Acta Limnologica Brasiliensia, 2012, vol. 24, no. 2, p. 160-166 http://dx.doi.org/10.1590/S2179-975X2012005000035

Characterization of effluents from bullfrog (*Lithobates catesbeianus,* Shaw, 1802) grow-out ponds

Caracterização de efluentes de viveiros de engorda de rã-touro (*Lithobates catesbeianus*, Shaw, 1802)

Fernanda de Freitas Borges, Luiz Augusto do Amaral and Marta Verardino De Stéfani

Centro de Aquicultura da UNESP – CAUNESP,
Universidade Estadual Paulista "Júlio de Mesquita Filho" – UNESP,
Via de Acesso Professor Paulo Donato Castellane, s/n, CEP 14884-900, Jaboticabal, SP, Brazil
e-mail: fernandafreitasb@ig.com.br; lamaral@fcav.unesp.br; martavs@fcav.unesp.br

Abstract: Aim: Current analysis characterizes the effluent from bullfrog-rearing ponds during the grow-out phase; **Methods:** Temperature, pH, dissolved oxygen, electric conductivity, turbidity, total phosphorus, $N\text{-}NH_3$, $N\text{-}NO_3$, BOD_5 and COD and the number of thermotolerant coliforms (*Escherichia coli*) of the inlet and outlet water of the ponds were analyzed twice a week. Assay consisted of a completely randomized experimental design with two treatments (inlet and outlet water) and six repetitions in a split-plot, coupled to collection over time as subplot; **Results:** All variables were significantly different ($p < 0.05$) between treatments and over time ($p < 0.05$). Average rates of temperature, pH and dissolved oxygen levels of the supply water were higher when compared to those of the effluent. The other variables such as conductivity, turbidity, total phosphorus, ammonia, nitrate, biological oxygen demand, chemical oxygen demand and *E. coli* were higher in the effluent when compared to rates in the supply water; **Conclusions:** The management during grow-out phase caused the deterioration of the water quality, with increasing levels of dissolved nutrients and the number of thermotolerant coliform. Ammonia and phosphorus levels in the effluent, caused by waste food, skin and feces, accelerate the eutrophication process of the receiving water body. Further studies on effluent treatment are required.

Keywords: eutrophication, nutrients, raniculture, water quality.

Resumo: Objetivo: Caracterizar a qualidade do efluente na fase de engorda da rã-touro; **Métodos:** Quinzenalmente foram mensurados a temperatura, pH, oxigênio dissolvido, condutividade elétrica, turbidez, PT, $N\text{-}NH_3$, $N\text{-}NO_3$, DBO_5, DQO e número de coliformes termotolerantes (*Escherichia coli*) da água de abastecimento e do efluente das baias de criação. O delineamento experimental utilizado foi o inteiramente casualizado com dois tratamentos (água de entrada e saída das baias) e seis repetições, em esquema de parcelas subdivididas, sendo as subparcelas as coletas no tempo; **Resultados:** Todas as variáveis apresentaram diferença significativa ($p < 0,05$) para os tratamentos e entre as coletas ($p < 0,05$). Os valores médios de temperatura, pH e oxigênio dissolvido da água de abastecimento das baias foram superiores aos do efluente. As demais variáveis; condutividade elétrica, turbidez, fósforo total, amônia, nitrato, demanda bioquímica de oxigênio, demanda química de oxigênio e *E. coli* foram superiores no efluente, em relação à água de abastecimento; **Conclusões:** O manejo realizado na fase de engorda de rã-touro deteriora a qualidade da água utilizada, aumentando as concentrações de nutrientes dissolvidos e o número de coliformes termotolerantes. As concentrações de amônia e fósforo, provenientes de restos de ração, peles e excretas, podem acelerar o processo de eutrofização do corpo d'água receptor, sendo necessário estudos sobre tratamentos desse tipo de efluente.

Palavras-chave: eutrofização, nutrientes, ranicultura, qualidade de água.

