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Effects of experimental eutrophization on zooplankton community

Efeitos da eutrofização experimental sobre a comunidade zooplanctônica

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Abstract: Aims: The present study evaluated the role that increased nutrient concentrations play on zooplankton community, by employing an experimental laboratory approach. **Methods**: Experiments were conducted in the laboratory, where three trophic state conditions were simulated, namely, mesotrophic, eutrophic and hypereutrophic. Each treatment was replicated three times and individuals of *Brachionus urceolaris* (10 individuals), *Hexarthra mira* (5) (Rotifera), *Latonopsis* sp. (10), *Moina minuta* (10) (Cladocera) and *Thermocyclops* sp. (5) (Copepoda) were introduced to each replicate. On the first experiment day, and at 7-day intervals for a 14-day period (totaling three evaluations), all water content was collected from each container and filtered to determine the densities of each zooplankton species. Two-way MANOVA and one-way ANOVA designs were used to determine zooplankton density fluctuations among treatments and throughout the study period. Further, Generalized Linear Models (GLMs) were employed to assess how environmental factors affected zooplankton numbers. Phytoplankton composition was also determined in the beginning and in the end of the experiment. **Results**: *B. urceolaris* and copepod nauplii, which are typical of eutrophic environments, showed higher densities on the eutrophic and hypereutrophic treatments. Furthermore, cyanobacteria such as *Aphanothece* sp. and *Merismopedia* sp. were recorded on the eutrophic and hypereutrophic treatments, respectively. **Conclusions**: Similarly to what is frequently observed in the wild, the eutrophic treatment showed higher densities of particular zooplankton species which are known to temporarily benefit from an increase in trophic concentrations. Positive or negative responses from zooplankton dynamics (but also phytoplankton species), provide an important bioindicator framework. Furthermore, results of the present study outline the need for implementing recovery measures on aquatic environments subject to constant nutrient inputs.

Keywords: zooplankton, trophic states, phytoplankton, laboratory experiments.

Resumo: Objetivos: O presente estudo avaliou os efeitos do aumento na concentração de nutrientes sobre a comunidade zooplanctônica, através de experimentos laboratoriais. **Métodos**: Os experimentos foram realizados em laboratório, onde três estados tróficos foram simulados, a saber, mesotrófico, eutrófico e hipereutrófico. Cada tratamento foi replicado três vezes, e indivíduos de *Brachionus urceolaris* (10 indivíduos), *Hexarthra mira* (5) (Rotifera), *Latonopsis* sp. (10), *Moina minuta* (10) (Cladocera) e *Thermocyclops* sp. (5) (Copepoda) foram introduzidos em cada réplica. Durante o primeiro dia de experimento, e a cada sete dias de intervalo durante 14 dias (totalizando três amostragens), toda a água foi coletada de cada aquário e filtrada, para a determinação das densidades de cada espécie de zooplâncton. MANOVAs duas vias e ANOVAs de uma via foram empregadas para a determinação das variações nas densidades de zooplâncton entre os tratamentos e ao longo do período de estudo. Ainda, Modelos Lineares Generalizados (MLGs) foram empregados para avaliar como fatores ambientais que influenciaram a densidade do zooplâncton. A composição do fitoplâncton foi determinada no ínicio e no final do experimento. **Resultados**: *B. urceolaris* e náuplios de copépodos, típicos de ambientes eutrofizados, apresentaram maiores densidades nos tratamentos eutrófico e hipereutrófico. Ainda, cianobactérias como *Aphanothece* sp. e *Merismopedia* sp. foram registradas nos tratamentos eutrófico e hipereutrófico, respectivamente. **Conclusões**: De maneira similar ao observado na natureza, o tratamento eutrófico apresentou maiores densidades de espécies do zooplâncton que se beneficiam do aumento na concentração de nutrientes. Respostas positivas ou negativas na dinâmica do zooplâncton fornecem uma ferramenta bioindicadora eficaz. Ainda, os resultados do presente estudo enfatizam a necessidade de implementar medidas reparadoras em ambientes aquáticos sujeitos a entradas constantes de nutrientes.

Palavras-chave: zooplâncton, estados tróficos, fitoplâncton, experimentos em laboratório.

1. Introduction

Eutrophization of lentic environments (i.e. reservoirs and lakes) is a natural process caused by a gradual increase in the concentration of nutrients, particularly, nitrogen and phosphorous (OECD, 1982; Esteves, 1998). Nevertheless, several human activities boost this process, modifying the environment at both landscape and community levels (see Kelly and Whitton, 1998; Figueirêdo et al., 2007).

