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DIURNAL DYNAMIC OF NITRITE AND AMMONIA GAS IN A NILE TILAPIA FISH POND IN SUBTROPICAL REGION

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ABSTRACT

The purpose of this paper is to describe the diurnal oscillations found in the concentrations of nitrite and gas ammonia in a fish pond of Nile Tilapia; identify the exact moment these substances reach inappropriate levels for cultivation, and show the best handling practices. The sampling was performed in 2006 from December 16th until December 20th, from 6 am till 8 pm every two hours. It was observed that the concentrations of nitrate and ammonia are affected by diurnal fluctuations, and that the higher levels of gas ammonia and nitrate can be found at 4 pm: 12.4 µg/L and 12.6 mg/L, respectively. The concentration of nitrite reached levels considered inadequate for cultivation. It was also observed that the parameters nitrite and ammonia are strongly influenced by subaqueous radiation and that the afternoon is the best period for obtaining the sampling for parameters. Results like that could be related to feeding management and mainly to the phytoplankton metabolism. The non-utilization of part of the ration spread contributed to the increment of forms of nitrogen into the system. It is suggested to adequate quantity and to improve quality of the food administered.

Key words: Aquatic metabolism, water quality, pisciculture, nitrite, gas ammonia

Dinámica diurna del nitrito y el gas amoníaco en un criadero de Tilapia del Nilo en una region subtropical.

RESUMEN

El objetivo de ese documento es describir las variaciones ocurridas durante el día en las concentraciones de nitrito y gas amoniaco en un criadero de Tilapia del Nilo (*Oreochromis* *niloticus*); identificar el momento exacto en el cual esas sustancias alcanzan niveles impropios para el cultivo y demostrar las mejores prácticas de manipulación. El muestreo fue realizado en el periodo de 16 al 20 de Diciembre de 2006, desde las 6 am hasta las 8 pm cada 2 horas. Se observó que las concentraciones de nitrato y amoniaco son afectadas por las variaciones ocurridas durante el día y que los niveles más altos de gas amoniaco y nitrato pueden ser encontrados a las 4 pm: 12.4 µg/L y 12.6 mg/L, respectivamente. La concentración de nitrito alcanzó niveles considerados impropios para el cultivo. De igual manera se observó que los parámetros nitrito y amoniaco son fuertemente influenciados por radiación subacuática y que en la tarde es el mejor periodo para obtener el muestreo para la comparación. Esos resultados pueden estar relacionados con el manejo de la alimentación y principalmente al metabolismo del fitoplancton. La ración desparramada y no utilizada contribuyó para el aumento de formatos de nitrógeno en el sistema. Se sugiere adecuar la cantidad y mejorar la calidad de la ración distribuida.

Palabras clave: metabolismo acuático, calidad del agua, cultivo de pescado, nitrito, gas amoniaco

INTRODUCTION

Water quality is the result of several interferences and mechanisms between biotic and abiotic environments that interact in a determined aquatic ecosystem. These interactions are particularly intense when cultivating fish in ponds, because the feeding levels, the manure practices and the phytoplankton metabolism influence in a peculiar way the dynamic of the water quality.

Many of such mechanisms of interaction between biotic and abiotic compartments that occur in pisciculture ponds in tropical countries are highly influenced by the photoperiod. Therefore, in order to evaluate the water quality, it is necessary to consider the fluctuation presented by the concentration at the parameter along the diurnal period.

The organic material (ration and dejection) not used will go through decomposition, assimilation, and mineralization processes, and part of it can be assimilated by the algae (Oláh & Szabó, 1986; Kochba, Diab & Avnimelech, 1994; Paerl & Tucker, 1995; Durborow, Crosby & Brunson, 1997). The production of reduced nitrogen compounds like ammonia is a result of aerobic and anaerobic decomposition of organic material.

The nitrite is a potentially toxic nitrogen compound that can accumulate in fish cultivation ponds. It is unstable in the presence of oxygen, and it is an intermediate form in the process of nitrification, where the ammonia is transformed (oxidized) into nitrite by bacteria, and then into nitrate in aquatic systems (Durborow et al. 1997; Piedras, Oliveira, Moraes & Bager, 2006).