1. Introduction

Frog culture is almost entirely based on the simple maintenance of the bullfrog, *Lithobates catesbeianus*, which originated in North America. The bullfrog requires between 60 and 70 days to develop from the larval stage to metamorphosis, whereas the growing phase needs three to four months (Lima and Agostinho, 1992). The most common type of installation in commercial frog cultures in Brazil is

the amphi-farm system, which consists of trough, shelter and pool arranged linearly (Lima and Agostinho, 1992; Lima, 1997).

The culture must have its own water source, which should be protected and free of pollution with enough water to meet the demand of the culture activities (Hipolito, 2006). As well as other aquatic organisms, frogs depends on water for fecal elimination, to control body temperature, breathing, reproduction and protection, thus making water quality extremely important for successful farming (Hipolito, 2004).

Ponds are dynamic ecosystems, characterized by shallowness and continuous water flow, which directly affect the limnological variables throughout the day. Water flow is extremely important due to its transport of nutrients, microorganisms, addition of oxygen to the medium and organic material (Boyd, 2003). Depending on flow rate and pond depth, continuous water flow may present a concentrating or diluting effect on the materials contained in the water (Sipaúba-Tavares et al., 2003).

The effluents from aquaculture can increase the quantity of suspended solids and promote the enrichment of nitrogen and phosphorus in aquatic ecosystems. To reduce the pollution on environment is important to formulate highly digestible diets and to treat the effluent to attend the legislative demands and release it into the aquatic ecosystem avoiding artificial eutrophication (Henry-Silva and Camargo, 2006).

Studies have revealed that bullfrogs may be a reservoir of *Escherichia coli* and the water is an important means of bacteria transmission to frogs, which, in turn, disseminate them within the environment (Gray et al., 2007), since bacteria is harbored in the animals' intestinal tract (Nataro and Kaper, 1998).

Since it became imperative to know the characteristics of this effluent, the objective of the present study is to analyze the physical, chemical and microbiological variables of the effluent of the grow-out pond of a bullfrog culture and evaluate the impact of culture in the water quality used.

2. Material and Methods

The study was conducted at the Frog Culture Sector (Figure 1), Aquaculture Center, Jaboticabal, SP, Brazil (21° 15' S and 48° 18' W), during the period from November, 2009 to January, 2010. The experiment lasted 77 days, until the animals reached the slaughtering weight of approximately 250 g.

Six covered grow-out ponds of 12 m^2 area, built with bricks according to Lima (1997) were used. Every pond had its own water inlet and outlet, equipped with continuous flow and volume of approximately 330 L. The water was supplied by an artesian well located outside the Aquaculture

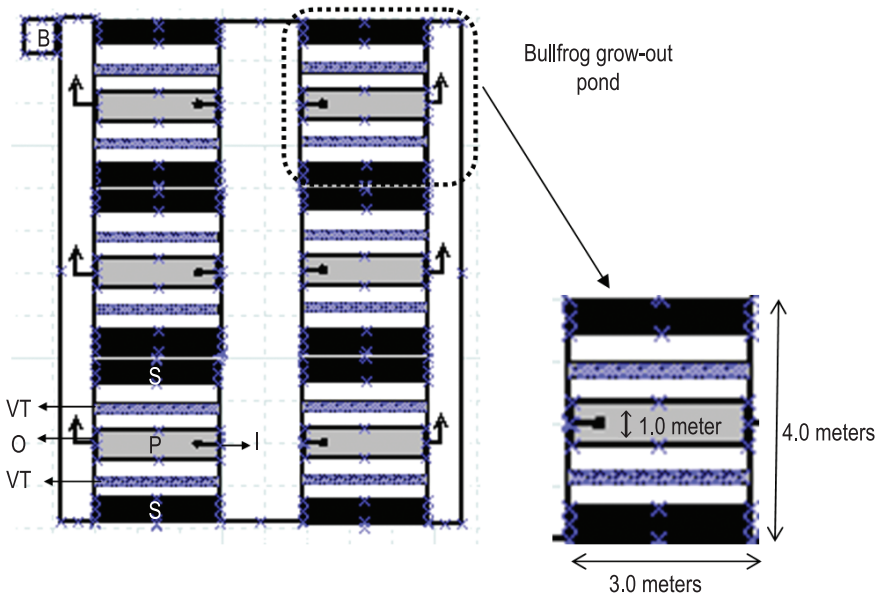

Figure 1. Scheme of Fattening Sector of the Frog Culture, Aquaculture Center at Unesp. The six ponds are composed of pool (P), shelters (S), vibrating trough for the feeding (VT), inlet (I) and outlet (O) water in continuous flow, arranged in a linear fashion. The effluent is taken out of the system according to the arrows which flows for an external receiver box (B).