When prompted by human activities, the origins of eutrophization are manifold, including urban, industrial and agricultural runoffs, which have profound effects on the physical, chemical and biological dynamics, and especially, on the trophic structure of the environment (Carpenter et al., 1985; Riegman, 1995). Therefore, eutrophization is a type of chemical pollution and, since increase in phytoplankton biomass is a direct effect of nutrients concentration, one of the primary effects of eutrophization is the rapid proliferation of algae, as acknowledged from several investigations (Anderson, 1989; Maresovic and Pucher-Petkovic, 1991; Scheffer, 1998; Silva, 1999; Anderson et al., 2002). As a consequence, this rapid phytoplankton growth may alter the overall trophic structure of the environment via trophic cascade interactions (Ravera, 1980; Seip, 1991; Scheffer, 1998) and, ultimately, drastically reduce water quality. In fact, a somewhat linear sequence is expected to occur with the rapid input of nitrogen and phosphorous, namely: 1) proliferation of diatoms, chlorophytes and cyanobacteria, 2) increase in the density of planktivores, and 4) decrease of water transparency and 5) decrease of dissolved oxygen (Esteves, 1998; Moss, 1998).

Several authors investigated the resulting cascade effects of increased nutrients on several environments worldwide (e.g. Maresovic and Pucher-Petkovic, 1991; Seip, 1991; Kelly and Whitton, 1998; Huszar et al., 1998; Forrester et al., 1999; Crispim et al., 2000; Bezerra-Neto, 2001; Anderson et al., 2002; Figueirêdo et al., 2007). These studies emphasize the central role played by zooplankton at transferring energy and, thus, linking primary producers to secondary and tertiary consumers. Furthermore, managing phytoplankton growth, particularly cyanobacterial blooms which present health risks for humans and livestock (Beasley et al., 1989; Aguiar and Azevedo, 1998), is a central objective in freshwater ecology.

The present study evaluated the effects of increased nutrient concentration (eutrophization) on some species of three major zooplankton groups (Rotifera, Cladocera and Copepoda) via experimental laboratory manipulations from different trophic states.

2. Material and Methods

2.1. Experimental design and lab procedures

The effects of increased nutrient concentrations (eutrophization) on the zooplankton community were experimentally tested under three simulated trophic states (mesotrophic, eutrophic and hypereutrophic).

Water used to conduct the experiments was collected on the Taperoá II reservoir, Taperoá city, Paraíba, Brazil (see Feliciano and Melo (2003) for detailed area description). Water was filtered through a 0.45 μm GFC membrane, and nutrients (nitrogen and phosphorus compounds) were subsequently added to each treatment. Given the natural mesotrophic conditions of the water collected from the reservoir, nutrients were not added to the mesotrophic treatment.

In field, subsamples of collected water were stored in one-liter-capacity PVC bottles and acclimatized in ice for subsequent determination of initial nutrient concentrations (i.e. total nitrogen and total phosphorus), and also to validate trophic states (Table 1). Nutrient concentrations were determined following the procedures described by Rodier (1975), Mackereth et al. (1978) and Clesceri et al. (1998). Furthermore, the addition of nitrogen and phosphorous as a means to simulate desired trophic conditions was recently tested by Vieira et al. (2011) and proved to be an efficient laboratory approach.

Table 1. Initial concentration levels of nitrogen and phosphate (μg/L) employed on experimental treatments simulating three trophic state conditions.

Experimental treatments	Compounds			
	Ammonia	Nitrite	Nitrate	Phosphate
Mesotrophic	105.3	20.2	363.6	108.5
Eutrophic	620.8	70.5	644.7	457.1
Hypereutrophic	2337.1	113.2	2923.9	479.1

Three treatments following a gradient of increased nutrient concentrations were prepared for the experiments, namely: mesotrophic (T1), eutrophic (T2) and hypereutrophic (T3). Each treatment was prepared on 300-mL-capacity PVC container and replicated three times. On each replicate, a total of 40 zooplankton individuals from five species were introduced at the following proportions: Rotifera (*Brachionus urceolaris* Müller, 1773: 10 individuals; *Hexarthra mira* (Hudson, 1871): 5 individuals), Cladocera (*Latonopsis* sp.: 10 individuals; *Moina minuta* Hansen, 1899: 10 individuals) and Copepoda (*Thermocyclops* sp.: 5 individuals). Species identification followed Koste (1978) and Stemberg (1979) for Rotifera, Elmoor-Loureiro (1997) for Cladocera, and Rocha and Matsumura-Tundisi (1976); Reid (1985); Silva (2003) and Silva and Matsumura-Tundisi (2005) for Copepoda.

