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# Effects of liming and development of Curimbatá *(Prochilodus lineatus)* larvae on the abundance of zooplankton in fish ponds

## Efeitos da calagem e desenvolvimento do Curimbatá (*Prochilodus lineatus*) na abundância do zooplâncton em viveiros de piscicultura

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**Abstract: Aim:** We aimed to evaluate the influence of the correction of the water alkalinity in the fish ponds on the density of zooplankton under a period they were stocked with larvae of *Prochilodus lineatus*, a neotropical fish called "Curimbatá". **Methods:** We used a factorial design completely randomized. In one plot (2 ponds) there was no correction of the alkalinity of the water (20 mg $CaCO_3.L^{-1}$) and in two others, this variable was adjusted weekly to values around 30 and 60 mg $CaCO_3.L^{-1\ 1}$, with two replicates each. Zooplankton was sampled weekly and the experiment lasted 63 days. **Results:** Significant differences in the density of the zooplankton over time ($F = 6.78$, $p < 0.05$) were found and there was a sharp decrease in zooplankton density from first to second week, and successive small increases in density from the fourth week until the end of the experiment. When considering the entire study period, alkalinity correction 60 mg $CaCO_3.L^{-1}$ resulted in higher density of zooplankton. Great changes in zooplankton composition occurred. Rotifera were dominant at the beginning of the experiment and Cladocera and Copepoda in the later weeks possibly due to an interplay of dilution by rain and fish predation in the initial part of the experiment and due to both alkalinity correction and predation by both fish and predatory copepods from the fourth week until the end. **Conclusion:** The experiment corroborated the hypothesis that correction of water alkalinity in ponds does favor zooplankton development but also highlighted biotic and abiotic interaction of factors operating simultaneously.

**Keywords:** fish predation, zooplankton, alkalinity correction, liming.

**Resumo: Objetivo:** Nosso objetivo foi avaliar a influencia da correção da alcalinidade da água em viveiros de piscicultura na densidade do zooplâncton em período em que foram estocados com larvas de *Prochilodus lineatus,* um peixe neotropical denominado "Curimbatá". **Métodos**: Foi utilizado um delineamento experimental fatorial, inteiramente causualizado. Em um tratamento (2 viveiros), não houve correção da alcalinidade da água, e em outros dois viveiros, a alcalinidade foi ajustada semanalmente para valores em torno de 30 e 60 mg $CaCO_3.L^{-1}$, com duas réplicas cada. Os organismos zooplanctônicos foram coletados semanalmente durante 63 dias. **Resultados:** Diferenças significativas foram observadas na densidade do zooplâncton ao longo do tempo ($F = 6,78$, $p < 0,05$) e um decréscimo acentuado na densidade do zooplâncton foi observado da primeira para a segunda semana, e pequenos aumentos sucessivos na densidade da quarta semana até o final do experimento. Ao considerar todo o período experimental, a alcalinidade corrigida para 60 mg $CaCO_3.L^{-1}$ resultou em maiores densidades de zooplâncton. Ocorreram grandes mudanças na composição zooplanctônica. Rotifera foram dominantes no início do experimento e Cladocera e Copepoda nas últimas semanas, possivelmente devido a uma interação da correção da alcalinidade e predação tanto por peixes quanto por copépodos predadores que ocorreram a partir da quarta semana até o fim. **Conclusão:** O experimento corroborou a hipótese que a correção da alcalinidade da água em viveiros favorece o desenvolvimento do zooplâncton, mas também destacou-se a interação de fatores bióticos e abióticos atuando simultaneamente.

