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Full Length Article

Growth and Metabolic Effects of Stocking Density in Bullfrog Tadpoles (*Rana catesbeiana*) under Greenhouse Conditions

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Abstract

The density-dependents and physiological effects in bullfrog tadpoles (*Rana catesbeiana* Shaw, 1802) was evaluated to asses optimum stocking density. During 70 days, five treatments (1/4, 1/3, 1/2, 1 and 2 tadpole L^{-1}) were evaluated under greenhouse conditions. Environmental variables, water quality parameters, biometric data, oxygen consumption and nitrogen excretion were measure every 3 weeks. At the end of experiment, the treatment of 1/4 tadpole L^{-1} obtained the highest weight, length and biomass. However, maximum survival (92.59%) and estimated final biomass (0.3051 g L^{-1}) was found in the treatment of 1/3 tadpole L^{-1}. Statistical differences were found in oxygen consumption (Kruskal-Wallis, H=19.935, P<0.001). Average values of QO_2 ranging from 6.59 (2 tadpoles L^{-1}) to 2.1 mg O_2 h^{-1} g^{-1} (1/4 tadpole L^{-1}). Statistical differences were also found in ammonium excretion (H=17.089, P<0.002), in which a maximum value of 0.366 mg NH_3-N h^{-1} g^{-1} corresponded to the density of 1/2 tadpole L^{-1}, and a minimum value of 0.122 mg NH_3-N h^{-1} g^{-1} was observed in the density of 1/3 tadpole L^{-1}. We suggest a stocking density of 1 tadpole per 3 L in order to minimize stress and optimize water. © 2015 Friends Science Publishers

Keywords: Growth performance; Water utilization; Metabolic rate; Relative density

Introduction

The increasing world population demands high consumptions rates in natural resources like water, energy and food (Beddington, 2011). New culture technologies that minimize environmental pollution and maximize biological synthesis of high quality protein with low saturated fatty acids are required. The culture of bullfrog (*Rana catesbeiana*) is a relative new promising option to satisfy these demands. However, due the complexity of its life cycle, a set of specific culture techniques must be applied in every life stage.

During tadpole stage, an adequate management is crucial to maintain high surveillance and reach metamorphose stage in the shortest possible time. The consequences are that early developed tadpoles are better candidates to achieve higher growth rates than lately developed ones (Browne *et al.,* 2003). In addition, the use of greenhouses in tadpole stages can help in many ways the farm performance, excluding predators and maintaining stable temperature and humidity (Rodriguez-Serna *et al.,* 1996).

To determine which factors are crucial to obtain well-developed tadpoles, several studies have been carried out. Evaluations that include temperature (Figueiredo *et al.,* 1999; Tavares-Braga and Lopes-Lima, 2001), photoperiod (Bambozzi *et al.,* 2004) and nutrition (Carmona-Osalde *et al.,* 1996; Olvera-Novoa *et al.,* 2007) are examples of work done in this field. In addition, stocking density is also an important factor in tadpole stage (Martínez *et al.,* 1996). Focus in this spotlight is, for example, the reported by Dash and Hota (1980), who studied density effects on the survival, growth rate, and metamorphosis size of *R. tigrina* tadpoles. As well as found that survival during larval period was independent of initial population density. They also found that the proportion of population that successfully completed metamorphosis was a negative exponential function of density. In other study, Browne *et al.* (2003) observed that high density rearing techniques with *Litoria aurea* tadpoles produces positive effects in development: high quality metamorphose, both shorten rearing time and metamorphosis period, and more efficient use of water, feed and space.

For practical purposes, some studies proposed optimal stock densities in bullfrog tadpoles. For example, Adams and Bruinsma (1987) proposed a range of 1 to 2 tadpoles L^{-1}, while Hayashi *et al.* (2004) proposed 2.5 tadpoles L^{-1} using net tanks. However, no one of the aforementioned studies

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takes into account the physiological tadpole response. To be more specific, no one determined the crowd-stress response. To achieve this goal, a common indirect method is to measure the oxygen consumption, because is a physiological parameter that is indirectly related to metabolic rate. In addition, the byproducts of nitrogen metabolism (like ammonia) can also be used to determine stress condition (Willmer *et al.,* 2004).