Center, surrounded by grazing cattle, horses and goats. The flow rates were calculated by the input and output speed of the water in the ponds, by measuring the time needed to fill up a 1-L container, expressed in liters/second.

We used 2,160 bullfrog froglets, weighing an average 19 g, that were distributed in six grow-out ponds, at a density of 30 animals/m^2, totaling 360 animals per pond. They were kept under the same conditions (temperature, light and humidity), management and feeding (commercial extruded feed containing 40% crude protein) until they reached average weight 288 g.

The experimental design was completely randomized (CRD) with two treatments (water inlet and outlet) and six repetitions in a split-plot, with the sub-plots collection over time.

The feed was supplied in vibrating troughs and the amount was adjusted according to table proposed by Lima et al. (2003) for the grow-out phase. Monthly weighing was performed on a sample equivalent to 10% of the frogs in each pond to monitor frog growth and adjust the amount of feed supplied.

The variables temperature, dissolved oxygen (Lutron potentiometric probe), pH and electrical conductivity (Phtek handheld devices) were analyzed *in situ*. Turbidity was determined using a portable Hach turbidimeter, model 2100. Total phosphorous was determined by Acid Hydrolysable method, ammonia by Nessler method and nitrate by Cadmio Reduction method, all were determined using a Hach spectrophotometer, model DR-2010 (APHA, 2005).

The chemical oxygen demand values (COD) were obtained by colorimetric method with acid digestion in potassium dichromate medium and catalysts using a digester block and Hach spectrophotometer, model DR-2010 (APHA, 2005). Biochemical oxygen demand was obtained using the BOD-Sensor equipment model AL 320, using potassium hydroxide solution 45% as a reagent and pressure sensors internal, according to the methodology of the Standard Methods for Examination of Water and Wastewater (APHA, 2005). The most probable number of *Escherichia coli* present in 100 mL of water sample was determined according to the multiple tube methodology (APHA, 2005).

Water sampling was carried out bi-weekly in the morning, totaling six collections. The inlet and outlet (effluent) water samples were collected directly from the taps and swimming pools into the ponds, respectively. The water was previously homogenized using a broom that was later used to clean the ponds. Each pond requires daily cleaning and has its own broom in order to avoid contamination and prevent spreading of disease. Water samples were collected in sterile and plastic bottles, for microbiological, physical and chemical analysis, respectively, placed in a cooler and transferred immediately to the laboratory.

Data of the limnological variables of ponds water were submitted to variance analysis (ANOVA) and means were compared by Tukey test at 5% probability, to check whether there was significant difference between treatments (inlet and outlet). Regression analysis was performed to determine the polynomial effect of collections over time, using the software SAS 9.1 (SAS, 2006).

3. Results

All water quality variables were significantly different between treatments ($p < 0.01$) and the polynomial effect showed the variation between collections ($p < 0.01$) over time (Table 1). The mean values of temperature, pH and dissolved oxygen were higher ($p < 0.01$) in the supply water compared to the effluent (Table 1).

Except for the initial collection, dissolved oxygen levels of the effluent were below 1.00 $mg.L^{-1}$, while the minimum value found was 0.32 $mg.L^{-1}$, due to the large amount of decomposing organic matter by aerobic organisms (Table 1).

The variables conductivity, turbidity, total phosphorous, ammonia, nitrate, biochemical oxygen demand, chemical oxygen demand and *E. coli* values were higher ($p < 0.01$) in the effluent compared to supply water (Table 1).

The mean ammonia and nitrate levels in the effluent were 6.94 $mg.L^{-1}$ and 2.37 $mg.L^{-1}$, respectively (Table 1). The low nitrate level may be explained by the lack of enough oxygen so the bacteria could perform the oxidation of ammonia. The Table 3 shows the load of total phosphorous, ammonia and nitrate (g) present in the water (inlet and outlet) per day of experiment.

