

## EFFECT OF FERTILIZATION IN WATER QUALITY AND IN ZOOPLANKTON COMMUNITY IN OPEN PLANKTON-CULTURE PONDS

### EFEITO DA FERTILIZAÇÃO NA QUALIDADE DA ÁGUA E NA COMUNIDADE ZOOPLANCTÔNICA EM TANQUES EXTERNOS DE CULTIVO DE PLÂNCTON

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**ABSTRACT:** The effect of swine manure fertilization on the water quality of zooplankton artificial culture in two ponds was measured in diel cycles on different months (October 1999, January and March 2000). Fertilization affected directly water quality; values for total phosphorus were above  $1.4 \text{ mgL}^{-1}$  and maximum rates for ammonia reached  $108 \text{ } \mu\text{gL}^{-1}$ . Zooplankton community comprised four species, namely, *Brachionus calyciflorus*, *B. falcatus*, *Moina* sp. and *Thermocyclops* sp. *B. falcatus* (Rotifera) and *Moina* sp. (Cladocera) were dominant respectively in January and in March. There was no difference in abundance of zooplankton between the two ponds ( $p>0.05$ ), although adult cyclopoid *Thermocyclops* sp was different between diel cycles ( $p<0.05$ ). There were significant differences ( $p<0.05$ ) in dissolved oxygen, ammonia, total phosphorus, orthophosphate, nitrite and nitrate between ponds. In fact, only dissolved oxygen and pH were significantly different ( $p<0.05$ ) between the ponds' surface and bottom. Organic fertilization also provided greater amount of nutrients and higher pH and conductivity, coupled to greater oscillations in the concentration of dissolved oxygen, directly affecting the zooplanktonic composition.

**KEYWORDS:** Pond. Water quality. Zooplankton. Manure. Management.

## INTRODUCTION

The necessity of high quality food to feed fish larvae has caused an increase in interest in the production of feed organisms in laboratory and/or in open systems. Rearing live feed (plankton) in laboratories requires great and specific efforts owing to the high costs demanded by the system's implementation and installation. Plankton production in outdoor ponds is a cheaper alternative, although it requires appropriate management in water quality and plankton production to maintain high nutritional rates (SIPAÚBA-TAVARES and BRAGA, 2007).

Ponds for rearing aquatic organisms usually display complex physical and chemical factors and biotic interactions that depend directly on the water quality. Fluctuations in these environments depend on the energy input and on the frequency and the nature of the nutrient load added to the system (SUMAGAYSAY-CHAVOSO and DIEGO-MC GLONE, 2003).

Fertilization of ponds to enhance alga growth and produce zooplankton suitable for larval fish is a common practice. Traditionally, organic fertilizers, such as animal manure, soy bean meal, alfalfa meal and yeast have been used. However, the excessive application of organic matter into ponds

may reduce dissolved oxygen and cause eutrophication (TEW et al., 2006).

The use of animal manure in semi-natural systems for large scale feed production for fish larvae and fingerlings requires thorough control of the nutritional quality of the plankton produced and that of water quality. However, under these circumstances it is difficult to control both environmental variables and temporal dynamics of the ponds' planktonic community. The community is usually very unstable due to the intense external load of organic matter and essential nutrients (SANTEIRO; PINTO-COELHO, 2000).

Since the addition of manure in plankton-culture ponds tends to accelerate the biological processes (i.e.: succession, production, etc.), it consequently provides a denser phytoplankton formation, followed by zooplankton development. This cycle, however, lasts only a couple of days. As a consequence, plankton ponds usually undergo intense fluctuations in the biomass of most plankton components. The above-mentioned management technique may cause the appearance of species inadequate to feed larvae and fish fingerlings. Low nutritional quality feed is related to the environment's eutrophication (SANTEIRO et al., 2006).

The plankton ponds' sustainable management strategy requires an optimal use of the available fertilizer. The excess of nutrient input is not only expensive but it is also responsible for nutrient enrichment in water bodies (BHAKTA et al., 2004). The development of a new set of procedures to attain sustainable management of large scale cultures of live plankton is one of the main objectives of modern aquaculture.

