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Nonlinear models for morphometric analysis in Bullfrog Tadpoles

Análise morfométrica através de modelos não-lineares para girinos de rã-touro

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SUMMARY

Biometric relationships are important to illustrate the growth of animals. When adjusted using nonlinear models, these relationships can provide important information that contributes to the improvement of breeding techniques. In this study, morphometric data as a function of weight obtained in four experiments involving bullfrog tadpoles were adjusted using Gompertz, Logistic, Von Bertalanffy and Brody nonlinear models and the best-fit model was determined. After fitting the parameters to the different models in each experiment, the models were compared based on confidence intervals (**α** = 0.05). The following criteria were used for selection of the best model: biological interpretation, residual mean square, coefficient of determination, graphic analysis, and number of iterations. Standard and total length data as a function of tadpole weight converged in the four models. The Logistic and Gompertz models had no biological interpretation for some datasets. The Brody model provided the lowest residual mean square and number of iterations for the variables studied in all experiments. The Brody relative growth rate (K) was lower for total length when compared to standard length, indicating a greater initial growth in standard length. The Brody model was the best to describe the growth in standard and total length of bullfrog tadpoles as a function of weight.

Keywords: biometric relationship, frog farming, growth, length

RESUMO

As relações biométricas são importantes para ilustrar o processo de crescimento dos animais. Estas, quando ajustadas a modelos não-lineares, podem revelar informações importantes para a melhoria das técnicas de criação. O conjunto de dados morfométricos em função do peso, de quatro experimentos com girinos de rã-touro, foram ajustados aos modelos não-lineares de Gompertz, Logístico, Von Bertalanffy e Brody, com objetivo de verificar o melhor ajuste. Após a adequação dos parâmetros para os diferentes modelos em cada experimento seguiu-se com a comparação dos mesmos, por meio dos intervalos de confiança (**α** = 0,05). Os critérios para selecionar os modelos foram: interpretação biológica, quadrado médio do resíduo, coeficiente de determinação, análise gráfica e o número de iterações. Os dados de comprimentos padrão e total em função do peso dos girinos convergiram nos quatro modelos. Os modelos Logístico e Gompertz não apresentaram interpretação biológica para alguns conjuntos de dados. O quadrado médio do resíduo e número de iterações em todos os experimentos e variáveis estudadas apresentaram os menores valores para o modelo de Brody. Os valores de K dos modelos de Brody para comprimento total, quando comparados ao padrão, apresentaram-se menores, indicando que os animais apresentaram um crescimento inicial maior em comprimento padrão. O modelo de Brody foi o mais indicado para descrever o comprimento padrão e total em função do peso para girinos de rã-touro.

Palavras-chave: comprimento, crescimento, ranicultura, relações biométricas

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INTRODUCTION

Bullfrogs have a complex life cycle which comprises two phases: aquatic and terrestrial. The aquatic stage consists of tadpoles that undergo a continuous process of morphophysiological changes over a short period of time (rapid growth) (WILBUR, 1980).

The body of tadpoles is divided into two parts. The head part comprises the body of the animal where all organs are found and the tail part mainly consists of muscles (ISHIZUYA-OKA et al., 2010). The latter is important for metamorphic climax and serves as a source of protein at the time of its absorption. One measure used to evaluate the growth of these parts is total length and standard length (ALTIG, 2007).

A model is said non-linear when no linearity with respect to the parameters and cannot be linearized by transformation. In general, these models are designed to describe an asymptotic trajectory of the independent variable in function of the independent variable. Generally, the difference between these models is given by defining the inflection point of the curve, which confers a sigmoid shape the same (SILVEIRA et al., 2011).

