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Longevity and survival curves of *Rhinella icterica* (Anura, Bufonidae) under laboratory conditions

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Abstract

Life tables and survival curves of tadpoles from *Rhinella icterica* species were studied in the laboratory, under abiotic conditions controlled by a purification filter, a timer and a chiller. The survival curve for larval stage confirms a great mortality trend in the initial stages, which decreases when reaching the mature morphological condition ($r = -0.94$). Stages 37, 38, 39, 40 and 41 showed gradual values for their age structures, while stages 42, 43 and 44 presented high variations. Based on the results under laboratory conditions, it can be concluded that the maturity of *R. icterica* tadpoles development between 37 and 44 stages has a negative correlation and their predicted life expectancy is a logarithmic growth curve ($y=-761.96Ln(x)+5298.5$).

Keywords: tadpole, life table, reproduction.

Longevidade e curva de sobrevivência de *Rhinella icterica* (Anura, Bufonidae) em condições de laboratório

Resumo

Tabelas de vida e curvas de sobrevivência em estágios larvais de girinos da espécie *Rhinella icterica* foram estudadas em laboratório, sob condições abióticas controladas com uso de filtro de depuração, timer e chiller. A curva de sobrevivência por estágio larval confirma a tendência de maior mortalidade para os estágios iniciais e menor mortalidade para o avanço da condição morfológica madura ($r = -0,94$). Os estágios 37, 38, 39, 40 e 41 apresentaram valores gradativos para sua estrutura etária, enquanto nos estágios 42, 43 e 44 ocorreram variações acentuadas. Com base nos resultados obtidos, nas condições de laboratório, pode-se concluir que a maturidade de desenvolvimento de girinos de *R. icterica* entre os estágios 37 e 44, apresenta correlação negativa e a predição de esperança de vida a uma curva logarítmica de crescimento ($y=-761,96Ln(x)+5298,5$).

Palavras-chave: girino, tabela de vida, reprodução.

1. Introduction

Most anuran species have a complex life cycle: aquatic tadpoles metamorphose into terrestrial adults, becoming typically different in overall morphology, physiology and behaviour (Duellman and Trueb, 1994). Environmental shifts potentially affect species, especially in life cycle stages most susceptible to ambient changing conditions. In this sense, the use of larval characters is very common among herpetologists – both for the diagnosis of species and for determination of intra-and intergeneric systematic relationships (Carneiro et al., 2004; Nascimento and Skuk, 2006; Casal and Juncá, 2008; Vieira and Arzabe, 2008).

The study of developmental change among anurans resulted in a staging table created by Gosner (1960), with the description of anuran embryos and larva through 46 developmental stages. However, the survival curves of tadpoles as well as their longevity still require deeper research.

Studies of structured populations (life expectancy tables) were initiated by Pearl et al. (1927), representing the life expectancy of a population by a numerical expression, which measured mortality and longevity of animal species submitted to certain conditions.

The most widely used tool applied in population modelling consists of a discrete age and stage-structured model, tracking dynamical aspects of a species (Freckleton et al., 2003; Gotelli, 2007).

The Bufonidae family is composed of 34 genera with a worldwide distribution, except for Antarctica and Australia (Zug et al., 2001; AmphibiaWeb, 2013). With the revision of Frost et al. (2006) the so-called *Bufo* genus was renamed *Chaunus*, but a new revision (Chaparro et al., 2007) rearranged this group and incorporated *Chaunus* and *Rhamphophryne* in *Rhinella* genus, which now has 86 species (Frost, 2013), being *R. icterica* from the group of *R. marina* (Linnaeus, 1758), comprising 10 species (Tolledo and Toledo, 2010; Frost, 2013) and *Bufo* remainig a valid genus in Eurasia (Chaparro et al., 2007; Frost, 2013).

Rhinella icterica (Spix, 1823) has a wide occurrence in southeast Brazil, in a large diversity of habitats (Amphibiaweb, 2013). According to Silvano et al. (2010) there are no known threats to this species that even adapts to human-altered habitats.

