QUALITY OF THE EFFLUENTS OF BULLFROG TADPOLE PONDS*

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ABSTRACT

The objective of this study was to determine the quality of the effluent from ponds populated with bullfrog tadpoles by evaluating the physical, chemical and microbiological parameters of the water. Samples were collected bi-weekly on the ponds located at the bullfrog culture sector in the Caunesp. The experimental design was completely randomized with two treatments (inlet and outlet water) and six repetitions (ponds). There was no difference (*P>0.01*) among treatments for conductivity, however, the values varied in different samples according to growth of tadpoles. The values of temperature, pH, dissolved oxygen and nitrate were higher (*P<0.01*) in the inlet, while total phosphorus, ammonia, *Escherichia coli*, chemical oxygen demand (COD), biochemical oxygen demand (BOD$_5$) and turbidity had increased concentrations in the outlet water. The total phosphorus and BOD$_5$ concentrations were higher than permitted by Brazilian environmental legislation. The management adopted to rear bullfrog tadpoles increased the nutrients in the water, with the highest concentrations of ammonia and total phosphorus deriving mainly from the uneaten feed and animal excretion, the latter being the main factor for water eutrophication.

Keywords: water quality; frog culture; phosphorus

QUALIDADE DE EFLUENTES DE TANQUES DE GIRINO DE RÃ-TOURO

RESUMO

O objetivo deste trabalho foi avaliar a qualidade do efluente de tanques de criação de girinos de rã-touro através da análise das variáveis físicas, químicas e microbiológica da água. As amostras foram coletadas quinzenalmente nos tanques do setor de ranicultura do Caunesp. O delineamento experimental utilizado foi inteiramente casualizado, com dois tratamentos (água de entrada e saída dos tanques) e seis repetições (tanques). Não houve diferença significativa (*P>0,01*) entre os tratamentos para condutividade, entretanto, os valores variaram nas diferentes coletas, conforme o crescimento dos girinos. Os valores de temperatura, pH, oxigênio dissolvido e nitrato foram maiores (*P<0,01*) na água de entrada, enquanto as concentrações de fósforo total, amônia, *Escherichia coli*, demanda química de oxigênio DQO, demanda bioquímica de oxigênio (DBO$_5$) e turbidez tiveram um aumento na água de saída. As concentrações de fósforo total e DBO$_5$ foram maiores do que o permitido pela legislação ambiental brasileira. O manejo realizado na criação de girinos provocou um aumento de nutrientes na água, sendo que as maiores concentrações de amônia e fósforo total foram provenientes, principalmente, da ração e das excretas dos animais.

Palavras chave: qualidade de água; ranicultura; fósforo

INTRODUCTION

In Brazil, frog culture has been gaining importance in the national scenario as an alternative production in the agricultural sector (FEIX *et al.*, 2006). Bullfrog is an excellent option for commercial rearing due to its high fecundity, rapid sexual maturation, large body size and climate adaptability (GRANT and BOTH, on line).

The successful rearing of tadpoles depends on the quality and quantity of feed, feed particle size and composition, and mainly the water quality used in the culture. These factors have a direct influence on growth rate of the animals (SIPAÚBA-TAVARES *et al.*, 2008).

Some studies showed that in water containing large amounts of nitrogenous pollutants, the development of anuran larvae may be slower while deformity occurrence rate may be higher (RUIZ *et al.*, 2010). Frog larvae, since they are aquatic, they mostly excrete nitrogen waste such as ammonia (BALINSKY, 1970). This residue being toxic to the organisms themselves must be constantly removed from the system.

In shallow ponds, such as those used for this kind of rearing, the continuous water flow, wind and rain often cause water circulation and transform it into a dynamic ecosystem (SIPAÚBA-TAVARES and MORENO, 1994). Therefore, various factors can influence these systems, including management and climate.

Already, the impact of the effluent quality on the receiving water body depends mainly on the species and density of the cultivated organisms, feeding management adopted and technological level of the farm (BOYD, 2000). Effluent pollutants come mainly from feeding, either directly in the form of uneaten or leached feeds, animal digesta and excretory products, or indirectly through water eutrophication and consequent increased natural productivity (TACON and FORSTER, 2003).

