Larviculture of Oreochromis niloticus Linnaeus, 1758 (Perciformes, Cichlidae) in ponds with different levels of water alkalinity.

ROJAS[1], N.E.T., MAINARDES-PINTO[1], C.S.R., ROCHA[2], O. & SILVA[1], A.L. DA

[1] Instituto de Pesca, SAA, Av. Francisco Matarazzo, 455, CEP 05001-900, Água Branca, São Paulo, SP, Brazil, niltonrojas@uol.com.br

[2] Universidade Federal de São Carlos, DEBE, Caixa postal 676, CEP 13565-905, São Carlos, São Paulo, Brazil.

ABSTRACT: Larviculture of Oreochromis niloticus Linnaeus, 1758 (Perciformes, Cichlidae) in ponds with different levels of water alkalinity. The growth of Oreochromis niloticus larvae and the limnological variables were studied in ponds with different levels of water alkalinity. The experiment, which was carried out over a period of 60 days, consisted of three treatments: A - without any correction of natural alkalinity; B - weekly alkalinity correction to 30 mg $CaCO_3$/L; C - weekly alkalinity correction to 60 mg $CaCO_3$/L. The following variables were monitored: maximum and minimum air and water temperature, alkalinity, hardness, calcium, pH, conductivity, dissolved oxygen, suspended solids, transparency, ammonia, nitrite, nitrate, total phosphorus, dissolved phosphate, potassium, chlorophyll a and organic fraction weight of zooplankton. The growth in total length and dry weight were determined, as well as the relationship between dry weight and total length of the larvae in the different treatments. The results indicate that the maintenance of tilapia larvae under mean values of alkalinity between 35.61±4.36 and 53.41±9.42 mg $CaCO_3$/L (treatments B and C), which provided calcium concentration between 5.95±1.08 and 10.31±2.43 mg Ca^{2+}/L, is recommended for promoting better growth in total length (8.50±0.98 and 8.51±0.97 cm) and dry weight (2.43±0.96 and 2.45±0.96 g), respectively.
Key-words: larviculture, alkalinity, fish growth, Oreochromis niloticus.

RESUMO: Larvicultura de Oreochromis niloticus Linnaeus, 1758 (Perciformes, Cichlidae), em viveiros com diferentes níveis de alcalinidade da água. O crescimento de larvas de Oreochromis niloticus e as variáveis limnológicas foram estudados em viveiros com diferentes níveis de alcalinidade da água. O experimento, conduzido durante 60 dias, consistiu de três tratamentos: A – sem correção da alcalinidade natural; B – correção semanal da alcalinidade para 30 mg $CaCO_3$/L; C – correção semanal da alcalinidade para 60 mg $CaCO_3$/L. Foram monitorados os valores de temperatura máxima e mínima do ar e da água, alcalinidade, dureza, cálcio, pH, condutividade, oxigênio dissolvido, sólidos em suspensão, transparência, amônia, nitrito, nitrato, fósforo total, fosfato dissolvido, potássio, clorofila a e peso da fração orgânica do zooplâncton. Foram determinados o crescimento em comprimento total e em peso seco e as relações peso seco-comprimento total das larvas nos diferentes tratamentos. Os resultados permitem concluir que a manutenção de larvas de tilápia sob valores médios de alcalinidade entre 35,61±4,36 e 53,41±9,42 mg $CaCO_3$/L (tratamentos B e C), que proporcionaram concentrações de cálcio entre 5,95±1,08 e 10,31±2,43 mg Ca^{2+}/L, são recomendados por promoverem melhor crescimento em comprimento total (8,50±0,98 e 8,51±0,97 cm) e em peso seco (2,43±0,96 e 2,45±0,96 g), respectivamente.
Palavras-chave: larvicultura, alcalinidade, crescimento de peixe, Oreochromis niloticus.

Introduction

The national production of fish derived from aquaculture in Brazil in 2001 was approximately 209,000 ton., 75% of which consisted of freshwater fish (Ibama, 2003).