Current research evaluates the effect of organic fertilization in water quality and zooplankton community in open plankton-culture ponds during diel cycles at three different periods (January, October and March). The ponds' plankton production serves as food for fish-larvae and fingerlings cultivated in the station.

### Study area

The study was carried out at Furnas Hydrobiology and Hatchery Station (20°40' S 46°19' W), Furnas Hydroelectric Plant (São José da Barra MG Brazil). The station is located on the Rio Grande's left bank, 2 km away from the Altinópolis reservoir which provides water to the FURNAS ponds. Two ponds (P<sub>1</sub> and P<sub>2</sub>), area 200 m<sup>2</sup>, volume 320 m<sup>3</sup>, 1.4 m deep, were used in the experiment. Continuous water flow provided 5% daily exchange rate of the rearing volume.

## MATERIAL AND METHODS

### Fertilization

Fertilization was applied by chemical fertilizer (25.6 kg of simple super phosphate and 48 kg of ammonia sulfate), added only at the beginning of the experiment, and by organic fertilizer, adding 100 kg of swine manure *in natura* at approximately 7-day intervals. Whereas no fertilizer was placed in Pond 1 (P<sub>1</sub>) during the collection week, Pond 2 (P<sub>2</sub>) was fertilized during the sampling week as a standard procedure adopted at this station.

### Physical and chemical parameters and zooplankton

Samples were collected in the two ponds during diel cycles, at regular 4-hour intervals during three periods, or rather, in October 1999 (start of the fish growing season), in January 2000 (high fish season) and March 2000 (end of the fish growing season). During these months plankton as food for fingerlings is highly demanded.

Water temperature, pH and conductivity were measured *in situ* by water quality checker Horiba U 10 at the water surface (0.10 m) and bottom (1.30 m). Nutrients in surface (0.10 m) water

were determined by spectrometer, according to Murphy and Riley (1962) and Mackereth et al. (1978) for nitrite, nitrate, ammonia, orthophosphate and total phosphorus. Chlorophyll-*a* (0.10 m) and dissolved oxygen (0.10 and 1.30 m) were measured according to Nush (1980) and Golterman et al. (1978), respectively.

Zooplankton sampled were obtained using a 58µm mesh size net and preserved in formalin/sucrose solution (4%). The zooplankton was sampled in vertical hauls of the water column with 45-L filtered total, calculated by net area and hauling depth (1.5m). The zooplankton samples were then placed in a Sedgewick-Rafter Cell, magnified 100 times and analyzed. Data were expressed in percentage of abundance.

### Statistical analysis

Limnological variables and zooplankton abundance were determined by Mann-Whitney's non-parametric test, or rather, the unilateral hypothesis test that defines H<sub>1</sub> higher or lower position. Significance level was p=0.05 (SIEGEL, 1975).

## RESULTS

### Physical and chemical parameters

As a rule, pH rates were higher at the surface (p<0.05), with greatest peak in P<sub>2</sub> (9.71) at 20:00 h. Rates of pH throughout the experiment ranged between 6.34 and 9.41 in P<sub>1</sub> and between 6.51 and 9.71 in P<sub>2</sub> (Tables 1 and 2).

Highest dissolved oxygen (DO) concentrations coincided with high pH rates in the water. DO was prone to decrease suddenly between 04:00 and 08:00 h in the morning, with lower concentrations in P<sub>2</sub>. As a rule, highest DO concentrations were reported at the surface (p<0.05) (Tables 1 and 2).

Chlorophyll-*a* was according to standard, or rather, higher concentrations during the daylight period and sudden fall between 20:00 and 04:00 h. Chlorophyll-*a* varied between 47.4 and 234 µgL<sup>-1</sup> in P<sub>1</sub> and between 50.2 and 299.9 µgL<sup>-1</sup> in P<sub>2</sub> with no significant difference (p>0.05) between the ponds (Tables 1 and 2).

**Table 1.** Diurnal variations of pH, temperature (°C), dissolved oxygen (DO; mgL<sup>-1</sup>), conductivity (µScm<sup>-1</sup>), chlorophyll-a (Chlo-a; µgL<sup>-1</sup>) and ammonia (Amm; µgL<sup>-1</sup>) in October, January and March at surface (S) and bottom (B) of two ponds, (P<sub>1</sub>= without fertilizer; P<sub>2</sub>= with fertilizer).

