



## Influence of the number of feeding environments on the performance of bullfrog tadpoles

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**ABSTRACT.** Feeding is one of the major factors that influences the growth and development of animals, justifying thus the present experiment that analyzed the effect of the number of feeding environments on the performance of bullfrog tadpoles through the growth (weight and length) and performance (food remains, average feed intake, average weight gain, and feed conversion). The treatments consisted of subunits of two, four, and eight feeding environments in the experimental units at disposal for the tadpole population, and one without subdivisions, comprising a single environment, during 60 experimental days. The design was completely randomized, with four treatments and three replications. The values were tested using an analysis of variance and Duncan test ( $\alpha = 0.05$ ). The growth in weight and length and the indices of animal performance were influenced by the number of feeding environments, with the best results found for the treatments with four and eight environments, and the worst, for the treatment with a single environment. The increased number of feeding environments may be an alternative to improve the performance of bullfrog tadpoles.

**Keywords:** performance, nutrition, frog farming, sustainable production.

## Influência do número de ambientes de alimentação no desempenho de girinos de rã-touro

**RESUMO.** Alimentação íntegra a um conjunto de fatores que influenciam o crescimento e o desenvolvimento dos animais. Com isso, foi realizado o experimento com o objetivo de verificar pelo crescimento (peso e comprimento) e o desempenho (sobras diárias de ração, consumo médio de ração, ganho de peso médio e conversão alimentar) dos girinos de rã-touro a influência do número de ambientes de alimentação no desempenho de girinos de rã-touro. Os tratamentos foram subdivisões em dois, quatro e oito ambientes de alimentação das unidades experimentais em relação à população de girinos e um sem subdivisões com único ambiente, durante 60 dias experimentais. O delineamento foi inteiramente casualizado, com quatro tratamentos e três repetições. Os valores encontrados foram submetidos à análise de variância e ao teste de Duncan ( $\alpha = 0,05$ ). O crescimento em peso e comprimento e os índices de desempenho zootécnicos foram influenciados pelo número de ambientes de alimentação, sendo que os melhores resultados foram para os tratamentos com quatro e oito ambientes e o piores para com único ambiente. O aumento do número de ambientes de alimentação pode ser uma alternativa para melhorar o desempenho dos girinos de rã-touro.

**Palavras-chave:** desempenho, nutrição, ranicultura, produção sustentável.

### Introduction

The farms of the bullfrog, *Lithobates catesbeianus*, have been growing steadily, due to the use of frog meat as therapeutic innovation, meat characteristics such as taste, biological value, and meat texture, but otherwise, the high cost for its production caused by the lack of zootechnical knowledge hinders its culture. Nevertheless the frog farm has a great potential as a tool for local development (NASCIMENTO et al., 2008).

Studies have aimed to develop economically viable methods for rearing tadpoles, emphasizing the nutrition (SECCO et al., 2005; SEIXAS FILHO et al., 2010, 2011), the performance (HAYASHI et al., 2004; LIMA et al., 2003), the water quality (SIPAÚBA-TAVARES et al., 2008), the behavior (SCHMIDT et al., 2011), and photoperiod (BAMBOZZI et al., 2004).

The growth, survival, and metamorphosis time (efficiency and effectiveness) are directly associated with factors like competition for food, amount and

quality of food (COLOMBANO et al., 2007; SEIXAS FILHO, et al., 2008), water quality and chemical inhibitors (BROWNE et al., 2003) and health (HIPOLITO et al., 2004, 2007).

In the natural environment, there is a great variety of organisms that can be used as a natural source of food, and the efficiency of its use along with the availability of soil minerals ensure the survival of the species (SCHIESARI et al., 2009). Tadpoles are considered herbivorous or detritivorous, being the algae the major food resource on the early developmental stages (SCHOONBEE et al., 1992).

Tadpoles accept every food class, from artificial to natural, and the combination of these provides satisfactory results besides reducing the cost of production (NASCIMENTO et al., 2008).

Behavioral aspects are directly related to the growth variation, since dominant animals have growth rates higher than the submissive, that is, heterogeneous growth, which may be influenced by genetic, social and population factors (BARBOSA et al., 2006).