Fitting a nonlinear equation that describes the lifespan of an animal is more informative for the study of growth since it condenses a dataset into a small group of biologically interpretable parameters (TURNER JUNIOR et al., 1976). The Gompertz (WINSOR, 1932), Von Bertalanffy (1957), Logistic (NELDER, 1961) and Brody (1945) nonlinear models are commonly used to fit datasets in animal production, particularly weight (g) as a function of time (days) (FREITAS, 2005; SARMENTO et al., 2006; DUMAS et al., 2010; KUHI et al., 2010). For animal populations, extrinsic factors such as climatic conditions clearly influence this relationship (ÁLVAREZ and NICIEZA, 2002; MACIEL and JUNCÁ, 2009). In these cases, ecological models (GAMITO, 1998) are indicated since weight gain rigorously reflects growth and its relationship with other variables that replace the time variable, such as length, height and width, often rules out environmental variations (SANTOS et al., 2007; GOMIERO et al., 2009).

The objective of the present study was to describe the morphometric growth of bullfrog tadpoles as a function of weight until metamorphosis by fitting four nonlinear mathematical functions using data from four rearing experiments.

MATERIAL AND METHODS

The dataset used was obtained from four experiments conducted at the Centro de Aquicultura (Aquaculture Center), Universidade Estadual Paulista (São Paulo State University), Jaboticabal, São Paulo, Brazil. Experiments 1 and 2 were carried out at the Laboratory of Aquatic Organisms Nutrition and experiments 3 and 4 at the Raniculture Section using bullfrog tadpoles (*Lithobates catesbeianus*).

A total of 3,240 bullfrog tadpoles at stage 25 (GOSNER, 1960), obtained from the same spawn, with an initial mean weight of 0.044 ± 0.001g, mean total length of 12.79 ± 0.19mm and mean standard length of 5.86 ± 0.18mm, were used. The animals were housed in eighteen 100-L tanks filled with 90L of water and equipped with an individual supply pipe and a direct bottom outlet.

The tadpoles were fed *ad libitum* powder food containing 26.23% digestible protein and 3,743.07kcal/kg digestible energy, three times per day. Leftovers were avoided so that the amount of food offered was considered to be same as that consumed (SOLOMON & TARUWA, 2011).

Total length (snout to tip of tail), standard length (snout to insertion of tail) and live weight were obtained at the beginning of the experiment and at 13, 23, 33, 42, 55 and 64 days. The last day of the experiment was defined when 20% of the animals had reached metamorphic climax (stage 42 according to GOSNER, 1960), a time when tadpoles cease feeding and use only accumulated reserves (WRIGHT et al., 2011). A sample of 10% of the animals of each tank was weighed on a digital scale (0.01g) and length measurements were obtained with a digital caliper (0.001mm).

The method used was the same as that described for Experiment 1, except that the animals were fed commercial ration containing 37.92% crude protein and 4,156.34kcal/kg crude energy.

A total of 2,700 bullfrog tadpoles at stage 25 (GOSNER, 1960), with an initial mean weight of 0.039 ± 0.001g, mean total length of 12.62 ± 0.08mm and mean standard length of 5.66 ± 0.17mm, were divided into six tanks outside (capacity of 500L). The animals were fed *ad libitum* a commercial powder diet ration containing 55% crude protein and 4,366.30kcal/kg crude energy.

Total and standard length and live weight were measured at the beginning of the experiment and at 12, 20, 29, 42, 54 and 63 days in 40 animals per chamber with a digital caliper (0.001mm) and a digital scale (0.01g), respectively.

A total of 2,700 bullfrog tadpoles at stage 25 (GOSNER, 1960) with an initial mean weight of 0.26 ± 0.02g, mean total length of 27.26 ± 0.25mm and mean standard length of 10.65 ± 0.28mm were used. The animals were divided into six tanks outside (capacity of 500L) and were fed *ad libitum* powder food containing 26.23% digestible protein and 3,743.07kcal/kg digestible energy, three times per day.

Total and standard length and live weight were measured at the beginning of the experiment and at 11, 22, 34, 45 and 55 days in 40 animals per tank with a digital caliper (0.001mm) and a digital scale (0.01g), respectively.

The water used in the experiments was obtained from a mine and 100% water changes were performed at intervals of 24h. For the maintenance of water quality, the tanks were cleaned on alternative days with a siphon to remove uneaten food and feces.