Period	Time	P <sub>1</sub>										P <sub>2</sub>									
		pH		Temperature		DO		Conductivity		Chlo-a	Amm	pH		Temperature		DO		Conductivity		Chlo-a	Amm
		S	B	S	B	S	B	S	B			S	B	S	B	S	B	S	B		
O	8:00	6.34	6.47	22.9	23.0	1.1	0.0	102	107	215.4	46	7.24	6.97	22.9	23.4	0.9	0.0	79	79	102.3	100
C	12:00	6.83	6.80	23.0	23.0	4.1	4.0	109	109	195.3	17	7.07	6.86	23.3	23.4	0.8	0.4	93	93	74.4	83
T	16:00	7.00	6.76	22.6	22.8	3.8	3.5	107	107	217.6	17	6.51	6.51	23.1	23.4	2.0	1.8	92	92	135.7	75
O	20:00	9.31	7.81	24.3	23.9	8.8	9.1	95	98	181.3	33	7.50	7.24	25.0	24.5	6.6	7.3	77	98	50.2	83
B	00:00	8.08	7.03	24.0	23.8	4.9	4.6	96	102	145.0	42	8.65	7.94	24.3	24.6	4.9	3.2	75	75	72.5	92
E	4:00	7.29	7.15	23.0	23.4	1.6	1.5	94	95	165.5	38	6.81	6.77	23.6	23.9	1.0	1.6	76	76	89.2	92
R	8:00	6.34	6.47	22.9	23.0	1.1	0.0	102	107	215.4	46	7.24	6.97	22.9	23.4	0.9	0.0	79	79	102.3	100
J	8:00	6.87	6.71	28.3	28.3	3.5	2.1	66	66	172.1	17	6.79	6.69	27.3	27.4	1.5	1.7	95	95	299.9	67
A	12:00	7.61	7.18	31.4	29.6	7.3	6.4	79	64	234.0	25	8.36	6.89	29.8	27.9	7.8	1.4	93	93	279.0	92
N	16:00	8.66	7.08	33.5	29.0	9.5	8.2	70	68	97.4	29	9.12	7.13	33.2	30.0	9.5	6.0	99	99	268.2	83
U	20:00	9.05	6.90	32.0	29.2	12.0	9.9	72	68	47.4	33	8.75	6.85	32.0	28.2	7.7	5.1	98	98	164.7	132
A	00:00	8.38	6.87	30.6	29.2	8.0	5.2	68	69	53.5	42	7.61	6.76	29.7	27.8	5.8	0.4	98	98	113.8	88
R	4:00	7.54	6.65	29.3	28.7	6.0	4.4	67	70	72.5	18	7.09	6.55	28.3	27.5	2.6	1.0	102	102	168.5	108
Y	8:00	6.80	6.74	28.6	28.8	3.5	4.2	68	68	62.9	17	6.63	6.53	27.4	27.6	0.8	0.8	98	98	299.0	75
M	8:00	7.77	7.78	26.6	26.7	4.3	3.4	75	74	111.6	10	7.44	7.43	25.8	25.8	4.0	3.5	218	218	252.5	33
A	12:00	8.96	8.69	28.2	27.5	5.5	5.2	88	78	92.6	15	8.75	7.74	28.1	26.4	5.5	3.6	218	218	251.1	33
R	16:00	9.33	8.96	27.8	27.8	6.0	4.5	90	78	95.9	25	9.33	7.73	27.1	26.2	7.3	5.1	222	222	220.4	33
C	20:00	9.41	9.33	27.4	27.4	5.7	6.1	83	78	86.4	15	9.71	7.24	26.6	26.0	7.6	5.8	221	221	137.6	29
H	00:00	8.83	8.87	26.7	26.9	4.7	4.4	80	77	77.0	15	8.40	8.38	25.7	25.9	2.8	2.8	220	220	176.7	21
	4:00	8.02	8.07	26.2	26.3	3.3	3.6	78	75	98.2	13	7.39	7.42	25.2	25.4	1.5	1.6	219	219	151.7	50
	8:00	7.59	7.58	25.7	25.9	3.6	3.7	75	75	120.5	70	7.23	7.25	25.0	25.1	2.1	2.2	218	218	287.3	74

**Table 2.** Results of analysis of water limnological parameters by Mann-Whitney's unilateral test between ponds (P<sub>1</sub>-P<sub>2</sub>) and depth (surface and bottom), where: Med= mean rate of variables; U= Mann-Whitney's test; \*= significance (p<0.05).