Treatments were maintained under controlled photoperiod (12L (light): 12D (dark)), and constant humidity and temperature (28 °C) within a culture chamber during the study period (i.e. 14 days). Microalgae collected on the reservoir and cultivated in the laboratory were used to feed the zooplankton. On each treatment, zooplankton cultures were fed with microalgae at concentrations of 6.416×10^3 ind.ml^{-1} at three-day intervals. In the beginning of the experiment and at four-day intervals, a 1 mL subsample was collected from each replicate to determine food concentration (algal density) as a means of estimating the necessary amount of food to be subsequently added and, thus, maintaining samples with constant food concentrations. In the beginning of the experiment, microalgae were observed at the following proportions on all treatments: *Scenedesmus acuminatus* (Lagerheim) (22.7% relative abundance), *Scenedesmus bijugatus* Kützing (38.5%), *Chorella* sp. (9.2%), unidentified algae (7.4%), *Aphanothece* sp. (15%), and *Chrococus* sp (7.2%). Algal densities were determined by counting individuals on a Fuchs Rosenthal counting chamber under proper magnification. Identification followed; Silva (1999) and Bicudo and Menezes (2006). During three intervals (days 1, 7 and 14) each replicate was entirely filtered and densities of each zooplankton species determined. On the first day, individuals of all species were introduced to each container. Densities of each zooplankton species were determined on the 7th and 14th days.

2.2. Data analysis

The effects of eutrophization on zooplankton community were evaluated by comparing zooplankton abundance among days and among treatments. Normality and homogeneity of the data were tested using Shapiro-Wilk's and Levene's tests (Royston, 1983), respectively, and, when necessary, data was $\log_{x+1}$-transformed. A two-way MANOVA design was employed to test the effects of trophic states (mesotrophic, eutrophic and hypereutrophic), sampling days (1st, 7th and 14th days) and their interaction terms on zooplankton density with data from all species pooled together. Also, univariate ANOVAs were conducted to test the abundance of each species individually amongst trophic states and sampling days. On both procedures, Tukey's HSD was used to assess post-hoc differences when significant p levels were detected.

To determine the contribution of environmental factors to the observed variance in zooplankton abundance, Generalized Linear Models (GLMs) were conducted. Abundances of zooplankton species were tested as dependent variables, whereas pH, water temperature and electric conductivity were tested as continuous independent variable and nutrient concentration was tested as categorical independent variable following concentration levels employed on the experimental treatments (mesotrophic, eutrophic and hypereutrophic).

3. Results

Density of *B. urceolaris* decreased on the 7th experiment day on all treatments and persisted declining on the mesotrophic treatment. However, on the eutrophic and hypereutrophic treatments, density of this species increased after the 7th day (Figure 1a). Nonetheless, no significant differences in the density of this species were detected among treatments and among sampling days (Table 2).

Density of *H. mira* decreased on the 7th experiment day on the hypereutrophic treatment and subsequently increased. On the other hand, the density of this species increased on the 7th day and subsequently decreased on the mesotrophic treatment (Figure 1b). Statistical analyses revealed significant differences among sampling days and for the interaction term between treatments and sampling days, but not amongst treatments alone (Table 2).

The cladoceran *Latonopsis* sp. showed a somewhat low variation in density on all treatments, but a slightly higher density was observed on the eutrophic treatment (Figure 1c), albeit statistical