In this study, we assume a direct relationship beetwen nitrogen excretion and oxygen consumption reflected in final wet weight.

Stocking density in bullfrog tadpoles culture could have different growth and metabolic effects, for this the aim of this work is to evaluate the growth and metabolic effects of stocking density in bullfrog tadpoles under greenhouse conditions. A thorough analysis of the data could determinate an adequate stocking density, taking into account a diminished stressful conditions combined with optimum growth performance.

Materials and Methods

Experimental Treatments

Location: The experiment facilities were located in the central part of México (100° 26' W, 20° 73' N, 1920 m.a.s.l.), in an experimental campus of the Querétaro State University. The experimental setup was located inside a plastic polyethylene greenhouse with a culture area of 214.5 m^2 (24.10 m × 8.90 m) with no ventilation, covered with a shade mesh to reduce the incidence of solar radiation.

Experimental setup: Ten circular polyethylene tanks (0.27 m depth, 0.61 m diameter) with capacity of 80 litres were placed in three different vertical levels. Five treatments of tadpole stocking density were used in this experiment (1/4, 1/3, 1/2, 1 and 2 tadpole L^{-1}), which represents an absolute density of 20, 27, 40, 80 and 160 tadpoles per tank, respectively (Fig. 1). No water exchange was done throughout the experiment, but in order to increase organic matter oxidation every tank was equipped with a 2-watts oxygen aquarium pump coupled with a diffuser stone. Water losses were replaced adding makeup water every week. The tadpoles were fed *ad libitum* four times a day with commercial balanced food minced containing 35% protein, 4% fat, 12% humidity, 10% ash and 5% fiber.

Environmental variables: Data of air temperature (°C) and relativity humidity (%) inside greenhouse were measured every 30 min with automatic data loggers (Spectrum technologies, Watchdog Model 1000, USA). Water temperature was measured every 30 min with temperature sensor placing in one tank per treatment. However, in the case of 1/2 tadpole L^{-1} the data was not recorded. Experimental was carried out from August 16 to October 24, 2011. At the end of the experiments the data were manipulated in Microsoft Excel © datasheets for its analysis.

Water quality: Data of water temperature was measured every 30 min. A total of 5 sensors (one per treatment) were placed inside water tanks. The sensors were connected to external data loggers (Spectrum technologies, Watchdog Model 1000, USA) to record data. Initial and final concentrations of ammonia, nitrate and nitrite were determined spectrophotometrically (Hach® model 2600, 8155 N-salicylate method, 8171 Cadmium reduction method and 8507 Diazotizatran method).

Biological Measurements

A total of 654 tadpoles were used in this experiment. All tadpoles were born in the same cohort 35 days before the experiment. Daily visual inspection was done at 8:00 am to check the correct function of the system and to report tadpole deaths and report atypical behaviour. To obtain meristic data, representative samples of 10% of total stocking density were taken every 17 days, with exception of the final measure, which was taken on day 19. Wet weight (g) and total length (mm) were measure using a digital scale (Startorius m. Prove Series AY303, Precision: ± 0.001 g, USA) and an electronic vernier (Mitutoyo, CD-6"PSX, Resolution: 0.01 mm, Precision: ± 0.02 mm, Japan).

Productivity indexes: Data of final biomass, specific growth rate, average daily weight and biomass density were estimated according with the methodology proposed by Alatorre-Jacome *et al.* (2012). In addition, total mortality and survival for each treatment were obtained at the end of experiment.

Metabolic performance: After 70 days, 2 individuals of each treatment were randomly measured for aerobic metabolism (oxygen consumed=QO_2) using a closed respirometric chamber technique with constant temperature and known water volume (Steffensen, 1989). Measurements of dissolved oxygen, temperature and ammonia excretion were taken every 4 h (09:00, 13:00, 17:00, 21:00, 01:00 and 05:00) during a 24 h cycle. Tadpoles were euthanized on ice with 40% ethanol solution immediately after finishing the experiment and dried for 96 h at 70°C in order to determine its dry weight for to get relation of the oxygen consumed by biomass (Barker *et al.,* 2002).