Parameters	PONDS		DEPTH	
	Mean	U	Mean	U
pH	7.6 – 8.0	17.5	8.4 – 7.1	41*
Temperature	28.9 – 28.1	20.0	27.4 – 27.0	58.5
Dissolved Oxygen	5.1 – 4.4	6.5*	16.5 – 3.8	35.5*
Conductivity	80.6 – 95.5	10.0	12.5 – 12.5	76.5
Chlorophyll- <i>a</i>	118.8 – 145.3	15.0		
Ammonia	21.0 – 85.5	5.0*		
Total Phosphorus	2.3 – 4.9	3.0*		
Orthophosphate	1.6 – 2.9	0.0*		
Nitrite	1.7 – 4.4	6.0*		
Nitrate	4.0 – 11.5	0.0*		

Nitrogen compounds were higher (p<0.05) in the fertilized pond during the sampling week (P<sub>2</sub>). Whereas ammonia was predominant (10 to 108 µg L<sup>-1</sup>) in ponds, nitrite was less abundant (0.8 to 6.7 µg L<sup>-1</sup>). In P<sub>2</sub> during March nitrite remained constant (1.7 µg L<sup>-1</sup>) throughout the hours in which analysis was undertaken. However, nitrate rate did not go beyond 19.2 µg L<sup>-1</sup>, with highest concentrations in March (from 8 to 19.2 µg L<sup>-1</sup>) in P<sub>2</sub> (Tables 1 and 2; Figure 1).

An increasing trend occurred in conductivity throughout the experiment in pond P<sub>2</sub>. Rates in March were over 218 µS cm<sup>-1</sup> and in pond P<sub>1</sub> they did not go beyond 109 µS cm<sup>-1</sup>. No significant differences occurred in conductivity (p>0.05) between the analyzed ponds (Tables 1 and 2).

Throughout the period under analysis temperature remained above 25°C, with direct influence from environmental conditions. In other words, higher rates were reported in the hottest period, in January (from 28.6 to 33.5°C), remaining the same throughout the day (p>0.05) (Tables 1 and 2).

A decreasing trend occurred in total phosphorus (TP) in P<sub>1</sub> throughout the experimental period directly related to chlorophyll-*a*. TP and orthophosphate (P-PO<sub>4</sub>) were higher in P<sub>2</sub> (p<0.05), with high concentrations of TP (9.9 mg L<sup>-1</sup>) and P-PO<sub>4</sub> (4.5 mg L<sup>-1</sup>) reported in March at 20:00 and 08:00 h respectively (Table 2; Figure 1).

