

## Bullfrog (*Lithobates catesbeianus*) farming system: water quality and environmental changes

Sistema de criação de rã-touro (*Lithobates catesbeianus*): qualidade da água e mudanças ambientais

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**Abstract: Introduction:** Frog farming, if not well managed, may cause environmental damages. The use of antibiotics, the organic discharge and the introduction of exotic species can disseminate risks such as eutrophication, changes in the water quality and organic pollution, factors that affect the human consumption. **Aim:** Evaluating the water quality of a bullfrog farming system, discussing their relations to production and the environment based on the current legislation. **Methods:** Sampling was performed on a monthly basis from November 2006 to March 2007 during growth and fattening phases of bullfrog (*Lithobates catesbeianus*). Sample sites were distributed according to the water flow: upstream from the mixing zone, affluent (supply water), bay, effluent, mixing zone and downstream from the mixing zone. In the field, pH, electrical conductivity, dissolved oxygen, temperature and turbidity were measured. In laboratory, nitrogen, phosphorus and chlorophyll *a* concentrations were analyzed. **Results:** The concentration of nutrients was determiner for water quality in the bay and its effluent. According to the current legislation, the effluent exceeded the limits for total phosphorus ( $> 0.030 \text{ mg L}^{-1}$ ) and total nitrogen ( $> 1.27 \text{ mg L}^{-1}$ ). Other variables presented acceptable values in light of the current laws. **Conclusion:** The high values of nutrients and other factors such as conductivity and turbidity are proportional to the animal growth due to the inadequate management practices evidenced by feed conversion rate. The following management options are proposed: maintaining the flow and decreased density of animals; maintaining the flow and density storage with adequate control of the food supply.

**Keywords:** management, aquaculture, phosphorus, nitrogen, effluent.

**Resumo: Introdução:** O cultivo de rãs, se não bem manejado, pode causar danos ambientais. Uso de antibióticos, descarga orgânica e introdução de espécies exóticas podem promover eutrofização, alteração das águas e poluição orgânica afetando o consumo humano. **Objetivo:** Avaliar a qualidade da água de sistema de criação de rã-touro discutindo sobre sua relação com a produção e o meio ambiente baseado na legislação vigente. **Métodos:** As amostragens foram mensais de novembro/2006 a março/2007, abrangendo as fases de crescimento e engorda de rã touro *Lithobates catesbeianus*. Os locais de amostragem foram de acordo com o fluxo hídrico: a montante da zona de mistura, afluente (abastecimento), baía de criação, efluente, zona de mistura e a sua jusante. No campo, foram medidos os valores de pH, condutividade elétrica, oxigênio dissolvido, temperatura e turbidez. No laboratório foram analisadas as séries de nitrogênio e fósforo bem como a clorofila *a*. **Resultados:** A concentração de nutrientes foi determinante da qualidade da água da baía e de seu efluente. Com relação à legislação vigente, o efluente excedeu os limites estabelecidos para fósforo total ( $> 0,030 \text{ mg L}^{-1}$ ) e nitrogênio total

(> 1,27 mg L<sup>-1</sup>). **Conclusão:** Elevados valores de nutrientes e de outros fatores como condutividade e turbidez foram proporcionais ao crescimento dos animais podendo ser relacionadas às práticas de manejo inadequadas evidenciadas pela taxa de conversão alimentar. As seguintes alternativas de manejo são propostas: manutenção da vazão e diminuição da densidade de animais; manutenção da vazão e da densidade de estocagem com controle adequado da oferta de alimento.

**Palavras-chave:** manejo, aquicultura, fósforo, nitrogênio, efluente.

## 1. Introduction

Over the last decades, several studies have been conducted aiming the improvement of the bullfrogs' production (*Lithobates catesbeianus*). The analyzed aspects are related to nutritional value, economic viability of commercial food, effects of toxicity, biochemistry and physiology of the species, pathologies and treatments, growth rate and molecular biology (Olvera-Novoa et al., 2007; França et al., 2008; Najiah et al., 2009; Pasteris et al., 2011; Castro et al., 2012; Mendonza et al., 2012).