Statistical Analysis

Non-parametric Kruskall-Wallis test was done to compare means between treatments. This test was used because there is no normality presented. Correlations were performed using the Pearson correlation coefficient and probability (Walpole, 1999). The statistical analysis was made using STATGRAPHICS software (Stat Point Inc. 2006).

Results

Environmental Performance

The hourly variation recorder for each treatment can be seen on Fig. 2. Daily average water temperature varies among treatment from 18.57 to 21.01°C. The highest average temperature was found in the tank with a 1/4 tadpole L^{-1} treatment and the lowest average temperature in the 1/3 tadpole L^{-1} treatment.

Water Quality

In all cases, the initial pH was 7.1. Initial ammonium concentrations were low in densities lower than 1 tadpole per litre (0.09 to 0.11 mg L^{-1}), and in the other two cases (treatments of 1 and 2 tadpole L^{-1}) higher ammonium concentrations were measure (0.48 and 0.32 mg L^{-1}, respectively). An initial nitrites level varied from 0.018 to 0.064 mg L^{-1}, and range of nitrate concentrations varied from 2.1 to 2.5 mg L^{-1}. At the end of the experiment, the pH value was 9.4 for all treatments. Ammonia concentration also increased, with a maximum value of 1.49 mg L^{-1} in treatment of 1 tadpole L^{-1}. However, a nitrite and nitrate concentration declined close to zero at the end of the experiments in all treatments.

Tadpole Growth Performance

Statistical differences were found in final individual length (mm) and final individual weight (g) (Table 3). Maximum values were found in treatment of 1/4 tadpole L^{-1}, whereas the lowest values were observed in treatment of 2 tadpoles L^{-1}. Lower densities (1/3 and 1/2 tadpole L^{-1}) presented high values of surveillance, in contrast to higher densities (1 and 2 tadpole L^{-1}) where low surveillance were presented.

The highest and lowest specific growth rate were observed on 1/4 tadpole L^{-1} and 2 tadpole L^{-1}, respectively. Similar data were observed in average daily weight gain, where treatment with 1/4 tadpole L^{-1} density presented the highest value and treatment of 2 tadpole L^{-1} presented the lowest value. The biomass increased in treatment 1/4 tadpole L^{-1} was 4.28 times greater than 2 tadpole L^{-1} treatment.

Fig. 3(A) shows weight performance for each treatment. Treatment of 1/4 tadpole L^{-1} presented higher weight than the other treatments. At the final of the experiment, significant differences were observed between treatments (H=39.5245, P<0.05). The higher weight all over the experiment was observed in the treatments with less density, with exception of the treatment of 1/3 tadpole L^{-1}. The lowest weight was observed the treatment of 2 tadpoles per litre.

In case of individual length measures, there were no significant differences at the beginning of the experiment. However, 17 days after initial culture, the values of 1/3 and 1/4 tadpole L^{-1} were higher than the other three treatments. From day 34 until the end of this study, significant differences were noted between the treatments (H=15.8063, P<0.05). At the end of the study, tadpoles cultivated in a density of 1/4 tadpole L^{-1} growth 173.9% more in length than tadpoles cultivated in a density of 2 tadpole L^{-1} (Fig. 3B).

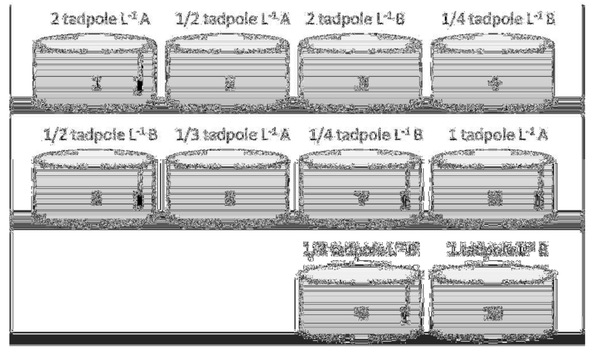

Fig. 1: Scheme of tanks distribution for the experiment. Temperature sensors were placed in tanks 1, 5, 7, 8 and 9

