

# Population dynamics of *Moina minuta* Hansen (1899), *Ceriodaphnia cornuta* Sars (1886), and *Diaphanosoma spinulosum* Herbst (1967) (Crustacea: Branchiopoda) in different nutrients (N and P) concentration ranges

Dinâmica populacional de *Moina minuta* Hansen (1899), *Ceriodaphnia cornuta* Sars (1886) e *Diaphanosoma spinulosum* Herbst (1967) (Crustacea: Branchiopoda) em diferentes faixas de concentração de nutrientes (N e P)

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**Abstract: Aim:** Aquatic environments of semi-arid regions are subject to changes in water quality and volume due to short and irregular rain seasons. Consequently, zooplankton composition changes in association to fluctuations in the trophic degree. To comprehend these processes, this study analyzed the influence of nutrient concentrations (N and P) on the population dynamics of three species of Cladocera – *Moina minuta*, *Ceriodaphnia cornuta*, and *Diaphanosoma spinulosum* – from the Taperoá II Dam, Paraíba, Brazil; **Methods:** Animals were experimentally submitted to different nutrient concentrations as a means to reproduce specific trophic conditions from natural environments. Zooplankton densities were evaluated once every three days, with food (i.e. algal cultures) being supplied in alternate days. To compare population growth, an ANOVA was performed, followed by Tukey's HSD post-hoc test; **Results:** The population growth of the three species differed significantly both among species and among treatments. *Moina minuta* populations showed higher growth rates under higher nutrient concentrations, whereas *Ceriodaphnia cornuta* grew more efficiently in intermediate concentrations, and *Diaphanosoma spinulosum* showed a better development in low nutrient concentrations; **Conclusions:** The results from the experimental approach, presented here, are similar to what is observed in the field, based on previous studies carried out in the Taperoá II Dam. Therefore, these results suggest that the hydrological cycle seems to be the major determinant of zooplankton population dynamics in aquatic semi-arid environments

**Keywords:** Cladocera, semi-arid, nutrient, trophic state.

**Resumo: Objetivo:** Os ambientes aquáticos de regiões semi-áridas sofrem alterações na qualidade da água e no seu volume devido à irregularidade do período chuvoso. Consequentemente, o zooplâncton sofre alterações em sua composição com a evolução do estado trófico. Para a compreensão desse processo, este trabalho analisou experimentalmente a influência da concentração de nutrientes (N e P) sobre a dinâmica populacional de três espécies de cladóceros: *Moina minuta*, *Ceriodaphnia cornuta* e *Diaphanosoma spinulosum*; **Métodos:** Os animais foram submetidos a diferentes concentrações de nutrientes (N e P), de modo a representar diferentes níveis tróficos. As suas densidades foram medidas a cada três dias, e alimentação feita com uma cultura pluri-algal em dias alternados. Para a comparação do desenvolvimento das populações, foi feito o teste ANOVA, seguido do teste de Tukey; **Resultados:** O crescimento populacional das três espécies diferiu significativamente tanto entre as espécies quanto entre os tratamentos. As populações de *Moina minuta* obtiveram maior crescimento nas concentrações mais elevadas de nutrientes e *Ceriodaphnia cornuta* em concentrações intermediárias, enquanto *Diaphanosoma spinulosum* se desenvolveu melhor no tratamento com baixa concentração de nutrientes;

**Conclusões:** Observa-se que, para as três espécies, o resultado obtido em laboratório é similar ao observado em campo, obtidos em estudos realizados anteriormente no local, e que o ciclo hidrológico parece ser o principal regulador da dinâmica desses organismos em ambientes aquáticos do semi-árido.

**Palavras-chave:** Cladóceros, semi-árido, nutrientes, estado trófico.

## 1. Introduction

Amongst the groups that comprise zooplankton communities, Cladocera is one of the most important and representative, due to its role in energy and matter transfer (Vakkilainen et al., 2004). These organisms show an intimate relationship to environmental conditions (Reid and Wood, 1976), their populations being strongly controlled by biotic factors, such as food availability and predation by fishes and larger invertebrates (Abrantes et al., 2006), and abiotic factors, such as pH, temperature, and concentration of several important compounds such as oxygen, ammonia (Phlips et al., 1997).