According to FAO (2009), as any other aquaculture activity, frog farming may cause environmental damages if not well managed. Factors such as organic discharge, use of antibiotics, introduction of exotic species, among others can disseminate risks such as eutrophication, changes in the water quality and organic pollution, affecting human consumption. This organization proposes mitigation strategies such as effluent treatment, its reuse in agriculture and the use of low-polluting food. As stated by FAO (2009) likewise, it is necessary the development of specific rules for frog farming, since it is an important activity in many regions. These rules should be based on general principles of the "Code of Conduct for Responsible Fisheries", articles 9 and 9.1.3 (FAO, 1995), that recommends that each State should elaborate and regulate aquaculture development strategies as a requirement to ensure its ecological sustainability, allowing the rational use of resources in different applications.

In Brazil, the interest in bullfrog *L. catesbeianus* farming is growing because of the taste and texture of the meat, making it a great potential in aquaculture (França et al., 2008; Sipaúba-Tavares, 2008).

However, research focused on environmental issues and water quality in aquaculture is scarce not only in Brazil, but worldwide, particularly in bullfrog farming. Borges et al. (2012) is the only study in Brazil about discharge of bullfrogs farming effluents. Nevertheless, it is possible to find some studies dealing with the issue indirectly,

for example the use of fertilizers in agriculture as a cause for amphibians decline in natural systems (Hamer et al., 2004).

Bishop et al. (1999) proposed that high nitrogen and phosphorus concentrations might affect the population and the community structure because the amphibians make use of water for reproduction. These authors demonstrated that exposure to nitrates can alter the feeding activity, mobility and reduce the growth and development processes of amphibian larvae. Flores-Nava (2000) reported some limnological parameters in water bodies at Yucatan City, Mexico, appropriate for growth of tadpoles and frogs and Flores-Nava et al. (1994) compared the water circulation arrangement in the cultivation of these animals. Benitez-Mandujano and Flores-Nava (1997) monitored the water quality in experiments that compared growth and metamorphosis. These authors observed that the phytoplankton primary productivity was essential to the tadpoles' growth since it accelerated the metamorphosis.

Sipaúba-Tavares (2008) in a study on feeding behavior and water quality in bullfrog tadpoles nurseries concluded that the use of diet, supplemented or not with plankton, provided satisfactory development, but with high nutrients concentrations and reduction of oxygen dissolved at the same time. Concerning this theme, it is important to promote studies to provide further information about water quality in bullfrog farming system. In addition, the description of environmental changes generated by the activity is also fundamental for sustainable management practices.

Considering the statements above and the principles mentioned in the Code of Conduct for Responsible Fisheries, this paper aimed the investigation of the water quality of a bullfrog farming system, discussing their relations with the production and the environment based on the current legislation. For comparisons, it was adopted the referential values of CONAMA Resolution 357/2005, which includes the acceptable limits of

various parameters of water quality and recommends the treatment of effluents.

## 2. Material and Methods

Sampling were performed in a commercial bullfrog farming system, located in Tremembé (São Paulo) in tropical region, 22° 57' 40" S, 45° 32' 24" W, running since 1997.

In the bullfrog farming was used the "amphifarm" system. The bay (12 m<sup>2</sup>) analyzed during of sampling had an average of 700 individuals (average density of 58 bullfrogs m<sup>-2</sup>), being the water constantly renewed in the system on an average flow input and output of 0.064 L s<sup>-1</sup>. It was used extruded feed for fish with 40% crude protein, indicated for carnivorous species.

Water samples were collected during five months, November 2006 until March 2007, comprising the stages of initial-fattening (7°g) (November, December and January) and fattening (200°g) (February and March). A creek headwater was dammed, supplying the water for the bullfrog farming system. Sampling activities included the following sites: Site 1 - Upstream (16°m) from the mixing zone (selected by the logistic position); Site 2 - Affluent (bay supply source); Site 3 - Breeding bay; Site 4 - Effluent (water outflow from the bay); Site 5 - Mixing zone (effluent discharge site into the receiving water body); and Site 6 - Downstream (24 m) from the mixing zone (Figure 1).

