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# Effect of two densities on the growth of bullfrog cultured in commercial continuous water-flow tank systems

# Efecto de dos densidades sobre el crecimiento de rana toro cultivadas en un sistema comercial de tanques de flujo continuo de agua

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## ABSTRACT

In commercial farming, growth performance is the most influential factor for achieving economic profitability. We evaluated the effects of stocking density on the growth performance of juvenile bullfrogs cultured in a continuous water-flow tank system on a commercial scale. 4,500 frogs were stocked at densities of 30 and 60 frogs/m$^2$, with three replicate ponds per density and an initial weight of 1.7. Frogs were fed an extruded diet and cultured for over 45 days. Student *t*-test, was used to evaluate the effect of culture density on growth parameters and to verify if *b* was significantly different from isometric growth value. The best growth corresponded to a density of 60 frogs/m$^2$ ($p$ – value = 2.2e-16). The densities did not influence the Absolute Growth Rate (AGR; $p$ = 0.2843) or survival ($p$ = 0.0779), but had a significant effect in feed intake ($p$ = 0.0461), Apparent Factor Conversation Rate (AFCR; $p$ = 0.0123), final weight ($p$ = 2.2e-16), final length ($p$ = 8.607e-08), and final biomass ($p$ = 0.0472). The results of the length-weight fitting results and the t-test suggested negative allometric growth. The bullfrogs grew successfully at both densities, but performance was better at a density of 60 bullfrogs/m$^2$.

**KEY WORDS:** Growth performance, stocking densities, *Lithobates catesbeianus*, commercial culture, bullfrog.

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**R E S U M E N**

En la acuicultura comercial, el crecimiento es el factor más influyente para lograr la rentabilidad económica. Se evaluaron los efectos de la densidad de siembra sobre el crecimiento de juveniles de rana toro cultivadas en un sistema de flujo de agua continua a escala comercial. Se sembraron 4,500 ranas en densidades de 30 y 60 ranas/$m^2$, con tres réplicas por cada densidad y peso inicial de 1.7 g. Las ranas se alimentaron con una dieta extruida y fueron cultivadas durante 45 días. Se utilizó la prueba *t* de Student para evaluar el efecto de la densidad de cultivo sobre parámetros de crecimiento y verificar si el parámetro *b* era significativamente distinto del valor de crecimiento isométrico. El mejor crecimiento correspondió a una densidad de 60 ranas/$m^2$ (Valor de $p$ = 2.2e-16). Las densidades no influyeron en la Tasa de Crecimiento Absoluto (AGR; $p$ = 0.2843) ó sobrevivencia ($p$ = 0.0779), pero fueron significativas en el consumo de alimento ($p$ = 0.0461), Factor de Conversión Alimenticia Aparente (AFCR; $p$ = 0.0123), peso final ($p$ = 2.2e-16), longitud final ($p$ = 8.607e-08) y biomasa final ($p$ = 0.0472). Los resultados del ajuste longitud-peso y la prueba *t* sugirieron un crecimiento alométrico negativo. Las ranas toro crecieron en ambas densidades, pero el mayor crecimiento se observó con una densidad de 60 ranas/$m^2$.

**P A L A B R A S   C L A V E :** Rendimiento del crecimiento, densidad de cultivo, Lithobates catesbeianus, cultivo comercial, rana toro.

## Introduction

Frog farming represents an alternative to world aquaculture. It is a developing technology with potential for food production and human consumption. Bullfrog (*Lithobates catesbeianus*) it's the most commercially important species for aquaculture; its size and muscle mass are part of the biological attributes that have increased its demand in international markets (Pasteris *et al.,* 2006). In Mexico, the National Aquaculture and Fisheries Commission (CONAPESCA) has promoted bullfrog farming to diversify the country's aquaculture sector. This is promoted as a viable alternative because it offers advantages for intensive commercial production, including rapid reproduction, a short fattening cycle, and fast growth, alongside increasing demand in both national and international markets. In this context, research on frog farming focuses on the bullfrog since it is one of the best prospects for its production in controlled systems (Flores-Nava, 2005). Some processes for its commercial and experimental cultivation have been documented: pathologies (Rodríguez-Serna *et al.,* 1996), production systems (Rodríguez-Serna *et al.,* 1996; Islas-Ojeda *et al.,* 2021), tadpoles density (Flores-Nava & Vera-Muñoz, 1999), and meat quality (Padilla-Cerezo *et al.,* 2022). The bullfrog is a carnivorous amphibian native to North America, introduced to southern Canada, Central and Northern Mexico (INAPESCA 2018). This species