Therefore, studies that result in the maximization of freshwater fish production greatly influence the national overall production. Among all freshwater species, tilapia is regarded as the most important one for aquaculture in the twenty-first century, due to its rusticity and the several ways in which it can be reared (Fitzsimmons, 2000).

Studies about pond limnology have been thought as fundamental to the development of aquaculture, since water is the most important natural resource for the producer. Weatherley (1988) believes that, for commercial reasons, the effects of acidification and liming over the fish have received more attention than the management of other abiotic factors, because such procedures can improve the survival of the individuals.

The use of some kind of carbonated product in liming is usually recommended. Some authors suggest the application of standard quantities of those products (Huet, 1973), while others propose a wide range of alkalinity correction (Boyd, 1990), or just the initial adjustment of water hardness before populating the pond (Rodrigues et al., 1991).

There is no recommendation in the literature for periodical adjustment of water alkalinity, nor any indication of specific values for this variable in tilapia larvae culture. Preliminary tests evidenced that the procedures commonly employed in the correction of water alkalinity or hardness before populating the pond (soil liming), do not provide a suitable alkaline reserve during the entire rearing period.

Thus, the aim of this study was to verify how the maintenance of water alkalinity levels can influence the growth of tilapia, Oreochromis niloticus, larvae and the limnological characteristics of fish ponds.

Material and methods

The experiment was carried out at the Aquaculture Center of the Fishery Institute - Pindamonhangaba, São Paulo, Brazil - in six 180m² earthen ponds (6X30 m each).

The treatments employed were (with one repetition): A - without any correction of natural alkalinity; B - weekly alkalinity correction to 30 mg $CaCO_3$/L; C - weekly alkalinity correction to 60 mg $CaCO_3$/L. Hydrated lime ($CaCO_3.2H_2O$), previously oxidized for 12 hours to avoid sharp pH variations, was used in alkalinity correction. The selection of correction levels was based on preliminary tests and on the natural alkalinity (around 20 mg $CaCO_3$/L). Before the beginning of the experiment, the ponds had been kept empty for seven days. They were then submitted to an initial liming with 100 g/m² of hydrated lime. The ponds were not fertilized. Afterwards, they were filled with water, taking the precaution of controlling the flow in order to keep a constant level and the selected alkalinity.

The larvae utilized in the treatments had been collected directly from the tilapia breeding ponds seven to ten days after hatching, and transferred to the experiment ponds. Larvae or juvenile total count, at the beginning and at the end of the study, was performed through sampling. An initial density between 128 and 131 larvae/m² was achieved. After the first week of experiment, the larvae started receiving, ad libitum and twice a day, 40% crude protein feed.

The values of maximum and minimum air and water temperature and water transparency (Secchi disk) were recorded daily. Zooplankton was collected weekly by trawling, using a 60 mm mesh plankton net all over the pond surface. The organisms were fixed in 4% formalin for posterior determination of the organic fraction weight, by gravimetric method. Chlorophyll a concentration was measured weekly, according to Golterman & Clymo (1969), with the modifications proposed by Wetzel & Likens (1991). Ammonia, nitrite, nitrate, total phosphorus and dissolved phosphate concentrations were determined every twelve hours according the methodology of APHA (1975). Electrical conductivity, pH, dissolved oxygen, alkalinity, hardness and calcium were measured every six hours, for a period of twenty-four hours, at intervals of one week. The solids in suspension were determined once a week.

Forty fishes from each pond were measured and weighed every fifteen days. The experiment lasted for sixty days.

The Kruskal-Wallis Test was initially used to identify any significant differences among the treatments concerning growth in total length and dry weight, chlorophyll a concentration, physical and chemical water variables and organic fraction weight of zooplankton. When the differences among the results were significant ($p<0.05$) a nonparametric Dunn Test was used (Zar, 1999). In order to contrast the relationships between dry weight and total length, an Analysis of Covariance (ANCOVA) was employed, through the Homogeneity Test (Ayres et al., 2000).