The following variables were measured (*in situ*) on a monthly basis at each site with multiparameter probe Horiba U-22 model: pH, conductivity, dissolved oxygen, temperature, turbidity. In the laboratory, the total suspended solids, alkalinity and total hardness were analyzed by titulometric

method (APHA, 2005). The concentrations of total nitrogen (TN) and total phosphorus (TP) were determined according to Valderrama (1981) and orthophosphate (P-PO<sub>4</sub>) according to Strickland and Parsons (1960). Chlorophyll *a* was estimated following Sartory and Grobellar (1984). Total ammoniac nitrogen (TAN) concentration was estimated by Nessler method (APHA, 2005), nitrate by cadmium reduction method (APHA, 2005) and the nitrite by the method described by Griess modified (Giné et al., 1980). All of them were determined using a spectrophotometer.

The nutrient load was determined by the product of the values of flow (L s<sup>-1</sup>) and concentrations of total phosphorus (TP) using the formula (1):

$$C = [N] * Q \quad (1)$$

Which C = nutrients load; [N] = nutrient concentrations and Q = flow rate of each treatment. The results were expressed in grams per day (g°day<sup>-1</sup>).

In order to characterize the zootechnical cycle environmental variables were described in terms of average value and confidence interval of 95%, both for sites and months. Whenever possible these means were compared with referential values defined by CONAMA resolution 357/2005. Temporal and site variations on the variables were analyzed through an ANOVA two-way without replication after log(x+1) transformation, followed by a Tukey test (Sokal and Rohlf, 1995). The level of significance adopted was 0.05.

## 3. Results

Spatio-temporal analysis showed different pattern among variable analyzed. Significant

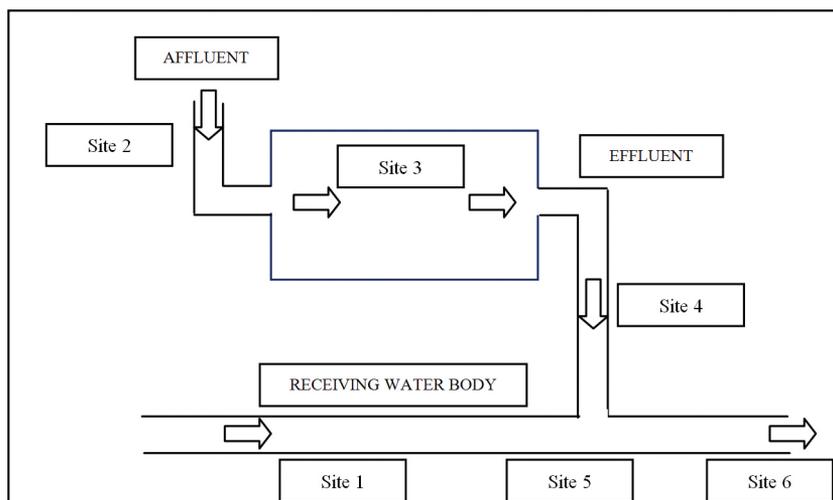


Figure 1. Representation of sampling sites in bullfrog farming system in Tremembé, São Paulo.

**Table 1.** Average values and confidence interval (95%) of the physical, chemical and biological variables analyzed at sites during the cycle of bullfrog farming system. Means followed by the same letters in the line do not differ by Tukey (0.05). Reference values for comparison of the results.