The goal of the present study was to build both a structured growth model and a larval stage-longevity curve for the toad *Rhinella icterica*, a species that is clearly tolerant to man-altered environments.

2. Material and Methods

Tadpoles were collected in the Cachimbaú River, in the Pinheiral municipality, State of Rio de Janeiro, Brazil. Voucher specimens are deposited in the collection Eugenio Izecksohn (EI 11 012). Whenever the school of tadpoles was found, sieves were used to collect them (400 × 700 mm mesh of 0.05 × 0.05 mm) until getting to the ideal initial experimental sample – 1,000 individuals in stage 37 (Lima and Peixoto, 2007). The number of individuals in subsequent stages corresponded to those tadpoles that survived and metamorphosed. When specimens were collected, dissolved oxygen (DO) was measured *in loco* with Quimis Q408 oximeter (precision 0.3 mg/l), pH was measured using a Hanna pHmeter and nitrite concentration with Alfakit 1307. Measurements were taken for five consecutive days and 4-hour intervals, from 6:00 AM to 12:00 PM.

2.1. Aqua-terrarium

An aqua-terrarium with chiller has been used for this study, in order to obtain an optimised control of abiotic conditions - providing similar variations to those of the environment under analysis. The aqua-terrarium measured 107 × 35 × 52 cm with an external filter system of 37 × 34 × 48 cm. Chiller compressor with 1.8 hp and timer for setting up the frequency of pump flow. Feed pump of 1200 l/h, return pump of 250 l/h and aeration pump 500 l/h.

2.2. Environmental control of the Aqua-terrarium

On a daily basis, dissolved oxygen was measured with a Q-408 QUIMIS oxygen meter (accuracy ± 0.3 mg/l), pH was measured with a HANNA pHmeter and nitrite concentration was measured with an ALFAKIT 1307. Temperature was measured using a submerged thermometer. The maintained these variables by the aeration pump and debugging tank with two biological filters, automatically debugging every 6 hours. The chiller was programmed to activate automatically in order to maintain the water temperature ranging between 23 and 25° C.

2.3. Mathematical model

For a better estimate of lifetimes, daily records were taken of the number of individuals per stage, among survivors, metamorphosed and dead. In the construction of life tables according to Silveira Neto et al. (1976) – adapted to the study of anuran larval development - the parameters below were arranged in columns according to the larval stage in unit time (x days). Lx is the fraction of survivors, on each "x" interval; dx is the fraction of individuals that metamorphosed or died between the ages "x" and "x+1" – this was estimated through the formula Lx-(Lx+1). The value of Ex (average survival probability among larval stages on successive metamorphosis) was calculated by formula Lx-(1/2(dx)), representing the age structure. Tx was estimated by the formula Σ Ex+(Ex+1)+Ew, where "w" is the maximum larval stage in weeks; life expectancy will be estimated by Tx/Lx. Mortality ratio (qx) by age interval was estimated by 1000qx(dx/Lx); data longevity of each stage was analyzed using the Pearson correlation coefficient and *t* variance test, for n-1 degrees of freedom (DF) and $\alpha = 0.05$.

The biotic potential was represented by a graph where individuals on stage 37 should reach stage 44. The biotic perpetuation was also represented by a graph, which measured how many individuals on stage 37 (in the beginning of the experiment) reached stage 44. The equations of both graphs were calculated and the differences were expressed on a mathematical model that predicts the life expectancy by larval stage.

3. Results and Discussion

Tables 1 to 8 show the parameters of life expectancy by larval stage. In the analysis of life table, the concept of survival is essential, thus the terms Ex, Tx and Lx express the age structure of population in a given time interval. Stages 37, 38, 39, 40 and 41 showed gradual values (five to eight days) for their age structures. However, stages 42, 43 and 44 presented high variations of eleven, fourteen and seventeen days.