Results

The O. niloticus larvae used at the beginning of the experiment exhibited an average of 1.15±0.13 cm total length; 13.10±7.77 mg wet weight and 2.22±1.10 mg dry weight (n=20).

The initial density of tilapia larvae ranged from 128 to 131 larvae/m² among the treatments. The highest juvenile final density (treatment B - alkalinity correction to 30 mg $CaCO_3$/L) corresponded to 56.88 juveniles/m² (10,238 juveniles/180 m² pond). The lowest values of juvenile final survival rate were found in treatment A, where alkalinity was not adjusted. The highest ones were obtained in treatment B (Tab. I).

Table I: Mean values of initial larvae stocking density, total larvae number at the beginning of the experiment, final juvenile number, survival rate, values (±S.D.) of total length, dry weight and wet weight, measured during the last week of the experiment with Oreochromis niloticus, kept in ponds under different levels of water alkalinity.

Treatment	Initial Density (larvae/m²)	Initial Number of Larvae	Final Number of Juveniles	Survival Rate (%)	Total Length (cm)	Dry Weight (g)	Wet Weight (g)
A	131	23,615	7,146	30.26	7.14±1.47	1.55±0.85	8.33±4.36
B	128	23,040	10,238	44.43	8.50±0.98	2.43±0.96	12.32±2.45
C	131	23,615	9,448	40.01	8.51±0.97	2.45±0.96	12.30±4.34

Fig. 1 shows adjusted curves with the results of increase in total length, dry weight and the relationship between dry weight and total length Oreochromis niloticus larvae submitted to the three treatments.

The Kruskal-Wallis Test indicated significant difference among the treatments. The nonparametric Dunn Test showed that the growth in total length and dry weight observed in the treatments B and C, were statistically similar. The comparison between the regression coefficients (b) and the relationship between dry weight and total length, showed no significant difference among the treatments. Therefore, the performance of the fishes with regard to growth in total length and dry weight was greater in B and C treatment ponds, where the larvae had been kept under levels of alkalinity between 35.61±4.36 and 53.41±9.42 mg $CaCO_3$/L, respectively.

The mean values of air and water temperature ranged from 20.2±2.0 to 32.7±3.5°C and 26.6±2.2 to 28.8±1.8°C, respectively. Alkalinity, calcium and hardness values were significantly different among the three treatments, subjecting the larvae to a wide range of alkalinity (26.00±3.87 to 53.41±9.42 mg $CaCO_3$/L), calcium (3.38±0.82 to 10.31±2.43 mg $CaCO_3$/L) and hardness (21.15±2.16 to 47.09±10.46 mg $CaCO_3$/L). In the B and C treatments, the water of ponds presents similar pH values (7.54±0.85 and 7.66±0.51, respectively), both of which were higher than the ones observed in treatment A (7.12±0.38). Electrical conductivity was significantly different among the three treatments due to the weekly addition of calcium carbonate. The values fluctuated between 51.50±11.87 mS/cm