In order to observe the environmental impacts caused by rearing management, the present study aimed to evaluate the water quality of the inlet (supply) and outlet (effluent) of ponds populated with bullfrog tadpoles (*Lithobates catesbeianus*), through physical, chemical and microbiological analysis. The results were compared with environmental legislation regarding to effluent quality for aquaculture.

MATERIAL AND METHODS

The study was conducted at the frog culture sector, in the Aquaculture Center of Unesp, Jaboticabal, SP (21°15'S and 48°19'W) from November 2008 to January 2009. The experiment lasted 76 days, until the tadpoles completed metamorphosis.

The experimental design was completely randomized (CRD), with two treatments (inlet and outlet water) and six replicates (ponds) in a split-plot, with the sub-plots collection over time. Water samples were collected in the morning at inlet (supply) and outlet (effluent) of the tadpole rearing ponds, every fifteen days, totaling six collections (C0, C1, C2, C3, C4 and C5).

Six excavated outdoor ponds, lined with polyethylene were used. Each pond had 1,600 L capacity, with dimensions of 2.0 x 1.2 x 0.70 m (length, width and depth), equipped with individual continuous flow water inlet and outlet (Figure 1).
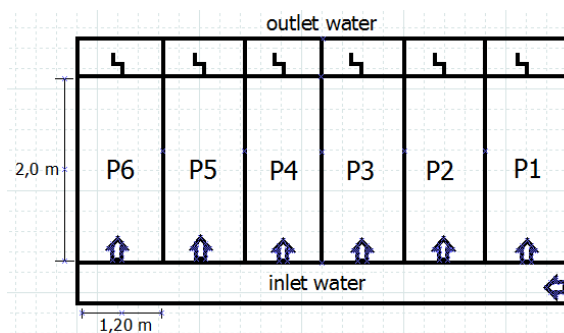
Figure 1. Layout of tadpole ponds (P1 – P6) with continuous water flow in the bullfrog culture sector.

The frog culture ponds were supplied with water from a pond located upstream from the Frog culture Sector. This pond is used to keep breeding fish and to receive the effluents from the parallel ponds and laboratories at Caunesp. The effluent is launched *in natura* directly into another pond downstream from this sector.

Four thousand and eight hundred bullfrog tadpoles (*L. catesbeianus*) were used. The tadpoles

were from the same spawn, in stage 25 of development (GOSNER, 1960), with an average initial weight of 0.21 ± 0.01 g and distributed at a stocking density of 0.5 tadpoles L⁻¹.

Ponds average flows were 9,504 and 6,912 L day⁻¹ for inlet and outlet water, respectively. The inlet and outlet water flow rates were calculated measuring the time needed to fill a one-liter recipient, and are expressed in liters per day.

Tadpoles were fed a commercial diet for carnivorous fish with 40% crude protein that was launched on the pond surface. The feed quantity was adjusted based on the biomass present in the pond, starting with 9% of total biomass and decreasing according to the weight of the animals (LIMA et al., 2003b). The biomass of each pond was obtained according to biometrics, by measuring monthly the total weight of 10% of tadpoles, using a digital electronic balance with 0.01 g precision to determine mean weight (g).

The physico-chemical and microbiological variables analyzed were temperature, dissolved oxygen (using a Lutron Dissolved Oxygen Meter), conductivity, pH (Phtek handset) and turbidity (Hach turbidimeter).

Total phosphorus (acid hydrolysis method), ammonia (colorimetric method), nitrate (cadmium reduction), biochemical oxygen demand (potassium hydroxide solution), chemical oxygen demand (colorimetric method) and *Escherichia coli* (most probable number - MPN) were evaluated according to Standard Methods for Examination of Water and Wastewater (APHA, 2005).

The nutrient load was calculated using the formula:

$$C = [N] \times Q,$$

which,

C = nutrients load;

[N] = nutrient concentrations; and

Q = flow rate of each treatment.

The results were expressed in grams per day (g day⁻¹).

