtration system were already encountered for *Rhinella icterica* (LIMA *et al*., 2014) with a maximum mortality rate of 7% in 2% of the ones found in this study. Given that the highest rate was 5%, it is similar to the result found in this study.

These findings illustrate the viability of the breeding of tadpoles in our system, marking the raise of experimentation that brings improvements of the system and adaptation to the needs of each experiment.

Experiments with tadpoles demand areas and free spaces for the distribution of breeding sites, for example, 24 polyethylene boxes corresponding to 14 m^2 for the evaluation of the photoperiod in *Lithobates catesbeianus* (BAMBOZZI *et al*., 2004), evaluation of gregarious growth of *L. catesbeianus* occupying 3 m^2 with three iterations, which amounted to 12 m^2 (SÃO PEDRO; SILVA; MANDUCA, 2008) .We calculated the area of use based on the information provided by the breeding equipment used for the tadpoles, there, the area for the researcher's circulation wasn't taken into account.

Regarding the 2 experiments (BAMBOZZI *et al*., 2004; SÃO PEDRO; SILVA; MANDUCA, 2008), it is evident that the occupied area in the laboratory could have been solved with our proposed methodology, using the exact same model with the same dimensions, by substituting the plastic trays and making the repetitions in each line with 4 operating units. In this respect, our model occupies a maximum area of 2 m^2 of flooring, considering the area for the researcher's circulation, thus vertically can be suitable to the laboratory conditions in accordance with the height available.

In another experiment, the evaluation of the quality of water for the development of *L. catesbeianus* in tanks had the partial renewal of water daily once a week (CASTRO; PINTO, 2000). The evaluation of pesticide toxicity in *Physalaemus cuvieri* required two researchers to completely change the water in the tank every two days (DA SILVA *et al*., 2013). If the experiments were developed using our methodology it wouldn't have been necessary to replace the water, since the filter would take care of removing the waste and other residues inexpedient to the experiment.

5 CONCLUSIONS

The advantages of using the model of breeding that we proposed are optimization of the spaces, allowing the increase of experimental repeatability for the chain of operating units, systemic autonomy to filtrate circulating impurities and the low cost since all the items are easy-to-use plastic and widely available on the national market.

6 ACKNOWLEDGMENT

We thank the interior designer Janice Bastianin Alvares for the collaboration in our illustrations.

REFERENCES

BAMBOZZI, A. C. *et al*. Efeito do fotoperíodo sobre o desenvolvimento de girinos de rã-touro (Rana catesbeiana Shaw, 1802). **Revista Brasileira de Zootecnia**, v. 33, n. 1, p. 1–7, fev. 2004.

CASTRO, J. C.; PINTO, A. T. Qualidade da Água em Tanques de Girinos de Rã-Touro, Rana catesbeiana Shaw, 1802, Cultivados em Diferentes Densidades de Estocagem 1 Quality of Water in Tanks of Tadpole of Bull Frog, Rana catesbeiana Shaw, 1802, Cultivated in Different Stock Densities. **Rev. bras. zootec**, v. 29, n. 6, p. 1903–1911, 2000.

DA SILVA, H. S. V. P. *et al*. Toxicidade aguda e genotoxidade do agrotóxico comercial Folusuper 600BR a girinos de Physalaemus cuvieri (Anura: Leiuperidae). **Pesticidas: Revista de Ecotoxicologia e Meio Ambiente**, v. 23, 31 dez. 2013.

DUBEUX, M. J. M. *et al*. Morphological characterization and taxonomic key of tadpoles (Amphibia: Anura) from the northern region of the Atlantic Forest. **Biota Neotropica**, v. 20, n. 2, p. 1–24, 2020.

DUELLMAN, W. E.; TRUEB, L. **Biology of Amphibians**. [s.l.] Johns Hopkins University Press, 1994.

GOSNER, K. L. A Simplified Table for Staging Anuran Embryos and Larvae with Notes on Identification. **Herpetologica**, v. 16, n. 3, p. 183–190, 1960.

GOTELLI, N. L. **Ecologia**. Planta, 2007.

GRAEFF, A.; PRUNER, E. N.; SPENGLER, M. M. Efeito da Niclosamida no Controle de Girinos de Anuros na Propagação de Pós-Larvas de Carpa Comum (Cyprinus carpio Linnaeus, 1758 Var. Specularis). **Revista Brasileira de Zootecnia**, v. 30, n. 6, p. 1664–1669, dez. 2001.

KOFFLER, S. *et al*. Influência da temperatura da água sobre a distribuição de girinos de Rhinella ornata (Amphibia: Bufonidae). **Prática da Pesquisa em Ecologia da Mata Atlântica - Ecologia da Mata Atlântica**, p. 1–5, 2011.

LIMA, M. S. C. S.; PEDERASSI, J.; SOUZA, C. A. DOS S. Longevity and survival curves of Rhinella icterica (Anura, Bufonidae) under laboratory conditions. **Brazilian Journal of Biology**, v. 74, n. 2, p. 438–443, maio 2014.

LIMA, M. S. S.; PEDERASSI, J. Introdução à Ecologia dos Anfíbios Anuros. In: LIMA, M. S. S.; CARVALHO, L. S.; PREZOTO, F. (Eds.). **Métodos em ecologia e comportamento animal**. Teresina: EDUFPI, 2015. p. 187–215.

MCDIARMID, R. W.; ALTIG, R. **Tadpoles: The Biology Of Anuran Larvae**. University of Chicago Press, 2000.

MIAUD, C. *et al*. Intra-specific variation in nitrate tolerance in tadpoles of the Natterjack toad. **Ecotoxicology**, v. 20, n. 6, p. 1176–1183, 30 ago. 2011.

MOURIÑO, J. L. P. *et al*. Isolamento de *Aeromonas hydrophila* em girinos de rã-touro na metamorfose. **Pesquisa Agropecuária Brasileira**, v. 41, n. 8, p. 1325–1327, ago. 2006.

PROVETE, D. B.; SILVA, F. R. Knowledge gaps and bibliographical revision about descriptions of free-swimming anuran larvae from Brazil. **Article in North-Western Journal of Zoology**, v. 8, n. 2, p. 283–286, 2012.

ROSSA-FERES, D. C. *et al*. Faculty Scholarship Collection Citation Rossa-Feres Terms of Use. **Herpetologia Brasileira**, v. 4, n. 2, p. 48–59, 2015.

SÃO PEDRO, V. DE A.; SILVA, C. D.; MANDUCA, E. G. O comportamento gregário de girinos de Lithobate catebeianus (Shaw, 1802) e sua relação com a temperatura. **Revista Brasileira de Zoociências** , v. 10, n. 2, p. 121–125, 2008.

VIEIRA, M. S. *et al*. Avaliação da dieta dos girinos de três corpos d'água temporários do monumento Natural Grota do Angico, Sergipe, Brasil. **Agroforestalis News**, v. 1, n. 1, p. 1–7, 2016.