**Palavras-chave:** predação por peixe, zooplânton, correção de alcalinidade, calagem.

## 1. Introduction

Zooplankton community is responsible for the transfer of a large fraction of energy in food chains, being the main link between primary producers and other levels of the food web in aquatic systems (Sipaúba-Tavares et al., 2010a). Its importance in fish culture is independent of the feeding habits of fish in adulthood, since fish in the larval stage and fingerlings feed mostly on zooplankton (Cestarolli et al., 1997). Thus one of the basic requirements for success in fish farming projects is to obtain zooplankton in abundance and good nutritional quality to provide food for fingerlings (Pinto-Coelho et al., 1997).

Despite efforts to completely replace live food by artificial diets, fish farmers still depend on the production and use of microorganisms for feeding aquaculture species (Guevara, 2003). Besides the nutritional supply, the diet must to be in an adequate distribution throughout the volume of water and preferentially to be slow moving organisms with colorful bodies to facilitate the capture by larval fish (Lavens and Sorgeloos, 1996).

The structure of plankton community is favored by adequate technical management, insofar as they provide the density of organisms that turns out to be friendly for the development of larvae and fingerlings, as well as to prevent the presence of toxins and algal blooms that may undermine the success of fish production (Macedo et al., 2005).

One of the useful techniques applied in the management of aquaculture is the liming (Valdenberg et al., 2006), which is the process of adding lime to the ponds. This improves the availability of nutrients for phytoplankton, helps to maintain the pH of the water at a more stable level and provides calcium for the normal development of zooplankton (Rojas et al., 2004). Moreover, the physiology of several species of fish is also affected by the pH of the water (Moraes, 2001) which is controlled by adding lime to the ponds. However, liming the ponds should be a well studied and controlled process, because field observations has revealed that there are some limitations to this procedure, which can cause long term problems, mainly when treatment is inadequate, by lack or excess of lime (Boyd and Daniels, 1993).

If used properly, liming supplies Ca and Mg to the environment; it helps with the release of ions which contribute to total alkalinity and total hardness; operates in the pH balance in water; increases the availability of carbon for photosynthesis; prevents the loss of $CO_2$ into the atmosphere and reduces the water turbidity (Boyd, 1979; Boyd and Daniels, 1993).

The good quality of water reflects positively on the living biomass of different trophic levels and therefore ecological studies on zooplankton communities can provide important information as subsidies for the management practices in nurseries that in turn may have a great effect on fish production.

The composition and abundance of zooplankton communities are affected by numerous physical, chemical and biological processes that operate simultaneously and interact to varying degrees, changing its structure through different paths (Rocha et al., 1995; Sampaio et al., 2002). Both quality and quantity abundance of plankton communities in fishponds are of great importance in managing successful aquaculture operations (Boyd and Daniels, 1993; Boyd, 1998; Bhuiyan et al., 2008).

The aim of this study was to experimentally analyze how the zooplankton community responds to the liming of fish ponds stocked with *Prochilodus lineatus* fingerlings, dealing with the hypotheses that liming favors the zooplankton production and that fish growth, and consequently the increase in fish biomass, progressively reduces zooplankton abundance.

## 2. Material and Methods

The experiment was conducted at the Aquaculture Fisheries Institute in Pindamonhangaba, SP, Brazil. For this study six ponds on land with 180 $m^2$ area (6 × 30 m) were selected. The experiment lasted 63 days.

Before the beginning of the experiment the ponds were kept without water for seven days and then subjected to an initial liming with 100 $g.m^{-2}$ of $CaCO_3$. The first two ponds did not receive a weekly correction of the alkalinity of the water. This was carried out only with an initial liming according to the procedure commonly employed by regional fish farmers. In this case, the alkalinity remained around 20 mg $CaCO_3.L^{-1}$ (T1 and T2 ponds). On the other ponds the alkalinity of the water was adjusted weekly to values around 30 mg $CaCO_3.L^{-1}$ (T3 and T4 ponds) and 60 mg $CaCO_3.L^{-1}$ (T5 and T6 ponds). For the correction of alkalinity, hydrated lime ($CaCO_3.2H_2O$) was used, previously oxidized for a period of 12 hours to avoid abrupt changes in water pH.