The quality and quantity of food consumed individually may be associated with the position taken by the individual within the social hierarchy that is specific to its group, and this position may come from both feeding behavior in the strict sense (DASH; HOTA, 1980), and feeding behavior in the broad sense, i.e., from inhibitory chemical excretions (ROSE, 1959). This is a complex of behaviors performed by almost all individuals, with several adaptive functions (SOMA et al., 2008).

In the competition for limited resources, it is essential to ensure the access to food and security, the reproductive success, and protection of offspring (DE ALMEIDA et al., 2005; KARL et al., 2004). The animals that win a territorial competition usually have a greater protection against predators, and better opportunities for foraging, which promote a rapid growth, conferring thus a higher probability of reproductive success and survival (JOHNSSON et al., 2002).

In this way, this study aimed at developing techniques to prevent the dominance or problems during the feeding of bullfrog tadpoles, in order to enable a rearing system to produce lots of tadpoles with better performance and with homogeneous sizes.

## Material and methods

The experiment was conducted at the Laboratory of Researches in Biology of the UNISUAM, Rio de Janeiro, Rio de Janeiro State, Brazil, during 60 days.

Three hundred and sixty bullfrog tadpoles at stage 25 of Gosner (1960) from the same spawning, were placed in 60% of volume of polypropylene boxes (50 liters), with a density of one tadpole L<sup>-1</sup>, compounding

an experimental unit, arranged side by side on a bench, and aerated by a porous stone at its end, and temperature controlled using a heater coupled to a thermostat, set at 25°C.

The animals received a commercial fish feed, with 36% crude protein (PB) supplied at pellets (2.5 mm diameter), once a day, placed in one, or in up to eight troughs, according to the treatments, in the following ratios: five grams per experimental unit, for the first thirty days, and ten grams per box, for the last thirty experimental days.

The feed remains, gathered every 24 hours, were dried in a forced air oven at 60°C, for 72 hours and then weighed, and the result values were transformed into percentage of the supplied amounts.

The biometric measurements were taken at the beginning, at 15, 30, 45 and 60 days, by registering: weight (g) using a digital scale accurate to 0.001 g, and length (mm), with a digital caliper accurate to 0.01 mm, performed individually.

The temperature of the experimental environment and of the water in the experimental units was measured daily. Also, the levels of NH<sub>4</sub> and NH<sub>3</sub> were determined by colorimetry, and the pH of the water in the experimental units was determined by means of a kit for controlling the water quality in aquaria (LABTEST®).

The treatments consisted of subunits of the experimental units containing the tadpole populations: treatment 1 (A1) a single experimental unit without division and with a single feeding trough, i.e., one feeding environment (Figure 1A); treatment 2 (A2) was formed by two environments by the arrangement of a divider which separated the unit into two environments, with two lateral aisles, and containing two feeding troughs, one at each subunit (Figure 1B); treatment 3 (A4) was the arrangement of four environments using two orthogonal dividers, with four side aisles, supplied with four feeding troughs (Figure 1C); treatment 4 (A8) consisted of eight environments designed by means of dividers placed in such a way to produce eight subunits with lateral aisles (similarly to the other treatments), with eight feeding troughs (Figure 1D).

The feeding troughs were arranged in such a way that if a tadpole was next to one of them, it could not visualize any other trough.