Variables	S1	S2	S3	S4	S5	S6	Reference values
pH	6.29 <sup>a</sup> ± 0.48	6.38 <sup>a</sup> ± 0.19	6.49 <sup>a</sup> ± 0.19	6.44 <sup>a</sup> ± 0.19	6.43 <sup>a</sup> ± 0.19	6.56 <sup>a</sup> ± 0.53	6.00-8.00**
Cond (µS cm <sup>-1</sup> )	180.40 <sup>a</sup> ± 322.96	30.60 <sup>a</sup> ± 11.25	80.20 <sup>a</sup> ± 28.91	94.20 <sup>a</sup> ± 40.26	36.80 <sup>a</sup> ± 10.70	64.80 <sup>b</sup> ± 12.27	≤ 150.00**
Turb (NTU)	136.86 <sup>a</sup> ± 198.29	32.48 <sup>a</sup> ± 42.65	186.48 <sup>a</sup> ± 171.80	71.74 <sup>a</sup> ± 63.14	202.76 <sup>a</sup> ± 356.13	94.66 <sup>b</sup> ± 106.64	≤ 100.00*
DO (mg L <sup>-1</sup> )	7.50 <sup>a</sup> ± 1.07	7.63 <sup>a</sup> ± 1.09	6.67 <sup>a</sup> ± 1.89	7.21 <sup>a</sup> ± 2.06	7.70 <sup>a</sup> ± 0.76	8.18 <sup>b</sup> ± 0.75	≥ 5.00*
T (°C)	24.56 <sup>a</sup> ± 1.38	24.01 <sup>bc</sup> ± 1.33	24.35 <sup>abc</sup> ± 1.53	24.54 <sup>ab</sup> ± 1.61	24.66 <sup>a</sup> ± 1.56	24.68 <sup>b</sup> ± 1.57	± 26.00***
TSS (mg L <sup>-1</sup> )	38.68 <sup>a</sup> ± 24.25	25.81 <sup>a</sup> ± 23.00	54.59 <sup>a</sup> ± 26.92	33.08 <sup>a</sup> ± 12.14	45.25 <sup>a</sup> ± 42.43	55.09 <sup>b</sup> ± 45.55	-
Alkalinity (mg L <sup>-1</sup> CaCO <sub>3</sub> )	17.32 <sup>a</sup> ± 6.96	12.49 <sup>a</sup> ± 2.34	30.94 <sup>a</sup> ± 10.31	32.20 <sup>a</sup> ± 19.67	14.27 <sup>a</sup> ± 5.18	17.82 <sup>b</sup> ± 7.19	10.00-80.00**
Hardness (mg L <sup>-1</sup> CaCO <sub>3</sub> )	11.65 <sup>a</sup> ± 3.47	8.81 <sup>a</sup> ± 1.21	31.05 <sup>b</sup> ± 17.98	26.78 <sup>bc</sup> ± 12.03	10.79 <sup>a</sup> ± 5.05	12.79 <sup>ac</sup> ± 2.92	10.00-80.00**
TAN (mg L <sup>-1</sup> )	1.54 <sup>abc</sup> ± 0.86	0.45 <sup>b</sup> ± 0.09	4.00 <sup>abc</sup> ± 4.22	4.83 <sup>c</sup> ± 3.96	1.23 <sup>abc</sup> ± 0.75	1.64 <sup>abc</sup> ± 0.78	≤ 3.70 if pH ≤ 7.5*
N-NO <sub>2</sub> (mg L <sup>-1</sup> )	0.007 <sup>abc</sup> ± 0.004	0.005 <sup>b</sup> ± 0.001	0.035 <sup>abc</sup> ± 0.036	0.085 <sup>c</sup> ± 0.088	0.012 <sup>ab</sup> ± 0.005	0.015 <sup>abc</sup> ± 0.007	≤ 1.000*
N-NO <sub>3</sub> (mg L <sup>-1</sup> )	0.26 <sup>abc</sup> ± 0.17	0.15 <sup>b</sup> ± 0.13	0.80 <sup>abc</sup> ± 0.75	1.05 <sup>c</sup> ± 1.08	0.20 <sup>ab</sup> ± 0.17	0.19 <sup>ab</sup> ± 0.20	≤ 1.00**
TN (mg L <sup>-1</sup> )	0.37 <sup>ac</sup> ± 0.22	0.30 <sup>c</sup> ± 0.29	1.91 <sup>b</sup> ± 1.79	2.81 <sup>b</sup> ± 2.75	0.45 <sup>ac</sup> ± 0.58	0.49 <sup>ab</sup> ± 0.27	≤ 1.27*
TP (mg L <sup>-1</sup> )	0.187 <sup>ac</sup> ± 0.154	0.035 <sup>c</sup> ± 0.028	2.061 <sup>b</sup> ± 1.421	2.591 <sup>b</sup> ± 1.928	0.116 <sup>ac</sup> ± 0.042	0.222 <sup>b</sup> ± 0.149	≤ 0.030*
P-PO <sub>4</sub> (mg L <sup>-1</sup> )	0.006 <sup>b</sup> ± 0.006	0.038 <sup>b</sup> ± 0.018	0.640 <sup>cd</sup> ± 0.841	0.729 <sup>acd</sup> ± 0.618	0.109 <sup>ab</sup> ± 0.054	0.094 <sup>acd</sup> ± 0.060	≤ 0.300**
CHL (mg L <sup>-1</sup> )	0.001 <sup>a</sup> ± 0.001	0.001 <sup>a</sup> ± 0.001	0.005 <sup>b</sup> ± 0.003	0.004 <sup>ab</sup> ± 0.004	0.002 <sup>ab</sup> ± 0.001	0.002 <sup>ab</sup> ± 0.002	≤ 0.030*

