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Success Rate of Metamorphosis of *Physalaemus cuvieri* (Anura, Leptodactylidae) in Laboratory Conditions

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ABSTRACT

Studies about the larval phase of anurocenose are incipient in Brazil. Even when it comes to common or high-occurrence species, there is still a lot to be researched. We analyzed the metamorphosis rate of *Physalaemus cuvieri* tadpoles in laboratory conditions of water level reduction simulation; similar to the temporary ponds where they develop. The population modeling applied was the Leslie matrix, where $\gamma > 1$ represents metamorphosis into the next stage and population increase; $\gamma < 1$ represents tadpole's death and population decrease, while $\gamma = 1$ represents the permanence of tadpole at the current stage, with no metamorphosis and no population increase. The correlation between metamorphosis rate and water level was estimated by the Pearson correlation. In laboratory conditions, the *P. cuvieri* tadpoles have a limited development due to the water volume availability. The model predicts that 50% of the tadpoles reach a complete metamorphosis and 50% hit the 42 stage followed by death. At least, 20% to 25% remain unchanged until death. The results suggest a great adaptation of the species' life cycle to the semi-arid conditions, reinforcing the need for more studies with tadpoles of Brazilian anurofauna.

Key Words: Amphibia; Leslie Matrix; Tadpoles; Reproductive Success; Semi-arid; Brazilian Cerrado

RESUMO

Estudos da fase larvar da anurocenose são incipientes no Brasil. Mesmo em se tratando de espécies comuns e de ampla ocorrência há muito que ser pesquisado. Analisamos aqui a taxa de metamorfose de girinos de *Physalaemus cuvieri* em condições laboratoriais de simulação de redução do nível de água, como ocorre nas poças temporárias onde se desenvolvem. A modelagem foi executada através da matriz de Leslie onde $\gamma > 1$ representa metamorfose para o estágio seguinte com aumento da população; $\gamma < 1$ representa óbito do girino e diminuição da população e $\gamma = 1$ representa a permanência do girino no mesmo estágio, sem metamorfose e com aumento populacional nulo. A correlação entre taxa de metamorfose e nível de água foi estimado pela correlação de Pearson. Os girinos de *P. cuvieri*, em condições laboratoriais, têm seu desenvolvimento limitado pelo volume de água disponível e a predição do modelo estabelece que 50% dos girinos alcançam a metamorfose completa e 50% atingem o estágio 42 com óbito nessa fase. No mínimo, 20 a 25% permanecem sem mudança de estágio até que ocorra o óbito. Os resultados sugerem uma grande adaptação do ciclo de vida da espécie à condição semiárida e reforçam a necessidade de mais estudos com girinos da anurofauna brasileira.

Palavras-chave: Amphibia, Matriz de Leslie, girinos, sucesso reprodutivo, semiárido, Cerrado brasileiro

INTRODUCTION

In Brazil, the study on tadpoles requires efforts to the comprehension of its natural history and ontogenetic development of their 46 stages (Gosner, 1960). This is especially true to the State of Piauí in the semi-arid Brazilian northeast where few researches have been made. Understanding the peculiarities in the development stages of anurans in many different environments is a sine qua non condition for the comprehension of their ecologic success that results in the great diversity of anurans in Brazil, with 913 species described so far (Segalla et al. 2012).

Among the variables that determine the success of the tadpole's development is the age structure, which affects the population growth because the frog just begins growing up after the metamorphosis (Gotelli 2007, Lima et al. in press). Ecological models regarding tadpoles infer that the environmental pressure, such as hydric stress, can accelerate metamorphosis (Beachy et al. 1999, Cano et al. 2004).

Some authors have developed ecological models that predict the effect of environmental pressure on metamorphosis, for example, for *Epidalea calamita* (Laurenti 1768) it was possible to estimate the survival rate and metamorphosis (Aubry et al. 2010). In Canada the impact on other species of introducing *Lithobates catesbeianus* (Shaw 1802), an exotic species, was evaluated based on the development and distribution rate of their tadpoles using a demographic matrix (Govindarajulu et al. 2005).