The maximum and minimum temperatures of the water in the tanks were determined daily with a digital thermometer. Dissolved oxygen (YSI oxygen meter), conductivity (handheld PHTEK device) and pH (handheld PHTEK pH monitor, model pH-100) were measured weekly. These parameters were obtained in all experiments. The mean maximum and minimum water temperatures were 26.0 and 24.2°C during experiments 1 and 2, and 31.4 and 27.8°C during experiments 3 and 4, respectively. The water temperature in outside tanks was higher, but was adequate for tadpole development (HOFFMANN et al., 1989). The mean dissolved oxygen (3.07 ± 0.92mg/L), electrical conductivity (38 ± 0.26µS/cm) and pH (6.17 ± 0.34) were adequate for frog farming (HAYASHI et al., 2004; HAILEY et al., 2006).

The following nonlinear functions were adopted to model standard and total length growth as a function of weight in tadpoles: Gompertz, $Y = A \exp(-B \exp(-K w))$; Von Bertalanffy, $Y = A (1 - B \exp(-K w))^3$; logistic, $Y = A (1+ B \exp(-K w))^{-1}$, and Brody, $Y = A (1 - B \exp(-K w))$, where Y = value of the measurement (cm); w = weight (g); A = total or

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standard length (cm) at metamorphosis; B = scale parameter without biological interpretation, and K = relative growth rate at metamorphosis. The model parameters were estimated by the modified Gauss-Newton method through nonlinear regression using the NLIN procedure of the SAS program (2001).

The following criteria were used for selection of the best model and description of the growth curve as a function of weight: biological interpretation of the parameters, residual mean square (RMS), graphic analysis of the models, and Akaike information criterion (AIC) (AKAIKE, 1974). In addition, the coefficient of determination (R^2) was calculated as the square of the correlation between observed and estimated weights, which corresponds to 1 – (RSS-TSS), where RSS is the residual sum of squares and TSS is the total sum of squares corrected for the mean (SOUZA, 1998).

RESULTS AND DISCUSSION

The total and standard length data as a function of tadpole weight converged in the four nonlinear models adopted.

The estimated A values were similar in three of the four experiments and for the two length variables. In the fourth experiment, the highest A value was estimated with the Brody model and was 104.1 cm for total length and 32.424cm for standard length. The K values estimated with the Brody model were the lowest in all experiments and for the two lengths studied (Tables 1 and 2).

The Brody model provided the lowest RMS and the smallest number of iterations of the variables studied in all experiments, indicating the best fit. The R^2 found was high and similar for all models (Table 1).

Graphical representation of the Brody model showed that the estimated values were close to the observed values for total length (Figure 1) and standard length (Figure 2) as a function of weight in the four experiments.

The Brody model was chosen to describe the morphometric relationships in bullfrog tadpoles since this model converged to the data, provided biological interpretation, and presented the lowest RMS and Akaike information criterion (AIC).

Values of four experiments were used, important to increase the reliability and choice of nonlinear model. The Logistic, Von Bertalanffy and Gompertz models underestimated the A values in experiments 1, 2 and 3 when describing morphometric growth as a function of weight. The same was observed for a Logistic model used to describe the growth in length of piracanjuba, *Brycon orbignyanus* (GOMIERO et al., 2009).

This tendency is related to the performance of models for these datasets. The Brody, Von Bertalanffy, Logistic and Gompertz models were found to adequately describe the morphometric growth of Nile tilapia as a function of body weight, with the Gompertz and Von Bertalanffy models being the most indicated (SANTOS et al., 2007). For piracanjuba, the best models were the Brody and Von Bertalanffy models (GOMIERO et al., 2009). Taken together, these findings suggest that each species possesses specific growth characteristics that lead to different results.