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is one of the largest frogs, reaching up to 20 cm in snout-vent length and up to 800 g in weight (FAO, 2006). In the international market, bullfrogs are a valuable commercial resource, prized for food (notably frog legs) and medicinal applications. As a result, bullfrog farming has emerged as a profitable global aquaculture sector, with 2020 production exceeding 147,000 tons and a value nearing 5 billion USD (FAO, 2022). This makes it a significant resource in countries like Mexico and across Latin America. Production statistics are scarce, and there is no regular compilation of its production in Mexico. However, estimates for 2018 indicate that aquaculture production reached 700 metric tons (INAPESCA, 2018). Given its high market value and demand (Islas-Ojeda *et al.,* 2021; Musigapan *et al.,* 2022), bullfrog farming offers an economically competitive production opportunity and is considered a new prospect for commercial aquaculture in Mexico.

Culture in continuous water-flow systems (CWFTS) is one of the aquaculture systems used to diversify aquaculture development (Álvarez-Lajonchere *et al.,* 2007; Hernández *et al.,* 2015), characterized by recycling water, reducing the negative impacts associated with aquaculture production. These systems have been used successfully for larval rearing of *Lutjannus guttatus* (Álvarez-Lajonchere *et al.,* 2012), including osteological development (Rodríguez-Ibarra *et al.,* 2015). In commercial farming, growth performance is the most influential factor for achieving economic and technical profitability (Jurado-Molina *et al.,* 2023). Despite the acquired knowledge about bullfrog growth, it is necessary to determine its limits and establish stock densities to optimize production. Stocking density is one of the key parameters in aquaculture, directly influencing growth, survival, production (Gonçalvez de Oliveira *et al.,* 2012), stress (Ellis *et al.,* 2002), and feeding rates (Rowland *et al.,* 2006). Maximizing production requires determining an optimal production density. This study evaluates the effects of stocking density on the growth performance of hatchery-reared juvenile bullfrogs cultured in continuous water-flow tank systems. The results of this study provide additional information to strengthen the knowledge of bullfrog farming in Mexico.

## Material and Methods

### Study area

We studied the growth of hatchery-reared bullfrog juveniles (Lithobates catesbeianus) from a single cohort on a farm in Sinaloa, Mexico (23.27429, -106.21546). The 25-day-old juveniles were obtained from Nutrifrogs (Mazatlan, Mexico), where they were produced using standard breeding and tadpole-rearing protocols.

### Experiment

During the pilot experiment, 4,500 juvenile frogs with an initial weight and length (1.7 ± 0.81 g; 2.54 ± 0.72 cm; mean ± SD) were randomly distributed and stocked in 16.5 $m^2$ in continuous water-flow tank systems with a depth of 20 cm; at densities of 30 and 60 frogs per $m^2$, with three replicate ponds for each density. We determined the lowest density based on the minimum density of bullfrogs cultured by the producer, and the highest density was determined by the

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goal of doubling the production per $m^2$. We used for the six tanks a special liner plastic with a 2” diameter PVC drainage tube. The tanks were housed within a metal-frame structure covered with diffuse white, UV-stabilized polyethylene plastic (200 μm thickness) to serve as a greenhouse. We cleaned the tanks once a day in the morning. We fed the frogs twice (0800 and 1600 h) with an extruded diet containing 45 % crude protein and 16 % crude lipids. Throughout the experiments, we used a 2 mm pelleted diet manufactured by High Quality Food Pedregal Company. We fed the frogs at 5 % of the estimated biomass. We sampled the frogs every seven days to evaluate their survival and increase in weight and length. We did samples of 150 frogs from each tank using dip nets, and the total length and weight of each frog were recorded in grams and centimeters. The daily feed was calculated and adjusted after each sampling. Water variables were recorded weekly in all tanks, including water temperature (ºC) via a partial immersion liquid thermometer and pH with a digital pH meter. Bullfrogs were reared for 45 days. The survival rate was estimated by daily inspection of tanks for dead bullfrogs. We calculated the number of surviving frogs by subtracting the number of dead bullfrogs from the initial number stocked; with this information, we calculated the survival percentage with the following equation:

$$\text{Survival (\%)} = (\text{harvest bullfrogs} / \text{stocked bullfrogs}) \times 100 \qquad \text{Equation 1}$$