Theories about the development of anurans in the northeast, particularly in semi-arid areas, empirically suggest that the temperature and water level gradient resulting from the regional rainfall regime makes the tadpoles develop rapidly its lifecycle to imago. In this study we examine this paradigm by evaluating the development of *Physalaemus cuvieri* Fitzinger 1826 in experimental laboratory conditions.

MATERIAL AND METHODS

Sampling Area

The Brazilian State of Piauí in the Northeast region consists of semi-arid climate to semi-humid, named equatorial (IBGE 2012). It is characterized by morphoclimatic areas of lowlands and transition on the border with the State of Maranhão (Ab'Sáber 2010). The city of Floriano is on the border of these states, being separated by the Parnaíba river (06°46'01"S; 43°01'22"W), at 140 meters above sea level.

Management Conditions

After collection in temporary ponds the tadpoles were transported during 40 min until the lab in thermic boxes with water at 7 cm depth (Bastos et al 2010). In the lab they were separated by stage of development, according to Gosner (1960). Allocated in aqua-terrarium of 31.5 cm^3 containing water with depth of 7 cm with stone aquarium arranged on the floor, totaling seven aqua-terrariums without aeration. The physical conditions of the aqua-terrariums were similar to those found in the field. The water temperature was held at 22 to 28°C, oxygen between 6.9 to 7.9 mg/L. Ammonia (NH_3) and pH were measured on a daily basis, in the morning, as a control against harmful levels of these elements in function of the excreta, being the reference zero to 30 mg/100 g of ammonia and pH between 5.6 and 8.6 (Castro and Pinto 2000, Mora 2010). As a source of food, ad libitum, was offered extruded commercial ration with 42% of crude protein. These proceedings were made thru the entire experiment (28 Nov 2012 to 29 Dez 2012).

Experimentation

Each aqua terrarium had randomized blocks of tadpoles by development stage (Gosner 1960), being distributed in 17 tadpoles in stage 25 and 55 in stage 30, composing a sample of 72 tadpoles. The experiment ended with the full metamorphosis or death of each individual in a total of 30 days of observation.

Ecological Model

There are three biotic conditions affecting the tadpoles: metamorphosis, i.e. develop until the following phase(s); death; and keeping unchanged the stage condition. Considering the transformation of these qualitative data into quantitative (Vanzolini 1993), we can consider the numerical score, respectively, 2, 1, and 0 resulting in the matrix (n x 3; being n the corresponding stage of the tadpole).

The matrix dynamics is described by the matrix projection equation, on which n represents the condition of the metamorphic stage for instant t, where any tadpole reaches its metamorphic condition in L. So the population can be divided into 46 age groups (cf. Gosner (1960), where each age group has L/n hr duration.

Matrix projection (1): $\mathbf{X^0} = \begin{bmatrix} \mathbf{X_1^0} \\ \mathbf{X_2^0} \\ \mathbf{X_n^0} \end{bmatrix}$

As in this study we know the number of tadpoles for the stage (age group) and successively the other stages, we can consider X_1 in the stage 25 and X_2 in stage 30, resulting in a vector of initial age distribution. This is, in the lab the initial stage was the 25 while in the environment it is zero according Gosner (1960). In other words, we have considered as the initial stage the more premature available to the study.

The process of metamorphosis or death of each tadpole can be described as (a_n) average number of tadpoles that died in stage n, being n = 1, 2, 3... 46; and (b_n) the fraction of tadpoles that survived and achieved the subsequent stage, where n = n+1.

The second matrix projection is defined by the vector $x^{(k)}$ of age distribution at instant t_k, where the tadpole in the n-th age range, stage 46 according Gosner (1960) in the moment t_k.