To verify the normality and homocedasticity of the data Shapiro-Wilks e Bartlett statistical tests were applied. The data of the limnological variables were submitted to variance analysis (repeated measures ANOVA) and, subsequently, to Student test at 1% probability, to check whether there was significant difference between treatments (inlet and outlet water) for each time (collection). Polynomial regression analysis was applied in each treatment to assess the variation in time. The software utilized was SAS 9.1 (SAS, 2006).

The limnological variables were compared with standards of effluent quality according to class II, established in Brazilian environmental legislation CONAMA Nº 357 Resolution (BRASIL, 2005).

RESULTS

There were no differences ($P>0.01$) among treatments for the variable conductivity and there were differences ($P<0.01$) for all other variables. In the sampling periods all variables there were difference ($P<0.01$) among time. Thus, temperature, pH and DO were higher in inlet than in outlet water, while the opposite was observed for the other variables (Table 1). The polynomial effect for the inlet and outlet water indicates the variation between collections, whereas the higher order demonstrates a greater time variation between the samples.

The average daily temperature varied between 25 to 27°C for both inlet and outlet water throughout the period and was higher in the inlet water in last collections, remaining in the ideal range for growth of organisms. The variation into collection was high (4º degree) for both treatments. In this study, weight gain was 9.4 ± 0.01 g, feed conversion 1.1 and survival rate 69%.

The values of pH showed small variation among treatments. The decrease observed in the effluent after collection three can be attributed to increase of respiration and size of organisms present in the water (phytoplankton and tadpoles). The variation into collection was high (5º degree) for both treatments.

Table 1. Mean values of the physical, chemical and microbiological variables for treatments (inlet and outlet water) at different collections. Means followed by the same letters in the column, for each variable, do not differ by Student 1%. The polynomial effect indicate the variation into the collections (over time).

Variables	TR	Collections (days)						Mean	Polynomial Effect
		0 (1)	1(16)	2 (31)	3 (46)	4 (61)	5 (76)		
Temp (°C)	I	26.50[a]	26.88[a]	27.37[a]	26.77[a]	25.60[a]	27.37[a]	26.75	4° Degree
	O	26.57[a]	26.70[a]	27.05[a]	26.58[a]	25.25[b]	26.97[b]	26.52	4° Degree
pH	I	7.92[a]	7.37[a]	7.70[a]	8.83[a]	8.28[a]	7.70[a]	7.97	5° Degree
	O	7.97[a]	7.35[a]	7.55[a]	8.23[b]	7.80[b]	7.48[b]	7.73	5° Degree
DO (mg L^{-1})	I	6.10[a]	7.14[a]	7.50[a]	8.34[a]	6.62[a]	5.28[a]	6.83	5° Degree
	O	6.63[a]	7.84[a]	6.50[b]	7.30[b]	4.70[b]	4.92[a]	6.15	5° Degree
EC (µS cm^{-1})	I	77.68[a]	52.00[a]	66.50[a]	69.00[a]	65.50[a]	72.00[a]	66.86[a]	4° Degree
	O	72.20[a]	57.33[a]	66.17[a]	66.83[a]	73.83[a]	72.83[a]	68.03[a]	3° Degree
Turb (NTU)	I	19.48[a]	14.27[a]	14.17[a]	15.47[a]	20.40[b]	14.42[b]	16.37	4° Degree
	O	13.00[b]	12.10[a]	15.38[a]	15.40[a]	26.16[a]	17.95[a]	16.43	5° Degree
E. coli (MPN 100mL^{-1})	I	39.17[a]	1,210[a]	84.17[a]	35.00[b]	1,026[a]	1,020[a]	569.25	4° Degree
	O	52.33[a]	1,308[a]	105.67[a]	338.83[a]	814.52[a]	991.23[a]	587.18	5° Degree
TP (mg L^{-1})	I	0.59[a]	0.83[b]	1.21[a]	0.59[a]	0.39[b]	0.77[b]	0.73	5° Degree
	O	0.58[a]	1.23[a]	1.17[a]	0.64[a]	1.88[a]	1.24[a]	1.12	5° Degree
N-NH$_3$ (mg L^{-1})	I	0.17[a]	0.18[a]	0.23[b]	0.34[b]	0.39[b]	0.30[b]	0.27	3° Degree
	O	0.16[a]	0.20[a]	0.33[a]	0.39[a]	0.82[a]	0.43[a]	0.40	5° Degree
N-NO$_3$ (mg L^{-1})	I	0.43[a]	0.37[a]	0.37[b]	0.45[a]	0.58[b]	0.53[a]	0.45	3° Degree
	O	0.36[a]	0.37[a]	0.48[a]	0.47[a]	0.68[a]	0.58[a]	0.49	5° Degree
BOD$_5$ (mg L^{-1})	I	11.00[a]	4.17[a]	3.00[b]	1.33[a]	3.17[b]	1.50[b]	4.03	3° Degree
	O	12.33[a]	6.33[a]	8.00[a]	2.00[a]	8.55[a]	4.67[a]	6.89	5° Degree
COD (mg L^{-1})	I	15.67[a]	17.50[a]	36.50[a]	16.50[a]	23.00[b]	26.50[a]	22.61	5° Degree
	O	17.50[a]	11.67[a]	33.50[a]	24.33[a]	50.50[a]	27.83[a]	27.56	5° Degree