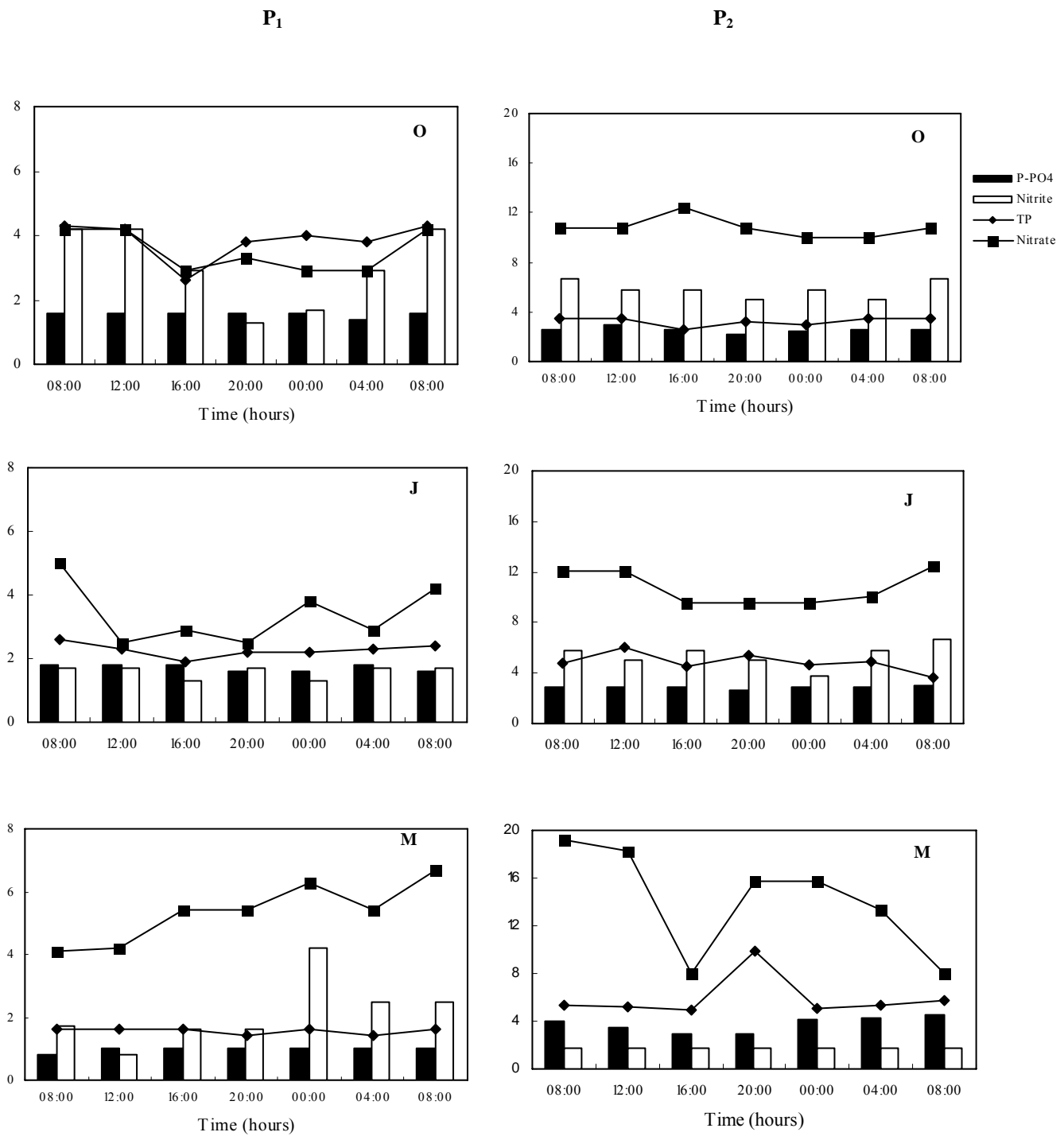
### Zooplankton

Zooplankton community presents few representatives of common cladocerans (*Moina* sp), small copepods (*Thermocyclops* sp) and loricate rotifers (*Brachionus calyciflorus* and *B. falcatus*). No significant difference (p>0.05) has been reported

between the two ponds. Only adult *Thermocyclops* sp was significantly different (p<0.05) throughout the day. *Moina* sp and adult *Thermocyclops* sp were present during the whole experimental period (Table 3; Figure 2). Highest *Moina* sp relative abundance was reported in October and March. *B. falcatus* was dominant in January, in fact the only month in which the two Rotifera and Cladocera species were reported in the two ponds. When the three development stages of *Thermocyclops* sp were present (adult, copepodid, and nauplii) in October and March, *B. falcatus* was absent. Nevertheless, *B. calyciflorus* was only present in P<sub>2</sub> in October, coinciding with abundance of copepodid *Thermocyclops* sp. An inverse relationship has been reported between adult *Thermocyclops* sp and Rotifera species, since *Moina* sp has been associated with the ponds' eutrophic conditions (Table 1; Figure 2).