Matrix projection (2): $\mathbf{X^k} = \begin{bmatrix} \mathbf{X_1^k} \\ \mathbf{X_2^k} \\ \mathbf{n} \end{bmatrix}$

Equation (1)

$$\mathbf{X_1^k = a_{1x_1^{(k-1)}} + a_{2x_2^{(k-1)}} + a_{nx_n^{(k-1)}}}$$
:

The tadpoles that remain in condition (age group) at the moment t_k shall be equivalent to:

Equation (2):

$$\mathbf{X_{n+1}^k = b_n\, x_n^{k-1}} \qquad n = 1, 2... n-1$$

Using matrix notation we can write:

$$\mathbf{L} = \begin{Bmatrix} \mathbf{a_1} & \mathbf{a_2} & \mathbf{a_3} & \cdots & \mathbf{a_{n-1}} & \mathbf{a_n} \\ \mathbf{b_1} & \mathbf{b_2} & \mathbf{0} & \cdots & \mathbf{0} & \mathbf{0} \\ \\ \mathbf{0} & \mathbf{b_2} & \mathbf{0} & \cdots & \mathbf{0} & \mathbf{0} \\ \mathbf{0} & \mathbf{0} & \mathbf{0} & \cdots & \mathbf{b_{n-1}} & \mathbf{0} \end{Bmatrix}$$

Leslie's Theorem

Leslie matrix has a single positive eigenvalue γ_1. This eigenvalue has multiplicity 1 and an associated eigenvector x_1 whose entries are all positive. The long term behavior of the population age distribution is determined by positive γ_1 and its eigenvector x_1.

If γ_1 is the one positive eigenvalue of a Leslie matrix (L and γ_1) and is any other real or complex eigenvalue of L, then $|\gamma_k| \leq \gamma_1$ (Stormowski 2008).

Considering the assumptions of the theorem of Leslie and the eigenvalue γ_1 we have three cases of initial array:

$\gamma_1 > 1$: metamorphosis to the next stage, increasing population;
$\gamma_1 < 1$: tadpole's death and population reduction;
$\gamma_1 = 1$: tadpole remains in the same metamorphic condition, zero population growth.

To estimate the correlation between the rate of metamorphosis and the water level in the aqua-terrarium, we used the index of correlation of Pearson and tested it with the t test for correlation.

RESULTS AND DISCUSSION

The air temperature ranged between 26 and 31°C and the water temperature ranged from 22 to 28°C. The average variation between water and air temperatures corresponded to 3.5 ± 0.5 ºC. The average variation of pH was 6.95 ± 1.5 and NH_3 = 0.001 ppm. When comparing these data with the ideal patterns for breeding *Lithobates catesbeianus*, we identified that the experimental framework was in a satisfactory condition, i.e., maximum allowable ammonia = 30 mg 100 g^{-1}; ideal pH = 7 (maximum 8 and minimum 5.6); ideal water temperature = 25 ºC (30 ºC maximum and 18 ºC minimum) (Mora 2010). Whereas there are no parameters for handling *P. cuvieri* and that the proposed values for *L. catesbeianus* meet the international parameters (Castro and Pinto 2000, Bastos et al 2010, FAO 2012) the values found in this study seem to represent satisfactory handling conditions.

The water level decrased by 2.7 cm, with the initial depth of 7 cm and end depth of 4.3 cm. These parameters corroborate the studies for the northeastern region, where the cubic volume of aqua terrarium was reduced by 0.13 cm^3 per day in average temperature of 26 to 31ºC, the equivalent to the studies developed in the semi-arid Northeastern (Silans et al. 2006, Lima et al. 2008).

When we submit the number of individuals metamorphosed and the water reduction to the Pearson correlation coefficient, we find a negative result ($r = -0.92$, 25 GL, $p<0.0001$; $t = -9.93$). This means that, as the water reduction occurs, death occurs and metamorphosis rate increases, which highlights that any stage can be affected by the reduction of the water.

In this experiment, from the sample of 72 (100%) tadpoles, 36 (50%) died when they reached the metamorphosis to stage 42; other 20 (27%) tadpoles reached the total metamorphosis, i.e. reached the stage 46, and 16 (22%) did not reach metamorphosis, staying in the same condition until death. When we submit these data to the Leslie matrix (Table 1) and generate the scatter plot, we observe a similar metamorphosis curve until the 19th day and a change in the curve from the 21st day, where the development condition of the sample of 72 tadpoles is plotted (Figure 1). When changing the Leslie matrix for the temporal conditions of 34 and 41 days (considering the same condition of reduced water volume - as determined in 27 days of experimentation and plotted in a scatter chart - Figure 2); we verify that, for the 34 days, the possibility of tadpoles achieving integral metamorphosis (i.e. develop until stage 46), is of 36 (50%) tadpoles. The possibility of achieving stage 42 and die is of equal absolute frequency of 36 (50%) tadpoles. The number of tadpoles that can remain in the same stage may vary between 15 and 31 (respectively 20 and 43%).