*I – inlet water; O – outlet water; TR - treatment; Temp – temperature; DO – dissolved oxygen; EC – electrical conductivity; Turb – turbidity; E. coli – **Escherichia coli**; TP – total phosphorus; N-NH₃ – ammonia; N-NO₃ – nitrate; BOD₅ – biochemical oxygen demand; COD – chemical oxygen demand.*

The dissolved oxygen of the inlet water ponds ranged from 5.28 to 8.34 mg L^{-1} and of the outlet water ponds from 4.70 to 7.30 mg L^{-1} during the period, not reaching critical levels for the tadpoles. The variation into collection was high (5° degree) for both treatments. However, lower values have been found for the bullfrog grow-out phase (Table 2).

Table 2. Mean values of six collections of limnological variables of the effluents from bullfrog tadpole and grow-out ponds.

Limnological variables	Bullfrog tadpole pond (present study) (n = 36)	Bullfrog grow-out pond (BORGES *et al.*, 2012)(n = 36)
Temperature (°C)	26.52	28.2
pH	7.7	7.2
Dissolved oxygen (mg L^{-1})	6.15	1.23
Conductivity (µS cm^{-1})	68	249
Turbidity (NTU)	16	66
Total phosphorus (mg L^{-1})	1.12	6.09
Ammonia (mg L^{-1})	0.39	6.94
Nitrate (mg L^{-1})	0.49	2.37
BOD$_5$ (mg L^{-1})	7	74
COD (mg L^{-1})	28	378
E. coli (MPN 100mL^{-1})	5.8×10^2	1.1×10^4

BOD₅ – biochemical oxygen demand; COD – chemical oxygen demand.

Conductivity lowest levels were 52 µS cm^{-1} and 57 µS cm^{-1}, and highest 78 µS cm^{-1} and 74 µS cm^{-1} for inlet and outlet water, respectively, indicating low presence of organic particles or salt ions. The higher variation into collection was in the inlet water (4° degree) compared to the outlet water (3° degree).

The effluent turbidity values ranged from 12 to 26 NTU, being close to the ones of water supply, between 14 to 20 NTU and was higher in the outlet water in last collections. The higher variation into collection was in the outlet water (5° degree) compared to the inlet water (4° degree). The turbidity of the inlet water may be associated with phytoplankton originated from pond that supplies the frog culture sector.

The thermotolerant coliforms number had some peaks both in the inlet water and effluent, 35 to 1,210 MPN 100mL^{-1} and 52 to 1,309 MPN 100mL^{-1}, respectively, and followed the same pattern among the two until the collection three, where there was a significant difference. After this, the pattern reversed, without significant differences between the treatments. The higher variation into collection was in the outlet water (5° degree) compared to the inlet water (4° degree).