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Table 1. Estimates of nonlinear growth function parameters for total and standard length as a function of weight, coefficient of determination (R^2), residual mean square (RMS), and Akaike information criterion (AIC) obtained for bullfrog tadpoles in four experiments

Modelos	A	B	K	QMR	R^2	AIC
	Total length					
	Experiment 1					
Gompertz	96.6544	1.5545	0.4627	24.2530	0.9947	5.2344
Von Bertalannfy	97.9737	0.4164	0.4025	20.6110	0.9955	5.0991
Logístico	94.2284	3.1757	0.6486	34.4466	0.9925	5.3455
Brody	102.300	0.8236	0.2873	13.3167	0.9853	4.9901
	Experiment 2					
Gompertz	98.2768	1.5564	0.4591	26.5350	0.9942	5.4432
Von Bertalannfy	99.6756	0.4144	0.3997	23.1510	0.9942	5.4321
Logístico	95.7072	3.1708	0.6444	35.4282	0.9922	5.6654
Brody	104.300	0.8309	0.2821	15.8665	0.9829	4.0100
	Experiment 3					
Gompertz	96.4507	1.5605	0.5707	28.5829	0.9943	6.3280
Von Bertalannfy	97.6187	0.4185	0.4968	24.4113	0.9951	6.0011
Logístico	94.1663	3.1843	0.8100	39.7980	0.9921	7.0908
Brody	101.300	0.8378	0.3536	15.7646	0.9830	5.7800
	Experiment 4					
Gompertz	96.0380	1.3093	0.5032	6.8343	0.9985	4.3760
Von Bertalannfy	97.9692	0.3646	0.4365	5.9421	0.9987	4.0099
Logístico	92.3858	2.3235	0.7048	9.6172	0.9979	5.0121
Brody	104.100	0.7774	0.3031	4.3049	0.9973	4.0760
	Standard length					
	Experiment 1					
Gompertz	31.4443	1.3543	0.5458	2.4465	0.9956	2.0499
Von Bertalannfy	31.6961	0.3723	0.4857	2.1209	0.9961	2.0048
Logístico	30.9433	2.5328	0.7336	3.3686	0.9934	2.1205
Brody	32.4396	0.7791	0.3684	1.4710	0.9834	1.7628
	Experiment 2					
Gompertz	30.3554	1.3290	0.6022	2.2972	0.9956	2.2211
Von Bertalannfy	30.5333	0.3670	0.5424	1.9869	0.9962	2.1566
Logístico	30.0137	2.4511	0.7867	3.1435	0.9940	3.0010
Brody	31.0845	0.7739	0.4226	1.3492	0.9839	1.9080
	Experiment 3					
Gompertz	30.8245	1.3556	0.6438	0.9145	0.9949	3.3422
Von Bertalannfy	31.0522	0.3732	0.5723	0.5723	0.9955	2.6900
Logístico	30.3885	2.5300	0.8753	0.8753	0.9931	3.1900
Brody	31.7161	0.7829	0.4349	0.4349	0.9791	2.5033
	Experiment 4					
Gompertz	30.9569	1.1636	0.5727	0.6306	0.9988	2.0098
Von Bertalannfy	31.3314	0.3301	0.5098	0.5631	0.9989	1.9088
Logístico	30.2040	1.9409	0.7634	0.8464	0.9984	2.4300
Brody	32.4240	0.7286	0.3844	0.4433	0.9908	1.6701

A = weight or length at metamorphosis; B = scale parameter without biological interpretation; K = relative growth rate at metamorphosis.

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Table 2. Lower and upper confidence limits of parameters of the Brody, Gompertz, Von Bertalanffy and Logistic functions for morphometric traits of bullfrog tadpoles