Monitoring growth performance relied on measuring the total length (TL; cm) and weight (g) once a week. We calculated the weight gain as follows:

$$\Delta W = W_f - W_i \qquad \text{Equation 2}$$

Where $\Delta W$ is the weight gain, $W_f$ is the final weight, and $W_i$ is the initial weight. We calculated the absolute growth rate (AGR, g/day) as follows:

$$AGR = (W_f - W_i) / t \qquad \text{Equation 3}$$

Where *t* is the number of days. The specific growth rate (SGR, %/day) was calculated with the following equation:

$$SGR = 100 \times (\ln W_f - \ln W_i) / t \qquad \text{Equation 4}$$

We calculated the apparent feed conversion rate AFCR as follows:

$$AFCR = \text{supplied feed} / \Delta W \qquad \text{Equation 5}$$

Later, we used total length and weight to determine the power relationship in the two stocking densities:

$$W = aL^b \qquad \text{Equation 6}$$

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Where *a* is the intercept and *b* is the allometric coefficient (Ricker, 1975). We fit equation (6) to the observed size and weight data using the *optim* subroutine from the statistical package R. Additionally, we used a t-test to verify whether *b* was significantly different from the isometric growth value ($b = 3$). Student *t*-test was also used to assess the effect of the culture density on some bullfrog's performance parameters (AGR, Survival, feed intake, AFCR, final weight, final length, and biomass) and water quality variables. In particular, we compared the total length and weight at 45 days. We used the R statistical package (R Core Team 2024) for all statistical analyses and graphs.

## Results and Discussion

Due to global challenges for food production, bullfrog farming is an alternative. In Mexico, bullfrog aquaculture has emerged as a significant industry in recent years, driven by growing domestic demand for stock (tadpoles) and meat (fattening frogs), as well as the potential to export live specimens to the United States for use in gourmet cuisine, medical applications, and academic research. Commercial bullfrog farming started in Mexico during the 1950s, a period that marked the beginning of more organized production and marketing efforts. Currently, it is a resource supported by the Mexican government to promote research on nutrition, reproduction, and fattening.

In this research, results from the t-test indicated that there were no differences in the mean water quality variables among the two continuous-flow tank systems at both densities. The t-test results indicated no differences in the mean water quality variables between the two continuous-flow tank systems at both densities. During the experimental period, the average water temperature remained within the appropriate parameters for the frog's growth in captivity, not exceeding 29 ºC under the built greenhouse with lateral air vents. These results are similar to the temperatures between 25 and 30 ºC reported by Braga & Lima (2001), but slightly higher than those by De Castro *et al.* (2012). The growth performance of bullfrogs in a continuous-flow commercial aquaculture system was the primary focus of this study. Our results indicated that density did not have a significant effect on survival rate (Table 1).

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**Table 1. Results of *t*-test analysis for growth performance in bullfrogs cultured for 45 days under two stocking densities in continuous flow-through tank systems.**

| Parameters | Densities | | ***P* – value** |
|---|---|---|---|
| | **30 Frogs/m³** | **60 Frogs/m³** | |
| Final weight | 45.2 ± 4.99 | 55.3 ± 6.91 | 2.2e-16 |
| Final length (cm) | 8.6 ± 0.65 | 9.0 ± 0.55 | 8.607e-08 |
| Final biomass (kg) | 149.44 | 361.41 | 0.0472 |
| Survival (%) | 90 | 93 | 0.0779 |
| Feed intake (kg) | 52.69 | 127.16 | 0.0461 |
| AFCR | 1.2 | 2.4 | 0.0123 |
| AGR (g/day) | 0.97 | 1.2 | 0.2843 |
| SGR (%/day) | 7.28 | 7.73 | 0.3708 |

Values represent means ± SD.