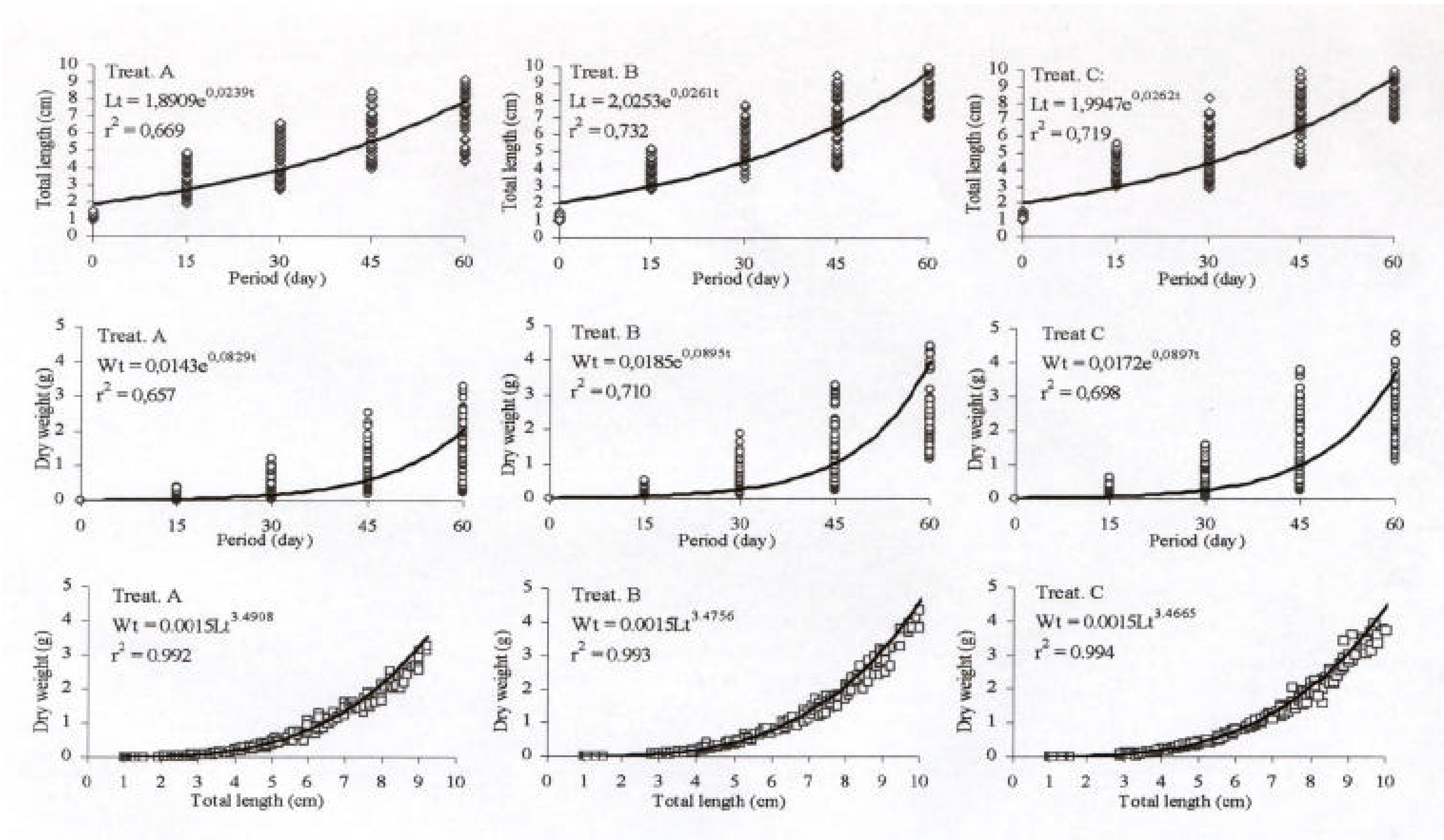

Figure 1: Growth in total length and dry weight and exponential equations of adjustment and relationship between dry weight and total length and potential equations of adjustment of the *Oreochromis niloticus* larvae, kept under three different levels of water alkalinity.

(treatment A) and 100.60±15.66 mS/cm (treatment C). The mean values of dissolved oxygen (›5.0 mg O_2/L) were significantly similar among the three treatments.

The content of suspended solids was significantly similar among the three treatments. However, transparency was higher in treatment B (58.10±15.27 cm), while in treatments A and C the values were similar (52.10±11.42 and 48.94±12.96 cm, respectively). There was no significant difference in the values of total ammonia, nitrite, nitrate, phosphorus, phosphate, chlorophyll a and dry weight of zooplankton among the three treatments.