Modelos	A		B		K	
	Inferior	Superior	Inferior	Superior	Inferior	Superior
Total length						
Experiment 1						
Gompertz	92.1230	101.2000	0.3884	0.5369	0.7740	1.1331
Von Bertalannfy	93.3240	102.6000	0.3918	0.4409	0.3387	0.4663
Logístico	89.8665	98.6001	2.6415	3.7098	0.5393	0.7579
Brody	97.1673	107.4000	0.8110	0.8542	0.2390	0.3285
Experiment 2						
Gompertz	92.9559	103.6000	0.3787	0.5396	0.7524	1.1466
Von Bertalannfy	94.0681	105.3000	0.3898	0.4391	0.3283	0.4710
Logístico	90.9207	100.5000	2.6513	3.6902	0.5347	0.7541
Brody	97.7566	110.8000	0.8085	0.8534	0.2287	0.3356
Experimento 3						
Gompertz	92.4090	100.5000	0.4712	0.6702	0.6333	0.9264
Von Bertalannfy	93.4903	101.7000	0.3889	0.4482	0.4134	0.5802
Logístico	90.2853	98.0474	2.5507	3.8179	0.6533	0.9666
Brody	96.8881	105.7000	0.8116	0.8640	0.2983	0.4089
Experimento 4						
Gompertz	91.5397	100.5000	0.4331	0.5734	0.4263	0.6449
Von Bertalannfy	93.5397	102.8000	0.3501	0.3790	0.3739	0.4990
Logístico	88.4324	96.3393	2.0929	2.5542	0.6088	0.8007
Brody	98.0433	110.2000	0.7613	0.7935	0.2540	0.3521
Standard length						
Experiment 1						
Gompertz	30.2892	32.5993	0.4607	0.6309	0.4200	0.6915
Von Bertalannfy	30.5405	32.8517	0.3496	0.3950	0.4117	0.5596
Logístico	29.7700	32.1165	2.1385	2.9272	0.6129	0.8563
Brody	31.2593	33.6200	0.7552	0.8030	0.3146	0.4221
Experiment 2						
Gompertz	29.2727	31.4380	0.5062	0.6981	0.3501	0.5946
Von Bertalannfy	29.4632	31.6034	0.3447	0.3893	0.4580	0.6267
Logístico	28.8933	31.1341	2.0811	2.8212	0.6550	0.9185
Brody	30.0336	32.1354	0.7599	0.7979	0.3609	0.4843
Experiment 3						
Gompertz	29.7339	31.9251	0.5324	0.7551	0.3449	0.6004
Von Bertalannfy	29.9506	32.1538	0.3454	0.4011	0.4769	0.6677
Logístico	29.2534	31.4636	2.0553	3.0046	0.7090	1.0416
Brody	30.6050	32.8271	0.7537	0.8122	0.3682	0.5017
Experiment 4						
Gompertz	29.9045	32.0094	0.5009	0.6446	0.1806	0.3484
Von Bertalannfy	30.2267	32.4361	0.3165	0.3438	0.4447	0.5749
Logístico	29.2374	31.1706	1.7625	2.1193	0.6683	0.8584
Brody	31.1313	33.7168	0.7108	0.7465	0.3310	0.4378

A = weight or length at metamorphosis; B = scale parameter without biological interpretation; K = relative growth rate at metamorphosis.