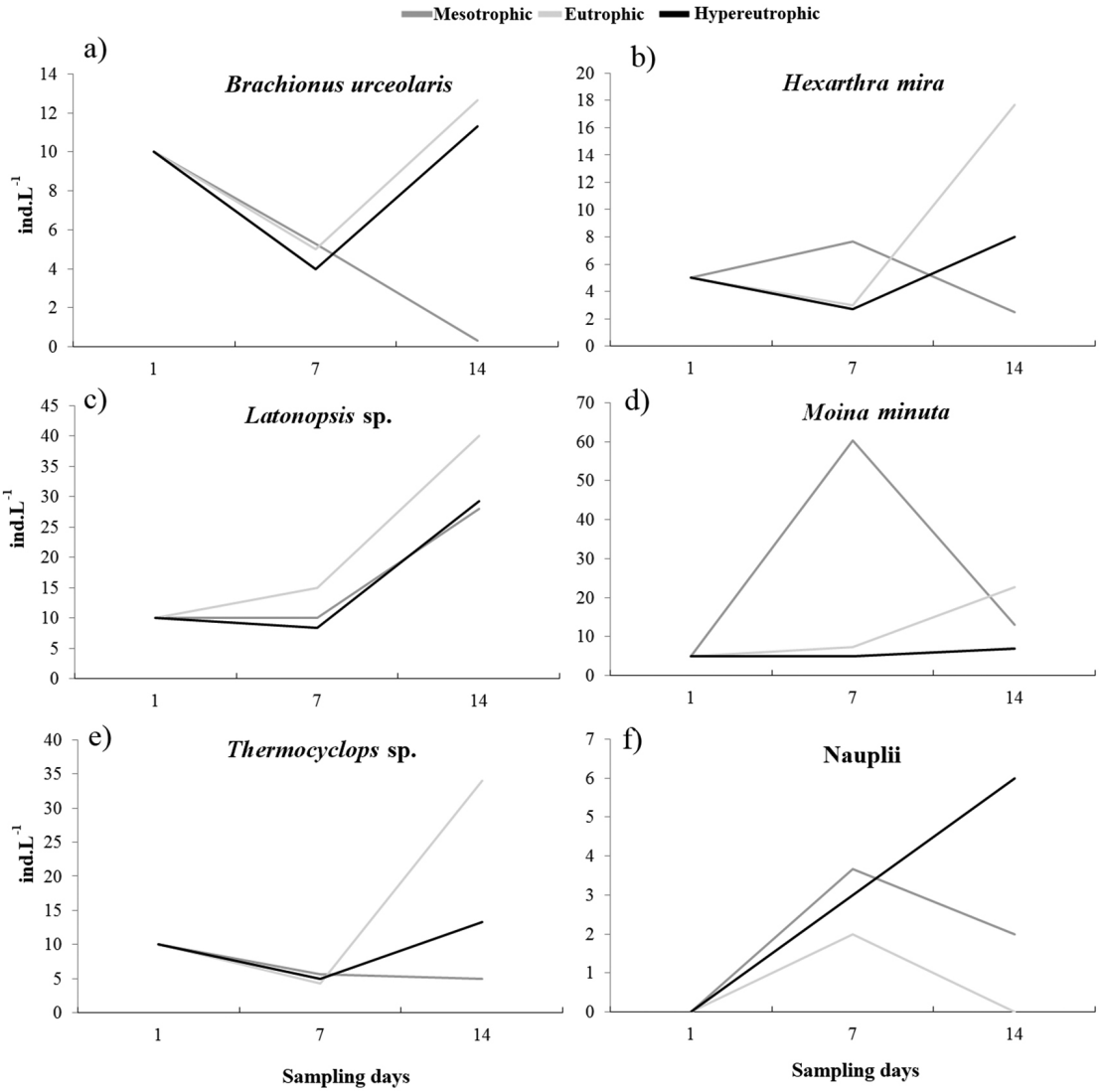

Figure 1. Temporal variations in the densities of *Brachionus urceolaris* (a), *Hexarthra mira* (b), *Latonopsis* sp. (c), *Moina minuta* (d), *Thermocyclops* sp. (e) and nauplii (f) on three experimentally simulated trophic conditions (mesotrophic, eutrophic and hypereutrophic).

analyses did not reveal significant differences (Table 2).

Density of *M. minuta* varied significantly among treatments and among sampling days (Figure 1d; Table 2). *M. minuta* showed a higher density on the 7th experiment day on the mesotrophic treatment, decreasing drastically afterwards, whereas higher density was observed on the 14th day on the eutrophic treatment. In hipereutrophic conditions densities remained similar along the experiment.

Density of the copepod *Thermocyclops* sp. initially decreased on all treatments, but subsequently increased on the eutrophic and hypereutrophic treatments (Figure 1e). On the mesotrophic treatment, density of *Thermocyclops* sp. decreased throughout the study period. Nonetheless, no significant differences were observed among treatments (Table 2).