The Kruskal-Wallis Test results expressed significant difference among the treatments, and the posterior application of the Dunn Test made it possible to conclude that the treatments employed influenced water quality (Tab. II).

Table II: Mean values (±S.D.) of abiotic and biotic variables of the water supply, and the three treatments of Oreochromis niloticus larviculture under different levels of water alkalinity.

Variable	**Water supply**	**Treatment A**	**Treatment B**	**Treatment C**
Alkalinity (mg $CaCO_3$/L)	21.31±3.38	26.00±3.87 a*	35.61±4.36 b	53.41±9.42 c
Calcium (mg Ca^{2+}/L)	2.13±0.62	3.38±0.82 a	5.95±1.08 b	10.31±2.43 c
Hardness (mg $CaCO_3$/L)	15.65±2.41	21.15±2.16a	30.12±3.96b	47.09±10.46c
pH	7.06±0.26	7.12±0.38 a	7.54±0.58 b	7.66±0.51 b
Conductivity (mS/cm)	41.02±8.28	51.50±11.87 a	69.35±11.31 b	100.60±15.66 c
Diss. oxygen (mg O_2/L)	5.24±0.67	5.56±1.95 a	5.86±2.35 a	5.36±2.44 a
Solids in Susp. (mg/L)	9.43±3.27	16.25±7.49 a	13.65±5.22 a	16.66±8.08 a
Transparency (cm)	-	52.10±11.42 a	58.10±15.27 b	48.94±12.96 a
Total ammonia (mg NH_3/L)	0.50±0.24	0.61±0.42 a	0.52±0.38 a	0.58±0.38 a
Nitrite (mg N-NO_2/L)	7.32±3.22	12.48±31.43 a	10.54±30.14 a	19.10±64.81 a
Nitrate (mg N-NO_3/L)	0.12±0.03	0.11±0.02 a	0.10±0.02 a	0.10±0.02 a
Phosphorus (mg P-PO_4/L)	69.26±28.14	128.63±42.13 a	129.07±49.76 a	150.07±69.08 a
Phosphate (mg P-PO_4/L)	26.04±12.15	42.01±14.91 a	45.46±21.38 a	49.24±23.82 a
Chlorophyll a (mg/L)	-	32.01±29.74 a	32.01±28.91 a	29.55±22.83 a
Zoopl. Dry Weight (g.10^2)	-	4.03±1.78 a	2.80±1.52 a	3.99±5.78 a

*Different letters indicate statistical difference ($p<0.05$) among treatments.

Discussion

The survival rate obtained in this study, between 30 and 44%, for a period of 60 days, may be considered as satisfactory. The best survival rate was found in the treatments B and C. However, larvae survival is also affected by predation, mishandling or bad weather, especially at the beginning of larviculture.

The mean values of maximum (32.7±3.5°C) and minimum air temperature (20.2±2.0°C), of maximum (28.8±1.8°C) and minimum water temperature (26.6±2.2°C) were within suitable limits for the development of tropical fish (Huet, 1973).

The growth in length and weight of O. niloticus larvae, found when alkalinity levels ranged from 35.61±4.36 to 53.41±9.42 mg $CaCO_3$/L (treatments B and C), corresponded to the best animal's performance. Duran & Loubens (1969) consider that length growth in fish is a reliable sign of development, because it is closely related to changes in bone structure.

In the treatment without alkalinity correction (26.00±3.87 mg $CaCO_3$/L), the most common practice in Brazilian fish culture, the lowest values of growth and survival rate of tilapia larvae were found.

No significant difference in the weight/length relationship was found in the three treatments, denoting similarity in the nutritional status of the larvae (Vazzoler, 1971; Weatherley, 1972).

Water alkalinity was significantly different among the treatments, being close to those predicted on the experimental design. The maintenance of alkalinity was hindered by the rain, which altered the concentration of carbonated substances in the water of the ponds.