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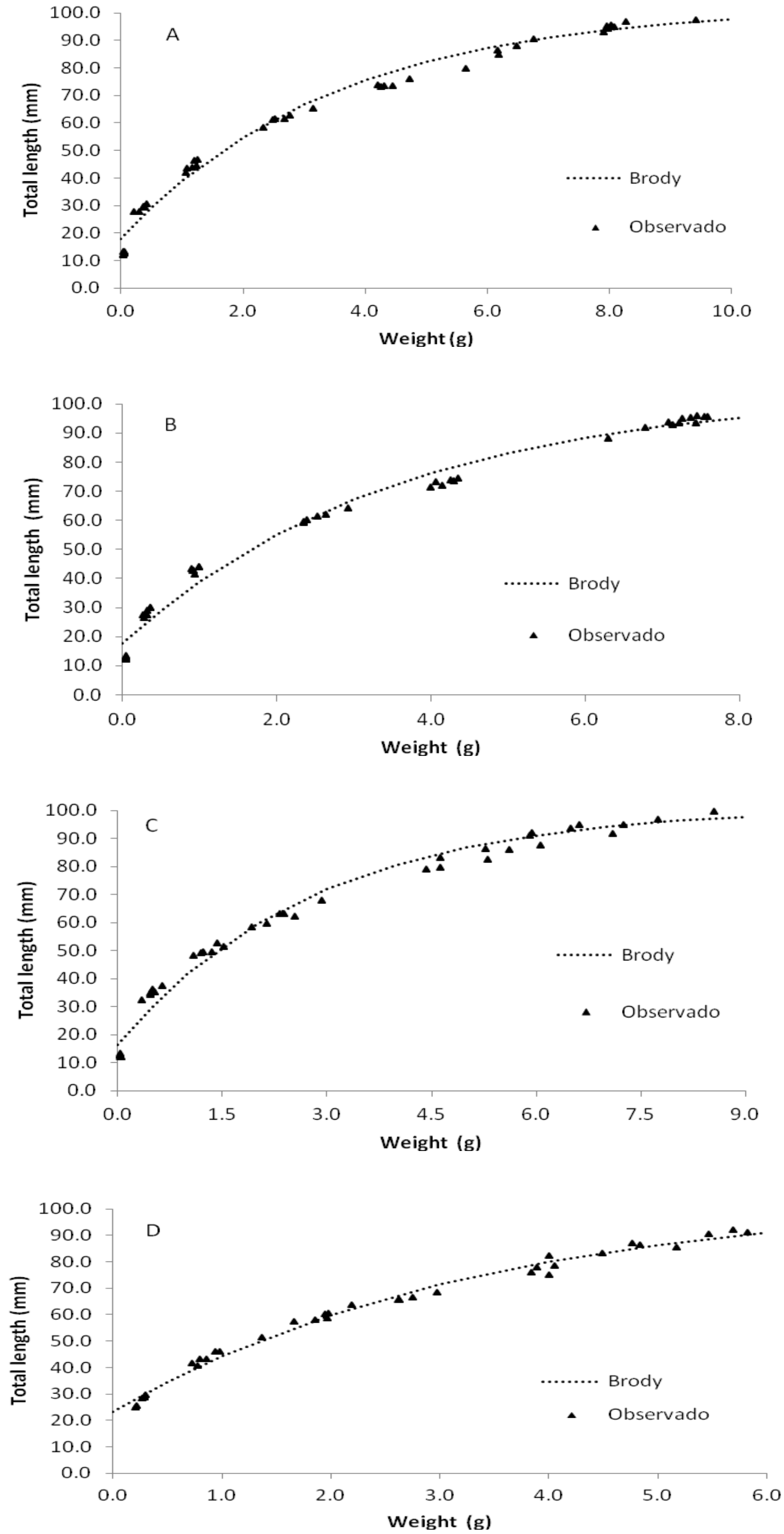

Figure 1. Brody growth curves of total length as a function of weight obtained for bullfrog tadpoles. Experiment 1 (A), experiment 2 (B), experiment 3 (C), and experiment 4 (D)