The nutrient load was calculated 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 ($g.day^{-1}$).

4. Discussion

The lowest temperature in the effluent (28 °C) was mainly induced by the shading of the water column, provided by covering the ponds. The lower values of pH (7.2) in the effluent are due to the decomposition process of organic matter.

At the end of the period the frogs weight gain on average 269 g, had 85% survival rate and feed conversion of 1.14, was different than other study with bullfrog, that the weight gain was 196 g, a feed conversion was 1.41, survival 95% where the medium environment temperature was 22 °C (Dias et al., 2008).

In bullfrog farming system with a water flow of 0.064 $L.seg^{-1}$, for the inlet and outlet of the ponds, reported higher level of dissolved oxygen in the effluent (4.37 $mg.L^{-1}$) (Pereira et al., 2007), which were higher than the flows in this study of 0.030 $L.seg^{-1}$ for supply water and 0.022 $L.seg^{-1}$ for the effluent and the less dissolved oxygen level (1.23 $mg.L^{-1}$). This low water flow associated to organic matter decomposition process by aerobic organisms may have contributed to the low levels of dissolved oxygen observed.

The highest conductivity values were 205 $\mu S.cm^{-1}$ and 302 $\mu S.cm^{-1}$ for the supply water and effluent, respectively, higher than the values reported by Pereira et al. (2007), of 44 $\mu S.cm^{-1}$ and 136 $\mu S.cm^{-1}$ for inlet and outlet water, respectively.

The turbidity values for effluent (66 NTU) were similar to that obtained by Henry-Silva and Camargo (2008) studying water pond grow-out *M. amazonicum* shrimp (62 NTU). However, in this study, the water volume and the residence time of shrimp pond of 200 m^2 with 17 hours were larger than the water volume in the grow-out pond of bullfrogs of 3.0 m^2 (330 L), with 12 hours of residence time. The stocking density for shrimps (85.7 $g.m^2$) was lower than for frogs (570 $g.m^2$). These factors favor the sedimentation of suspended solids and the dilution of nutrients.

The frog culture also displayed higher levels of dissolved nutrients, mainly concentrations of phosphorus, ammonia and conductivity, when compared to those in other aquaculture activities,

Table 1. Means of the physical, chemical and microbiological variables of the inflow (I) and outflow (O) water in the grow-out ponds at different collections. Means followed by the same letters in the column do not differ by Tukey 5%. The polynomial effect indicates the variation into collection, whereas the higher order demonstrates a greater time variation between the samples.