In the second simulation of 41 days, the possibility of tadpoles achieving integral metamorphosis is of 38 (52%) tadpoles; the possibility of reaching stage 42 and die is of equal absolute frequency of 38 (52%) tadpoles. The number of tadpoles that can remain in the same stage may vary between 18 and 31 tadpoles (respectively 25 and 43%).

According to the experiment, we can infer that, under laboratory conditions, *P. cuvieri* in the form of tadpoles in stages 25 and 30 have their development limited by the water volume available. Even when we submitted it to the ecological model, Leslie matrix, 27 days in laboratory conditions and 34 and 41 days, simulated in the matrix, the success rates for the development of tadpoles of *P. cuvieri* reached 50% for complete metamorphosis; 50% for development until stage 42 (followed by death) and at least 20 to 25% remain in the same morphological condition until death. According to Leslie matrix there is reduction in the population of tadpoles ($\gamma_1 < 1$), being this reduction more pronounced in the earlier stages.

The extreme environmental condition of the northeast semi-arid, in relation to the life cycle of *P. cuvieri*, may favor the selection of individuals more adapted to the ephemeral condition of their water bodies. This selection pressure seems to be more pronounced in earlier stages of development, thereby tadpoles in advanced stages are better able to reach metamorphosis to imago when the water body starts to dry. However, the confirmation of the trend evidenced by this study lacks research in regions with different environmental condi-

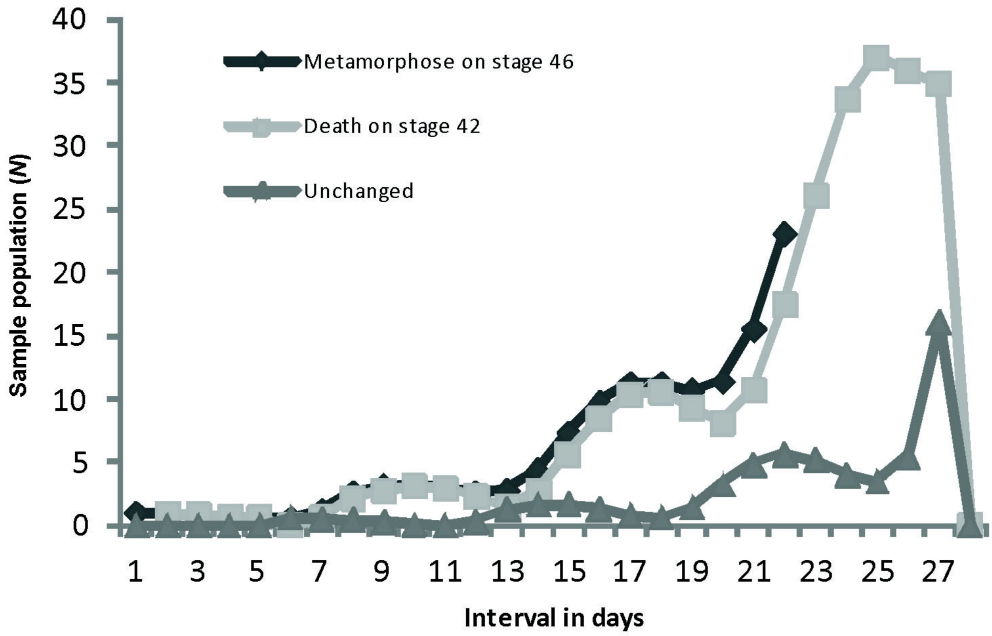

Figure 1. Scatter plot for the 27 days of laboratory observation.