The estimated life expectancy (Ex), considering "day" a time measurement unit (x) is represented in Tables 1 to 8 below:

Rhinella icterica age monitoring and its biological cycle were described by Rosa (1951). That study did not set the range of days between the last larval stage and the young stage (imago); also, the stages proposed by Gosner (1960) were not known. However, we found similarities relating to the metamorphosis cycle proposed by Rosa (1951) and ours, that is, the older stages takes longer to reach the next stage. As for the differences between Rosa (1951) results

Table 1. Life expectancy for stage 37 of *Rhinella icterica* under controlled laboratory conditions. x= age interval in days; Lx= fraction of survivors on each "x" interval; dx: the fraction of individuals that metamorphosed or died between the ages "x" and "x+1"; Ex= age structure; Tx= total number of tadpoles of "x" age beyond the "x" age; ex: life expectancy; qX= risk ratio by age.

x	Lx	dx	Ex	Tx	ex	1000qX
1	1000	0	1000	3193	3.193	0
2	1000	176	912	2193	2.193	176
3	824	256	696	1281	1.554612	310.6796
4	568	267	434.5	585	1.02993	470.0704
5	301	301	150.5	150.5	0.5	1000

Table 2. Life expectancy for stage 38 of *Rhinella icterica* under controlled laboratory conditions. x= age interval in days; Lx= fraction of survivors on each "x" interval; dx: the fraction of individuals that metamorphosed or died between the ages "x" and "x+1"; Ex= age structure; Tx= total number of tadpoles of "x" age beyond the "x" age; ex: life expectancy; qX= risk ratio by age.

x	Lx	dx	Ex	Tx	ex	1000qX
1	870	0	870	2736	3.144828	0
2	870	176	782	1866	2.144828	176
3	694	227	580.5	1084	1.56196	284.5677
4	467	267	333.5	503.5	1.078158	497.409
5	200	130	135	170	0.85	565.5
6	70	70	35	35	0.5	870

Table 3. Life expectancy for stage 39 of *Rhinella icterica* under controlled laboratory conditions. x= age interval in days; Lx= fraction of survivors on each "x" interval; dx: the fraction of individuals that metamorphosed or died between the ages "x" and "x+1"; Ex= age structure; Tx= total number of tadpoles of "x" age beyond the "x" age; ex: life expectancy; qX= risk ratio by age.

x	Lx	dx	Ex	Tx	ex	1000qX
1	780	15	772.5	3193	4.09359	15
2	765	68	731	2420.5	3.164052	69.33333
3	697	120	637	1689.5	2.42396	134.2898
4	577	180	487	1052.5	1.82409	243.3276
5	397	118	338	565.5	1.424433	231.8388
6	279	191	183.5	227.5	0.815412	533.9785
7	88	88	44	44	0.5	780

Table 4. Life expectancy for stage 40 of *Rhinella icterica* under controlled laboratory conditions. x= age interval in days; Lx= fraction of survivors on each "x" interval; dx: the fraction of individuals that metamorphosed or died between the ages "x" and "x+1"; Ex= age structure; Tx= total number of tadpoles of "x" age beyond the "x" age; ex: life expectancy; qX= risk ratio by age.

x	Lx	dx	Ex	Tx	ex	1000qX
1	710	0	710	2718	3.828169	0
2	710	118	651	2008	2.828169	118
3	592	135	524.5	1357	2.29223	161.9088
4	457	162	376	832.5	1.821663	251.6849
5	295	88	251	456.5	1.547458	211.7966
6	207	120	147	205.5	0.992754	411.5942
7	87	72	51	58.5	0.672414	587.5862
8	15	15	7.5	7.5	0.5	710

Table 5. Life expectancy for stage 41 of *Rhinella icterica* under controlled laboratory conditions. x= age interval in days; Lx= fraction of survivors on each "x" interval; dx: the fraction of individuals that metamorphosed or died between the ages "x" and "x+1"; Ex= age structure; Tx= total number of tadpoles of "x" age beyond the "x" age; ex: life expectancy; qX= risk ratio by age.

x	Lx	dx	Ex	Tx	ex	1000qX
1	630	0	630	2249	3.569841	0
2	630	22	619	1619	2.569841	22
3	608	133	541.5	1000	1.644737	137.8125
4	475	367	291.5	458.5	0.965263	486.7579
5	108	44	86	167	1.546296	256.6667
6	64	15	56.5	81	1.265625	147.6563
7	49	49	24.5	24.5	0.5	630