The bullfrog's survival rates were above 90 %, as described by De Castro *et al.* (2012), but 20 % higher than those reported by Rodríguez-Serna *et al.* (1996). Throughout the experiment, mortality was consistently observed and linked to predatory cannibalism by larger individuals. An overall survival rate of 70 % can be considered satisfactory in aquaculture (Benetti *et al.,* 2002). In this study, the high survival rates were likely due to a combination of factors: maintaining good water quality through continuous supply and exchange, using high-quality proven feed, providing adequate aquaculture resources, and performing regular tank maintenance throughout the culture period. Rodríguez-Serna *et al.* (1996) mention that the bullfrog is very sensitive to high densities and recommend a density between 50 and 100 bullfrogs/m$^2$ in vertical production systems. Our results suggest we can stock a CWFTS with an initial density of 60 bullfrog/m$^2$ without compromising survival. Our results also indicate that stocking density significantly affected bullfrog growth over the 45-day culture period. The results also showed that stocking density did not significantly affect absolute growth rate or survival but did significantly influence feed intake, final length, and final biomass. Notably, the final weight (and consequently biomass) was lower at 30 frogs/m² than at 60 frogs/m² after the 45-day culture period (Figure 1).

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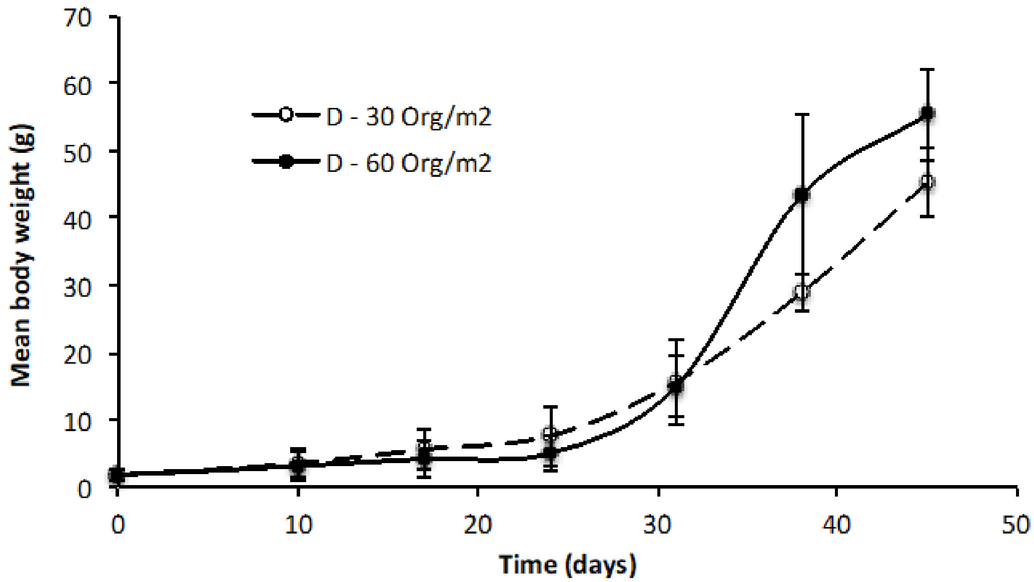


**Figure 1. Weight gain of bullfrogs reared at two stocking densities (30 vs. 60 frogs/m²) in a continuous water-flow system over a 45-day period.**

For fattening issues, density comparisons are relevant to determine their relative weight behavior and plan harvest. On a commercial scale using CWFTS, bullfrogs exhibited successful growth at both stocking densities, with significantly better performance observed at 60 bullfrogs/m². The final average weight was similar during the first 30 days of age; however, from 32 to 45 days, the density of 60 frogs/m$^2$ presented a substantial weight and size increase compared to the density of 30 frogs/m$^2$ (Figure 1). Studies in frog farming have shown that the body size of aquaculture organisms increases with increasing population density. Our study suggested a positive influence when the culture density was increased to 60 frogs/m². This aligns with the direct positive relationship reported for bullfrogs reared in vertical systems at 50 and 100 frogs/m² (Rodríguez-Serna *et al.,* 1996) and for bullfrog tadpoles (Flores-Nava & Vera-Muñoz, 1999). The AGR (Absolute growth rates) of hatchery-reared bullfrogs in this study was similar to that reported by Braga & Lima (2001) for bullfrogs (mean 1.01 g/day). In the present study, juvenile bullfrogs (starting weight: 1.70 g) cultured for 45 days at two densities achieved an SGR greater than 7 %/day. This value far exceeds the SGR range of 4.9–5.5 %/day reported by Medeiros-Maia *et al.* (2022) for bullfrog tadpoles fed diets formulated from different protein sources. Although these results are not conclusive, the observed difference could be attributed to both the higher initial weight and the more efficient culture conditions in our experiment. De Castro et al. (2012) reported an average AFCR of 1.80 over 60 days, while Braga and Lima (2001) noted an AFCR range of 1.40–1.46 for growing-phase bullfrogs (mean weight increase from 37.5 to 90.12 g over 45 days). Our results are consistent with these findings. At the lower density of 30 frogs/m², we recorded an