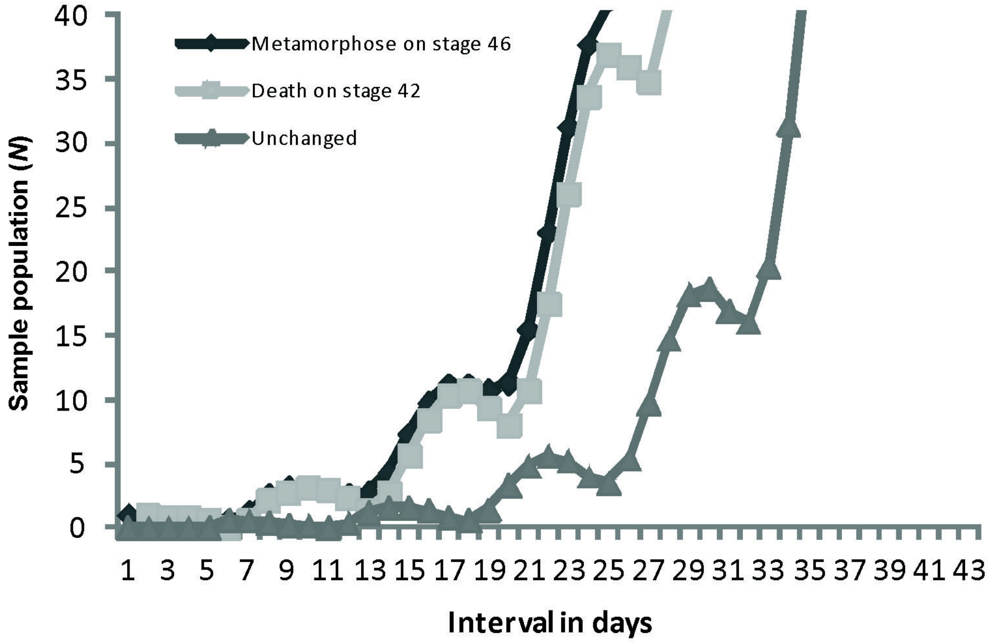

Figure 2. Scatter plot extrapolated to the 43rd day of the tadpole development.

tions. As *P. cuvieri* is a stenoecius species, it represents an excellent model once it presents populations adapted to all Brazilian biomes with different abiotic conditions throughout its range.

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REFERENCES

Ab'Sáber, A. 2010. A Obra de Aziz Nacib Ab'Sáber, São Paulo, BECA. 588 pages.

Aubry, A.; Bécart, E.; Davenport, J. and Emmerson, M.C. 2010. Estimation of survival rate and extinction probability for stage-structured populations with overlapping life stages. Population Ecology 51: 437-450.

Bastos, L.E.; Shimoda, E.; Maranhão, H.P.; Azevedo, M.R.G. and Yasui, G.S. 2010. Logística de transporte de girinos de rã-touro gigante (*Rana catesbeiana*): otimização da densidade. Sistemas & Gestão 5(3):149-160.

Beachy, C.K., Surges, T.H., Reyes, M. (1999. Effects of developmental and growth history on metamorphosis in the gray treefrog, *Hyla versicolor* (Amphibia, Anura). Journal of Experimental Zoology. 283: 522-530.

Cano, J.M., Laurila, A.; Palo, J. and Merila J. 2004. Population Differentiation in G Matrix structure due to natural selection in *Rana temporaria*. Evolution 58: 2013-2020.

Castro, J.C. and Pinto, A.T. 2000. Qualidade da água em tanques de girinos de rã-touro, *Rana catesbeiana* Shaw, 1802 cultivados em diferentes densidades de estocagem. Revista Brasileira de Zootecnia 29(6): 1903-1911.

FAO. 2012. Organização das Nações Unidas para a Alimentação e Agricultura. Available at https://www.fao.org.br/. Acessed on 18 Nov 2012.

Gosner, K.L. 1960. A simplified table for staging anuran embryos and larvae with notes on identification. Herpetologica. 16: 183-190.

Gotelli, N.J. 2007. Ecologia. Londrina. Editora Planta. 260 pages.

Govindarajulu, P.; Altwegg, R. and Anholt, B.R. 2005. Matrix model investigation of invasive species control: Bullfrogs on Vancouver Island. Ecological Applications 15: 2161–2170.