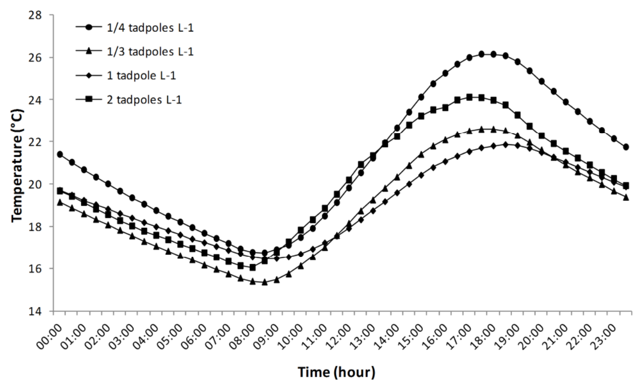

Fig. 2: Average water temperatures of tanks at each stocking density under greenhouse conditions during a 24-h cycle

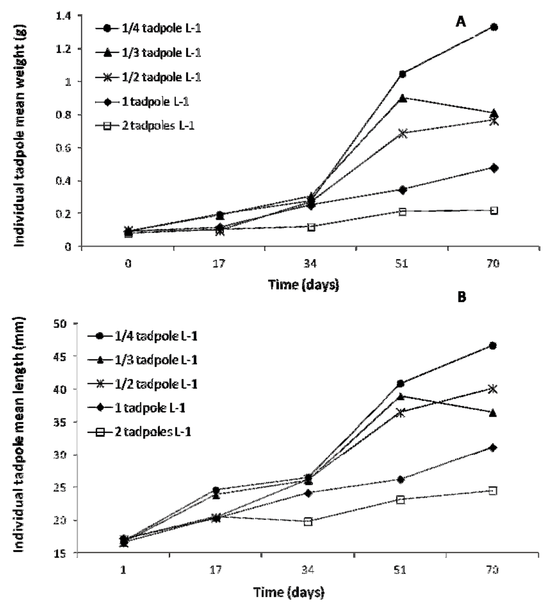

Fig. 3: Average tadpole weight (A) and length (B) during the experimental time

Table 1: Average daily water temperature, greenhouse temperature and relativity humidity

Temperature (° C)	1/4 tadpole L^{-1}	1/3 tadpole L^{-1}	1/2 tadpole L^{-1}	1 tadpole L^{-1}	2 tadpole L^{-1}	Environment	Relative Humidity (%)
Average daily minimum	17.54 ± 0.33	15.56 ± 0.26	*	15.07 ± 0.19	16.72 ± 0.18	20.22 ± 0.43	52.99 ± 2.15
Overall mean	21.01 ± 0.42	18.57 ± 0.32	*	19.21 ± 0.25	20.16 ± 0.35	23.54 ± 1.02	42.06 ± 1.08
Average daily maximum	24.02 ± 0.81	21.86 ± 0.65	*	21.99 ± 0.46	22.56 ± 1.13	25.66 ± 1.85	91.08 ± 3.8

*No data recorded

Table 2: Initial and final pH and nitrogen metabolites measured in culture water

	1/4 tadpole L^{-1}		1/3 tadpole L^{-1}		1/2 tadpole L^{-1}		1 tadpole L^{-1}		2 tadpole L^{-1}	
Parameter	Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final
pH	7.1	9.4	7.1	9.4	7.1	9.4	7.1	9.4	7.1	9.4
Ammonia (mg/L)	0.09	0.54	0.11	1.23	0.1	0.35	0.48	1.49	0.32	1.31
Nitrate (mg/L)	2.1	0	2.4	0.2	2.5	0	2.3	0.2	2.5	0
Nitrite (mg/L)	0.018	0.001	0.019	0.006	0.025	0.006	0.058	0.006	0.064	0.068

Table 3: Parameters measured in stock density treatments of *Rana catesbeiana* tadpoles