*Thermocyclops* sp nauplii disappeared completely in P<sub>2</sub> during January but appeared again in March. This fact did not occur in P<sub>1</sub> since it was present throughout the whole period (Figure 2).

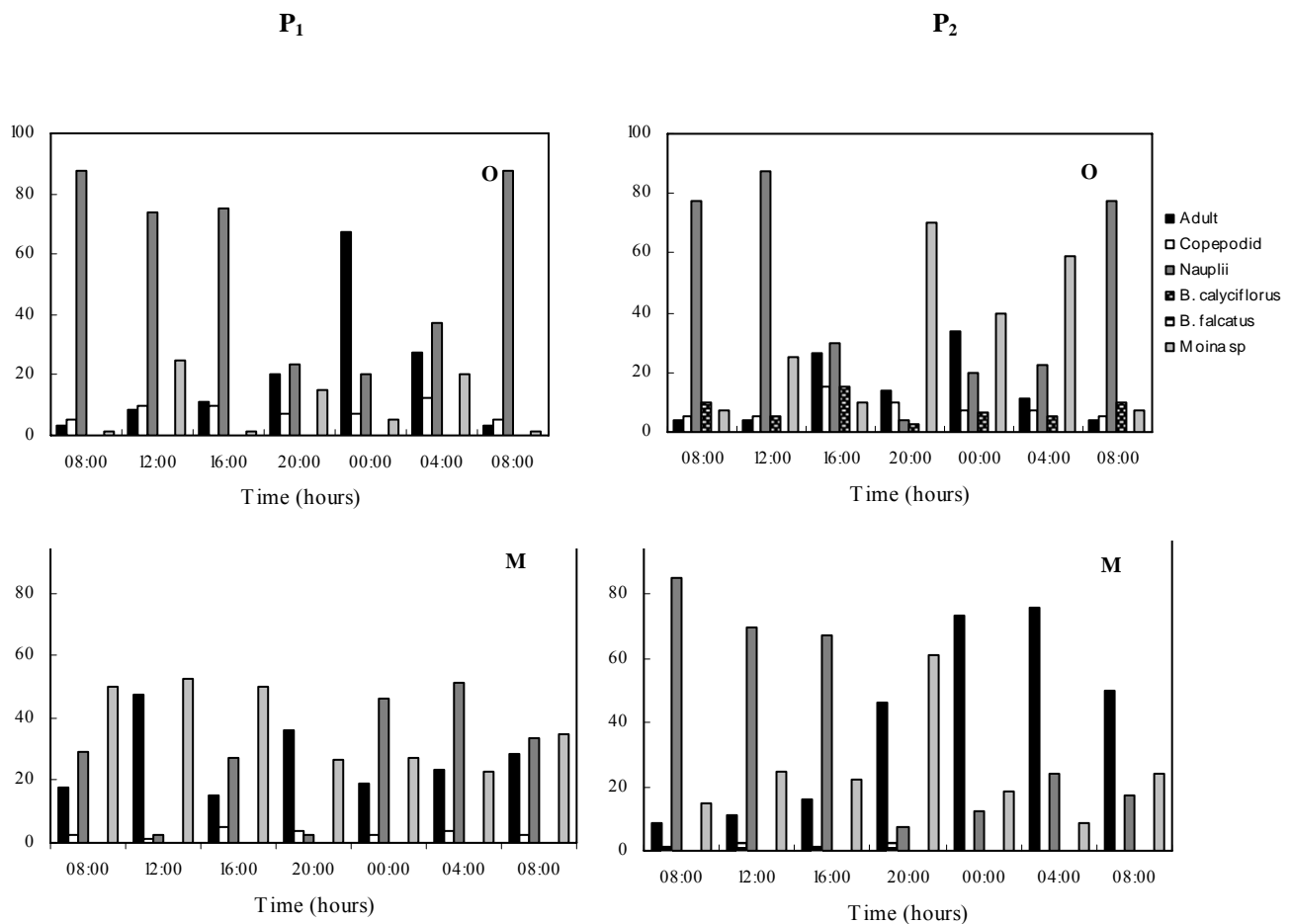
High densities of *B. falcatus* and *B. calyciflorus* in the two ponds in January coincided with high chlorophyll-*a* concentrations in the ponds (Figure 2; Table 1). In fact, planktonic species in the ponds tended to increase their density from the afternoon (16:00 h) till the early hours of the following day (till 04:00 h) (Figure 2).