Cond = conductivity; Turb = Turbidity; DO = dissolved oxygen; T = water temperature; TSS = total suspended solids; TAN = total ammoniac nitrogen; N-NO<sub>2</sub> = nitrogen nitrite; N-NO<sub>3</sub> = nitrogen nitrate; TN = total nitrogen; TP = total phosphorus; P-PO<sub>4</sub> = orthophosphate; CHL = chlorophyll *a*; \*Brasil (2005); \*\*Ferreira (2003); \*\*\*Ferreira et al. (2002).

variations among sites were found in water temperature ( $F = 4.47$ ;  $P = 0.007$ ), hardness ( $F = 8.46$ ;  $P < 0.001$ ), total ammoniac nitrogen ( $F = 3.08$ ;  $P = 0.032$ ), nitrite ( $F = 4.94$ ;  $P = 0.004$ ), nitrate ( $F = 4.65$ ;  $P = 0.006$ ), total nitrogen ( $F = 10.49$ ;  $P < 0.001$ ), total phosphorus ( $F = 16.93$ ;  $P < 0.001$ ), orthophosphate ( $F = 14.27$ ;  $P < 0.001$ ) and chlorophyll *a* ( $F = 3.73$ ;  $P = 0.015$ ) (Table 1). Among months, pH ( $F = 4.98$ ;  $P = 0.006$ ), conductivity ( $F = 3.02$ ;  $P = 0.042$ ), turbidity ( $F = 5.90$ ;  $P = 0.003$ ), dissolved oxygen ( $F = 2.93$ ;  $P = 0.047$ ), water temperature ( $F = 189.37$ ;  $P < 0.001$ ), total suspended solids ( $F = 5.60$ ;  $P = 0.003$ ), nitrate ( $F = 4.62$ ;  $P = 0.008$ ) and total nitrogen ( $F = 28.56$ ;  $P < 0.001$ ) presented significant differences (Table 2).

Considering the current legislation CONAMA resolution 357/2005 (Brasil, 2005) (Class II,

lentic environment), nutrient concentrations in effluent (Site 4) resulted in very large values, much higher than it is recommended. The maximum concentration recommended for total phosphorus in effluent is  $0.030 \text{ mg L}^{-1}$ . However, in this study the values obtained ranged from  $0.26 \text{ mg L}^{-1}$  (November 16<sup>th</sup>, 2006) to  $5.0 \text{ mg L}^{-1}$  (February 15<sup>th</sup>, 2007). The total nitrogen concentrations in effluent were between  $0.18 \text{ mg L}^{-1}$  (November 16<sup>th</sup>, 2006) and  $6.11 \text{ mg L}^{-1}$  (February 15<sup>th</sup>, 2007), in accordance with CONAMA, whose maximum acceptable value is  $1.27 \text{ mg L}^{-1}$  (Table 1).

The average concentrations of total phosphorus (TP) and flow in the water (inlet and outlet) and the load produced per day were compared between different systems of animals' production (Table 3).

**Table 2.** Average values and confidence interval (95%) of the physical, chemical and biological variables analyzed monthly during the cycle of bullfrog farming system. Means followed by the same letters in the line do not differ by Tukey (0.05).