The total phosphorus concentration in the outlet was significantly higher (0.58 to 1.88 mg L^{-1}) than in the inlet water (0.39 to 1.21 mg L^{-1}) at the collections one, four and five. In the collection 4 a decrease in the temperature was observed, which may have affected the tadpoles food consumption, increasing remains and, consequently, the phosphorus concentrations in the water. The variation into collection was high (5° degree) for both treatments.

The same was observed for ammonia (varying from 0.17 to 0.39 mg L^{-1} for inlet and 0.16 to 0.82 mg L^{-1} for outlet water) and nitrate (0.37 to 0.58 mg L^{-1} for inlet and 0.36 to 0.68 mg L^{-1} for outlet water). The higher variation into collection was in the outlet water (5° degree) compared to the inlet water (3° degree) for both variables.

The biochemical oxygen demand varied from 1 to 11 mg L^{-1} in the inlet water and from 2 to 12 mg L^{-1} in the effluent and there was difference between treatments in collection two, four and five.

The chemical oxygen demand was relatively low in both treatments (16 to 36 mg L^{-1} in the inlet and 12 to 50 mg L^{-1} in the outlet water), throughout the period, increasing significantly in the effluent in collection four when it reached 50.50 mg L^{-1}. The higher variation into collection was in the outlet water (5° degree) compared to the inlet water (3° degree) for BOD$_5$ and the variation into collection was high (5° degree) for both treatments for COD.

Table 3 shows the mean concentrations of total phosphorous, ammonia and nitrate (mg L^{-1}) present in different phases of bullfrog rearing and the respective load (g day^{-1}). Despite the relatively low values of ammonia and nitrate when compared with the load of these nutrients, they are considered to be representative and when compared to the grow-out phase, it is observed that phosphorous is the most representative between them.

Table 3. Mean concentrations of total phosphorus (TP), ammonia (N-NH$_3$) and nitrate (N-NO$_3$) in different phases of bullfrog rearing and the load produced per day.

Variables	Bullfrog tadpole ponds (present study) (n = 36)		Bullfrog grow-out ponds (BORGES *et al.*, 2012) (n = 36)	
	Concentration (mg L^{-1})	Load (g day^{-1})	Concentration (mg L^{-1})	Load (g day^{-1})
Total P	1.12	7.74	6.09	11.57
N-NH$_3$	0.39	2.69	6.94	13.19
N-NO$_3$	0.49	3.39	2.37	4.50

C = [N] x Q, which: C = nutrients load; [N] = nutrient concentrations; and Q = flow rate of each treatment.

In Table 4, were compared the medium values of the limnological variables of the water supply and effluent of bullfrog tadpoles ponds during the experimental period, with the maximum values allowed by Resolution Nº 357 of the National Environmental Council (CONAMA) of 2005 to freshwater (Class II), for aquaculture and fishing activity (BRASIL, 2005).

Table 4. Means of biotic and abiotic variables of treatments (inlet and outlet water) over the timer compared with the values showed in environmental legislation to freshwater class II.

Limnological Variables	Medium values and standard treatments deviation		CONAMA Nº 357/2005 (BRASIL, 2005)
	Inlet	Outlet	Class II
Temperature (ºC)	26.75 ± 0.66	26.52 ± 0.80	< 40
pH	7.97 ± 0.50	7.73 ± 0.34	6.0 a 9.0
DO (mg L⁻¹)	6.83 ± 1.14	6.15 ± 1.15	> 5.0
EC (μS cm⁻¹)	66.86 ± 14.66	68.03 ± 10.0	-
Turbidity (NTU)	16.37 ± 3.16	16.43 ± 4.94	< 100
TP (mg L⁻¹)	0.73 ± 0.38	1.12 ± 0.58	< 0.030
Ammonia (mg L⁻¹)	0.27 ± 0.08	0.39 ± 0.22	2.0 to 7.5 < pH ≤ 8.0
Nitrate (mg L⁻¹)	0.45 ± 0.10	0.49 ± 0.12	< 10.0
BOD₅ (mg L⁻¹)	4.03 ± 3.77	6.89 ± 4.55	< 5.0
COD (mg L⁻¹)	22.61 ± 11.84	27.56 ± 6.90	-
E. coli (MPN 100mL⁻¹)	569.3 ± 768.9	587.2 ± 705	< 1,000

*DO - dissolved oxygen; EC - electrical conductivity; TP - total phosphorus; BOD₅ - biochemical oxygen demand; COD - chemical oxygen demand; E. coli - **Escherichia coli**.*

Only the total phosphorus concentrations, for inlet and outlet water, and BOD₅, for outlet water, were higher than permitted by Brazilian environmental legislation.