**Figure 1.** Diel variation of total phosphorus (TP), orthophosphate (P-PO<sub>4</sub>) (mgL<sup>-1</sup>), nitrate and nitrite (µgL<sup>-1</sup>) in open plankton-culture ponds (P<sub>1</sub> and P<sub>2</sub>), during October (O), January (J) and March (M).

**Table 3.** Results of zooplankton abundance from unilateral Mann-Whitney's test between ponds ( $P_1 - P_2$ ) and time of day, where Med=mean rate of zooplankton abundance; U= Mann-Whitney's test, \*= significance ( $p < 0.05$ ).

ZOOPLANKTON	PONDS		DAY PERIOD	
	Mean	U	Mean	U
<i>Thermocyclops</i> (adult)	14.4 – 15.7	17	6.9 – 26.9	5*
<i>Thermocyclops</i> (copepodid)	1.5 – 2.5	7	3.8 – 5.0	10
<i>Thermocyclops</i> (nauplii)	2.5 – 45	8.1	70 – 20	8
<i>Moina</i> sp	15.3 – 21.9	18	25 – 14.4	16



**Figure 2.** Diel variation and relative abundance of Rotifera, Cladocera, and Copepoda - *Thermocyclops* sp (adult, copepodid, and nauplii) in open plankton-culture ponds ( $P_1$  and  $P_2$ ), during October (O), January (J) and March (M).

## DISCUSSION

Management and environmental conditions affected directly the zooplankton community since the few species extant were characterized by environments with high nutrient concentrations, mainly nitrogen and phosphorus, low transparency due to organic turbidity (high chlorophyll-*a*

concentrations), high electrical conductivity and sudden oscillations of DO in the water.

Although chlorophyll-*a* rate was higher during the light period (between 8:00 and 16:00 h), fertilization triggered mainly the sudden decrease of DO during the critical period (from 00:00 to 08:00 h) in pond  $P_2$ .

Pinto-Coelho (1998) found a significant correlation between *Daphnia* and total phosphorus. Positive relationship between chlorophyll-*a* and total phosphorus is chiefly interpreted as a direct influence of the nutrient's availability.

According to Sipaúba-Tavares et al. (2006), higher phosphorus contents ( $>1\text{mgL}^{-1}$ ) in ponds favored species with fast reproduction rate and a short life span of r- strategists organisms, such as Rotifera. In this study, the high relative abundance of *Brachionus falcatus* and *B. calyciflorus* in January also coincided with high concentrations of ammonia, total phosphorus and high temperature. Santeiro et al. (2006) observed that the lowest phosphorus values in zooplankton were detected in outdoor plankton-culture ponds when the community was represented mainly by Rotifera.

An inverse relationship between Copepoda and Rotifera has been detected in current experiment and evidence the omnivorous habits of *Thermocyclops* sp. The above-mentioned species is a characteristic of eutrophic environments, especially those fertilized by organic matter, and thus resistant to the habitat's adverse conditions (FREGADOLLI, 2003).

The significant presence of Cyclopoida-Copepoda *Thermocyclops* sp in the ponds may be associated to the latter's conditions (high total phosphorus, conductivity and chlorophyll-*a*). Cyclopoid organisms are resistant to hyper-eutrophic condition of water and to low oxygen concentrations (PINTO-COELHO, 1998).

*Moina* sp is a common species in South America (HARDY and DUNCAN, 1994) and generally is a zooplanktonic organism that tolerates poor water quality. In current study, *Moina* sp populations were more abundant in March, or rather, during a period of low adult *Thermocyclops* sp incidence.

Differences in zooplanktonic population abundance may be attributed to the frequency and type of fertilization used in plankton production. Santeiro & Pinto-Coelho (2000) concluded that the organic fertilization (swine manure) in the ponds affected water quality and thus the composition of zooplankton species. Community structure changes gradually as a function of nutrient availability in water. Since the organic fertilizer dissolves slowly in water and increases bacterial and algal productivity, the level of the soil's organic matter improves (GOYAL et al., 2005).