VAR.	TR	Collections (days)						Mean	Polyn. effect
		0 (1)	1 (16)	2 (31)	3 (46)	4 (61)	5 (76)		
TEMP. (°C)	I	29.53^a	30.35^a	30.02^a	29.75^a	30.50^a	29.98^a	30.02	4º order**
	O	28.43^b	27.38^b	28.68^b	27.93^b	28.62^b	27.32^b	28.23	5º order**
pH	I	7.90^a	8.00^a	8.00^a	8.02^a	8.10^a	8.07^a	8.01	4º order*
	O	7.37^b	6.98^b	7.18^b	7.23^b	7.33^b	7.32^b	7.24	5º order*
DO ($mg.L^{-1}$)	I	5.47^a	5.02^a	4.88^a	5.11^a	4.63^a	4.34^a	4.89	5º order*
	O	5.34^a	0.57^b	0.64^b	0.74^b	0.38^b	0.32^b	1.23	4º order**
COND. ($\mu S.cm^{-1}$)	I	200.50^a	203.00^b	204.33^b	205.00^b	197.33^b	199.67^b	201.64	5º order*
	O	201.00^a	230.50^a	231.33^a	258.00^a	271.00^a	301.67^a	248.92	1º order**
TURB. (NTU)	I	0.34^b	0.30^b	0.28^b	0.57^b	0.59^b	0.44^b	0.42	3º order**
	O	8.63^a	31.12^a	44.00^a	61.30^a	76.36^a	232.93^a	66.14	4º order**
E. coli (MPN $100Ml^{-1}$)	I	1.04^b	0.93^b	1.02^b	0.77^b	1.89^b	1.49^b	1.19	NS
	O	8.799^a	9.632^a	11.453^a	13.102^a	12.887^a	15.846^a	11.953	4º order*
TP ($mg.L^{-1}$)	I	0.06^a	0.07^b	0.10^b	0.08^b	0.09^b	0.05^b	0.07	NS
	O	0.07^a	3.65^a	7.67^a	8.66^a	7.07^a	9.41^a	6.09	2º order**
$N\text{-}NH_3$ ($mg.L^{-1}$)	I	0.03^a	0.02^b	0.008^b	0.12^b	0.04^b	0.02^b	0.04	5º order**
	O	1.01^a	3.89^a	6.07^a	7.26^a	8.72^a	16.80^a	6.94	3º order*
$N\text{-}NO_3$ ($mg.L^{-1}$)	I	0.07^a	0.08^b	0.15^b	0.15^b	0.22^b	0.20^b	0.14	1º order**
	O	0.18^a	1.90^a	2.32^a	2.54^a	2.48^a	4.83^a	2.37	3º order**
BOD_5 ($mg.L^{-1}$)	I	2.50^b	0.50^b	0.50^b	1.00^b	0.67^b	0.50^b	0.94	3º order**
	O	40.17^a	52.00^a	66.67^a	93.33^a	95.67^a	96.67^a	74.08	5º order*
COD ($mg.L^{-1}$)	I	3.83^a	6.17^b	11.33^b	13.17^b	21.50^b	4.00^b	10.00	4º order**
	O	70.67^a	175.50^a	242.50^a	384.83^a	580.67^a	816.00^a	378.36	2º order*

**significant at 1%; *significant at 5%; NS – not significant; TR – treatment; TEMP – temperature; DO – dissolved oxygen; COND – conductivity; TURB – turbidity; *E. coli* – *Escherichia coli;* TP – total phosphorus; $N\text{-}NH_3$ – ammonia; $N\text{-}NO_3$ – nitrate; BOD_5 – biochemical oxygen demand; COD – chemical oxygen demand.

such as fish and shrimp culture (Table 2). The daily rate of water renewal in frog culture is 100% and the swimming pool area in the pond is 3 m^2. In the case of shrimp and fish culture, the daily rate of water renewal is 10%, with shrimp and fish ponds area respectively of 200 m^2 and 1000 m^2. The feeding of frogs is carried out in pelleted ration containing 40% crude protein and the initial stocking density reached 570 $g.m^2$. In the case of shrimps, the pelleted diet contains 32% CP and initial stocking density reaches 86 $g.m^2$. On the other hand, pelleted ration for fish contains 28% CP and the density is 48 $g.m^2$. Data show that there is a dilution in the shrimp and fish ponds, contrastingly to the frog ponds with a higher concentration of nutrients due to a smaller water volume.

The high occurrence of *Escherichia coli* (mean 1.1×10^4 MPN 100 mL^{-1}) in the effluent can be explained by a previous contamination of froglets that came from a commercial frog culture. Already their proliferation in the pond may have been due to external contributions and enrichment of water by nutrients and organic matter creating an environment favorable for the bacterial growth. Since bullfrogs are infected the bacteria multiply, and then are released in large number back to the environment (Stitt et al., 2009). In a study in the frog culture sector, Aquaculture Center of Unesp, Jaboticabal, showed that the isolated pathogen that caused the mortality of bullfrog froglets was in fact the *E. coli* bacteria (Mouriño et al., 2005).

The mean value for total phosphorous in the present study 6.09 $mg.L^{-1}$ was higher than the studied in one the sampling site frog culture of 2.23 $mg.L^{-1}$ (Caruso, 2009). The feed and its ingredients must have low phosphorous content and be easily digestible, since the organisms retain only about 25% of the phosphorous and that most is excreted as feces (insoluble phosphorous) or urine (soluble phosphorous) (Hardy, 2000).