Fish exposed to high concentrations of nitrite may present a reduction in the hemoglobin concentration due to its transformation into metahemoglobin; thus decreasing its capability of transporting oxygen to the tissue. As a consequence fish may perish of hypoxia even when oxygen is highly dissolved into water (Sampaio, Pissetti & Morena 2006).

Ammonia gas is considered one of the main parameters to be monitored during the cultivation due to its toxic effects to the fish like: a) elevation of blood pH; b) influence on fish permeability and reduction of internal concentration of ions; c) increase the consumption of oxygen by the tissues, damage the gill and reduce blood capability to transport oxygen d) histological changes mainly in kidney and dull; e) increase of sensibility to diseases (Hargreaves & Kucuk, 2001; Ismiño-Orbe, Araujo-Lima & Gomes, 2003; Cavero, Pereira-Filho, Bordinhon, Fonseca, Ituassú, Roubach & Akifumi Ono, 2004).

The purpose of this paper is to describe the diurnal oscillations found in the concentrations of nitrite and gas ammonia in a fish pond of Nile Tilapia; identify the exact moment these substances reach inappropriate levels for cultivation, and show the best handling practices.

MATERIAL AND METHODS

The experiment was developed at Agencia Paulista de Tecnologia dos Agronegócios – APTA, Regional Vale do Paraíba, district of Pindamonhangaba, situated at 22º 55’ 50” S, 45º 27’22” W, in the State of São Paulo.

The fish pond object of this analysis is dimensioned as follows: 33 x 80 x 29 meters, a superficial area of 2480 m^2, and average depth of 1.0 meter (Fig. 1), and pool volume of 3144 m^3.

A total of 3750 Nile Tilapia, *Oreochromis niloticus* (Linnaeus), young individuals were introduced into the lake. The first population occurred on 20/10/2006 with 2585 individuals (average weight = 118.15 g) and the second one happened on 23/10/2006 with 1166 individuals (average weight = 262.86 g). On December 6th, 2006, due to bird attacks the estimated figure pointed to 3500 fish and average weight of 335.32 g.

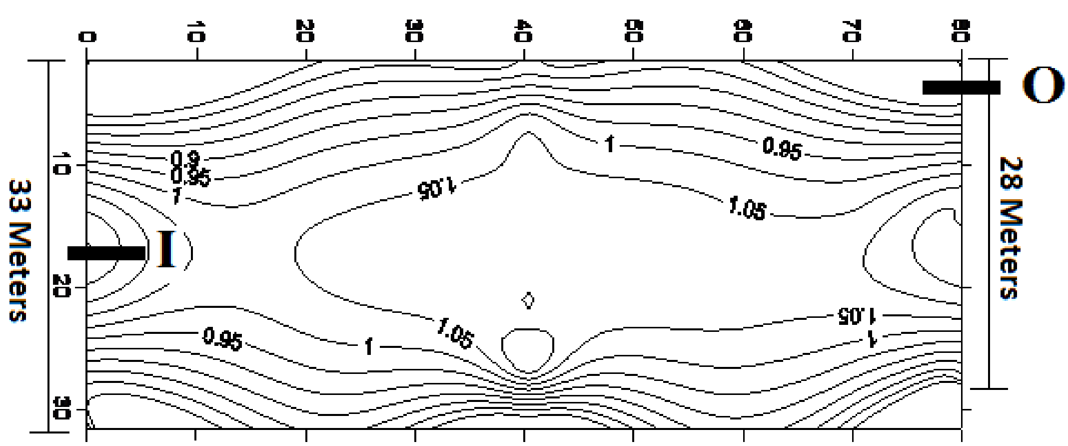

Figure 1 – Diagram with isolines representing the pond depth where I = inflow and O = outflow.
Diagrama con isolineas que representan la profundidad del criadero (I = afluencia y O = flujo).

Feeding was based on the population biomass and maturity phase, considering a rate of 2 % of the fish average weight, or 23.5 kg of extruded ration daily, twice a day. The first feeding was at 7:00 am and the second one at 3:00 pm.