Low abundance of organisms in the ponds suggested inadequate management. The reduced number of species reported in current study was probably related to the management technique used

(the weekly addition of swine manure into the ponds). Optimal fertilization rate is the amount of organic matter that may be cost effective and utilized in a pond ecosystem without any harmful effect on water quality.

Sipaúba-Tavares et al. (2006) suggested that inorganic fertilizers (NPK) produce adequate plankton (mainly Chlorophyceae and Calanoida-Copepoda) with only slight water quality degradation and economic efficiencies when compared to organic fertilizers. Although fertilization is necessary to obtain plankton with adequate nutritional value, water quality should be controlled to maintain species with high nutritional rates (SANTEIRO et al., 2006).

Further, Santeiro et al. (2006) observed lower lipid contents (9.14%DW) in the outdoor plankton culture pond when *Thermocyclops* sp was dominant in the zooplankton.

High concentration of phosphorus, extreme pH, high conductivity, nutrients and sudden oscillations of dissolved oxygen at certain periods of the day established a poor zooplankton community in the ponds under analysis, with the prevalence of *Thermocyclops* sp a clearly inadequate species (i.e: small and low nutritional value) as food for post-larvae fish and fingerlings (SIPAÚBA-TAVARES and BRAGA, 2007).

Food availability and quality also influenced the diversity of zooplankton species. Extremely high concentrations of chlorophyll-*a* (maximum  $299.9\ \mu\text{gL}^{-1}$ ) were reported in the ponds, which is a characteristic of environments favoring abundant algae and jeopardizing water conditions with the appearance of species that may survive within the habitat's adverse conditions.

Optimal fertilization rate is the amount of organic matter that may be cost effective and utilized in a pond ecosystem without any harmful effect on the water quality and aquatic organisms (BHAKTA et al., 2004).

Fertilization management in ponds for the production of live feed was inadequate owing to low plankton density and to species with low nutrition rates, improper to feed fish larvae. Interaction in the fertilization processes, density and zooplankton biomass quality between ponds under analysis suggests a decrease in the frequency of fertilization, or rather, an over 7-day period, so that excess of organic matter and a decrease in the ponds' water quality may be avoided.

The development of new techniques of fertilization in open pond systems for compatible high zooplankton production featuring better quality

(higher diversity, large organisms with better nutritional status) is still urgently needed.

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**RESUMO.** Este estudo avaliou os efeitos da fertilização com esterco de suíno na qualidade da água de dois tanques utilizados para produção de zooplâncton. Foi realizada análise da variação nictemeral, em três períodos distintos do ano (Outubro de 1999, Janeiro e Julho de 2000). A fertilização afetou diretamente a qualidade da água com concentrações de fósforo total acima de  $1,4 \text{ mgL}^{-1}$  e amônia com máximo de  $108 \text{ } \mu\text{gL}^{-1}$ . O zooplâncton foi representado por quatro espécies *Brachionus calyciflorus*, *B. falcatus*, *Moina* sp e *Thermocyclops* sp. *B. falcatus* (Rotifera) foi dominante no mês de janeiro e *Moina* sp (Cladocera) em março. Abundância zooplanctônica entre tanques não foi diferente ( $p > 0,05$ ), porém *Thermocyclops* sp adulto foi diferente ao longo do dia ( $p < 0,05$ ). Foram observadas diferenças significativas ( $p < 0,05$ ) entre viveiros para variáveis oxigênio dissolvido, amônia, fósforo total, ortofosfato, nitrito e nitrato, sendo que somente oxigênio dissolvido e pH apresentaram diferenças significativas ( $p < 0,05$ ) entre superfície e fundo dos viveiros. O fertilizante orgânico promoveu altas concentrações de nutrientes, pH, condutividade e oscilações bruscas de oxigênio dissolvido, refletindo diretamente na composição zooplanctônica.

**PALAVRAS-CHAVE:** Tanques. Qualidade da água. Zooplâncton. Esterco. Manejo.

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