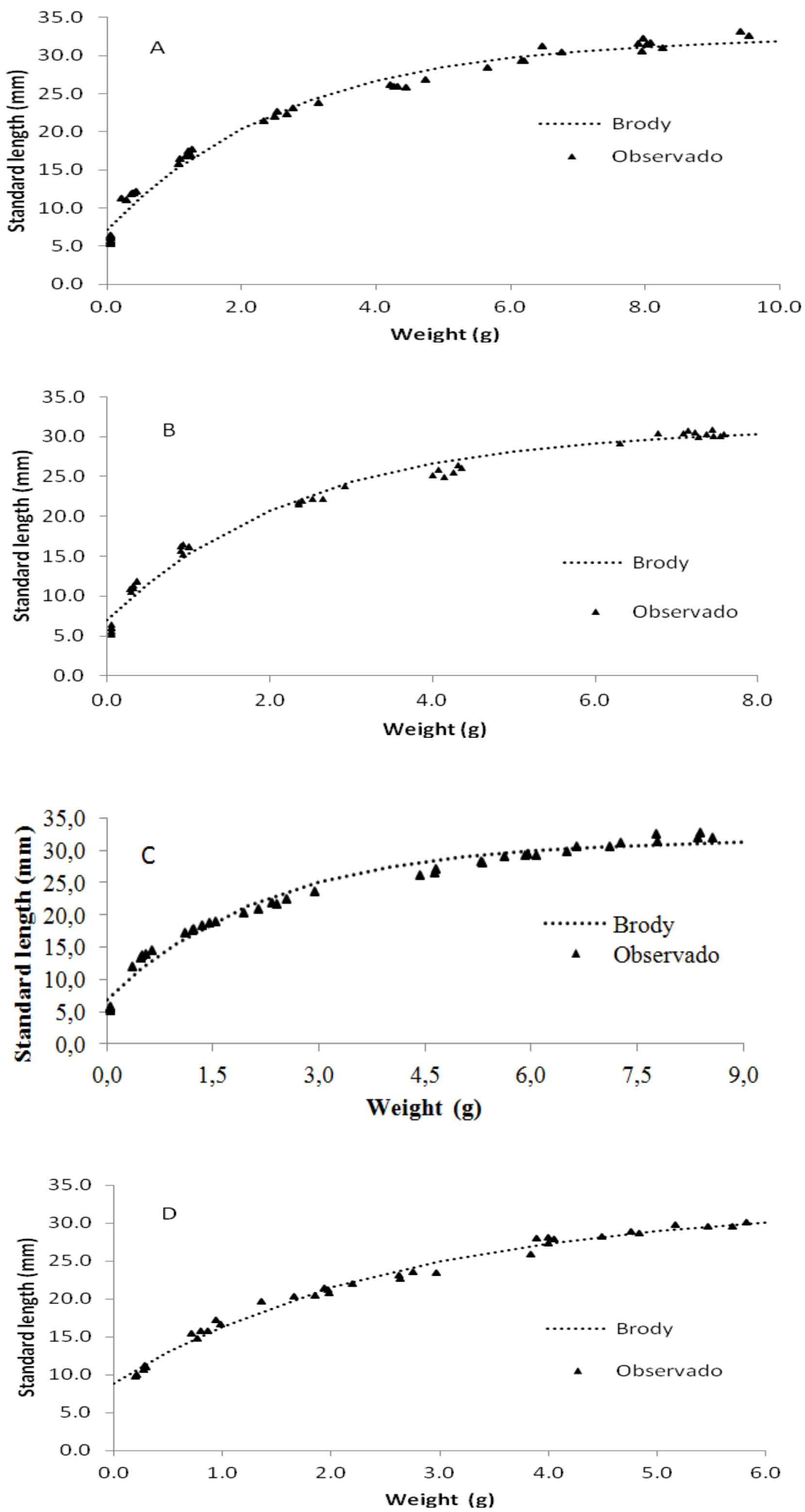

Figure 2. Brody growth curves of standard length as a function of weight obtained for bullfrog tadpoles. Experiment 1 (A), experiment 2 (B), experiment 3 (C), and experiment 4 (D)

In some cases, the Brody model was not adequate to describe the growth of domestic animal species such as sheep (SILVEIRA et al., 2011), Guzerá cows (OLIVEIRA et al., 2000), or lamb (GUEDES et al., 2004). However, this model was found to be adequate for beef cows of different biological types (SILVA et al., 2011), *Salvelinus alpinus* fish (LOEWEN et al., 2010), and *Panopea generosa* mollusks (CALDERON-AGUILERA et al., 2010). These results show that a given model may or may not be adequate to fit a dataset and this type of study is therefore always necessary for different farm species.

The K values of the Brody model were lower for total length as a function of weight when compared to standard length as a function of weight in all experiments, indicating a greater initial growth in standard length, i.e., the head and organs (corresponding to standard length) develop first, followed by growth of the tail, muscle tissue and reserves for consumption during metamorphosis (MANSANO et al., 2014). Different K values (relative growth rate at maturity) for different parts of the body have also been reported for Nile tilapia (SANTOS et al., 2007) and piracanjuba (GOMIERO et al., 2009), demonstrating heterogenous growth.

The Brody model is the best to describe the growth in standard and total length of bullfrog tadpoles as a function of weight.

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