Collections were realized from December 16th until December 20th, 2006 (rainy/hot summer) with the purpose to consider higher temperatures and larger photoperiod. Everyday and every two hours from 6:00 am until 8:00 pm samples were taken from the pond water column subsurface with a polyethylene container. Then the contents were poured into smaller polyethylene bottles of approximately 300 mL each. The concentration of nitrite was found out by Griess modified method (Giné, Bergamin, Zagatto & Reis, 1980) and findings about ammonia gas followed the method described by Nessler (APHA, 1992) for total ammonia, and finally results were submitted to equation 1. The analyses were performed at the research laboratory of Centro de Estudos de Bacias Hidrográficas no Instituto de Pesca in São Paulo.

$$[NH_3] = \frac{K_2\,[NA_T]}{K_2 + [H^+]} \qquad (1)$$

Where: $[NH_3]$ = ammonia gas concentration

$[NA_T]$ = total ammoniacal nitrogen concentration

$[H^+]$ = hydrogen ion concentration

K_2 = constant of equilibrium for the temperature observed at the sampling.

A light meter (Data Looger LI – 1400) was used to register the light radiation on the water column and the multiparameter sound Horiba U – 22 was used to evaluate the dissolved oxygen parameters - DO and pH. Both equipments were placed 30 cm deep into the center of the pond.

ANOVA statistical analysis and linear correlations were performed considering the level of significance 0,05.

RESULTS AND DISCUSSION

Along the five-day sampling campaign, nitrite (NO_2^-) presented an average value of 5.15 mg/L and a pattern deviation of 1.84 mg/L, except for the value of 12.63 mg/L (Table 1) observed during the first day of collection. During the experiment it was observed a periodicity in the fluctuation of NO_2^- concentrations (anova $p = 0.16$). The behavior of nitrite was described as presenting a little increment in the morning and then a fall around 2:00pm followed by another increment, where the higher observed values occur at 4:00pm (Fig. 2). This behavior is related to a synergism of several factors, however feeding management and mainly the phytoplankton metabolism of the pond are the main ones. The ration contributed to this increment because fish do not eat all the offered food, thus promoting the nitrogen compounds (NO_2^- among them) lixiviation to the pond waters. Baccarin, Frascá-Scorvo & Novato (2000) studying the environmental impact of *Oreochromis niloticus* (tilapia) cultivation over different feeding habits concluded that the highest values for nitrite were reached in ponds where extruded ration was used.

Another aspect that contributes to the nitrite accumulation in pisciculture ponds is related to nitrification bacteria requiring a slightly alkaline pH (7 to 8.5) for an optimum growth, (Hargreaves, 1998). In this analysis average values of pH equal to 6.22 (minimum 4.9 and maximum 7.41) were observed. Therefore, maybe the *Nitrobacter* bacteria was inhibited more than the *Nitrosomonas* ones, thus resulting in an accumulation of nitrite.

Wada & Hattori (1971) working with ocean phytoplankton state that 50 - 63% of the absorbed nitrate (NO_3^-) was released as NO_2^- under low radiation conditions. Notice at figure 2 that from 2:00pm until 4:00pm there was a significant fall of the subaquatic radiation and at the same period it is observed an increment of the nitrite concentrations.

The values observed for nitrite are considered high. Piedras *et.al.* (2006) found out during a laboratory experiment that 45.6% of Cará (*Cichlasoma facetum*) fingerlings died after being exposed during 96 hours to a concentration of 6.68 mg/L. Oliveira, Souza, Nunes, Carvalho, Menezes, Marcon, Akifumi Ono & Affonso (2008) determined that LC_{50} to cardin

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Table 1 – Average, minimum and maximum (between parenthesis) values for the parameters observed during the experiment. *Mediana, valores mínimos y máximos (entre paréntesis) para los parámetros observados durante el experimento.*