Due to their ecological importance, sensitivity to environmental changes, and easy handling (Brancelj et al., 1997), experimental studies with cladocerans are widely spread, with several aspects of their life histories, such as nutrition, predation, competition, ecotoxicologic tests, as well as tolerance to changes in water quality, being documented (see Ringelberg, 1997).

Among experimental studies, the influence of nutrients concentration (N and P) and the overall trophic state on zooplankton community composition is amongst the most studied aspect, given its importance in elucidating the ecological mechanisms responsible for matter and energy transfer. Studies conducted by Vakkilainen et al. (2004) and Esteves and Sendacz (1988) showed an increase in the biomass of rotifers, protozoans and small cladocerans, with no influence on filter feeders, such as larger cladocerans and calanoid copepods. Similar observations were made by Kozłowsky-Suzuki and Bozelli (2002) and Sterza et al. (2002).

In environments where trophic conditions change seasonally, understanding how this process affects the ecological succession is extremely important in order to understand the dynamics of these ecosystems. This is the case for aquatic environments in the northeastern semi-arid region of Brazil, including the ecologically important Taperoá II Dam, evaluated in the present study. At these particular environments, yearlong high temperatures (25-30 °C) predominate, whereas rainfall is scarce (400 mm.year<sup>-1</sup>) and highly irregular (Barbosa et al., 2002). Due to these severe climatic

conditions, a deficit in water balance is typical of the region, turning the majority of water bodies in these areas temporary. This large cyclical variation in water volume, highly dependent on adequate rainfall rates, affects water quality, which consequently prompts corresponding variations in trophic conditions of these environments (Perez-Martinez et al., 1991; Crispim and Watanabe, 2000).

Studies with cladoceran populations in semi-arid environments of Paraíba state indicate a clear ecological succession among the three most common species, which is strongly dependent on the hydrological regime of the region. For example, during the rainy season, *Moina minuta* Hansen 1899 is the first species to colonize the environment, soon decreasing its density and being replaced by *Ceriodaphnia cornuta* Sars 1886 and *Diaphanosoma spinulosum* Herbst 1967 (Freitas and Crispim, 2005; Crispim et al., 2003; Crispim and Watanabe, 2001). Due to these aspects, variations in environmental conditions, caused by changes in hydrological regimes, should be critical for the dynamics of these species. Therefore, evaluating these relationships is of major importance to understand the factors responsible for the population dynamics of cladoceran species in semi-arid aquatic environments.

## 2. Material and Methods

Subject species – *M. minuta*, *C. cornuta*, and *D. spinulosum* – were originally isolated from Taperoá II Dam, in Taperoá city, Paraíba state, Brazil. One single individual of each species was selected to produce replicated clones.

Individuals were submitted to three treatments, in order to reproduce a gradient of nutrients concentrations (NH<sub>3</sub>, NO<sub>2</sub>, NO<sub>3</sub>, and PO<sub>4</sub>) from three general trophic degrees (oligotrophic, mesotrophic, and eutrophic), as periodically observed in the study area. Data provided by Project PELD – Caatinga/CNPq, which also integrates the present study, was used to estimate these trophic conditions. Concentration ranges employed here are shown in Table 1. For this purpose, stock solutions were prepared for each nutrient following

**Table 1.** Concentration gradients adopted during experimental procedures in laboratory.

Nutrient/ Trophic state	Ammonia ( $\text{CINH}_4$ 1000 $\text{mg.L}^{-1}$ )	Nitrite ( $\text{NaNO}_2$ 100 $\text{mg.L}^{-1}$ )	Nitrate ( $\text{NaNO}_3$ 1000 $\text{mg.L}^{-1}$ )	Orthophosphate ( $\text{K}_2\text{PO}_4$ 1000 $\text{mg.L}^{-1}$ )
Oligotrophic	20 $\mu\text{g.L}^{-1}$	10 $\mu\text{g.L}^{-1}$	20 $\mu\text{g.L}^{-1}$	10 $\mu\text{g.L}^{-1}$
Mesotrophic	100 $\mu\text{g.L}^{-1}$	50 $\mu\text{g.L}^{-1}$	100 $\mu\text{g.L}^{-1}$	50 $\mu\text{g.L}^{-1}$
Eutrophic	1800 $\mu\text{g.L}^{-1}$	100 $\mu\text{g.L}^{-1}$	2000 $\mu\text{g.L}^{-1}$	500 $\mu\text{g.L}^{-1}$