Variables	November 2006	December 2006	January 2007	February 2007	March 2007
pH	6.33 <sup>ab</sup> ± 0.32	6.34 <sup>ab</sup> ± 0.08	6.13 <sup>a</sup> ± 0.10	6.70 <sup>b</sup> ± 0.24	6.66 <sup>b</sup> ± 0.15
Conductivity ( $\mu\text{S cm}^{-1}$ )	52.50 <sup>ab</sup> ± 9.69	38.50 <sup>a</sup> ± 18.52	56.50 <sup>ab</sup> ± 32.96	61.17 <sup>ab</sup> ± 30.25	197.17 <sup>b</sup> ± 225.94
Turbidity (NTU)	36.72 <sup>ab</sup> ± 20.59	16.30 <sup>a</sup> ± 4.56	144.78 <sup>b</sup> ± 71.70	105.57 <sup>ab</sup> ± 130.37	300.78 <sup>b</sup> ± 256.52
DO ( $\text{mg L}^{-1}$ )	7.11 <sup>ab</sup> ± 0.63	7.20 <sup>ab</sup> ± 1.23	8.58 <sup>a</sup> ± 0.47	6.41 <sup>b</sup> ± 1.41	8.11 <sup>ab</sup> ± 0.70
T (°C)	23.72 <sup>a</sup> ± 0.31	24.65 <sup>c</sup> ± 0.14	22.73 <sup>b</sup> ± 0.28	24.43 <sup>c</sup> ± 0.07	26.82 <sup>d</sup> ± 0.44
TSS ( $\text{mg L}^{-1}$ )	55.72 <sup>ab</sup> ± 27.75	29.83 <sup>a</sup> ± 20.89	76.76 <sup>b</sup> ± 25.98	23.54 <sup>a</sup> ± 1.81	24.57 <sup>a</sup> ± 2.27
Alkalinity ( $\text{mg L}^{-1} \text{ CaCO}_3$ )	24.23 <sup>a</sup> ± 4.55	19.31 <sup>a</sup> ± 2.99	22.67 <sup>a</sup> ± 16.09	23.57 <sup>a</sup> ± 14.49	14.42 <sup>a</sup> ± 4.30
Hardness ( $\text{mg L}^{-1} \text{ CaCO}_3$ )	15.51 <sup>a</sup> ± 3.53	9.57 <sup>a</sup> ± 1.56	21.12 <sup>a</sup> ± 13.39	16.83 <sup>a</sup> ± 9.44	21.87 <sup>a</sup> ± 14.17
TAN ( $\text{mg L}^{-1}$ )	1.67 <sup>a</sup> ± 0.75	0.54 <sup>a</sup> ± 0.06	3.24 <sup>a</sup> ± 2.80	3.96 <sup>a</sup> ± 3.59	1.98 <sup>a</sup> ± 1.24
N-NO <sub>2</sub> ( $\text{mg L}^{-1}$ )	0.013 <sup>a</sup> ± 0.004	0.010 <sup>a</sup> ± 0.004	0.039 <sup>a</sup> ± 0.036	0.059 <sup>a</sup> ± 0.071	0.016 <sup>a</sup> ± 0.021
N-NO <sub>3</sub> ( $\text{mg L}^{-1}$ )	0.30 <sup>ab</sup> ± 0.11	0.15 <sup>a</sup> ± 0.12	0.13 <sup>a</sup> ± 0.08	0.94 <sup>b</sup> ± 0.94	0.68 <sup>ab</sup> ± 0.41
TN ( $\text{mg L}^{-1}$ )	0.17 <sup>a</sup> ± 0.07	0.16 <sup>a</sup> ± 0.07	1.83 <sup>b</sup> ± 1.80	2.18 <sup>b</sup> ± 1.84	0.95 <sup>b</sup> ± 0.53
TP ( $\text{mg L}^{-1}$ )	0.304 <sup>a</sup> ± 0.161	0.826 <sup>a</sup> ± 0.962	1.261 <sup>a</sup> ± 1.388	1.495 <sup>a</sup> ± 1.778	0.459 <sup>a</sup> ± 0.657
P-PO <sub>4</sub> ( $\text{mg L}^{-1}$ )	0.099 <sup>a</sup> ± 0.046	0.063 <sup>a</sup> ± 0.040	0.352 <sup>a</sup> ± 0.498	0.307 <sup>a</sup> ± 0.304	0.525 <sup>a</sup> ± 0.684
CHL ( $\text{mg L}^{-1}$ )	0.002 <sup>a</sup> ± 0.002	0.001 <sup>a</sup> ± 0.001	0.003 <sup>a</sup> ± 0.001	0.005 <sup>a</sup> ± 0.004	0.002 <sup>a</sup> ± 0.002

DO = dissolved oxygen; T = water temperature; TSS = total suspended solids; TAN = total ammoniac nitrogen; N-NO<sub>2</sub> = nitrogen nitrite; N-NO<sub>3</sub> = nitrogen nitrate; TN = total nitrogen; TP = total phosphorus; P-PO<sub>4</sub> = orthophosphate; CHL = chlorophyll *a*.