Amongst the most commonly recommended liming procedures, Huet (1973) proposes the addition of 100 to 1,500 kg/ha calcium oxide in order to eradicate parasites and provide suitable calcium carbonate reserves. This author recommended a liming method for the supply water using a mill or waterwheel, which releases the carbonated substance at a rate of 50 to 1,000 kilograms per week. Boyd (1990) considers levels of alkalinity between 25 and 100 mg $CaCO_3$/L as acceptable, and states that the demand for lime can be determined by the water pH and the kind of soil, resulting a range from 895 to 14,320 kg/ha of lime.

According to Rodrigues et al. (1991) the liming procedures are not performed correctly, due to the alkalinization caused by microbial activity during water-soil interactive. These authors suggest an initial adjustment of lime to correct the value of water hardness to 25 mg $CaCO_3$/L. Kubitza (1999) recommend the adjustment of acidity based on the pH value which could result in a range between 750 and 2,200 kg/ha of hydrated lime, altering the alkalinity to 30 mg $CaCO_3$/L. Alkalinity should be checked and corrected, if necessary, one or two weeks after pond filling, employing 500 to 1,000 kg/ha of lime (Kubitza, 1999). Recently, the application of agricultural calcium carbonate in the pond water was recommended (Boyd & Queiroz, 2004) in order to improve water and effluent quality whenever the alkalinity is lower than 20 mg $CaCO_3$/L. In our study, in the treatment with no weekly correction of alkalinity (treatment A), an initial liming with 100 g/m^2 (1,000 kg/ha) of hydrated lime was used.

Since no recommendations for periodical correction neither any indication of specific values of water alkalinity were found in literature, the results of our study suggest that, for O. niloticus, water alkalinity should be adjusted to and kept between 35.61±4.36 and 53.41±9.42 mg $CaCO_3$/L (treatments B and C), and the values of calcium should range from 6 to 10 mg Ca^{2+}/L. However, some important relationships between water alkalinity and other variables were established by Sipaúba-Tavares et al. (1999a), suggesting that the alkalinity fluctuations are connected to the ions in the environment and can also due to high bacterial decomposition producing great quantities of CO_2, that increase the solubility of calcium carbonate in the sediment. Besides, the pH held its alkalinity showing significant differences during the period with the aerator functioning when compared without and after the use of aerator (Sipaúba-Tavares et al., 1999b).

Townsend et al. (2003) observed higher survival rate for Rhamdia quelen larvae under 30 mg $CaCO_3$/L water hardness, and greater biomass gain under 30 and 70 mg $CaCO_3$/L. So, hardness affects the native Brazilian fish development. Similar results were found in the present study for O. niloticus larvae, since larger growth was found under hardness values between 30 and 47 mg $CaCO_3$/L.

Some species used in rearing systems have specific requirements for alkalinity and calcium. A twenty-five percent higher production of Tilapia aurea was obtained in ponds with higher alkalinity (between 28.5 and 38.1 mg $CaCO_3$/L). Alkalinity also reduced the discrepancy in fish production among the ponds (Arce & Boyd, 1975). The same species, when reared in ponds without calcium in water, also needs a suitable amount of this substance in their food (0.17 and 0.65% of Ca^{+2}) in order to present growth and bone mineralization (Robinson et al., 1984). Furthermore, O'Connel & Gatlin III (1994) attested that calcium is more important than vitamin D3 for Oreochromis aureus kept under low concentration of that ion. Hwang et al. (1994) affirmed that during the embryonic phase, O. mossambicus larvae keep the levels of sodium, potassium and calcium constant in their bodies, but ten days after hatching, the levels of calcium are sixty times higher. The maintenance of suitable levels of calcium in the environment is necessary, so that the larvae can obtain it with lower energy expense. Hwang et al. (1996) reported that the hatching rate and growth of O. mossambicus larvae were not affected by different concentrations of calcium in the water (0.8 and 87.2 mg Ca^{2+}/L), but the larvae growth occurred only three days after hatching. Nevertheless, the present study evidenced that