the procedures described in *Standard Methods* (Clesceri et al. 1998). For each treatment, the culture was prepared with 900 mL of water from Taperoá Dam, 25 mL of stock solution from each specific nutrient, for each proposed concentration value. Prior to these procedures, water was filtered and analyzed to evaluate its original nutrient content.

Ten neonates with about  $22 \pm 2$  hours of life were added to the aquariums at the beginning of the experiment, and the abundance of populations was observed once every three days. The experiment was conducted in a laboratory with a controlled temperature of  $25 \pm 1$  °C and 12h30:11h30 (day:night) photoperiod, with animals being fed every two days. Cladocerans were fed  $1,5 \times 10^6$  cells per mL of a mixed culture of chlorophyceae (*Scenedesmus*, *Chlorella*, *Kirchneriella*), in Conway culture. The experiment lasted three weeks, with three replicates for each treatment. Population growth rate was calculated following (Equation 1):

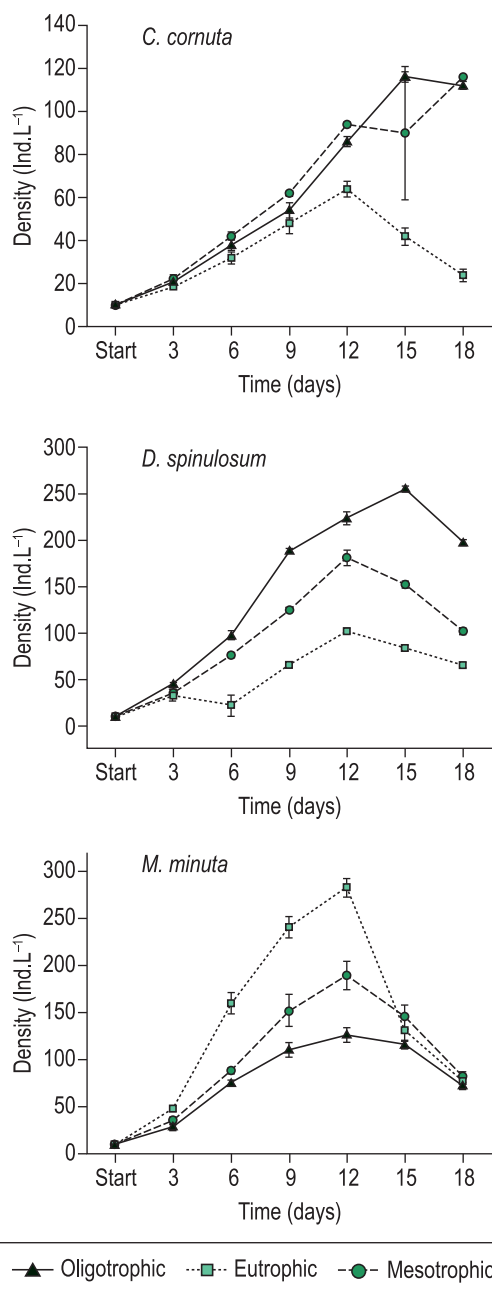
$$r = \frac{\ln N_t - \ln N_0}{\Delta t} \quad (1)$$

where  $N_0$  = initial density and  $N_t$  = density after  $\Delta t$  (days), (Krebs, 1998).

To compare the population growth of each species in three different treatments one-way analyses of variance (ANOVA), followed by Tukey's HSD test were carried out.