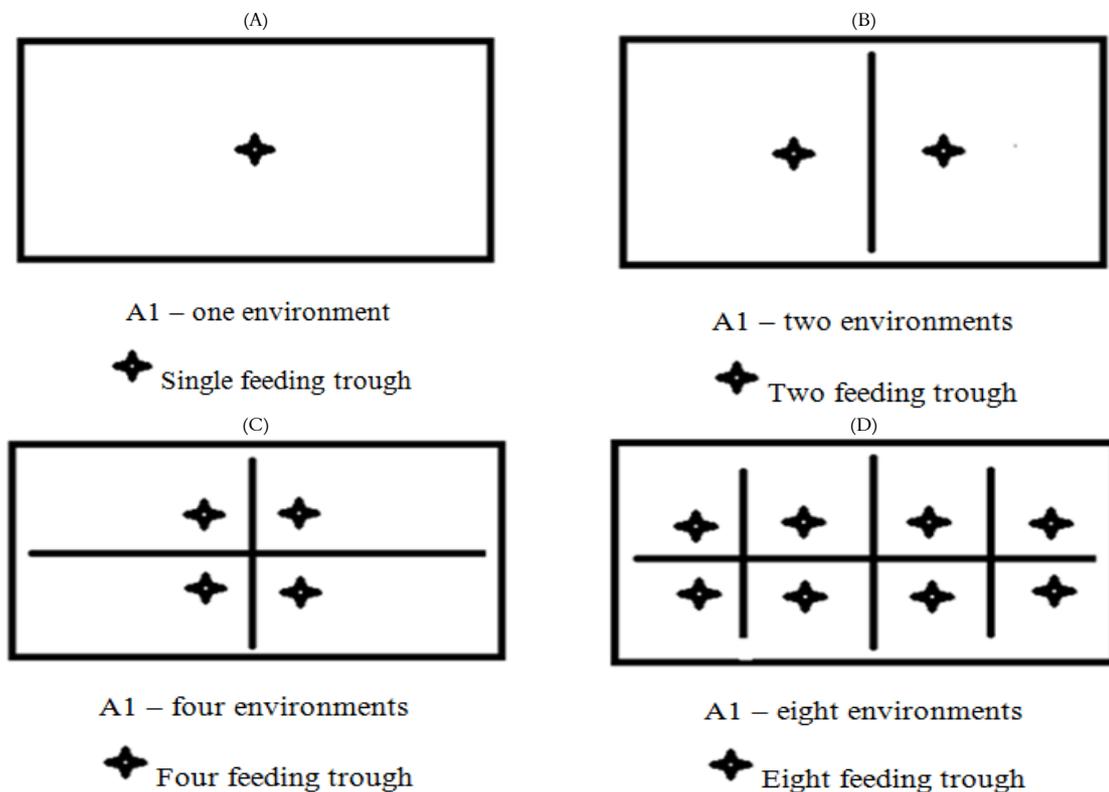
The design was completely randomized, with four treatments and three replications. The parameters evaluated were average weight and average total length at the beginning, at 15, 30, 45, and 60 days, and also the feed remains on a daily scale, average feed intake, average weight gain, and feed conversion for the periods of 0-15 days, 16-30, 31-45, 46-60 days. The values were then subjected to an analysis of variance and Duncan test at 5% probability using the software Statistical Analysis System (SAS, 2001).

## Results and discussion

The average room temperature was  $25.3 \pm 0.6^\circ\text{C}$ , and the average temperature of the rearing water was  $24.4 \pm 0.4^\circ\text{C}$ . The daily levels of  $\text{NH}_4$  and  $\text{NH}_3$  were  $1.0 \pm 0.7 \text{ mg L}^{-1}$  and  $0.0004 \pm 0.0005 \text{ mg L}^{-1}$ , respectively. The water pH of the boxes was on average  $6.6 \pm 0.3$ . The average value observed for the water temperature is according to indicated for this species at this developmental stage (HAYASHI et al., 2004; LIMA et al., 2003). The pH value is within the range

recommended for tadpoles (between 6 and 8) (SIPAÚBA-TAVARES et al., 2008), that is, the conditions were optimal for the tadpole growth.

The number of feeding environments has influenced ( $p < 0.05$ ) the growth in weight and length of bullfrog tadpoles (Table 1), the treatments with 4 and 8 environments were the best throughout experimental period for weight and length, the treatment with one trough had the worst results for weight, and the treatment with two troughs, the worst for length.



**Figure 1.** Sketch of the subdivisions of the area called A1 (A), A2 (B), A4 (C) and A8 (D) of the experimental units for behavioral assessment in relation to the feeding of bullfrog tadpoles.

**Table 1.** Average body weight and average body length of bullfrog tadpoles according to the number of feeding environments and the time elapsed.