O. niloticus larvae have their development impaired if the levels of calcium in the water are below 6 mg Ca^{2+}/L.

Water transparency was higher than 30 to 40 cm, in all the treatments. According to Rodrigues et al. (1991), above this range the plankton production may still be raised through fertilization to increase fish production. However, the fish growth found in the ponds with alkalinity correction to 30 and 60 mg $CaCO_3$/L (treatments B and C) suggest that water transparency was no related only with plankton concentration. The utilization of natural food production by the larvae would also be related to the management of other variables, such as water alkalinity. Thus, the determination of water quality for each species would lead to a better physiological performance and consequent larger growth and better food use. Therefore, the primary attempts to develop aquaculture should concern the enhancement of knowledge about water quality. Since live and inert feed availability was the same in every pond, alkalinity, hardness, calcium, pH and conductivity in treatments B and C (significantly higher than the ones found in treatment A) may be considered suitable to promote a better physiological performance.

The dissolved oxygen in the experimental pond water was recorded within the limits of 4 to 10 mg O_2/L recommended by Boyd (1990). Accordingly, the relationships between carbon dioxide, carbonate and bicarbonate were similar in all ponds, despite the different alkalinity levels tested. Furthermore, fish density was low, the amount of daily feed was small and the ponds had not been fertilized.

The mean concentration of total ammonia was above to the maximum limit of 0.7 mg NH_4^+/L recommended by Kubitza (1999). Nevertheless, analyzing the values of mean and standard deviation amount, it was verified that the limit was exceeded in all the treatments. This nitrogen compound may have originated from the pond soil (Zimba et al., 2003), because the ponds were built over 20 years ago. On the other hand, the concentration of non-ionized ammonia (NH_3), calculated from the highest concentration of total ammonia, found in treatment A, reached 0.01 mg/L (below 0.20 mg/L Arana, 2004). The small concentration of non-ionized ammonia and similar values of total ammonia, nitrite and nitrate, in all the treatments, occurred because dissolved oxygen is present in the water (Arana, 2004).

The solids in suspension were below the range of 25 to 80 mg/L proposed by Alabaster & Lloyd (1980), in all the ponds. In the three treatments, the water pH ranged from 6 to 8 and the values of nitrite and nitrate were below 1.0 mg/L and 10 mg/L, respectively, as recommended by Boyd (1990).

In fish culture, there are few studies relating the concentration of total phosphorus and dissolved phosphate to fish productivity. Sipaúba-Tavares et al. (1999) – in pacu rearing - observed total phosphorus values ranging from 140.0 to 829.0 mg/L when using two kinds of feed with different protein content and three stock densities. Fish can excrete soluble and particulate forms of phosphorus and affect water quality directly, increasing the primary productivity and the phytoplankton growth. Nevertheless, despite the smaller variation of total phosphorus (between 128.63 and 150.07 mg/L - similar among the treatments), the present study showed a higher increase on algal growth since the chlorophyll a biomass index ranged from 29.55 to 32.01 mg/L, while Sipaúba-Tavares et al. (1999) had found values between 4.8 and 11.5 mg/L.

Chlorophyll a concentration is an indicator of phytoplankton biomass and is therefore related to zooplankton production. In spite of that, in the ponds where alkalinity had been corrected to 30 and 60 mg $CaCO_3$/L (treatments B and C), and where the best performance in growth was obtained, the values of organic fraction weight of zooplankton were not higher than the ones found in treatment A.

Thus, the level of water alkalinity acquired by the procedure traditionally recommended for fish culture - only one initial soil liming, or correction of water alkalinity before populating the pond - did not result in higher growth for Oreochromis niloticus larvae. The management of water quality in larviculture ponds with O. niloticus should be carried out through periodical corrections of alkalinity to values between 35.61±4.36 and 53.41±9.42 mg $CaCO_3$/L, corresponding to 5.95±1.08 and 10.31±2.43 mg Ca^{2+}/L.

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