### 3. Results

Positive growth rates were observed for all species at the three experimental treatments, but somewhat distinct patterns were observed (Figure 1). Significant differences in zooplankton density among species were observed on the oligotrophic (ANOVA;  $F_{2,6} = 137.4$ ;  $p < 0.001$ ), mesotrophic (ANOVA;  $F_{2,6} = 28.6$ ;  $p < 0.001$ ), and eutrophic ( $F_{2,6} = 343.2$ ;  $p < 0.001$ ) treatments, during the 12<sup>th</sup> day of experiment. Densities were significantly different among all species on the oligotrophic treatment (Tukey's HSD test,  $p < 0.001$  in all cases), *D. spinulosum* (mean = 224 individuals) showing significantly higher densities than *M. minuta*



**Figure 1.** Mean  $\pm$  SE densities of three cladoceran species (*Moina minuta*, *Ceriodaphnia cornuta* and *Diaphanosoma spinulosum*) at three experimental treatments following a trophic gradient (oligotrophic, mesotrophic and eutrophic).

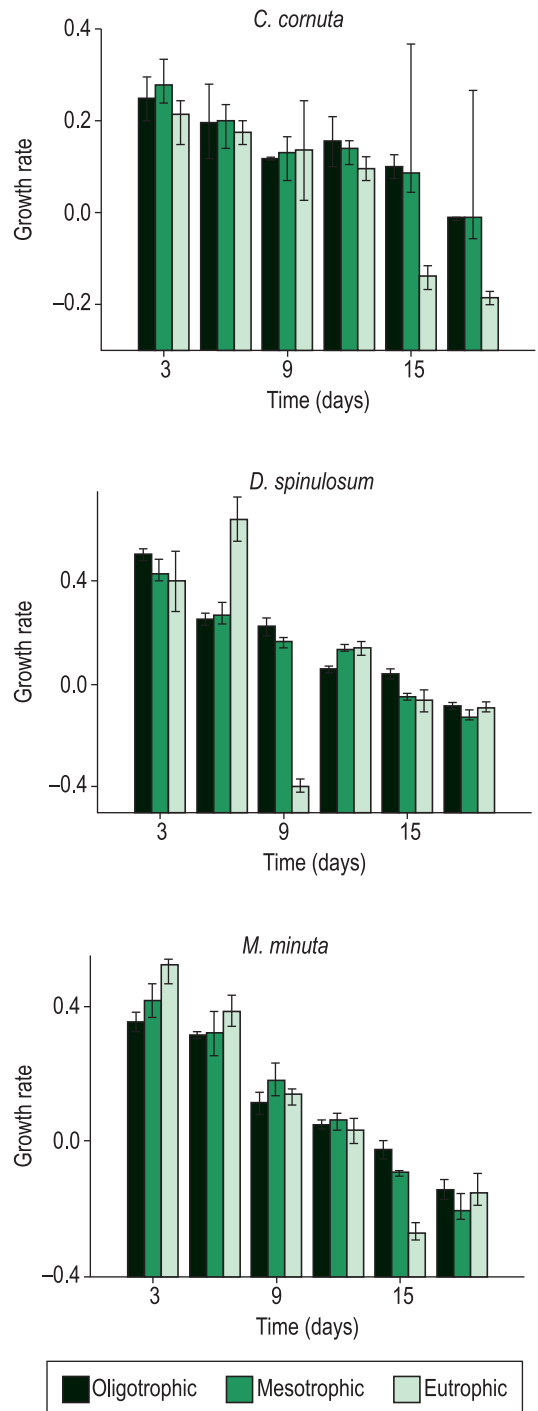
(mean = 127), which, in turn, showed significantly higher values than *C. cornuta* (mean = 86). On the mesotrophic treatment, significant differences were observed between the densities of *M. minuta* (190) and *C. cornuta* (94) (Tukey's HSD test,  $p < 0.01$ ), and between *D. spinulosum* (181.3) and *C. cornuta* (Tukey's HSD test,  $p < 0.01$ ), *M. minuta* and *D. spinulosum* showing similar values (Tukey's HSD test,  $p > 0.05$ ). On the eutrophic treatment, densities of the three species were significantly different (Tukey's HSD test,  $p < 0.05$  in all cases), *M. minuta* (283) significantly showing higher values than *D. spinulosum* (102.7), which, in turn, was significantly higher than those of *C. cornuta* (64).