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AFCR of 1.4; at 60 frogs/m², the AFCR increased to 2.4. This trend aligns with the AFCR values reported by Rodríguez-Serna et al. (1996) at densities of 50 and 100 bullfrogs/m².

Regarding the length-weight relationship, our results suggested bullfrog exhibits negative allometric growth (Figure 2; Table 2) characterized by length growth being slower than weight growth and organisms becoming slender as length increases, these results coincide with Pauly (1984) suggestions for length-weight estimates for fish in tropical water. The effects of captivity on fish morphology, characterized by a greater increase in weight than in length ($b \geq 3$), have been previously demonstrated (e.g., Benetti *et al.,* 2002). This pattern is likely due to reduced energy expenditure and lipid accumulation in captive organisms. For example, Hernández *et al.* (2016) reported a condition factor (b) of 3.1 for spotted rose snapper cultured in cages, supporting this general finding. The frogs' developmental stage may explain the negative allometric growth observed in this study. They had recently completed metamorphosis (R1-R4 larval period) to become imagos (average weight: 1.7 g), a stage characterized by tail reabsorption and internal organ accommodation. As described by Coppo (2003), frogs in this stage and up to ~50 g undergo substantial weight gain, primarily through increased muscle mass and fat—a phase known as pre-fat gain. After the imago period, amphibians enter a fattening stage to reach slaughter weight (Mansano *et al.,* 2017). Identifying the optimal stocking density during this phase is therefore critical in aquaculture, as it directly supports the primary objective of accelerating growth for commercial profit (Jurado-Molina *et al.,* 2023). To this end, it has been identified that, among the production parameters, weight gain is the main factor affecting economic profitability. Therefore, optimizing culture density is one of the most valuable control tools to generate strategic planning and maximize economic benefits; so, these initial results provide additional information that could help to improve economic benefits for aquaculturists. However, these results should not be considered conclusive but rather an incentive to conduct new studies with a broader range of densities to identify suitable productive strategies for this species. To our knowledge, these are the first studies on weight and length growth estimates for this species. These findings enhance our understanding of bullfrog behavior under production conditions and provide key insights for developing improved strategies to optimize their growth and yield.