Table 6. Life expectancy for stage 42 of *Rhinella icterica* under controlled laboratory conditions. x= age interval in days; Lx= fraction of survivors on each "x" interval; dx: the fraction of individuals that metamorphosed or died between the ages "x" and "x+1"; Ex= age structure; Tx= total number of tadpoles of "x" age beyond the "x" age; ex: life expectancy; qX= risk ratio by age.

x	Lx	dx	Ex	Tx	ex	1000qX
1	555	29	540.5	3544.5	6.386486	29
2	526	7	522.5	3004	5.711027	7.385932
3	519	10	514	2481.5	4.78131	10.69364
4	509	38	490	1967.5	3.865422	41.43418
5	471	74	434	1477.5	3.136943	87.19745
6	397	96	349	1043.5	2.628463	134.2065
7	301	65	268.5	694.5	2.307309	119.8505
8	236	51	210.5	426	1.805085	119.9364
9	185	108	131	215.5	1.164865	324
10	77	31	61.5	84.5	1.097403	223.4416
11	46	46	23	23	0,5	555

Table 7. Life expectancy for stage 43 of *Rhinella icterica* under controlled laboratory conditions. x= age interval in days; Lx= fraction of survivors on each "x" interval; dx: the fraction of individuals that metamorphosed or died between the ages "x" and "x+1"; Ex= age structure; Tx= total number of tadpoles of "x" age beyond the "x" age; ex: life expectancy; qX= risk ratio by age.

x	Lx	dx	Ex	Tx	ex	1000qX
1	480	53	453.5	2573	5.360417	53
2	427	79	387.5	2119.5	4.9637	88.80562
3	348	53	321.5	1732	4.977011	73.10345
4	295	18	286	1410.5	4.781356	29.28814
5	277	31	261.5	1124.5	4.059567	53.71841
6	246	32	230	863	3.50813	62.43902
7	214	53	187.5	633	2.957944	118.8785
8	161	32	145	445.5	2.767081	95.40373
9	129	26	116	300.5	2.329457	96.74419
10	103	48	79	184.5	1.791262	223.6893
11	55	16	47	105.5	1.918182	139.6364
12	39	13	32.5	58.5	1,5	160
13	26	13	19.5	26	1	240
14	13	13	6.5	6.5	0.5	480

Table 8. Life expectancy for stage 44 of *Rhinella icterica* under controlled laboratory conditions. x= age interval in days; Lx= fraction of survivors on each "x" interval; dx: the fraction of individuals that metamorphosed or died between the ages "x" and "x+1"; Ex= age structure; Tx= total number of tadpoles of "x" age beyond the "x" age; ex: life expectancy; qX= risk ratio by age.

x	Lx	dx	Ex	Tx	ex	1000qX
1	450	35	432.5	3522	7.826667	35
2	415	35	397.5	3089.5	7.444578	37.95181
3	380	48	356	2692	7.084211	56.84211
4	332	21	321.5	2336	7.036145	28.46386
5	311	22	300	2014.5	6.477492	31.8328
6	289	10	284	1714.5	5.932526	15.57093
7	279	19	269.5	1430.5	5.12724	30.64516
8	260	18	251	1161	4.465385	31.15385
9	242	14	235	910	3.760331	26.03306
10	228	37	209.5	675	2.960526	73.02632
11	191	21	180.5	465.5	2.437173	49.47644
12	170	84	128	285	1.676471	222.3529
13	86	19	76.5	157	1.825581	99.4186
14	67	49	42.5	80.5	1.201493	329.1045
15	18	0	18	38	2.111111	0
16	18	7	14.5	20	1.111111	175
17	11	11	5.5	5.5	0.5	450

versus our results, when submitting them to the *t* test, no significant discrepancies were noted (see Figure 1).