Throughout the experiment, growth rate differed significantly among the three species in the oligotrophic (ANOVA;  $F_{2,6} = 10.7$ ;  $p < 0.05$ ), mesotrophic (ANOVA;  $F_{2,6} = 8.5$ ;  $p < 0.05$ ), and eutrophic (ANOVA;  $F_{2,6} = 6.7$ ;  $p < 0.05$ ) treatments (Figure 2). On the oligotrophic treatment, significant differences were observed between the growth rate of *M. minuta* and *C. cornuta* (Tukey's HSD test,  $p < 0.05$ ) and *D. spinulosum* and *C. cornuta* (Tukey's HSD test,  $p < 0.05$ ), *M. minuta* and *D. spinulosum* showing no significant differences (Tukey's HSD test,  $p > 0.05$ ). On the mesotrophic treatment, growth rate was significantly different between *M. minuta* and *C. cornuta* (Tukey's HSD test,  $p < 0.05$ ), but neither between *M. minuta* and *D. spinulosum* (Tukey's HSD test,  $p > 0.05$ ), nor *D. spinulosum* and *C. cornuta* (Tukey's HSD test,  $p > 0.05$ ). On the eutrophic treatment, growth rate was only significantly different between *M. minuta* and *D. spinulosum* (Tukey's HSD test,  $p < 0.05$ ), but were similar between *M. minuta* and *C. cornuta* (Tukey's HSD test,  $p > 0.05$ ), and between *D. spinulosum* and *C. cornuta* (Tukey's HSD test,  $p > 0.05$ ).

#### 4. Discussion

The subject species of the present study are not only the three most common cladoceran species of semi-arid dams in Paraíba state, but are also the most representative in tropical water bodies (Sarma et al., 2004; Dumont, 1994; Shrivastava et al., 1999). *Moina minuta* populations showed, in general, a faster growth rate and reached higher densities than populations of *Ceriodaphnia cornuta* and *Diaphanosoma spinulosum*. However, we observed that the population of the former species declined faster than those of the latter. Mangas-Ramírez et al. (2002) also observed a similar difference studying cultures of related *Moina* species.

These results support field observations from the Taperoá II Dam, which suggest that *M. minuta* is the first species to colonize the environment. Typically, this species shows a somewhat fast increase



**Figure 2.** Mean  $\pm$  SE growth rates of three cladoceran species (*Moina minuta*, *Ceriodaphnia cornuta* and *Diaphanosoma spinulosum*) at three experimental treatments following a trophic gradient (oligotrophic, mesotrophic and eutrophic).

in population density, which is followed by a corresponding decrease until it virtually disappears from the water column.

Population growth of *M. minuta* was positively influenced by higher nutrient concentrations. Nevertheless, it was also under these higher concentrations that population declines were more pronounced, whereas lower nutrient concentrations resulted in moderate mortality curves. Benider et al. (2002), in a similar experiment with *Moina macrocopa*, observed the same rapid development when cultures were submitted to high nutrients concentrations. This rapid increase in zooplankton density is easily explained by a trophic cascade effect, given that higher filtration rates are observed where algal biomass is larger due to an increased availability of nutrients from external sources (Jana and Pal, 1985; Bonou et al., 1991). This should also be the case for *M. minuta* in the present study.

Nandini and Sarma (2003) observed a higher population growth of *M. macrocopa* under higher concentration of algae than *C. dubia*, which showed higher growth under lower concentrations of algae. These authors associated these findings to the rapid life cycle of *Moina* (which seems to be an attribute of the genus). As a consequence, these species require a larger amount of food, for example, when compared to species of *Ceriodaphnia* (Nandini and Sarma, 2003). Sipaúba-Tavares and Bachion (2002) found similar results studying *Moina micrura* and *Diaphanosoma birgei*, where they observed a higher food requirement for the former species.