DISCUSSION

The temperature remained around 26ºC while the ideal mean temperature for frog culture is 25ºC (FERREIRA *et al.*, 2002). According to LIMA *et al* (2003a), water temperature influences directly tadpole metabolism due to their being ectothermic, promoting faster growth and weight gain in ideal thermal conditions.

The performance parameters found in this study were considered satisfactory for production (LIMA and AGOSTINHO, 1992). According to the authors a feed conversion average of 2.0:1.0 and survival around 70% can be adopted in commercial frog farms.

SIPAÚBA-TAVARES *et al.* (2007) while working with sequential ponds for semi-intensive fish production at Caunesp observed during the rainy season (summer) a mean conductivity value of 46 μS cm⁻¹ in the pond that supplies water to the tadpole rearing ponds and 113 μS cm⁻¹ in the pond that receives the effluent from the frog culture ponds. The authors stated that high and low conductivity values indicate high degree of decomposition and sharp primary production, respectively. Since the values found were within the range reported by the author, they are considered characteristic of Caunesp ponds and associated with high production of phytoplankton organisms associated with the increase of feed in the water.

The maximum turbidity values for inlet and outlet water were 20 and 26 NTU, respectively, which indicated the presence of clays and colloidal dissolved organic matter (SIPAÚBA-TAVARES, 1994) or a phytoplankton, that serve for a natural food for the tadpoles.

A biodegradable effluent should have a COD/BOD ratio lower than five (VALENTE *et al.*, 1997). In this study, the mean COD/BOD ratio of the effluent during the period was four, thus indicating the biodegradability of the resulting wastewater.

Biochemical oxygen demand (BOD_5) was higher in the pond effluent compared to supply water and was above permitted by CONAMA Resolution N° 357/2005 (BRASIL, 2005); however, this value was about ten times lower than the grow-out phase (BORGES *et al.*, 2012), showing that the nutrient load in this stage is smaller and there is a larger water renewal.

The high values of *E. coli* can occurred probably due to rainfall over sampling period, with average values of 82 mm in November, 279 mm in December and 238 mm in January. It should also be noted that some *E. coli* contamination occurs previously in the pond that supplies water to the tadpole ponds. There is a correlation between the high number of fecal coliform during the warmest and rainiest days of the year, with the input of allochthonous organic matter and resuspending of sediment of the pond loaded with fecal coliform (PIANETTI *et al.*, 2004).

CASTRO and PINTO (2000) reported pH values around 7.33, towards the alkaline range, using the same stocking density (0.5 tadpoles L^{-1}). There is a tendency for lower pH values as the animals grow, however, in collection three there was an increase in inlet water pH that may have influenced the outlet water.

Pond water flow rates provided good oxygenation of the water, keeping in general, mean dissolved oxygen values at approximately 6 mg L^{-1}. Decreasing dissolved oxygen level in the water is related to both the respiration of the animals and the decomposition of organic matter by bacteria. In the case of aquaculture activities, including frog farming, the organic matter increase is due primarily to the addition of artificial feed in the pond owing to need to increase food according to animal growth.

The inlet water of the tadpole ponds came from the upstream pond that receives the effluent from the sequential ponds used to rear fish; and therefore, entered the ponds with mean total phosphorus level of 0.73 mg L^{-1}. The average total phosphorus (1.12 mg L^{-1}) found in the effluent may also be related to high crude protein content (40%) of the feed. The inlet and outlet water had phosphorus concentrations above those permitted by legislation.