Data regarding daily pluviosity were obtained from Agriculture Department (CATI) at Pindamonhangaba city. The ponds were not fertilized and, before the insertion of larvae, they were filled with water, with flow controlled to maintain both the water level and the desired alkalinity.

The Curimbatá (*Prochilodus lineatus*) larvae were kept in incubators for three days after hatching and an estimated number of larvae were transferred to each pond. After the first week, they began receiving diets containing 40% crude protein ad libitum. twice daily. The larvae of *P. lineatus* used at the beginning of the experiment were, on an average, 0.701 ± 0.029 cm in total length, 1.0 ± 0.2 mg wet weight and 0.10 ± 0.04 mg dry weight ($n$ = 20). Total length and dry weight (mg. $ind^{-1}$) were measured weekly from a random sample of 20 larvae.

Zooplankton was collected weekly by horizontally hauling across the major axis of the pond, using a 60 µm-mesh net and preserved in formaldehyde solution (4%). The preserved zooplankton samples were analyzed using a Sedgewick-Rafter counting cell with a compound binocular microscope (Zeiss Axioscope 40/A1 model) at 200× magnification. Cladocera and Copepoda were counted in a reticulated chamber under a Zeiss modelo Stemi 2000 stereoscopic microscope, counting sub-samples or until the entire sample for rare organisms. For taxonomical identification specialized literature was employed as: Koste (1978); Reid (1985), Segers (1995), Elmoor-Loureiro (1997), Silva and Matsumura-Tundisi (2005), besides other more specific literature.

For the experiment, a completely randomized factorial scheme (9 × 3) with nine sampling times and three levels of alkalinity (20, 30 and 60 mg $CaCO_3.L^{-1}$) with two replicates (ponds) was used. To test the differences among zooplankton densities between periods and between treatments an analysis of variance was performed and a Tukey test was applied to determine the effect of liming, independently of period. Correlation tests between the size of fish (length and weight), or time period, and zooplankton density were performed density were performed by using Statistic 7.0 program.

## 3. Results

A significant difference in the density of the zooplankton community was observed over time ($F = 6.78$; $p < 0.05$), with a great decrease of zooplankton density from first to second week. From the second week onwards the density of zooplankton ranged in a non-regular manner for each treatment, but successive increases occurred in the density of zooplankton throughout the experimental period (Figure 1).

Regarding the influence of liming treatments on zooplankton density, there were no significant differences in density when each period was considered separately ($F = 0.82$; $p > 0.05$). Although there was no significant difference in density between the treatments comparing each week separately, densities tended to be higher in the treatments with higher levels of lime, for more weeks and particularly after the 6th week onwards (Figure 1).

In order to determine the influence of liming treatments, regardless of the weeks examined, the Tukey test was applied and it showed that there were significant differences in the density of zooplankton when the average densities for the three treatments were considered. The treatment with 20 $mg.L^{-1}$ $CaCO_3$ alcalinity level had a lower density of zooplankton in relation to treatment with 60 $mg.L^{-1}$ $CaCO_3$ (Table 1).

In relation to fish development the best performance of *P. lineatus* larvae was found in the intermediate alkalinity level, of 30 $mg.L^{-1}$ de $CaCO_3$ (Figure 2).

Correlation analysis of fish larvae body weight and length versus time revealed that along the experimental time there was a positive increase in weight ($r = 0.7646$) and length ($r = 0.93$) of

**Table 1.** Mean density of zooplankton (individuals $L^{-1}$) with different concentrations of $CaCO_3$.

| Concentration of $CaCO_3$ ($mg.L^{-1}$) | Zooplankton density ($individuals.m^{-3}$) |
|---|---|
| 20 | 7414[a] |
| 30 | 10966[ab] |
| 60 | 14792[b] |
| **C.V. (%)** | **6,7** |

Means followed by the same letter do not differ in Tukey test at 5% probability.