Time	Nitrite (mg/L)	Ammonia (µg/L)	DO* %	pH	Radiation ($mmol.s^{-1}.m^{-2}$)
06:00	3,98 (3,30-5,84)	0,20 (0,03-0,31)	68,9 (53,0-80,0)	5,55 (4,90-5,84)	4,6E-5 (2,9E-5 - 6,0E-5)
08:00	4,66 (3,86-6,41)	0,18 (0,14-0,23)	81,8 (72,8-92,1)	5,60 (5,50-5,74)	2,3E-4 (2,0E-4 - 2,6E-4)
10:00	4,66 (3,58-6,41)	0,37 (0,20-0,43)	97,7 (81,3-116,9)	5,87 (5,65-6,01)	3,9 (3,4 - 3,5)
12:00	5,22 (4,15-8,1)	2,78 (1,70-4,81)	115,8 (104,6-26,9)	6,65 (6,41-6,94)	4,6 (3,7 - 5,5)
14:00	3,81 (3,3-4,71)	6,44 (0,94-10,14)	114,0 (97,5-120,7)	6,93 (6,21 -7,22)	4,2 (3,4 - 5,0)
16:00	7,77 (6,41-12,63)	5,36 (1,21-12,39)	114,0 (98,4-125,1)	6,77 (6,25-7,30)	1,7E-4 (4,4E-4 - 2,6E-4)
18:00	6,52 (4,99-8,67)	3,67 (0,45-10,0)	106,8 (92,4-118,4)	6,55 (5,83-7,28)	1,2E-5 (4,7E-6 - 2,7E-5)
20:00	4,66 (3,58-6,69)	0,40 (0,25-0,51)	103,23	5,85 (5,64-6,00)	0,0 (0,0 - 0,0)