Tadpole feces release high rates of ammonia and phosphorus into the medium (FLORES-NAVA and GASCA-LEYVA, 1997). The animals assimilate approximately 25% of phosphorus contained in the feed and the remainder is excreted in the feces (insoluble form) or urine (soluble form). Therefore, the diet ingredients should have low phosphorus levels and be easily digestible (HARDY, 2000) since the nutrients that are not assimilated either end up in the water as dissolved nitrogen and phosphorus or are incorporated into the sediment pond as particulate matter (Bergheim *et al.*, 1991 *apud* HENRY-SILVA and CAMARGO, 2008).

Other studies indicate higher concentrations of ammonia but water renewal and the effect of nutrient dilution must be taken into account. CASTRO and PINTO (2000), using the same stocking density, feed with 45% crude protein and 20% daily water renewal, found ammonia values between 0.25 and 0.78 mg L^{-1} (average 0.54 mg L^{-1}), higher than the present study (average 0.39 mg L^{-1}), indicating the relationship between feed and water flow, which promotes dilution and ammonia nitrification.

SIPAÚBA-TAVARES *et al.* (2008) comparing treatments with and without feed concluded that using artificial feed causes an increase of ammonia and reduction of nitrate levels, the first being more toxic for the organisms. This was not observed in the present study, where both had lower concentrations, probably due to the highest water renewal rate in the ponds (432%) compared for the author (5%).

As shown in BORGES *et al.* (2012) the fattening phase of bullfrogs have greater load of ammonia, nitrate and phosphorus. The load is associated to a nutrient concentration with the water flow. In the tadpole stage the volume of water in ponds is greater and the nutrient concentration is lower, even then the load is lower than in the growing phase.

CONCLUSIONS

For the conditions of this study, suitable stocking density, good feed conversion and high water flow, it can be concluded that the main problem observed is the high phosphorus load, since this component is primarily responsible for

artificial eutrophication of water bodies. Although pond inlet water already has high phosphorus concentration, there was still a sizeable increase under the experiment conditions. It is, therefore, necessary to remove phosphorus compounds from the effluent before releasing it to the environment. Management techniques aiming at optimizing water use in frog farms should be adopted and studies to test different methods of removal of dissolved nutrients should be carried out to adjust the bullfrog rearing to Brazilian environmental law.

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REFERENCES

APHA - American Public Health Association. 2005 *Standard methods for examination of water and wastewater*. 21ed. USA: Washington. 1085p.

BALINSKY, J.B. 1970 Nitrogen metabolism in amphibians. In: CAMPBELL, J.W. (Ed.) *Comparative Biochemistry of Nitrogen Metabolism*. USA: New York. p.519-637.

BORGES, F.F.; AMARAL, L.A; STÉFANI, M.V. 2012 Characterization of effluents from bullfrog (*Lithobates catesbeianus*, Shaw, 1802) grow-out ponds. *Acta Limnologica Brasiliensia*, 24(2): 160-166.

BOYD, C.E. 2000 *Water quality: an introduction*. Kluwer Academic Publishers. USA: Massachusetts. 291p.

BRASIL, 2005 RESOLUÇÃO CONAMA Nº 357, de 17 de março de 2005. Dispõe sobre a classificação dos corpos de água e diretrizes ambientais para o seu enquadramento, bem como estabelece as condições e padrões de lançamento de efluentes, e dá outras providências. *Diário Oficial da União*, 18 de março de 2005, Nº 53, Seção 1, p.58-63.

CASTRO, J.C and PINTO, A.T. 2000 Qualidade da água em tanques de girinos de rã-touro, *Rana catesbeiana* Shaw, 1802, cultivados em diferentes densidades de estocagem. *Revista Brasileira de Zootecnia*, 29(6): 1903-1911 (Suplemento 1).

FEIX, R.D.; ABDALLAH, P.R.; FIGUEIREDO, M.R.C. 2006 Resultado econômico da criação de rã em regiões de clima temperado, Brasil. *Informações Econômicas*, 36(3): 70-80.

FERREIRA, C.M.; PIMENTA, A.G.C.; PAIVA NETO, J.S. 2002 *Introdução à ranicultura*. São Paulo: Boletim Técnico do Instituto de Pesca, 33: 15p.