Parameter	1/4 tadpole L^{-1}	1/3 tadpole L^{-1}	1/2 tadpole L^{-1}	1 tadpole L^{-1}	2 tadpoles L^{-1}
Number of tadpoles per tank	20	27	40	80	160
Initial Mean Individual Length (mm) ± SEM	16.72 ± 0.82	16.57 ± 0.83	16.66 ± 0.41	17.22 ± 0.22	17.11 ± 0.18
Final Mean Individual Length (mm) ± SEM	46.71 ± 0.45[a]	36.45 ± 1.83[a]	40.11 ± 1.92[a]	31.14 ± 0.65[b]	24.50 ± 0.138[b]
Initial Mean Individual Weight (g) ± SEM	0.093 ± 0.002	0.090 ± 0.001	0.099 ± 0.011	0.094 ± 0.002	0.081 ± 0.005
Final Mean Individual Weight (g) ± SEM	1.33 ± 0.000[a]	0.813 ± 0.042[a]	0.763 ± 0.092[b]	0.477 ± 0.003[c]	0.220 ± 0.000[c]
Survival (%)	85.0 ± 10.0	92.59 ± 7.41	92.50 ± 2.50	74.38 ± 1.88	72.5 ± 13.8
Specific Growth Rate (%)	3.63	3	2.78	1.77	1.31
Average Daily Weight Gain (g day^{-1})	0.0169	0.009	0.009	0.003	0.001
Percentage of Biomass increment (%)	1430.1	903.33	770.7	341.192	261.52

[a, b] Means in the same row with different superscripts differ ($P < 0.05$) according to Kruskal-Wallis test

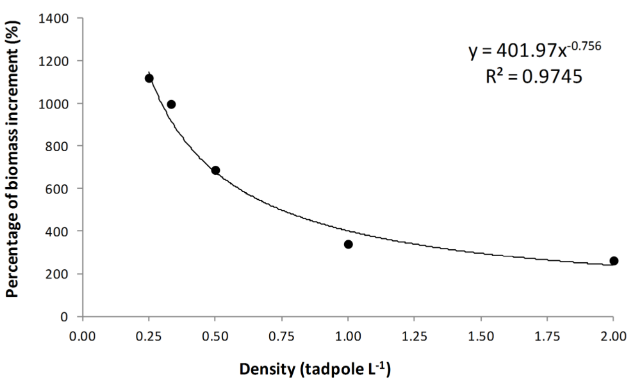

Fig. 4: Percentage of biomass increment (%) as a function of relative tadpole density

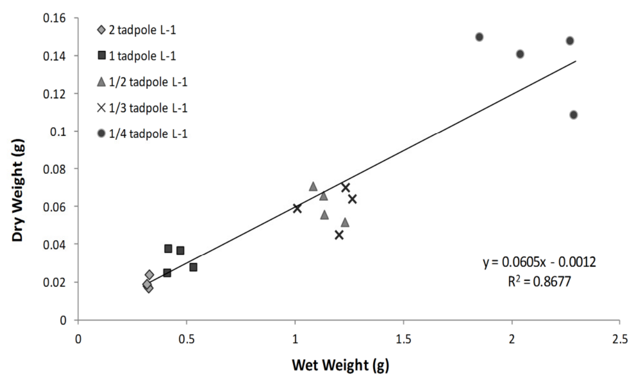

Fig. 5: Correlation of wet weight and dry weight in tadpoles after 70 days treatment for each density

In addition, a direct relationship of percentage of biomass increment and relative density was found (Fig. 4). The performance of this parameter shows a potential function of the form $y = a\,x^b$ (R^2=0.9745). It can be seen that biomass increment trends to increase if the density decreased. In contrast, a linear relation was found among wet weight and dry weight (Fig. 5). Three clusters were formed in direct relation with the tadpole density.

Metabolic Performance

Values of QO_2 ranged from 1.25 to 15.90 mg O_2 h^{-1} g^{-1} (Table 4). Maximum values were observed in treatment of 2 tadpoles L^{-1} whereas the minimum value is present in 1/2 tadpole L^{-1}. Treatments with low densities (1/4, 1/3 and 1/2 tadpole L^{-1}) presented stable QO_2 values during all day cycle. Higher densities showed variability on QO_2 values during all day cycle.