In shrimp grow-out ponds (*Macrobrachium rosenbergii*), the total phosphorous load in the effluent during the months of November, December and January, was 1.20 $g.day^{-1}$; 1.08 $g.day^{-1}$ and 1.37 $g.day^{-1}$, respectively. There is a 1.22 $g.day^{-1}$ during the whole cycle (Biudes, 2007), lower than in the bullfrog ponds that displayed mean of 11.57 $g.day^{-1}$ in the cycle (Table 3). The impact of aquatic organisms culture in pond water quality depends on crop species, stocking density, feed management and technology employed (Boyd, 2003). In frog culture the stocking density of the animals is increased and the volume of water used is less when compared to shrimp culture. The daily handling is also different, with total exchange of swimming pool water when compared to other aquaculture systems with an average 10% renewal.

Table 2. Comparison of limnological characteristics of the effluents from frog, shrimp and fish cultures.

Limnological variables	Bullfrog culture (present study)	Shrimp culture (Henry-Silva and Camargo, 2008)	Fish culture (Sipaúba-Tavares et al., 2006)[1] (Henry-Silva and Camargo, 2006)[2]
Temperature (°C)	28.2	26.5	25.7 [1]
pH	7.2	8.1	7.2 [1]
Dissolved oxygen ($mg.L^{-1}$)	1.23	5.10	5.40 [1]
Conductivity ($\mu S.cm^{-1}$)	249	68	62 [1]
Turbidity (NTU)	66	62	26 [2]
Total phosphorus ($mg.L^{-1}$)	6.09	0.23	0.20 [1]
Ammonia ($mg.L^{-1}$)	6.94	0.02	0.07 [1]
Nitrate ($mg.L^{-1}$)	2.37	0.16	0.16 [1]

Shrimp culture – breeding pond of *M. amazonicum*; Fish culture[1] – mean values among three semi-intensive fishponds placed in series with *P. mesopotamicus*, *C. macropomum*, *B. cephalus*, *O. niloticus and C. ocellaris;* [2]pond populated with *Oreochromis niloticus*.

Table 3. Mean concentrations of total phosphorus (Total P), ammonia ($N\text{-}NH_3$) and nitrate ($N\text{-}NO_3$) in the water (inlet and outlet) during the experimental period and the load produced per day.

Variables	Inlet		Outlet	
	Concentration ($mg.L^{-1}$)	Load ($g.day^{-1}$)	Concentration ($mg.L^{-1}$)	Load ($g.day^{-1}$)
Total P	0.07	0.18	6.09	11.57
$N\text{-}NH_3$	0.04	0.10	6.94	13.19
$N\text{-}NO_3$	0.14	0.36	2.37	4.50

Peaks of ammonia and nitrate, 4.38 mg.L^{-1} and 1.06 mg.L^{-1}, respectively, were reported in the other frog culture effluent (Caruso, 2009). Similar average level for ammonia 6.70 mg.L^{-1} and highest levels for nitrate 5.20 mg.L^{-1} were found in ponds of sport fishing farms in the Mogi-Guaçu river (Eler and Espíndola, 2006).

The urea excreted by adult frogs is rapidly converted into ammonia. Which then undergoes the process of nitrification in the water of the ponds (Odum, 1988). This whole process depletes the oxygen present in the water, thus approaching anoxic levels.

In environments with pH between 7 and 8, temperatures from 25 to 35 °C and appropriate oxygen level, ammonia rapidly oxidizes to nitrate by bacteria (Vinatea-Arana, 1997), which was not observed in this study due to insufficient dissolved oxygen. Compared to other agro-industrial effluents, the mean value (74 mg.L^{-1}) for BOD measured in the effluent of frog grow-out ponds (Table 1) is similar to the slaughterhouse effluents that are treated by stabilization pond systems and range from 71 to 78 mg.L^{-1} (Bezerra et al., 2002).

An effluent is considered biodegradable when the ratio chemical oxygen demand (COD)/biological oxygen demand (BOD) is less than 5 (Valente et al., 1997). During the grow-out phase of bullfrogs, as the animals grow, water quality deteriorates, thus increasing this ratio. During the studied period, the mean value of the ratio COD/BOD was 5.0, indicating that the effluent is biodegradable, although it rich in suspended solids (378 mg.L^{-1} COD). The organic matter present in the water results from uneaten feed, feces and animal skin, which constitute the solids that settle on the bottom of the ponds and undergo the decomposition process.