IBGE. 2012. Instituto Brasileiro de Geografia e Estatísitca. Available at <http://www.ibge.gov.br.> Accessed 15.09.2012.

INMET. 2012. Estação Meteorológica de Observação de Superfície Automática. Available at: <http://www.inmet.gov.br/portal/index.php?r=estacoes/estacoesAutomaticas.> Acessed on 31.10.2012.

Lima, M.S.C.; Pederassi, J. and Souza, C.A.S. (in press): Longevity and survival curves of *Rhinella icterica* (Anura, Bufonidae) under laboratory conditions. Brazilian Jounal of Biology.

Lima, V.T.A., Campeche, D.F.B, Pereira, L.A. 2009. Caracterização da água de açudes com peixes no semi-árido pernambucano. Revista da Academia de Ciencias Agrárias e Ambientais 7: 395-405.

Table 1. Leslie matrix of *Physalaemus cuvieri* in larval stages 25 and 30. Eigenvalues (γ) for 27 days of experimentation. The Eigenvalues for the first day of experimentation is crosshatched

1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**	**25**	**26**	**27**
1	0	0	0	0	0	0.6	1.6	0.9	0.6	0.1	0	0.3	1.9	3.5	3.4	2.8	1.5	0.8	1.8	5.4	9.5	11.3	10.6	7.8	5.8	8.1
0	0.9	0	0	0	0	0	0.5	1.3	0.8	0.5	0.1	0	0.3	1.7	3.2	3.1	2.5	1.3	0.7	1.7	4.9	8.5	10.2	9.6	7.0	5.2
0	0	0.8	0	0	0	0	0	0.5	1.3	0.7	0.5	0.1	0	0.3	1.5	2.9	2.8	2.3	1.2	0.8	1.5	4.4	7.7	9.1	8.6	6.3
0	0	0	0.7	0	0	0	0	0	0.4	1.2	0.6	0.4	0.1	0	0.3	1.4	2.6	2.5	2.0	1.1	0.6	1.3	3.9	6.9	8.2	7.7
0	0	0	0	0.7	0	0	0	0	0	0.4	1.0	0.6	0.4	0.1	0	0.2	1.2	1.3	2.2	1.8	1.0	0.5	1.2	3.6	6.2	7.4
0	0	0	0	0	0.6	0	0	0	0	0	0.3	0.9	0.5	0.4	0.1	0	0.2	1.1	2.1	2.0	1.7	0.9	0.5	1.1	3.2	5.6
0	0	0	0	0	0	0.5	0	0	0	0	0	0.3	0.8	0.5	0.3	0.1	0	0.2	1.0	1.9	1.8	1.5	0.8	0.4	1.0	2.9
0	0	0	0	0	0	0	0.4	0	0	0	0	0	0.3	0.7	0.4	0.3	0.1	0	0.1	0.8	1.5	1.5	1.2	0.6	0.3	0.8

Mora, R.I.S. 2010. Manual de Producción de Rana Toro. Centro de Investigación y Desarrollo Del Estado de Michoacán. Michoacán. 69 pages.

Nogueira, P. 2012. O censo dos girinos. UNESPCiência. 3: 32-35.

Segalla, M.V.; Caramaschi, U.; Cruz, C.A.G.; Garcia, P.C.A.; Grant. T.; Haddad, C.F.B. and Langone, J. 2012. Brazilian Amphibians: List of espécies. Available at http://www.sbherpetologia.org.br. Sociedade Brasileira de Herpetologia. Accessed 17 Dec. 2013.

Silans, A.P.; Silva, F.M. and Barbosa, F.A.R. 2006. Determinação *in loco* da difusividade térmica num solo da região de Caatinga (PB). Revista Brasileira de Ciencias do Solo 30: 41-48.

Stormowski, V. 2008. Estudando matrizes a partir de transformações geométricas. Dissertação de Mestrado em Matemática. Porto Alegre, UFRGS. 144 pages.

Vanzolini, P.E. 1993. Métodos estatísticos elementares em sistemática zoológica. Hucitec, São Paulo. 130 pages.

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