Table 4: Overall oxygen consumption in tadpole density treatments during a 24-hour cycle

Treatment	1/4 tadpole L^{-1}	1/3 tadpole L^{-1}	1/2 tadpole L^{-1}	1 tadpole L^{-1}	2 tadpole L^{-1}
n	12	12	12	12	12
Minimum	1.399	1.893	1.254	1.621	2.077
Maximum	2.959	4.876	3.941	5.925	15.909
Mean ± SEM (mg g^{-1} h^{-1})	2.100±0.15	3.433±0.26	2.633±0.27	3.687±0.47	6.594±1.20
Range	1.559	2.982	2.687	4.303	13.831
Standar Deviation	0.531	0.912	0.962	1.573	4.162
Coefficient of variation (%)	24.6	26.56	36.53	42.67	63.13

Table 5: Statistical data of tadpole ammonia excretion by treatment

Treatment	1/4 tadpole L^{-1}	1/3 tadpole L^{-1}	1/2 tadpole L^{-1}	1 tadpole L^{-1}	2 tadpole L^{-1}
n	5	5	5	5	5
Minimum	0.062	0	0.304	0.193	0
Maximum	0.188	0.183	0.514	0.43	0.199
Mean ± SEM (mg g^{-1} h^{-1})	0.138±0.02	0.115±0.03	0.365±0.03	0.328±0.04	0.121±0.03
Range	0.126	0.183	0.21	0.237	0.199
Standar Deviation	0.048	0.075	0.086	0.097	0.076
Coefficient of variation (%)	34.73	65.51	23.63	29.58	61.99

Hourly recorders of QO_2 are illustrated in Fig. 6. Treatments of 2, 1, and 1/3 tadpole L^{-1} showed both the higher oxygen consumption (Fig. 6A, B and D). These treatments tend to decreased oxygen consumption from 1:00 to 9:00 h. Densities of 1/2 tadpole L^{-1} and 1/4 tadpole L^{-1} show lower oxygen consumption than other treatments (Fig. 6C, and E). At 17:00 hour tadpole increased O_2 consumption in treatment of 2, 1, 1/3 and 1/4 tadpoles L^{-1}. In some cases, this variation was more pronounced. For example, in the treatment of 2 tadpoles L^{-1} the variation was high during the experiment; mainly at 17:00 hours (Fig. 6A). In contrast, treatment of 1/2 tadpoles L^{-1} maintain constant levels of QO_2 all day long. In general, higher levels of QO_2 were observed during the evening and afternoon time (Fig. 6F). On the other hand, the ammonia excretion by hour during a day indicates that lowest ammonia excretion occurred at 21:00 h and there was an increasing tendency within 21:00 and 5:00 hours (Fig. 7A). However, there was no statistical differences between h ($P <0.05$). Fig. 7B shows QO_2/NH_3-N ratio. Treatments of 1, 1/2 and 1/4 tadpoles L^{-1} shows a low ratio QO_2/ NH_3-N ratio, while 1/3 and 2 tadpole L^{-1} treatment has a high ratio.

Lowest ammonia excretion was noted in treatment of 1/3 tadpoles L^{-1} (0.115 mg h^{-1} g^{-1}), meanwhile treatment of 2 and 1/4 tadpoles L^{-1} presented low ammonia excretion rates. However, in treatments 1/3, 1/4 and 2 tadpoles L^{-1} no statistical differences were found. Treatments of 1/2 and 1 tadpoles L^{-1} had a high ammonia excretion, in which the highest ammonia excretion was noted in treatment of 1/2 tadpoles L^{-1} (0.366 mg h^{-1} g^{-1}).

Finally, there existed remarkable differences among estimated ratios of oxygen consumption and ammonia excretion. For example, in the treatment of 1/2 tadpoles L^{-1} the calculated ratio was 7.2, meanwhile in the treatment of 2 tadpoles L^{-1} was 54.46. This means a difference of the metabolic nitrogen efficiency of 756%. However, the density was not a determinant factor in this case (Fig. 7b).

