## Bullfrog growth parameters in continuous water-flow tanks systems on a commercial scale

# Parámetros del crecimiento de rana toro en sistemas de tanques de flujo continuo de agua a escala commercial

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#### Abstract

The growth parameters of hatchery-reared bullfrog (*Lithobates catesbeianus*) reared at commercial continuous water-flow tanks systems were studied and discussed. We stock 3.000 juvenile with a density of 60 frogs/m<sup>2</sup> (mean weight 16 ± 1.82 g) into three 16.5 m<sup>2</sup> continuous water-flow tank systems with a depth of 12 cm flooded height. Bullfrog's were fed twice daily (8:00 and 16:00 hours) with a reference floating extruded diet (45 % crude protein and 16 % crude lipids). After 50 day the mean weight was 26.03 ± 2.72 g. The apparent feed conversion rate (AFCR) was 1.5. A multi-model approach was used for select the best growth model for juvenile bullfrog under culture conditions. Two variations of Schnute model and Ruiz-Velazco model were used as a candidate model. With a weight of evidence of 76.94% according to Akaike weight, case 3 of Schnutte model was selected as the best model to describe bullfrog growth in continuous water-flow tank systems. The results about the best model obtained in this experiment are only applicable for the interval from 16 to 32 g.

Key words: bullfrog; growth model; raniculture; commercial I

#### Resumen

Se estudiaron y discutieron los parámetros del crecimiento de rana toro (*Lithobates catesbeianus*) criada en un laboratorio y cultivada en un sistema comercial de tanques de flujo continuo de agua. Se sembraron 3,000 juveniles con una densidad de 60 ranas/m<sup>2</sup> (peso promedio 16 ± 1,82 g) en tres sistemas de tanques de flujo continuo de agua con capacidad de 16,5 m<sup>2</sup> y una profundidad inundable de 12 cm. Las ranas fueron alimentadas diariamente (8:00 y 16:00 horas) con una dieta extruída flotante (45 % de proteína cruda y 16 % de lípidos crudos). Después de 50 días el peso promedio fue 26,03 ± 2,72 g. El factor de conversión alimenticia aparente FCAA fue de 1,5. Se utilizó en enfoque multimodelo para seleccionar al que mejor describa el crecimiento de rana toro cultivada. Con un peso de evidencia de 76,94 % según el peso de Akaike, el caso 3 del modelo de Schnutte fue seleccionado como el mejor modelo para describir el crecimiento de rana toro en sistemas de flujo continuo de agua. Los resultados sobre el mejor modelo obtenido en este experimento solo son aplicables a intervalos de de entre 16 y 32 g.

Palabras clave: scale rana toro; modelo de crecimiento; ranicultura, escala comercia

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Raniculture represents a sustainable alternative in world aquaculture. It is a culture with potential for frog's production. In Mexico, aquaculture research on frogs has focused on bullfrogs (Litobathes castebeianus), particularly on growth performance, another important factor is its high productive performance and low operating cost compared to other aguaculture resources that are produced in land-based systems. Farming of bullfrogs began in Mexico in the 1925's (INAPESCA, 2018). It is one of the cultures with the greaters development given the controlled production of it its culture cycle; form eggs to fattening and other technological advances such as automation in feeding systems. The bullfrogs have been identified by the government of Mexico as one of the prospects for commercial cultivation. Some biological and technological processes have been well documented (Islas-Ojeda *et al.*, 2021; Hernández-López and Hernández-Yau 2023; Padilla-Cerezo et al., 2022).

Today, in commercial aquaculture the growth performance is the most influential factor for achieving technical profitability (Jurado-Molina et al., 2023). Despite the knowledge acquired about the growth of this resource, its evaluations have been in no commercial environments, so under this assumption it is important to know its limits and establish a growth model and length-weight relationship that maximizes productive efficiency of the resource on a commercial scale. For this purpose, today different models are available for the estimation of individual growth, which estimates the average body growth of an individual (Baer et al., 2011). A priori, today the model that is mostly applied in aquaculture is the von Bertalanffy growth model (Katsanevakis and Maravelias, 2008) however there are other models such as Ruiz-Velazco (2011) and Logistic model (Ricker, 1975). Model selection requires the use on the Akaike information criterion (BIC) as an alternative to select more reliable model that fists the data as a better alternative that describes the growth in weight or length of a resource. The objective of the present study was to compare the performance of the Ruiz-Velazco model; von Bertalanffy model and the Logistics model on the growth of bullfrogs cultured in continuous water flow tanks systems and determine the allometric coefficient b (length-weight relationship). The main rationale behind the selection of these objectives was to generate more knowledge of the growth of bullfrogs in culture to maximize the profitability of their culture on a commercial scale.