At Taperoá Dam, despite typical lower algal densities during rainy seasons, productivity rates are higher (Barbosa et al., 2002), which may provide food in abundance for *M. minuta*.

*C. cornuta* showed lower densities under higher nutrients concentrations. Villalobos and González (2006), in their study on the biology of *C. cornuta*, claimed that this species shows optimal growth rates on waters with trophic conditions varying between mesotrophic and oligotrophic, and that chlorophyceae, diatoms and detritus are their primary source of their food (Ferrão-Filho et al., 2003; González, 2004; Wylie and Currie, 1991).

Populations of *D. spinulosum* showed higher growth rates under lower concentration of nutrients. Shrivastava et al. (1999) observed similar results in an experiment with *Diaphanosoma celebensis*, which also showed the largest population growth under low food conditions. The same was observed by Crispim and Boavida (2002) in competition experiments involving three species of Cladocera.

These authors found that higher population growths of *Diaphanosoma brachyurum* were observed at manipulated samples with low food concentrations ( $3,5 \times 10^3$  cél.ml<sup>-1</sup>). For *C. cornuta* and *D. spinulosum*, the largest variations of growth rates as well as the highest decreases were observed under eutrophic conditions.

Boikova (2005) stated, from results of her experiment on *D. brachyurum*, that populations showed lowest growth rates and late post-embryonic development under conditions of high food levels. According to Wacker et al. (2001), *Diaphanosoma* is a genus of tropical cladocerans with low nutritional requirements and low tolerance to eutrophication (i.e. a stenotrophic species).

Within the population dynamics of the three species, as observed from our experimental data, a similar pattern to that of previous field investigations in the Taperoá II Dam was observed (Vieira et al., 2009). Populations of *M. minuta* reach peaks of densities under situations where mesotrophic and eutrophic conditions, and consequently, higher food availability, predominate in the dam, but soon after, population densities of this species start to decline. Further, this population decline is followed by an increase in the population growth of *D. spinulosum* and *C. cornuta* in the dam. This strategy of rapid growth, or “explosive growth” according to Romanovsky (1985), was reported by Benider et al. (2002), Ferrão-Filho et al. (2003), and Duncan (1989); their studies suggest that, after a period of population growth, these species invest almost all their energy on sexual reproduction, in order to guarantee an adequate stock of resting eggs in the sediment, with the consequential effect of continued species existence (Lynch, 1980; Rojas et al., 2001). This pattern is common at several aquatic ecosystems of semi-arid regions in Paraíba state, where, depending on environmental stability, *M. minuta* may produce different proportions (high or low) of ephippial females, soon after their density peaks (Crispim et al., 2003). However, it is noteworthy that as trophic levels increase, a change in phytoplankton diversity follows, thus turning typically abundant algal species into rare species. As a consequence, this change in phytoplankton diversity is usually followed by a corresponding change in zooplankton composition, particularly due to the fact that highly selective zooplankton predators, such as cladocerans, are most affected (Pace, 1986; Esteves and Sendacz, 1988; Sarma et al., 2003).



Regarding *C. cornuta* populations, this species shows a peak of density in the dam at prevailing oligotrophic to mesotrophic conditions, and this observation is supported by our experimental results. Similar observations were made by Ferrão-Filho et al. (2005) and Conde-Porcuna et al. (2002), which stated that *Ceriodaphnia* species grow better under moderate trophic conditions. Romanovsky (1985) ranked *Ceriodaphnia* as a genus of “low growth”, but with great tolerance to starvation, toxic algae and mineral limitation of N and P. With regards to *D. spinulosum* populations, observations of the present experimental study were also similar to a previous study conducted at the dam (Vieira et al., 2009). *D. spinulosum* showed higher population densities when the dam was under oligotrophic conditions. Other studies conducted with congeners as subject species showed that the highest densities were recorded under oligotrophic conditions (Neves et al., 2003; Gulati et al., 1990, 1990; Mengestou and Fernando, 1991). Boikova (2005), in her study on *D. brachyurum*, noted that populations thrived best at low trophic conditions, showing density variations under higher trophic conditions, as well as changes in embryonic and post-embryonic development. Similar results were also found by Malhotra and Langer (1993).