The survival curve per larval stage confirms the trend of higher mortality in the early stages and lower mortality as advancing on the mature morphologic condition (see Figure 2). When assessing the statistical dependence between the larval stage and mortality, we evidenced, based on Pearson's correlation coefficient, a negative correlation (r = –0.94), implying that a greater maturity tends to a lower mortality.

Under laboratory-controlled conditions, nitrite was kept between 0.25 and 0.50 ppm, pH between 6.8 and 7.4, dissolved oxygen between 8 and 10.16 mh/l and temperature between 23 and 25° C. (see Figure 3). External measurements: pH 6.7 (SD = 0.7), dissolved oxygen 8.3 mh/l (SD = 2.2), 0.21 ppm nitrite (SD = 0.2). Among the measured abiotic factors, nitrite is the only one that presented differences (0.9 ppm) between aqua-terrarium and external conditions. We believe that although the water was in continuous flux through the use of pumps, the aqua-terrarium environment offers little dilution of nitrite, which may raise mortality rates.

Based on the results obtained, on the conditions in which the study was developed, we conclude that even in controlled laboratory conditions mortality occurs and the curve is negatively expressed, that is, the longer the maturity stage, the lower the mortality. When submitting the values for the biotic potential of the population, along with the biotic perpetuation on a life expectancy curve, differences were found for all larval stages (37 to 44) and this range of numerical differences was called environmental resistance (see Figure 4).

On each larval stage, the differences found between the number of individuals that should morph into the next stage

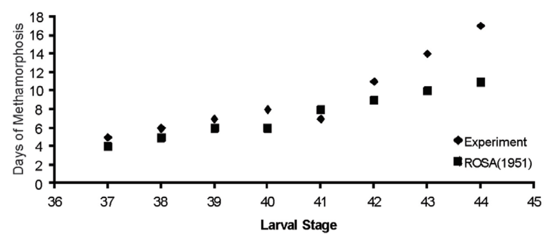

Figure 1. Comparison between the results found by Rosa (1951) and this experiment regarding the developmental stages of *Rhinella icterica*.

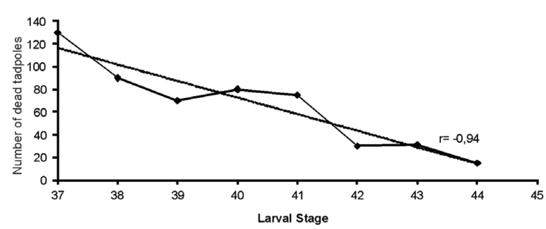

Figure 2. Correlation between larval stage and mortality.

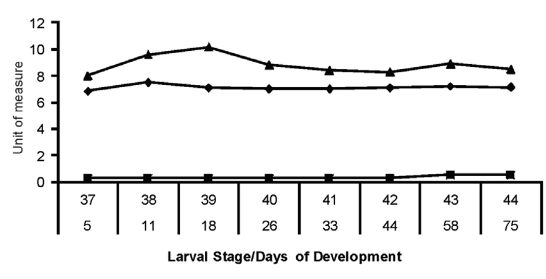

Figure 3. Abiotic variation of aqua-terrarium under controlled laboratory conditions.

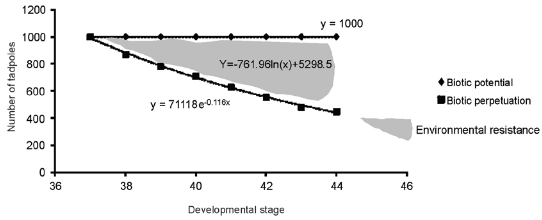

Figure 4. Life expectancy curve.

and the number of individuals that actually metamorphosed correspond to an exponential ($y = 71118e^{-1155x}$) in contrast to the biotic potential which should correspond to $Y = 1000$. The difference between these two curves, expected and achieved, predicts the logarithmic model of life expectancy corresponding to $y = -761.96Ln(x)+5298.5$.

Based on the results obtained in laboratory conditions, it can be concluded that the development maturity of tadpoles of *R. icterica* between stages 37 and 44 is negatively correlated and its life expectancy prediction is a logarithmic growth curve.

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