Hatchery-reared bullfrogs juvenile with an initial mean weight of  $16 \pm 1.82$  g was obtained from a frog farm in Sinaloa, Mexico,

using the breeding and tadpole production protocols. In total 3,000 juveniles were randomly distributed and stocked at density of 60 frogs / m<sup>2</sup> into three 16.5 m<sup>2</sup> continuous waterflow tank systems with a depth of 12 cm flooded height. Each tank has a PVC systems for lateral overflow drainage that allows water exchange the volume as required. Each tank has a metal structure covered with white UV-protected plastic to form a greenhouse. Bullfrog's were fed twice daily (8:00 and 16:00 hours) with a reference floating extruded diet (45 % crude protein and 16 % crude lipids). Feed was supplied at 4 % of biomass per day during the experiment. Samples for monitoring bullfrog growth and making adjustments for feeding were taken every week. A sample of 180 bullfrog's was obtained from each tank weighed (g) with a Toro Rey brand digital scale. Water variables temperature (via a partial immersion liquid thermometer, °C) and pH (with a digital pH meter) was recorder daily in both tanks. Bullfrog's were reared for 50 days; the survival rate was estimated by checking tanks daily for dead bullfrogs with the following equation: Survival (%) = (harvest bullfrogs / stocked bullfrogs) x 100. Growth metric calculated were: absolute growth rate (AGR; q / d) or (*Wf – Wi*) / t, where Wf = final weight, Wi = initial weight, t = time (d); specific growth rate (SGR (percent body weight/d) or 100 (log e Wf log e W//t; apparent feed conversion rate (AFCR) = supplied feed/increase in bullfrog's weight. We modeled growth with three weight models. The two cases of Schnute (1981) model's were:

Case 1,  $a \neq 0$  y  $b \neq 0$ 

$$P(t) = \left[Y_1^{\ b} + \left(Y_2^{\ b} - Y_1^{\ b}\right) \frac{1 - e^{-a(t - \tau_1)}}{1 - e^{-a(\tau_2 - \tau_1)}}\right]^{\frac{1}{b}}$$
(1)

Case 3, a = 0 y  $b \neq 0$ 

$$P(t) = \left[Y_1^{\ b} + \left(Y_2^{\ b} - Y_1^{\ b}\right) \frac{t - \tau_1}{\tau_2 - \tau_1}\right]^{\frac{1}{b}}$$
(2)

Where P (t) is the individual weight in grams at age t, Y<sub>1</sub> y Y<sub>2</sub> are the total weight at the beginning and end of the time interval t<sub>1</sub>- t<sub>2</sub>, a is a growth parameter with units of years, *b* is related to the inflection point of an "*S*" shaped growth curve.

We used a different growth model (Ruiz-Velazco, 2011). The new growth model in aquaculture is:

$$w_{t} = w_{i} + (w_{f} - w_{i}) \left(\frac{1 - k^{t}}{1 - k^{c}}\right)^{3}$$
(3)

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Where  $w_i$  is the initial weight,  $w_f$  is the final weight, k refers to the speed at which the weight changes from its initial value to its final value and c is the duration of the crop. We estimated the model parameters from equation 1-3 and their uncertainties using the maximum likelihood estimation (MLE) method (Kimura, 1980); thus, the objective function was log-likelihood (LL) (Haddon, 2001).

$$LL = \left(-\frac{n}{2}\right) \left(Ln(2\pi) + 2Ln(\sigma) + 1\right) \tag{4}$$

To select the best model, the one with the lowest Akaike index, corrected for small samples (AIC<sub>c</sub>), was chosen.

$$AIC_{c} = 2(k - LL) + (2k(k + 1)/(n - k - 1))$$
(5)

Akaike's weight was estimated as:

$$W_i = \frac{e^{-0.5\Delta AIC}}{\sum_{c=1}^4 e^{-0.5\Delta AIC}}$$
(6)

where *c* is each case of Schnute model and the

 $\Delta AIC = AIC_c - AIC_{small}$ 

Water temperatures ranged from 25-29 °C (mean 27.9 ± 1.2)