Treatment	Experimental period (days)				
	0	15	30	45	60
Average body weight (g)					
A1	$0.175 \pm 0.018^a$	$1.037 \pm 0.056^b$	$2.244 \pm 0.156^b$	$4.596 \pm 0.173^c$	$8.836 \pm 0.526^c$
A2	$0.169 \pm 0.003^a$	$1.040 \pm 0.082^b$	$2.684 \pm 0.272^b$	$5.211 \pm 0.060^b$	$11.096 \pm 0.288^b$
A4	$0.176 \pm 0.013^a$	$1.399 \pm 0.113^{ab}$	$3.696 \pm 0.129^a$	$7.483 \pm 0.109^a$	$13.584 \pm 0.394^a$
A8	$0.178 \pm 0.006^a$	$1.166 \pm 0.046^a$	$3.456 \pm 0.227^a$	$7.313 \pm 0.194^a$	$13.776 \pm 0.356^a$
CV (%)	11.80	11.83	11.84	4.10	5.92
P-value	0.9691	0.0366	0.0043	0.0001	0.0001
Average body length (mm)					
A1	$8.825 \pm 0.360^a$	$15.992 \pm 0.308^b$	$21.685 \pm 0.132^b$	$26.338 \pm 0.392^b$	$33.152 \pm 0.999^b$
A2	$8.808 \pm 0.049^a$	$16.230 \pm 0.526^b$	$22.349 \pm 0.474^b$	$26.857 \pm 0.382^b$	$34.929 \pm 0.643^b$
A4	$8.902 \pm 0.287^a$	$18.071 \pm 0.330^a$	$24.898 \pm 0.552^a$	$31.044 \pm 0.351^a$	$37.085 \pm 1.099^a$
A8	$8.849 \pm 0.126^a$	$16.634 \pm 0.436^b$	$24.243 \pm 0.508^a$	$30.468 \pm 0.215^a$	$37.785 \pm 0.253^a$
CV (%)	4.71	4.23	3.36	2.07	3.98
P-value	0.9851	0.0298	0.0020	0.0001	0.0208

A1 – one environment; A2 – two environments; A4 – four environments; A8 – eight environments. Different letters in the same column for each parameter indicate significant differences (Duncan test,  $p < 0.05$ ). Mean  $\pm$  Standard Error.

Daily feed remains, average feed intake, average weight gain, and feed intake were influenced ( $p < 0.05$ ) by the number of feeding environments (Table 2), the treatments with 4 and 8 environments presented the best results for these parameters, except the period of 16-30 days for daily feed remains where the treatment with 4 environments was the best one. The treatment with one environment presented the worst values for the parameters during the studied periods.

**Table 2.** Daily feed remains, average feed intake, average weight gain, feed conversion of bullfrog tadpoles according to the number of feeding environments and the time elapsed.