Based on the aforementioned considerations, it becomes clear that the effects of nutrients concentrations on cladoceran populations are of an indirect nature, formerly influencing their food supply through an algal dynamics and consequently, the overall zooplankton community, via trophic cascades. Some of the compounds studied, such as ammonia and nitrite, have known toxic effects on organisms, (Esteves, 1998). However, concentrations used during the experiment did not reach harmful levels to organismal development and, therefore, had no apparent negative effect on individual-level fitness. Similar results were observed in studies carried out by Elser et al. (1988), Sterza et al. (2002), Benider et al. (2002), Makino et al. (2002) and Kozłowski-Suzuki and Bozelli (2002).

Environments subject to a unidirectional increase in nutrients concentrations show a permanent shift in phytoplankton community composition, which tends to be replaced by communities with low richness and high dominance of few species, particularly cyanobacteria and euglenids (i.e. decreased diversity) (Smith et al., 1999; Sterza et al., 2002; Boersma and Vijverberg, 1996). Furthermore, during these shifts, microzooplankton

species (rotifers and protozoans) tend to substitute or become more abundant than macrozooplankton ones (cladocerans and calanoid copepods). Nevertheless, when the nature of this process is cyclical, as observed at Caatinga environments, a return to the former, and preferred conditions regarding community development occurs, this being a common annual cycle triggered by the periodic hydrological regimes and made available by ephippial eggs deposited on the sediments (Crispim and Watanabe, 2000).

The somewhat large cyclical fluctuations, typical of semi-arid environments, enable the so-called “ideal” conditions for the development of particular species from different periods, given the changes on the environmental quality, which is directly influenced by the hydrological cycle. In other words, there is a cyclical ecological succession, or a cyclical temporal succession which is dependent upon changes in environmental quality. According to Mageed and Heikal (2006), a change in the water’s chemical composition, and consequently its trophic state, is the main factor responsible for the seasonal fluctuations of zooplankton composition at tropical environments, as opposed to what is observed at temperate water bodies, in which temperature is, by far, the most important determinant (Sarma et al., 2005).

Particularly for cladoceran populations, the influence of water quality is more pronounced, given the fact that these animals show a lower tolerance to environmental fluctuations than do other zooplankton groups (Goldman et al., 1979; Esteves and Sendacz, 1988; Elser et al., 1988; Conde-Porcuna et al., 2002; Kappes and Sinsch, 2005). Therefore, this explains, at least partially, the low densities of this group when compared to others recorded in similar dams (Crispim and Watanabe, 2003; Vieira et al., 2009).

Based on the results shown in the present study (laboratory analyses), along with historical field analyses, we state that the three subject species are important indicators of trophic conditions at the dam, providing an indirect, albeit consistent, measure of its water quality. These relationships explain the sequence of species replacement observed in dams of semi-arid regions in Paraíba state, where, during the beginning of the rainy season, *M. minuta* is the first species to colonize the environment, followed by *C. cornuta* and, finally, *D. spinulosum*. In fact, soon after the beginning of the first rains, an increase in phytoplankton primary productivity takes place (Dantas, 2009), due to

the dilution of stressful conditions (such as the decrease in pH and electrical conductivity values, which are typically high during the dry season and a limiting factor for some species) coupled with an input of nitrogen compounds caused by water discharge and sediment lixiviation (Crispim et al., 2003). As a consequence, this increase in algal biomass, will provide food in abundance to the overall heterotrophic aquatic community. The particularly noteworthy positive effects of increased primary productivity on the density of *M. minuta*, given that this species reached higher densities than the other evaluated species (Crispim et al., 2003), comes about from its strong herbivorous pressure. The resulting decrease (or stability) in food levels, favor the presence of *C. cornuta* and *D. spinulosum*. These latter species, along with calanoid Copepoda species, are more abundant around two to three months subsequent to the beginning of the rainy season at these ecosystems.

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