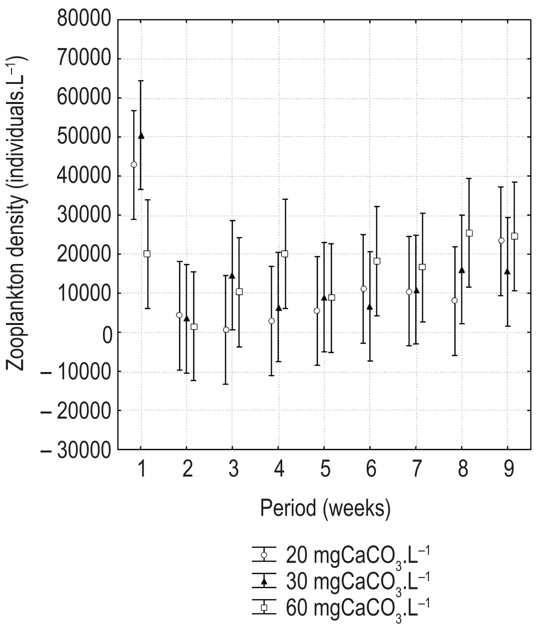


**Figure 1.** Variation in zooplankton density (individuals. $L^{-1}$) over time for three different alkalinity levels ($F = 1.35$, $p = 0.236$). Vertical bars denote 95% confidence intervals.

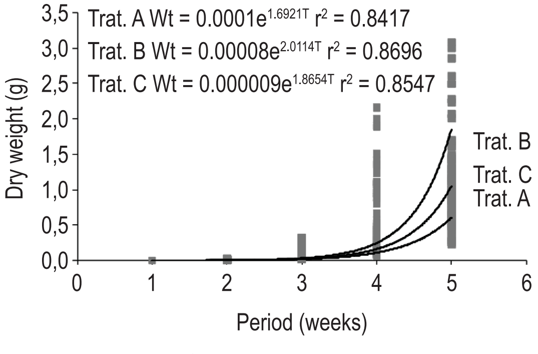


**Figure 2.** *Prochilodus lineatus* (curimbatá) larvae growth expressed as the weight (DW) increases over time, for the three experimental liming treatments: Treat. A = 20 $mg.L^{-1}$ $CaCO_3$; Treat B = 30 $mg.L^{-1}$ $CaCO_3$ and Treat C = 60 $mg.L^{-1}$ $CaCO_3$).

*P. lineatus* ($p \leq 0.001$). On the other hand, a similar analysis showed a decrease in the densities of rotifers ($r = -0.44$), cladocerans ($r = -0.64$) and a great increase in copepods density, this latter exclusively belonging to the Cyclopoida order. The overall relationship between zooplankton density and time was negative and significant ($r = -0.464$; $p = 0.0098$).

A sharp reduction occurred in the density of zooplankton in the second week. In order to evaluate if other environmental factor such as rainfall could be interfering in the zooplankton densities the pattern of rainfall during the experimental period was also examined (Figure 3).

No significant changes were found between the zooplankton densities in the first two weeks. Similar results were found when the whole data set for the nine weeks was considered, although there were significant differences in the density of the zooplankton community over time ($F = 2.71$; $p < 0.05$), but no significant effect of liming on the density of zooplankton was found when each week was tested against the other separately ($F = 5.78$, $p > 0.05$).

In addition to the density variation, changes were also observed in the proportion of different zooplankton groups throughout the experiment. Rotifera was dominant at the beginning of the experiment and Cladocera was also very abundant. Copepoda occurred in very small numbers (Figure 4). The dominant species found in the ponds are listed in Table 2. Among the Rotifera, the taxa that occurred in highest densities were *Conochilus* sp. and *Hexarthra intermedia* and among Cladocera, *Moina minuta*. In the Copepoda group only one species, *Thermocyclops decipiens* was found, with nauplii as the most representative stage. The adult stage occurred only after the fifth week.