Some of the parameters measured in the inlet water were higher than expected. The well water is pumped to a water tank from where it is distributed to the entire campus, therefore contamination can occur on this route and, also, infiltration of rainwater and seepage of nutrients to the soil near the well, taking into account the characteristics of its surroundings.

5. Conclusions

The management adopted to prevent diseases during frog grow-out phase includes constant water renewal and daily cleaning of the ponds. Consequently, the main negative impact during this phase is the effluent quality, where the decomposition of uneaten food, feces and skin releases nutrients into the water by leaching and decomposition, thus increasing ammoniacal nitrogen, phosphorous and particulate organic matter levels and decreasing dissolved oxygen level that results in almost anoxic water.

The conditions under which the study was conducted lead us to conclude that the management adopted in the bullfrog grow-out ponds changed the water quality. In contrast to other aquatic organism cultures (fish and shrimp), the effluent of frog culture has a greater potential to cause eutrophication in receiving water bodies.

Further studies should be conducted to test the methods that are appropriate to treat the raniculture wastewater. The best aquaculture practices (BAP) should also be recommended to frog farms in order to prevent water pollution and animal contamination (food biosecurity).

Acknowledgements

Thanks are due to Fapesp (Research Foundation of the State of São Paulo) for the financial support and to CNPq (National Research Council) for the graduate scholarship (Master's).

References

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

BEZERRA, LP., BARBEDO, AGA., IDE, CN., IMOLENE, LM., OLIVEIRA, KRF. and CASTRO, RA. 2002. Efluentes agroindustriais em Mato Grosso do Sul – características. In *Anais do II Simpósio de Recursos Hídricos do Centro Oeste*, 2002. Porto Alegre: Associação Brasileira de Recursos Hídricos. Available from: <TTP://www.abrh.org.br/novo/ii_simp_rec_hidric_centro_oeste_campo_grande72.pdf>. Access in: 02 abr. 2010.

BIUDES, JFV. 2007. *Uso de wetlands construídas no tratamento de efluentes de carcinicultura*. Jaboticabal: Universidade Estadual Paulista. [Tese de Doutorado em Aquicultura].

BOYD, CE. 2003. Guidelines for aquaculture effluent management at the farm-level. *Aquaculture*, vol. 226, no. 1-4, p. 101-112.

CARUSO, NPP. 2009. *Ensaios ecotoxicológicos para avaliação da qualidade da água em um sistema de ranicultura*. São Paulo: Instituto de Pesca. [Dissertação de Mestrado em Aquicultura e Pesca].

DIAS, DC., STÉFANI, MV., FERREIRA, CM. and FRANÇA, FM. 2008. Uso de probióticos em ração de rã-touro (*Rana catesbeiana*): desempenho

produtivo. *Archivos de Zootecnia,* vol. 57, no. 220, p. 449-455.

ELER, MN. and ESPÍNDOLA, ELG. 2006. *Avaliação dos impactos de pesque-pague*: uma análise da atividade na bacia hidrográfica do rio Mogi-Guaçu. São Carlos: Ed. Rima. 116 p.

GRAY, MJ., RAJEEV, S., MILLER, DR., SCHUMUTZER, AC., BURTON, EC., ROGERS, ED. and HICKLING, GJ. 2007. Preliminary evidence that American Bullfrogs (*Rana catesbeiana*) are suitable hosts for *Escherichia coli* O157:H7. *Applied and Environmental Microbiology*, vol. 73, no. 12, p. 4066-4068. http://dx.doi.org/10.1128/AEM.02905-06

HARDY, RW. 2000. Advances in the development of low pollution feeds for salmonids. *Global Aquaculture Advocate*, vol. 3, no. 2, p. 63-74.