A somewhat high density of nauplii was observed on the hypereutrophic treatment, particularly on the 14th day, when compared to the other treatments (Figure 1f). Furthermore, the mesotrophic treatment, with low densities of adults and copepodites, showed higher nauplii density than the eutrophic treatment. Albeit to a smaller extent, a similar tradeoff between adults and nauplii was also observed on the hypereutrophic treatment. Statistical analyses revealed significant differences throughout the study period and among treatments regarding nauplii density (Table 2).

The hypereutrophic treatment showed lowest pH values, but in general, pH values were always

Table 2. Results of one-way ANOVAs testing the effects of treatments (mesotrophic, eutrophic and hypereutrophic), experiment days (days 1, 7 and 14) and their interaction term on the density of zooplankton.

Zooplankton	ANOVA results			
	Effect	**F**	**df**	***p***
Moina minuta	Treatments (T)	19.82	2	< 0.001
	Days (D)	18.22	2	< 0.001
	T x D	15.89	4	< 0.001
	Residual		17	
Latonopsis sp.	Treatments (T)	2.27	2	*ns*
	Days (D)	32.57	2	< 0.001
	T x D	0.71	4	*ns*
	Residual		17	
Brachionus urceolaris	Treatments (T)	11.92	2	< 0.001
	Days (D)	12.37	2	< 0.001
	T x D	13.33	4	< 0.001
	Residual		17	
Thermocyclops sp.	Treatments (T)	3.57	2	*ns*
	Days (D)	12.43	2	< 0.001
	T x D	4.57	4	< 0.05
	Residual		17	
Hexarthra mira	Treatments (T)	2.8	2	*ns*
	Days (D)	5.31	2	< 0.05
	T x D	10.73	4	< 0.001
	Residual		17	
Náuplio	Treatments (T)	18.93	2	< 0.001
	Days (D)	139.04	2	< 0.001
	T x D	9.90	4	< 0.001
	Residual		17	

ns: non-significant.

alkaline, and tended to increase throughout the study period (Figure 2).

With data from all species pooled together, the two-way MANOVA procedure revealed significant differences among sampling days and among treatments. Also, the interaction between these two factors was significant, suggesting that the observed variation in trophic concentrations were related to temporal variations (Table 3).

Results of univariate ANOVAs are shown in Table 2. Density of *Latonopsis* sp. varied only among sampling days. Density of both *H. mira* and *Thermocyclops* sp. varied significantly among sampling days and for the interaction term between treatment and sampling days, but not for treatments. Further, density of *Latonopsis* sp. varied significantly only among sampling days (Table 2).

Results of Generalized Linear Models suggest that pH and trophic states were major determinants of zooplankton concentrations (Table 4).

Analysis of the relative abundances of algae species in the end of the experiment revealed the following patterns: mesotrophic treatment (*Scenedesmus acuminatus*: 64%; *Scenedesmus bijugatus*: 20%; *Chorella* sp.: 10.2% and an unidentified algae: 5.8%); eutrophic treatment (*Aphanotece* sp.: 42.2%; *Chrococus* sp.: 28.6%; *Scenedesmus* sp.: 15.2% and *Chlorella* sp.: 14%) and hypereutrophic treatment (*S. bijugatus*: 41%; *Chlorella* sp.: 22%; *Merismopedia* sp.: 10% and an unidentified algae: 27%).

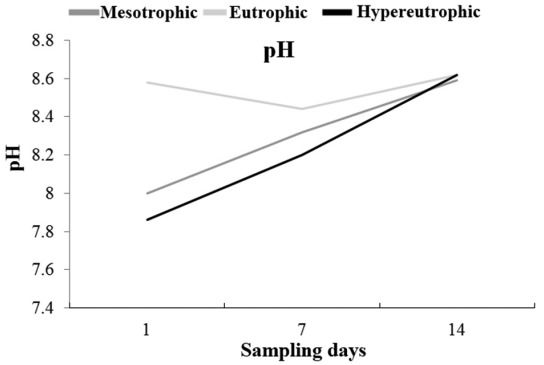

Figure 2. Temporal variations of pH on three experimentally simulated trophic conditions (mesotrophic, eutrophic and hypereutrophic).

Table 3. Results of factorial MANOVA testing the effects of treatments (mesotrophic, eutrophic and hypereutrophic), experiment days (days 1, 7 and 14) and their interaction term on the density of zooplankton species (*Moina minuta*, *Latonopsis* sp., *Brachionus urceolaris*, *Hexarthra mira* and *Thermocyclops* sp.) and nauplii pooled together.