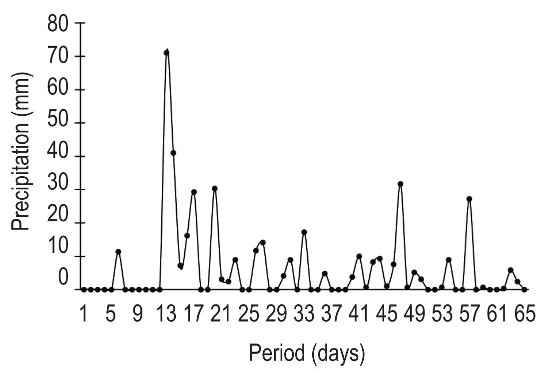


**Figure 3.** Daily variation of precipitation (mm) during the study period, Pindamonhangaba-SP - Source: Institute for Fisheries Pindamonhangaba.

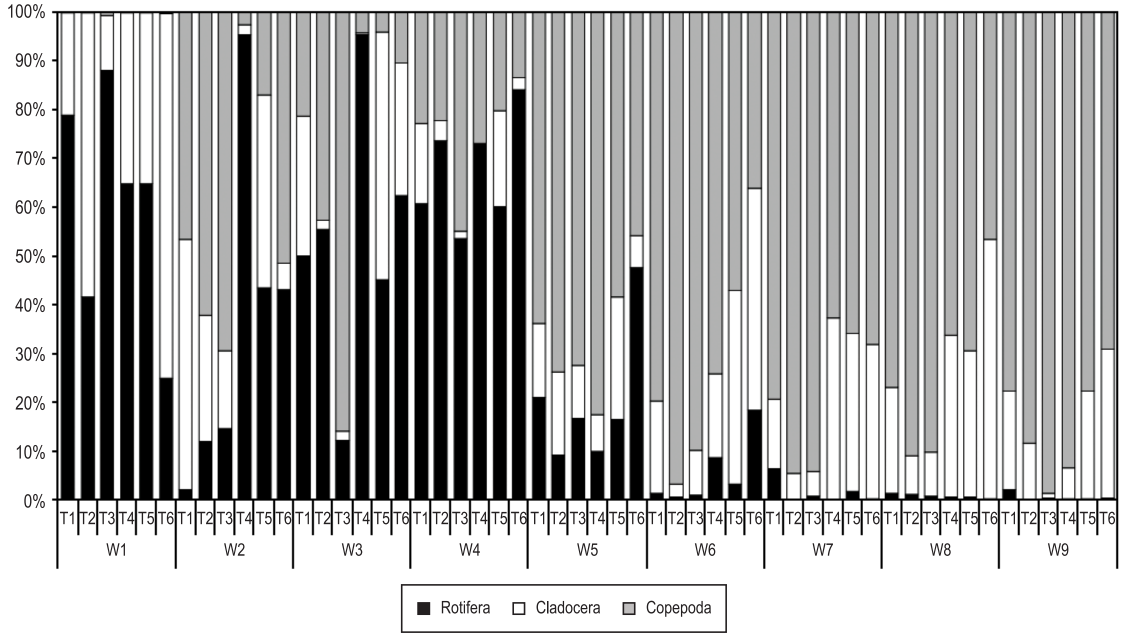


**Figure 4.** Relative abundance (%) of zooplankton found in the fish ponds ($T_1 – T_6$), during the study period.

**Table 2.** Composition of zooplankton community in the fish ponds during the study period.

| | |
|---|---|
| **ROTIFERA** | |
| *Anureopsis* sp. | *Horaella thomassoni* Donner, 1949 |
| *Asplanchna sieboldi* Leydig, 1854 | *Keratella americana* Carlin, 1943 |
| *Brachionus angularis* Gosse, 1851 | *Keratella cochlearis* Gosse, 1851 |
| *Brachionus calyciflorus* Pallas, 1766 | *Lecane bulla* Gosse, 1886 |
| *Brachionus caudatus* Barros e Daday, 1894 | *Lecane cornuta* Müller, 1786 |
| *Brachionus quadridentatus* Hermann, 1783 | *Lecane luna* Müller, 1776 |
| *Cephalodella* sp. | *Lecane lunaris* Ehrenberg, 1832 |
| *Collotheca* sp. | *Lecane papuana* Murray, 1913 |
| *Conochilus coenobasis* Skorikov, 1914 | *Lecane quadridentata* Ehrenberg, 1832 |
| *Conochilus dossuarius* Hudson 1885 | *Plationus patulus* Müller, 1953 |
| *Conochilus natans* Seligo, 1990 | *Platyias quadricornis* Ehrenberg, 1832 |
| *Conochilus unicornis* Rousselet, 1892 | *Polyarthra* sp. |
| *Epiphanis* sp. | *Sinantherina* sp. |
| *Euchlanis dilatata* Ehrenber, 1832 | *Synchaeta* sp. |
| *Gastropus* sp. | *Testudinella patina* Hermann, 1783 |
| *Hexarthra intermedia* Weiszniewski, 1929 | *Trichocerca cylindrica* Sudzuki, 1956 |
| *Hexarthra mira mira* Hudson, 1871 | *Trichocerca* sp. |
| **CLADOCERA** | |
| *Alona* sp. | *Daphnia gessneri* Birge, 1978 |
| *Bosmina hagmanni* Stingelin, 1904 | *Diaphanosoma birgei* Korineck, 1981 |
| *Bosmina tubicen* Brehm, 1939 | *Diaphanosoma brevireme* Sars, 1901 |
| *Bosminopsis deitersi* Richard, 1895 | *Diaphanosoma spinulosum* Herbest, 1967 |