FLORES-NAVA, A. and GASCA-LEYVA, E. 1997 Use of artificial grazing substrates in bullfrog tadpole culture. *Aquaculture*, 152(1-4): 91-101.

GOSNER, K.L. 1960 A simplified table for staging anuran embryos and larvae with notes on identification. *Herpetologica*, 16(3): 183-190.

GRANT, T. and BOTH, C. (*on line*). *Rã-touro no Brasil*. Available at: <http://www.ib.usp.br/grant/Ra-touro_no_Brasil/Potenciais_Impactos.html> Access on: 9 Apr. 2013.

HARDY, R.W. 2000 Advances in the development of low pollution feeds for salmonids. *Global Aquaculture Advocate*, 3(2): 63-74.

HENRY-SILVA, G.G. and CAMARGO, A.F.M. 2008 Impacto das atividades de aquicultura e sistemas de tratamento de efluentes com macrófitas aquáticas – relato de caso. *Boletim do Instituto de Pesca*, 34(1): 163-173.

LIMA, S.L. and AGOSTINHO, C.A. 1992 *A tecnologia de criação de rãs.* Viçosa: UFV. 168p.

LIMA, S.L.; CASALI, A.P.; AGOSTINHO, C.A. 2003a Desempenho zootécnico e percentual de consumo de alimento de rã-touro (*Rana catesbeiana*) na fase de recria (pós-metamorfose) do sistema anfigranja. *Revista Brasileira de Zootecnia*, 32(3): 505-511.

LIMA, S.L.; CASALI, A.P.; AGOSTINHO, C.A. 2003b Desempenho zootécnico e tabela de alimentação de girinos de rã-touro (*Rana catesbeiana*) criados em sistema anfigranja. *Revista Brasileira de Zootecnia*, 32(3): 512-518.

PIANETTI, A.; BRUSCOLINI, F.; SABATINI, L.; COLANTONI, P. 2004 Microbial characteristics of marine sediments in bathing area along Pesaro-Gabicce coast (Italy): a preliminary study. *Journal of Applied Microbiology*, 97: 682-689.

RUIZ, A.M.; MAERZ, J.C.; DAVIS, A.K.; KEEL, M.K.; FERREIRA, A.R.; CONROY, M.J.; MORRIS, L.A.; FISK, A.T. 2010 Patterns of development and

abnormalities among tadpoles in a constructed wetland receiving treated wastewater. *Environmental Science & Technology*, 44: 4862–4868.

SAS 9.1. 2006 *Business analytics software for windows 2006*. North Carolina: Institute Inc. Cary.

SIPAÚBA-TAVARES, L.H. 1994 *Limnologia aplicada à aquicultura.* Jaboticabal: Boletim Técnico do CAUNESP. 70p.

SIPAÚBA-TAVARES, L.H. and MORENO, S.Q. 1994 Variação dos parâmetros limnológicos em um viveiro de piscicultura nos períodos de seca e chuva. *Revista UNIMAR*, 16(4): 229-242.

SIPAÚBA-TAVARES, L.H.; GUARIGLIA, C.S.T.; BRAGA, F.M.S. 2007 Effects of rainfall on water quality in six sequentially disposed fishponds with continuous water flow. *Brazilian Journal of Biology*, 67: 643-649.

SIPAÚBA-TAVARES, L.H.; MORAIS, J.C.L.; STÉFANI, M.V. 2008 Comportamento alimentar e qualidade de água em tanques de criação de girinos de rã-touro *Lithobates catesbeianus*. *Acta Scientiarum Animal Science*, 30: 95-101.

TACON A.G.J. and FORSTER I.P. 2003 Aquafeeds and the environment: policy implications. *Aquaculture*, 226(1-4): 181–189.

VALENTE, J.P.S.; PADILHA, P.M.; SILVA, A.M.M. 1997 Oxigênio dissolvido (OD), demanda bioquímica de oxigênio (DBO) e demanda química de oxigênio (DQO) como parâmetros de poluição no ribeirão Lavapés/Botucatu – SP. *Eclética Química*, 22: 49-66.