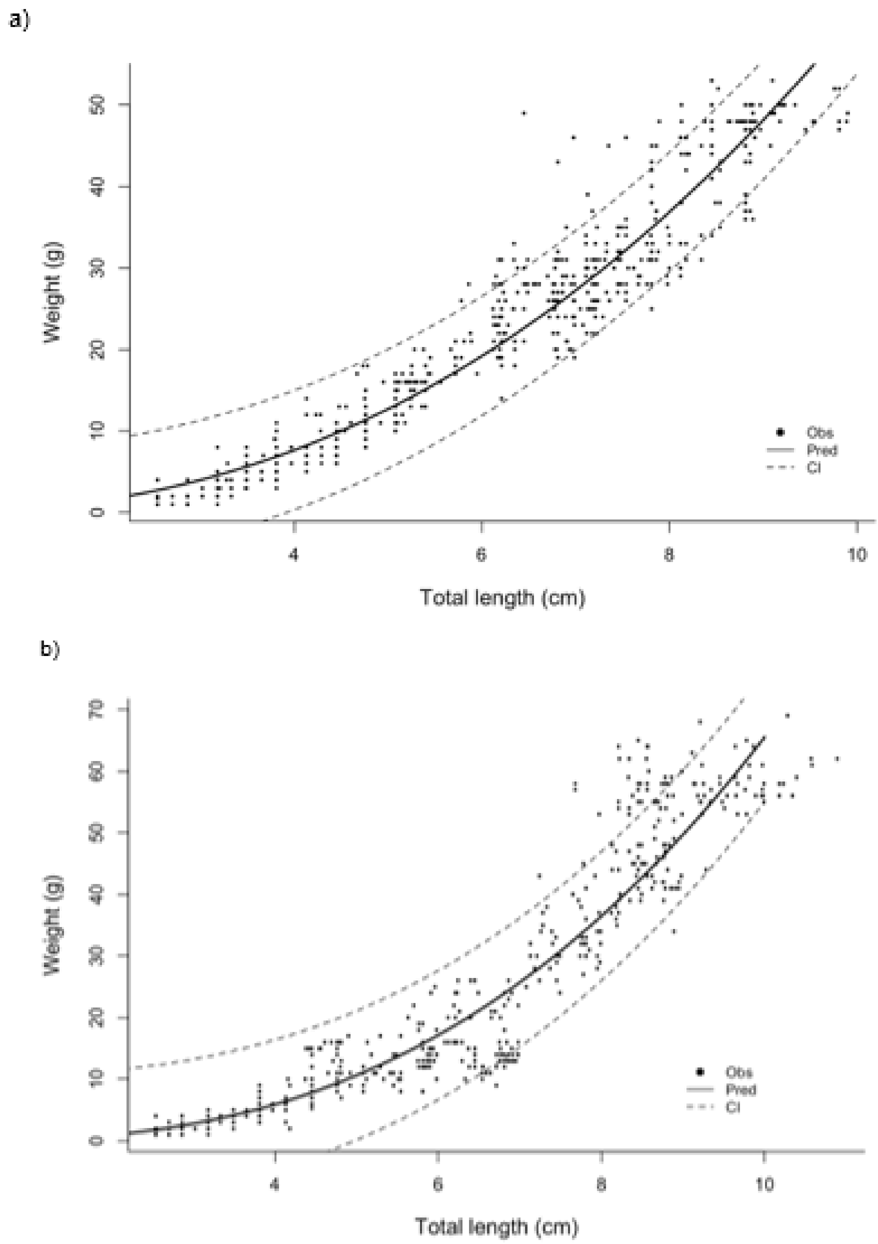


**Figure 2. Length-weight relationship for hatchery-reared bullfrogs cultured in a continuous-flow water tank system.**

Data are shown for two stocking densities: 30 org/m² (a) and 60 org/m² (b). Solid circles represent observed values; the solid line represents the predicted model fit; and the shaded band represents the 95 % confidence interval (CI).

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**Table 2. Results of the length-weight for bullfrogs cultured in continuous water-flow tank systems for 45 d**

| Density | Parameter | Estimate | Standard error | *t*-value | *P* (>\|*t*\|) |
|---|---|---|---|---|---|
| 30 frogs/m$^2$ | *a* | 0.33014 | 0.01787 | 18.48 | $<2 \times 10^{-16}$ |
| | *b* | 2.26778 | 0.02626 | 86.36 | $<2 \times 10^{-16}$ |
| 60 frogs/m$^2$ | *a* | 0.15574 | 0.01404 | 11.09 | $<2 \times 10^{-16}$ |
| | *b* | 2.62417 | 0.04205 | 62.41 | $<2 \times 10^{-16}$ |

## Conclusions

This study demonstrates that selecting an optimal stocking density significantly improves growth performance in bullfrog aquaculture. Production at 60 frogs/m² resulted in an 18.3 % higher final weight and approximately 58.6 % greater biomass gain compared to 30 frogs/m². These findings indicate that using an adequate culture density is a key technical factor for enhancing the viability and productivity of commercial frog farming.

## Authors contribution

Conceptualization of the work (CHHL, JJM). Methodology development (CHHL, JJM). Software management (JJM, CHHL). Experimental validation (CHHL, JAHY). Results analysis (CHHL, JJM). Data management (CHHL, JAHY). Manuscript writing and preparation (CHHL, JJM, JMJ). Writing, revising, and editing (CHHL, JJM, JMJ). Project manager (CHHL). Acquisition of funds (CHHL, JAHY).

All authors of this manuscript have read and accepted the published version of the manuscript.

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## Conflict of interest

The authors declare that they have no conflicts of interest.

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