**Table 3.** Average concentrations of total phosphorus (TP) and flow in the water (inlet and outlet) and the load produced per day in different systems of animals' production.

	Inlet			Outlet			
	Concentration ( $\text{mg L}^{-1}$ )	Flow ( $\text{L s}^{-1}$ )	Load ( $\text{g dia}^{-1}$ )	Concentration ( $\text{mg L}^{-1}$ )	Flow ( $\text{L s}^{-1}$ )	Load ( $\text{g dia}^{-1}$ )	
Bullfrog farming	0.03	0.06	0.21	0.19	0.06	14.30	This study
Bullfrog farming	0.07	0.03	0.18	6.09	0.02	11.57	Borges et al. (2012)
Tilapia farming	0.42	2.76	9.70	2.45	2.76	49.10	Pereira et al. (2008)
Trout farming	72.26	40.61	233.33	99.69	40.61	343.67	Moraes et al. (2013)

The average productivity of the bullfrog farming system was 3.4 kg of meat of bullfrog  $m^{-2}$  and the feed conversion ratio was 5.31:1.

#### 4. Discussion

Compared to the usual monitoring water quality parameters for production, the results demonstrated acceptable values of pH, dissolved oxygen, water temperature, alkalinity and hardness respectively.

The lower values of pH during the production cycle were, probably, due to the decomposition process of organic matter, corroborated by the values of conductivity. Other authors (Seixas-Filho et al., 2011; Borges et al., 2012) have observed the same situation. The pH remained mildly acidic, being it an optimum condition for frogs (Ferreira, 2003). Additionally, Sipaúba-Tavares et al. (2008) suggested a pH range between 6.5 to 7.0, but observed values of 6.0 to 8.0 without harm to animals.

High electrical conductivity values and turbidity were observed, especially at the end of the fattening period. These parameters are directly related to the input of food into the water that promotes an increase of ions and suspended particles (Tacon and Foster, 2003; Baccarin and Camargo, 2005).

The highest values of total suspended solids were observed in the river, 119  $mg L^{-1}$  and 116  $mg L^{-1}$  upstream (Site 1) and downstream (Site 6), respectively, during January, probably due to the carrying of inorganic and organic particles related to rainy season.

Regarding the turbidity, high values were obtained upstream (Site 1), in the bay (Site 3) and in mixing zone (Site 5) in the last three months. According to the criterion of CONAMA Resolution 357/2005 (Brasil, 2005), values up to 100 NTU are acceptable, indicating that the results in these sites were not in accordance with the legislation. The stream catchment crossed a landfill that may have caused the high values obtained upstream. Sipaúba-Tavares (1994) discusses that high levels of turbidity may also be related to the presence of clays, colloidal or dissolved organic matter.

CONAMA Resolution 357/2005 (Brasil, 2005) recommends that the minimum limit for dissolved oxygen is 5  $mg L^{-1}$ . The values during the production cycle were acceptable, probably due to the fact that the bullfrog in growing and fattening phases presents predominantly air breathing, addition to the maintenance the continuous water flow and high renewal rate in the bay.

The weight gain is influenced by environmental conditions since the metabolism of bullfrogs may

vary according to the temperature, because frogs are poikilotherms (LIMA ET AL., 2003). Thus, the production of bullfrogs is performed during the warm season, in view of a better adaptation and development of the animals, at an average temperature of 26 °C (Ferreira et al., 2002). The water temperature varied between 22 °C and 27 °C during the production cycle. The temporal variation observed was considered adequate and it is within the standards for production. In study of Sipaúba-Tavares et al. (2008) the average water temperature of the bullfrog farming system ranged from 24 °C to 26 °C, similar to the present study, providing adequate condition for the proper development of the animals.

The presence of calcium in the food composition had a direct relationship with the increase alkalinity and hardness verified in the breeding bay (Site 3) and in effluent (Site 4). Therefore, the feeding altered these values; however, the observed values remained within the values already observed (between 10 and 80  $mg L^{-1} CaCO_3$ ) without apparent damage throughout the production cycle (Ferreira, 2003).