HENRY-SILVA, GG and CAMARGO, AFM. 2006. Efficiency of aquatic macrophytes to treat Nile tilapia pond effluents. *Scientia Agricola*, vol. 63, no. 5, p. 433-438. http://dx.doi.org/10.1590/S0103-90162006000500003

HENRY-SILVA, GG. and CAMARGO, AFM. 2008. Tratamento de efluentes de carcinicultura por macrófitas aquáticas flutuantes. *Revista Brasileira de Zootecnia*, vol. 37, no. 2, p. 181-188. http://dx.doi.org/10.1590/S1516-35982008000200002

HIPOLITO, M. 2004. Manejo sanitário no cultivo de rã. In RANZANI-PAIVA, MJT., TAKEMOTO, RM. and LIZAMA, MAP., orgs. *Sanidade de organismos aquáticos*. São Paulo: Ed. Varela. p. 330-351, 426 p.

HIPOLITO, M. 2006. Qualidade dos produtos da TTPcanra – ranicultura. In SILVA-SOUZA, A. T., org. *Sanidade dos organismos aquáticos no Brasil*. Maringá: Associação Brasileira de Patologia de Organismos Aquáticos - Abrapoa. p. 353-367, 426 p.

LIMA, SL. and AGOSTINHO, CA. 1992. *A tecnologia da criação de rãs*. Viçosa: Folha de Viçosa. 168 p.

LIMA, SL. 1997. *Criação de rãs* (*Sistema Anfigranja*). Viçosa: CPT. Manual Técnico, no. 3, 48 p.

LIMA, SL., CASALI, AP. and AGOSTINHO, CA. 2003. 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*, vol. 32, no. 3, p. 505-511. http://dx.doi.org/10.1590/S1516-35982003000300001

MOURIÑO, JLP., URBANO, T., MARTINS, ML., FENERICK JUNIOR, J., SCHOCKEN-ITURINO, RP. and STÉFANI, MV. 2005. Isolamento e caracterização de possível agente patogênico causador de mortalidade em imagos de *Rana catesbeiana* Shaw, 1802. *Ars Veterinária*, vol. 21, p. 160-163. Suplemento.

NATARO, JP. and KAPER, JB. 1998. Diarheagenic *Escherichia coli*. *Clinical Microbiology Reviews*, vol. 11, no. 1, p. 142-201.

ODUM, EP. 1988. *Ecologia*. Rio de Janeiro Ed. Guanabara. 434 p.

PEREIRA, JS., MERCANTE, CTJ., LOMBARDI, JV., CARUSO, NPP., OSTI, JAS., MIASHIRO, L. and EVANGELISTA, LCS. 2007. Caracterização da qualidade da água, através de fatores abióticos, da entrada e saída de um sistema de produção de rãs (*Rana castebeiana Shaw*). In *Anais do VIII Congresso de Ecologia do Brasil*, 2007. Caxambu.

SAS. 2006. *SAS 9.1 for windows*. Cary: Institute SAS Inc.

SIPAÚBA-TAVARES, LH., BARROS, AF. and BRAGA, FMS. 2003. Effect of floating macrophyte cover on the water quality in fishpond. *Acta Scientarium. Animal Sciences*, v. 25, no. 1, p. 12-24.

SIPAÚBA-TAVARES, LH., BACHION, MA. and COLUS, DSO. 2006. Estudos limnológicas em três viveiros de criação de peixes com fluxo contínuo de água. *Boletim Técnico do CEPTA*, vol. 19, p. 35-47.

STITT, T., NORDIN, R. and IWASAWA, S. 2009. *Examining the potential impacts of TTPcan bullfrogs* (*Rana catesbeiana*) *on drinking water quality in the greater Victoria water supply area*. Centre for Coastal Health – Capital Regional District Water Services. Reports. 79 p. Available from: <TTP://www.crd.bc.ca/reports/regionalwatersupplyc_/2009_/crdbullfrogreportsep/crdbullfrogreportsep.pdf>. Access in: 13 jun. 2012.

VALENTE, JPS., PADILHA, PM. and SILVA, AMM. 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*, vol. 22, p. 49-66. http://dx.doi.org/10.1590/S0100-46701997000100005

VINATEA-ARANA, L. 1997. *Princípios químicos de qualidade da água em aquicultura*. Florianópolis: Ed. UFSC. 166 p.

Received: 11 November 2011
Accepted: 22 August 2012