Discussion

Differences in water temperature between treatments can be attributed to vertical tanks positions (Soto-Zarazúa *et al.,* 2011), with more light incidence in upper tanks. However, in this study a higher temperature was recorded in a tank located in the middle level of the experiment (1, 1/2 and 1/4 tadpole L^{-1}) (Fig. 1). These results suggested a possible vertical temperature gradient. Nevertheless, during the experiment the daily tank temperature performance was very similar, and there were no statistical differences in hourly analysis ($P>0.05$). Moreover, the observed performance was similar to temperature curves described in other productive systems (Goudriaan and Van Laar, 1992). In general, during the experimental period the average water temperature was lower than optimum values for frog production (Brown *et al.,* 2003). It is also known that temperature affected greatly both tadpole development and metamorphosis (Álvarez and Nicieza, 2002). In this case, results suggested no noise factor caused by temperature in the results.

No water exchange was carried out in this experiment, because bacterial organic matter consumption was assumed (Avnimelech, 2006). In order to optimized water consumption, we assume biological filtration and nitrogen intake by algae and bacteria (Ebeling *et al.,* 2006). However, higher levels of pH and ammonia were observed at the end of the experiment. This combination is very harmful for almost all aquaculture species, and in some circumstances it could be lethal (Kuo-Feng and Kuo-Lin, 2004). Some behavioural signs can be observed if ammonia intoxication occurs, like lack of motion, side-swimming or loss of sensorial response (Alatorre-Jácome *et al.,* 2012).

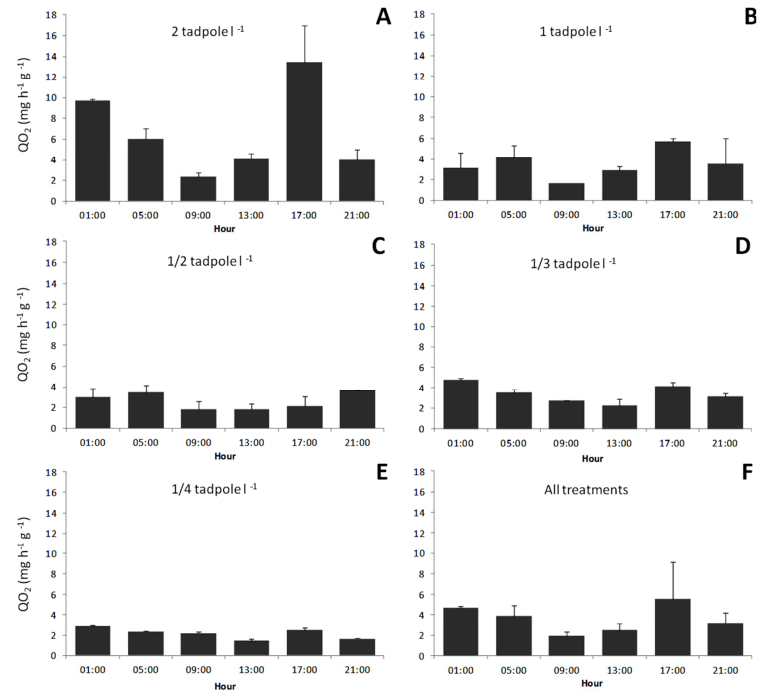

Fig. 6: Oxygen consumption by treatment during a 24-h cycle

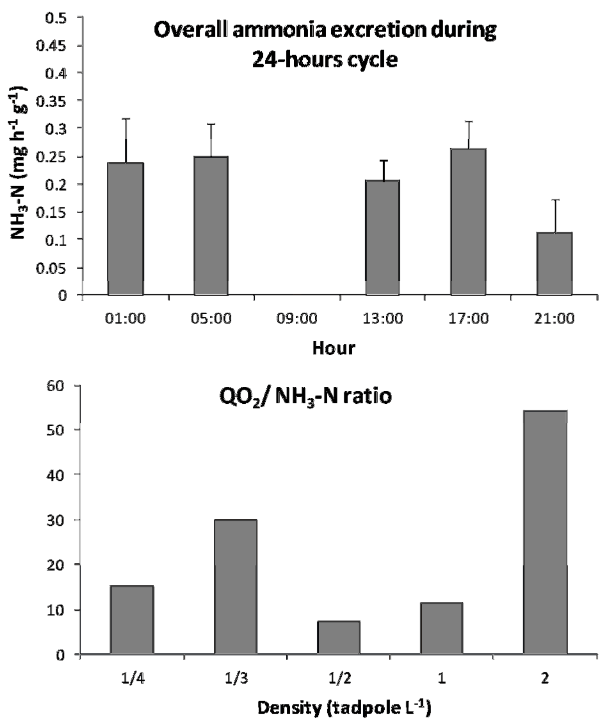

Fig. 7: Tadpole ammonia excretion by time (A). QO_2/NH_3-N ratio (B)

In this experiment none of the aforementioned symptoms were observed. It could be explained by the observations made by Wright and Wright (1996), who found that tadpoles are capable to metabolize environmental ammonia into urea.