Treatment	Experimental period (days)			
	0-15	16-30	31-45	46-60
<b>Daily feed remains (%)</b>				
A1	33.9±1.46 <sup>a</sup>	22.3±1.35 <sup>ab</sup>	29.9±1.09 <sup>a</sup>	21.0±1.03 <sup>a</sup>
A2	29.0±0.72 <sup>b</sup>	20.4±6.63 <sup>a</sup>	24.1±2.11 <sup>a</sup>	11.1±2.06 <sup>b</sup>
A4	22.9±0.87 <sup>c</sup>	9.1±0.81 <sup>c</sup>	10.1±2.92 <sup>b</sup>	6.5±0.40 <sup>b</sup>
A8	23.2±1.02 <sup>c</sup>	13.2±1.74 <sup>b</sup>	12.2±1.66 <sup>b</sup>	6.6±0.88 <sup>b</sup>
CV (%)	6.72	24.17	18.79	19.20
P-value	0.0002	0.0371	0.0003	0.0001
<b>Average feed intake (g)</b>				
A1	1.65±0.03 <sup>b</sup>	1.95±0.02 <sup>b</sup>	3.66±0.05 <sup>b</sup>	5.49±0.17 <sup>b</sup>
A2	1.88±0.05 <sup>a</sup>	2.04±0.16 <sup>ab</sup>	4.27±0.11 <sup>a</sup>	6.69±0.25 <sup>a</sup>
A4	1.97±0.04 <sup>a</sup>	2.31±0.07 <sup>ab</sup>	4.59±0.06 <sup>a</sup>	6.36±0.17 <sup>ab</sup>
A8	1.96±0.04 <sup>a</sup>	2.24±0.04 <sup>a</sup>	4.81±0.31 <sup>a</sup>	6.79±0.43 <sup>a</sup>
CV (%)	4.36	7.66	6.96	7.76
P-value	0.0044	0.0842	0.0078	0.0430
<b>Average weight gain (g)</b>				
A1	0.86±0.04 <sup>b</sup>	1.21±0.14 <sup>b</sup>	2.33±0.08 <sup>b</sup>	4.25±0.46 <sup>b</sup>
A2	0.86±0.08 <sup>b</sup>	1.62±0.19 <sup>b</sup>	2.54±0.21 <sup>b</sup>	5.98±0.27 <sup>a</sup>
A4	0.98±0.12 <sup>a</sup>	2.18±0.08 <sup>a</sup>	3.79±0.23 <sup>a</sup>	6.12±0.34 <sup>a</sup>
A8	1.22±0.04 <sup>a</sup>	2.29±0.18 <sup>a</sup>	3.77±0.06 <sup>a</sup>	6.12±0.18 <sup>a</sup>
CV (%)	14.40	14.95	9.29	10.25
P-value	0.0454	0.0042	0.0003	0.0101
<b>Feed conversion (g)</b>				
A1	1.92±0.09 <sup>a</sup>	1.64±0.18 <sup>a</sup>	1.57±0.04 <sup>a</sup>	1.31±0.11 <sup>a</sup>
A2	2.22±0.26 <sup>a</sup>	1.28±0.14 <sup>ab</sup>	1.70±0.10 <sup>a</sup>	1.12±0.02 <sup>ab</sup>
A4	1.64±0.15 <sup>a</sup>	1.01±0.06 <sup>b</sup>	1.22±0.09 <sup>b</sup>	1.04±0.02 <sup>b</sup>
A8	2.00±0.10 <sup>a</sup>	1.04±0.11 <sup>b</sup>	1.27±0.06 <sup>b</sup>	1.11±0.04 <sup>ab</sup>
CV (%)	14.90	18.88	9.84	9.34
P-value	0.1868	0.0374	0.0088	0.0684

A1 – one environment; A2 – two environments; A4 – four environments; A8 – eight environments. Different letters in the same column for each parameter indicate significant differences (Duncan test,  $p < 0.05$ ). Mean ± Standard Error.

From the obtained results, it became evident the importance of the interactions of the animal with the environment, once the behavior patterns were set by a stimuli that triggered a response, corroborating Freake and Phillips (2005) or a sequence of responses, similar to reports of Ruchin (2003). The food intake was considered within the behavioral standards that can be changed by biotic or abiotic factors, behaviors already mentioned by Santos et al. (2004) and Silva et al. (2009).

In the experiments with bullfrog tadpoles a problem to be considered is the wide intragroup variance that hinders the detection of significant differences between the experimental treatments, this variance may be assigned to the genetic

variability of the species that underwent few processes of genetic selection (BARBOSA et al., 2005) which still are unable to influence responses to the treatments imposed to the population, and also, part of this intragroup variation comes from the social behavior from the dominance hierarchy in all groups of gregarious animals, but with the results revealed herein, the increased number of feeding environments may have reduced the influence of dominance hierarchy and thus the weight variability was lower and allowed growth results and animal indices to be higher than mentioned in literature (BARBOSA et al., 2005; SECCO et al., 2005; SEIXAS FILHO et al., 2010).

Our results were similar to those for high populations of *Rana tigrina* tadpoles (DASH; HOTA, 1980), for *Litoria aurea* tadpoles (BROWNE et al., 2003) and for *Litoria moorei* (MATSON et al., 2010), where the reduced values of average weight, survival, and increase in weight variation of the lots were related to the autecology of the species, such as competition for food, and social factors, like competition for space and hierarchy.

## Conclusion

The increase in the number of feeding environments for bullfrog tadpoles may be an alternative to improve the performance of bullfrog tadpoles in commercial frog farms, allowing the acquisition of larger and more homogeneous froglets.

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*Received on April 11, 2012.*

*Accepted on May 29, 2012.*

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