and pH ranged from 6.2 to 7 (mean 6.7  $\pm$  0.3). Survival rate was above 90 %, mortality was observed continuously into three tanks throughout the experiment; it related to high sings of cannibalism. In the first 20 days of cultivation a sustained growth of 3.29 g was observed, during the next 10 days the growth decreased to a gain weight of 1.87 g and in the last 10 days, the growth in weight gain accelerated again to 2.72 g (Figure 1). The final mean weight was  $25.95 \pm 4.42$  g, the AGR was 0.22 g / d, the SGR was 0.99 (%/d) and the AFCR was 1.5. Other growth parameters are shown in Table 1. The growth in weight of hatchery-reared bullfrogs cultured in continuous water-flow tank systems with a density of 60 frogs / m<sup>2</sup> during 50 days was successful. We tested the fit of three of growth models to data on growth of bullfrogs. The case 3 of Schnute growth model was selected the best model for bullfrog's growth in continuous water-flow tank systems with a density of 60 frogs / m<sup>2</sup> with a weight of evidence of 76.94 % according to Akaike weight (Table 2). The case 1 of Schnute growth model was also supported to some extent by data (22.73 % Akaike weight), while Ruiz-Velazco model had considerably less support with a weight of evidence of 0.33 % according to Akaike weight. However, despite the numerical differences in Akaike weight when comparing the graphs of the three frogs growth model, it was observed that the three models are very identical to the grow curve (Figure 2).



Figure 1. Growth in weight of Bullfrogs stocked at 60 frog/m<sup>2</sup> in continuous water-flow tank systems.



Growth Variable	Values
Initial mean weight (g)	16.00 ± 1.82
Final mean weight (g)	26.03 ± 2.26
Weight gain (g)	10.03 ± 2.72
Initial biomass (Kg)	48
Final biomass (Kg)	71.1
Survival (%)	91
AFCR	1.5
AGR (g/d)	0.22
SGR (%/d)	0.99

Figure 2. Model fits to observed mean weight for bullfrogs rearing in continuous water-flow tank systems at density of 60 frogs/m<sup>2</sup>. Table 1. Growth performance of Bullfrogs in Continuous Water Flow-Tank Systems.

Table 2. Estimated parameters for each candidate model with their respective AIC values and Akaike weight.

Case	AIC	۵AIC	e <sup>(0.5*∆AIC)</sup>	Wi
Ruíz Model	4086	10.90	4E-03	0.33
Case 1 Schnute Model	4078	2.44	3E-01	22.73
Case 3 Schnute Model	4075	0.00	1E+00	76.94

In general terms, bullfrog's growth data are acceptable, which indicates that this resource can be an option for increasing animal protein form aquaculture. However, and like any emerging resource, the generations of greater growth information must be considered, especially in stages of greater weight to consolidate knowledge of the entire growth curve. Water guality is considered normal within aguaculture. These results are similar to the temperature between 25 and 30 °C (Braga y Lima, 2001; Rodríguez-Serna et al., 1996). Considering that this stage evaluated in this experiment is the one with the highest mortality associated with cannibalism, the survival recorded in this work is adequate for organism in aquaculture, and overall survival rate of 70 % can be considered satisfactory (Rodríguez-Serna et al., 1996). The growth rates AGR of hatchery-reared bullfrogs raised in this study (mean 0.22 g/day) were inferior to those reported by (Braga y Lima, 2001; Rodríguez-Serna et al., 1996) for bullfrogs (mean 1.01 g/day), but with frogs that weigh more than 40 g, just the stage where the bullfrog begins its accelerated growth. Despite the oscillating nature of the bullfrog's growth data in culture, the Ruiz-Velazco model (specially for use in aquaculture) did not have good support in the data according to the Akaike information index even when it alone requires the estimation of three parameters and not four and three like cases y and three of Schnute models (1981). The situation is due to the lack of older specimens and weight close to their maximum value (350 - 400 g) so that the model would have information on the

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asymptotic growth phase. When adjusting Schnute's case 3 model to the growth data of bullfrog juveniles, they resulted in an adjustment close to 80 %, this is because linear growth is presented to a greater extent because there is only data from initial stages, for example. Therefore, the results of these models cannot be used to estimate a value of the infinite growth parameter and any extrapolation must be handled with caution, although officially there are no precedents on bullfrog's growth modeling, and the results of this experiment are the first to use a multimodel approach in this resource. It is clarified that although the data represent only part of the bullfrog growth curve, these models can be valid to apply only in weight intervals of 14 to 32 g.

#### **Conflict of interest**

The author declared that he has no conflict of interest.

#### Author's contributions

Carlos Humberto Hernández-López, Heimy Franceline Martínez-Sánchez, Alfredo Emmanuel Vázquez-Olivares and Jorge Flores-Olivares; conceptualization, writing and editing, development of methodological design, acquisition of financing, data collection and data analysis.

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