* DO - Dissolved Oxygen

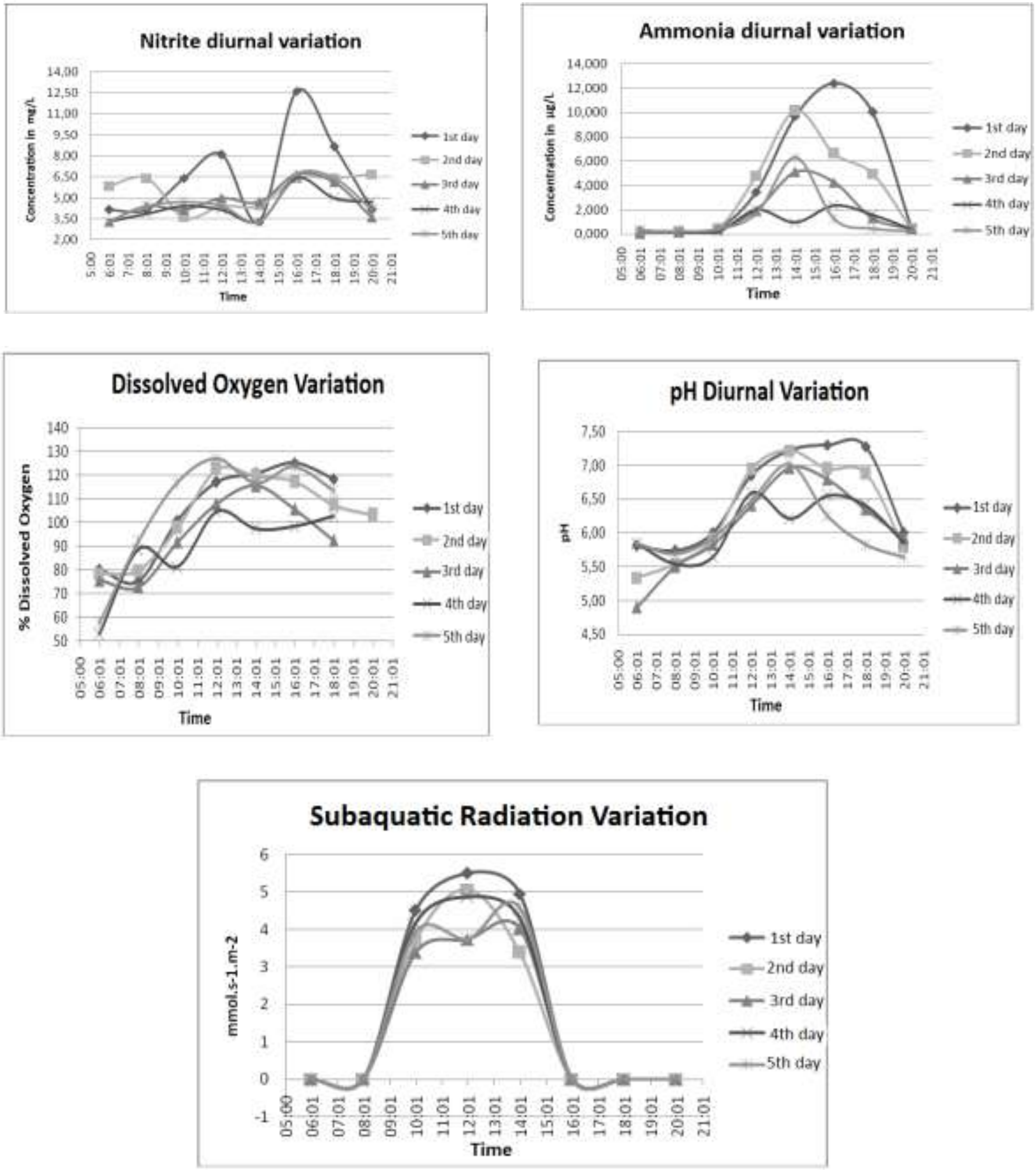

Figure 2 – Diurnal variation of parameters evaluated during the experiment. *Variación diurna de los parámetros evaluados durante el experimento.*

(*Paracheirodon axelrodi*) is 1.1 mg/L of nitrite. Corrêia, Neto, Lazzari, Veiverberg, Bergamin, al tetra Pedron, Ferreira, Emanuelli & Ribeiro (2009) evaluated the growing of Hungarian carps (hornbeam) and *jundiás* in a water circulation system and found concentrations of 0.11 mg/L nitrite into the pond.

During the five-day campaign, ammonia gas (NH_3) showed an average value of 2.43 μg/L and a pattern deviation of 3.31 μg/L (Table 1). Ammonia gas presented a daily fluctuation pattern during the experiment, presenting concentration peaks between 2:00 pm and 4:00 pm (Fig. 2), (ANOVA p = 0.13). The behavior of ammonia gas is related to the pH variation into the environment (as described at Materials and Methods), presenting a linear correlation of 0.86 with it. The diurnal oscillations of subaquatic radiation cause alterations in pH due to the phytoplankton metabolism.

Consuming carbon dioxide (CO_2), the photosynthesis community provokes the increase of the environment pH, due to a displacement of equation 2 to the left, in addition to the carbonate ion hydrolysis (HCO_3^-) that generates hydroxyl ions (OH^-), as per equation 3.

$$CO_2 + H_2O \leftrightarrow HCO_3^- + H^+ \quad (2)$$

$$CO_3^{-2} + H_2O \leftrightarrow HCO_3^- + OH^- \quad (3)$$

The concentrations of NH_3 determined during the experiment are below the critical values pointed out by lots of authors. Acute exposure to 0.6 mg/L NH_3-N is lethal to most fish, while chronic exposure to 0.6 mg/L NH_3-N damages respiratory and hepatic tissue thus reducing growth for it causes cerebral dysfunction and reduces blood oxygenation capacity (Duborow *et al.*, 1997). Piedras *et al.* (2006) observed that pejerrey, *Odontesthes bonariensis,* larvae exposed to un-ionized ammonia during 96 hours presented 50% mortality at 0.71 mg/L NH_3-N.

The ammonia toxicity potential is still lower when the concentration of dissolved oxygen is considered. Foss, Evensen, Vollen & Oiestad (2003) reports that ammonia toxicity rises as the level of oxygen decreases; and adds that tolerance to ammonia for fish cultivation can be raised to levels above the normal saturation, using water oversaturated with oxygen. Figure 2 shows that the time the highest concentrations of ammonia were observed coincides with the time the highest percentage of dissolved oxygen saturation was noticed, presenting a positive linear correlation of 0.59, thus decreasing the ammonia gas toxicity potential.

CONCLUSIONS

1. During the experiment, it was confirmed the periodic oscillation of the concentration of the parameters as a consequence of the subaquatic radiation availability.
2. The concentration of nitrite reached values that could put into risk the fish cultivation in the afternoon.
3. The nitrite and ammonia sampling should be realized in the afternoon preferably. This behavior is related to a synergism of several factors, however feeding management and mainly the phytoplankton metabolism of the pond are the main ones. The ration contributed to this increment because fish do not eat all the offered food, thus promoting the nitrogen compounds (NO_2^- among them) lixiviation to the pond waters.
4. It is suggested to adjust the ration quantity and quality so that the use can be improved and consequently promote the system equilibrium.

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