Considerable differences in growth performance were observed in this study. The growth curves for weight and length showed a remarkable attenuation caused by density (Fig. 3), as has been reported in other similar studies. However, in these studies the reported final weights are higher than the values found in this work. For example, Hayashi *et al.* (2004) reported weights of 9.8, 9.77, 10 10.43 and 11.08 g at 2.5, 2, 1.5 1 and 0.5 tadpoles L^{-1}, respectively. In other experiment, Browne *et al.* (2003) reported individual final weights of 2.1, 1.9, 1.65 and 1.42 g per tadpole in densities of 40, 80, 120 and 160 tadpoles L^{-1}, respectively. In general it can be difficult to make an accurately comparisons among studies, because Brown *et al.* (2003) studied *L. aurea* in Australia and Hayashi *et al.* (2004) studied *R. catesbeiana* in Brazil. Besides, factors must to be taken into account, like temperature variations, differences in experimental set-ups, feeding rates and, water quality management.

In addition, the relative amount of growth could be described as a function of relative density. A negative exponential function describes this behaviour. It is remarkable than $-\mathrm{b} \approx \frac{3}{4}$, similarly to other allometric relationships (West *et al.,* 1997) like 3/4 power law for metabolic rates. It is in agreement with this theory if we considered the density instead weight for metabolic rate estimation. The implications are that the percentage of biomass increment is highly related with metabolic rate, which is a major topic to discuss in ecological synthesis (Willmer *et al.,* 2004). Other relationship was found among wet weight and dry weight. This function indicated that water content in tadpole is proportional to dry weight. During experimental period, any treatment had to metamorphosis because the photoperiod at this latitude was not appropriate for that to take place.

The results obtained in the respirometric measures suggested that higher densities consumed more oxygen. Oxygen consumption is a physiological parameter that is indirectly related to metabolic rate. Under stress conditions, metabolic rate is high and physiological functions are affected (Okie, 2012). On the other hand, ammonia excretion indicates a low approach of protein intake. When ammonia excretion is high, nitrogen of food intake is not assimilated into organic tissue, and growth performance is decreased. In addition, it is well-known that high concentrations of un-ionized ammonia could be toxic for a large range of aquatic animals (Alatorre-Jácome *et al.,* 2011). In this case, the addition of carbohydrates to water culture can help to reduced un-ionized ammonia concentration (Olvera-Olvera *et al.,* 2009). Carbohydrates promote of development of heterotrophic bacteria population able to process the inorganic nitrogen, organic matter, phosphate and other wastes of tadpole culture produced by the unconsumed food and tadpole excretion (Dewedar *et al.,* 2006).

High densities of tadpole culture causes stress conditions that affects growth (metamorphic size, metamorphic time), oxygen consumption and survival (Browne *et al.,* 2003). In this experiment, the treatment of 1/3 tadpole L^{-1} presented high growth performance and survival, meanwhile its oxygen consumption and ammonia excretion was low. It would be advisable to use this density for initial periods of culture to promote tadpole growth and once getting the right size for metamorphosis, increasing crop density to force metamorphosis. Based on the obtained evidence, density of 1/3 tadpole L^{-1} could be considered a metabolic-comfortable density for growth and survival of bullfrog tadpoles.

In conclusion, stocking density had an effect on individual growth performance and metabolic rate of tadpoles of *R. catesbeiana* under greenhouse conditions. Higher densities (2 and 1 tadpole L^{-1}) of culture had a low growth and high metabolic rate which indicate a stress condition. Density of 1 tadpoles per 3 L is recommended in order to reduce metabolic stress and maximum growth performance in greenhouse conditions.

Acknowledgments

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