Effect	Wilk's lambda	F	df	*p*
Treatments (T)	0.03	10.58	12	< 0.001
Days (D)	0.01	36.99	12	< 0.001
T × D	0.01	5.18	24	< 0.001

Table 4. Results of Generalized Linear Models (GLMs) testing the effects of pH and trophic states on the total density of five zooplankton taxa (*Moina minuta*, *Latonopsis* sp., *Brachionus urceolaris*, *Hexarthra mira* and *Thermocyclops* sp.) pooled together.

Predictors	GLM Results			
	Wilk's lambda	F	df	*p*
pH	0.69	1.43	1	*ns*
Trophic states	0.23	3.44	2	< 0,01

ns: non-significant.

4. Discussion

The significant decrease in the density of *B. urceolaris* throughout the study is likely to be an effect of their negative acclimation to the culture mediums. It is well known that zooplankton species may not always ideally cope with laboratory conditions, given stress-induced factors interfering with reproductive rates and, thus, density growth (Sarma and Nandini, 2001). As in the present investigation, several studies also found positive correlations between *Brachionus* species and eutrophic conditions (Gannon and Stemberger, 1978; Sládeck, 1983; Blancher, 1984; Berzins and Pejler, 1989; Matsumura-Tundisi et al., 1990; Pontin and Langley, 1993; Torres-Orozco and Zanatt, 1998).

Furthermore, results of the present study suggest that Rotifers are typical of nutrient-rich environments, as previously acknowledged on a tropical semiarid reservoir (Vieira et al., 2009).

The observed distribution patterns of the cladoceran *Latonopsis* sp. may be attributed to its ticoplanktonic habits, therefore closely associating to substrates where it feeds on particulate organic matter (Lansac-Tôha et al., 2004). Given that the culture mediums favored algal and organic matter deposition, this species may have been positively influenced by these processes. Nonetheless, the eutrophic treatment showed higher density concentrations than did the other treatments.

Peak densities decrease of *M. minuta* observed on the mesotrophic treatment (i.e. after 60 ind.L^{-1}) may have been due decrease in available food (due high density) and limited space. Since population growth is constrained by the physical space available on each culture medium this observed decrease may be easily justified (Vieira et al., 2011).

As with other cyclopoids, *Thermocyclops* are most abundant in nutrient-rich environments and results of the present study are consistent with those observed at semi-arid reservoirs (e.g. Leitão et al., 2006). Cyclopoids actively feed on rotifers (Rao and Kumar, 2002), and their increase is likely to be an effect of higher rotifer densities observed on the eutrophic and hypereutrophic treatments. Density of nauplii also increased on nutrient-rich samples. The effect of high nutrient concentration increasing the density of cyclopoids is well known (Silva et al., 2009). Our results suggest that nauplii growth, a consequence of cyclopoid reproduction, reduced on samples with high adult population densities, particularly on the eutrophic treatment. Therefore, the experiment supports what is observed in the field, in which cyclopoids initially benefit from an increase in trophic concentration, as revealed by the rapid increase in nauplii density, and, subsequently, the high population density constrains an ongoing reproduction (Blancher, 1984; Matsumura-Tundisi et al., 1990; Torres-Orosco and Zanatt, 1998). These observations suggest that longer experimental periods would result in a shift from a high nauplii concentration to a high adult concentration.

The increase in pH levels on the three treatments may have been due to the increased photosynthetic activities of algae consuming carbon dioxide and influencing pH levels (Esteves, 1998).

Comparison of algae composition between the beginning and the end of the experiment reveals community-level modifications. For example, containers subject to higher trophic levels showed higher abundance of cyanobacteria. Individuals of *Aphanotece* sp. dominated, and on the hypereutrophic treatment, *Merismopedia* sp. showed a somewhat high abundance (i.e. 10% of total abundance), albeit it was not detected in the beginning of the study. Therefore, algal density

played an important role, affecting (positively or negatively) different zooplankton species.

Furthermore, zooplankton species which are positively influenced by eutrophization, are ecological valuable at indicating pollution (Avila et al., 2009). Given that, nutrients play a direct role on primary production and algal density, affecting zooplankton via trophic cascades, it is possible to predict zooplankton numbers, and to a certain extent, overall zooplankton composition, from samples with different nutrient concentrations (Horppila, 1998). Our experiment highlights these well-documented patterns in aquatic ecology using experimental manipulation approaches conducted under controlled laboratory simulations.

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