| *Ceriodaphnia cornuta cornuta* Sars, 1886 | *Ilyocryptus spinifer* Herrick, 1882 |
| *Ceriodaphnia cornuta intermedia* Sars,1886 | *Macrothrix* sp. |
| *Ceriodaphnia cornuta rigaudi* Sars, 1896 | *Moina minuta* Hansen, 1899 |
| *Ceriodaphnia silvestrii* Daday, 1902 | *Simocephalus latirostris* Stingelin, 1906 |
| **COPEPODA** | |
| *Thermocyclops decipiens* Kiefer, 1929 | |
| Nauplii cyclopoida | |
| Copepodid cyclopoida | |

## 4. Discussion

The demand of fish larvae by live food as the zooplanktonic organisms is influenced by environmental variables, in addition to the management practices adopted in each system (Sá-Junior and Sipaúba Tavares, 1997; Sipaúba-Tavares, 2010b). In semi-intensive aquaculture the natural food production is very important and therefore any advance in the understanding of ecological interactions and processes that can influence zooplankton community composition and production are relevant to future advances in aquaculture.

The high density of rotifers at the beginning of the experiment indicate the predominance of them in the water that supplied the fish ponds utilized in this study, as previously shown by Pereira et al. (2004). However, one week after the increase of the alkalinity and the stocking of fish larvae in the fish ponds resulted in a sharp decrease in zooplankton densities in all ponds, independently of the alkalinity level reached. This fast response was probably influenced by three factors; firstly and partially by the heavy rains that occurred close to the end of the second week ($13^{th}$ and $14^{th}$ days) possibly diluting zooplankton populations; secondly and probably the preponderant factor, by the heavy predation pressure on the live food after the introduction of *P. lineatus* larvae and thirdly by the possible adverse effect of higher alkanity levels on rotifer abundances.