In regard to nutrients, the values were significantly different among sites, with increased in the breeding bay (Site 3) and effluent (Site 4), with consequent dilution of concentrations in the mixing zone (Site 5) and downstream from mixing zone (Site 6).

Total ammoniac nitrogen values were higher than the acceptable levels for production described by Ferreira (2003), which may cause damage to animals when above 0.7  $mg L^{-1}$ . The increase observed in the breeding bay (Site 3) and in effluent (Site 4) was related to feeding and excretion corroborated by study performed by Seixas-Filho et al. (2011), where treatments with feed showed higher concentrations of TAN compared to treatments without feed.

Benitez-Mandujano and Flores-Nava (1997) in experiments about growth and metamorphosis of *Rana catesbeiana*, obtained relative low values of nitrite, ranging from 0.08 to 0.10  $mg L^{-1}$ , higher than values obtained in the present study and within the interval recommended for production (Ferreira, 2003).

High values of nitrate, total nitrogen, total phosphorus and orthophosphate were obtained in the breeding bay (Site 3) and in effluent (Site 4). During the production cycle, an increase in the concentrations of TAN, N-NO<sub>2</sub>, N-NO<sub>3</sub>, TN, TP and P-PO<sub>4</sub> were noted. High concentrations of nutrients in effluent are associated with feed

management during grow-out phase that causes deterioration of the water quality (Borges et al., 2012), as the eutrophication process. Bullfrogs show better performance when fed with crude protein diets near 40% and carbohydrates, both for tadpoles (Stéfani et al., 2001) as in the fattening stage (Stéfani et al., 2002; Olvera-Novoa et al., 2007; Seixas-Filho et al., 2013), because the amphibians are carnivorous in post-metamorphic phase. Protein levels between 35 and 40% have a ratio of C:N smaller than 10:1 and are easily decomposed by bacteria. The large amount of decomposing organic matter by aerobic organisms reflects in a reduction of oxygen content in water (McIntosh, 2000), probably promoting the increase of nutrients.

According to FAO (2009), like in any other aquaculture activity, if not well managed it can cause several problems, mentioning organic discharges due to the excessive release of nutrients into the water, causing eutrophication and ecological changes.

During the period studied, the concentration of chlorophyll *a* reached a maximum value of 11  $\mu\text{g L}^{-1}$ , being the maximum value acceptable 30  $\mu\text{g L}^{-1}$  (Brasil, 2005). The constant renewal of water in the bay to removing excreta in the water and skin debris coming from the constant exchanges of animals (Ferreira et al., 2002) hindered the establishment of phytoplankton.

In this study, the increased flow resulted in higher loads of TP in comparison with Borges et al. (2012), who had the highest concentrations of this nutrient. The water inflow controlled in the production system directly reflects in the nutrient loads in effluent, fact also observed in tilapia and trout farming systems (Pereira et al., 2008; Moraes et al., 2013, respectively).

Considering the area of the bay and density of animals, the average productivity of bullfrogs (3.40  $\text{kg m}^{-2}$ ) in "amphifarm" system was similar to Fontanello et al. (1993), who obtained a productivity of 3.86  $\text{kg m}^{-2}$  with the lowest number of animals per area of production (50 bullfrogs  $\text{m}^{-2}$ ).

The bullfrog farming system showed high nitrogen and phosphorus concentrations coming from the excessive food and excreta of animals and according to the current legislation, this system is irregular with respect to the discharge of effluent with high nutrients concentrations, a potential inducer of the eutrophication process. It is important to note that this increase in nutrients and other factors such as conductivity and turbidity is proportional to the animal growth, due to the inadequate feeding

management practices evidenced by feed conversion rate being possible to posit that a part of the feed was discharged in effluent.

A constant renovation of the water in the breeding bay is necessary to avoid the toxic effect for bullfrogs. However, it may promote higher loads of nutrients. To improve the quality of effluent aiming to reduce nutrient loading, besides treatment of effluent, are proposed the following management options: a) maintaining the flow and decreased density of animals; b) maintaining the flow and density storage with a better control of the food supply, its quality and digestibility.

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