Although the heavy rainfall that occurred during this same period could also have adversely affected the density of zooplankton due to the dilution effect of the rainwater as shown for zooplankton in small tropical reservoirs by Talamoni and Okano (1997), the rainfall in our experiment was probably not intense enough to surpass the strong influence that larval fish predation pressure can exert upon the emergent zooplankton in nursery ponds as shown by Valdenberg et al. (2006) and Milstein (2008). It has been shown that both the density of fish larvae and the stocking time are of great importance for the successful maintenance of zooplankton population at stable levels in fish ponds (Morris and Mischke, 1999; Valdenberg et al., 2006)

After this great initial impact that lasted at least three weeks, zooplankton densities tended to increase slowly and there was a noticeable change in zooplankton composition. From the $4^{th}$ week onwards, the zooplankton community that was strongly dominated by rotifers changed for a community dominated by crustaceans, particularly the Cyclopoida copepod *Thermocyclops decipiens.*

In this case the interaction of two factors can be hypothesized: the increasing predation pressure on rotifers by *P. lineatus* fingerlings that were growing exponentially as evidenced by the results simultaneously obtained from a parallel study (Rojas et al., 2004) and a possible adverse effect of the increase in alkalinity upon the Rotifera.

The increase in the density of adult *Thermocyclops decipiens* resulted in the decreased density of other zooplankton groups, which may be explained by predation pressure this cyclopoid exerts on other zooplankton populations. According to Clarke (1978) and Carvalho (1984), *Thermocyclops decipiens* feeds not only on algae but also on their own nauplii, rotifers and cladocerans, which can contribute to further delay in total zooplankton growth.

The liming treatment used with weekly reinforcement of alkalinity correction could have indeed triggered a change in zooplankton species composition, as already evidenced in some studies. A greater frequency of rotifers and small-sized crustaceans in water bodies of low to moderate alkalinity and a tendency to increase the abundance of species of large body sizes in high alkalinity lakes was found in natural lakes by Tessier and Horowitz (1990) from the analyses of physical and chemical characteristics and zooplankton composition of 146 lakes of three categories of alkalinity in United States northern territory. They found an increase in small rotifers with decreasing water hardness. This trend was also recently reinforced by the findings of McCarthy and Irvine (2010) for six Irish lakes of contrasting hardness.

Usually the zooplankton response to the increase of alkalinity by liming is positive, that is there is an increase in zooplankton density as shown Rabman and Hussain (2008) that found positive and significant correlations of calcium alkalinity and zooplankton densities in a three years monthly study in fish farming ponds in India. An increase in zooplankton biomasses with increasing doses of lime is related to the improvement in the availability of nutrients to phytoplankton, the main food source for zooplankton and also by the availability of calcium required for the normal development of important zooplankton groups such as the micro-crustaceans (Sipaúba-Tavares, 1995). According to Cairns and Yan (2009) the freshwater crustaceans demand higher concentrations of calcium than other groups because they have heavily calcified exoskeletons and due to their regular molt cycle. When their requirements for calcium are matched by the external environment they can possibly out-compete small rotifers.

Besides influencing the density of zooplankton, the alkalinity also influences the growth of fish, since it affects the availability of food (Sipaúba-Tavares, 1995). In the present study, although the highest dose of lime (60 mg.$L^{-1}$ de $CaCO_3$) resulted in the best development of the zooplankton it did not favor the optimal development of *P. lineatus* larvae, which grew better in the treatment with intermediate alkalinity (30 mg.$L^{-1}$ de $CaCO_3$). Therefore the right amounts of liming can be of extreme importance for the development of fish during its early stages of development, since fish larvae rely almost entirely on the zooplankton as a food source till they grow old enough to be able to eat from other food sources (Cestarolli et al., 1997)

From our study the zooplankton community structure appears to have been influenced by both physical and chemical factors and biological interactions. It appears therefore that besides well-known factors influencing zooplankton composition as food availability and selective predation by fish and other invertebrates, the water chemistry factor alkalinity will deserve a better attention in fish pond management research aiming aquaculture improvement.

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