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Planktonic heterotrophic nanoflagellate abundance along a trophic gradient in subtropical reservoirs: influence of a bottom-up or top-down control mechanism?

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ABSTRACT. The aim of the current study was to assess the variability in heterotrophic nanoflagellate abundance attributable to variables responsible for the influence of bottom-up and top-down control mechanisms in the plankton food web along a trophic gradient in subtropical reservoirs (Paraná State, Brazil). In particular, we hypothesised that food resources were the main determinant for the abundance of heterotrophic nanoflagellates (HNF) along a trophic gradient. Results showed that HNF communities were weakly influenced by the bottom-up mechanism. Moreover, there was evidence of a slight influence from a top-down control mechanism on this community. Therefore, the results of the present study did not corroborate the hypothesis initially proposed, and further studies are needed to elucidate the complex outcomes of trophic gradients on planktonic HNF communities, including other predictor variables related to abiotic interactions, such as morphometrics, hydrology, priorities uses and climate conditions.

Keywords: plankton, protozoa, food resource availability, cascading effect, freshwater environments.

Abundância de nanoflagelados heterotróficos ao longo de um gradiente trófico em reservatórios tropicais: influência do mecanismo de controle *bottom-up* ou *top-down*?

RESUMO. O objetivo do presente estudo foi investigar a variabilidade da abundância de nanoflagelados heterotróficos (NFH) planctônicos atribuída a variáveis responsáveis pela influencia dos mecanismos de controle *bottom-up* e *top-down* na cadeia alimentar planctônica ao longo de um gradiente trófico em reservatórios subtropicais (Estado do Paraná, Brasil). Nós avaliamos a hipótese de que a abundância de NFH aumenta ao longo de um gradiente trófico principalmente devido ao efeito *bottom-up*. Os resultados mostraram importantes relações presa-predador entre NFH e bactéria e fioplâncton. Entretanto, a ausência de um padrão claro de aumento da abundância de NFH em direção aos reservatórios eutróficos sugere que o controle *bottom-up* não foi relacionado ao gradiente trófico. Além disso, apesar de significante, um fraco efeito *bottom-up* e um sinal do mecanismo de controle *top-down* sugerem que o recurso alimentar não foi o principal fator regulador da dinâmica de NFH nos reservatórios estudados. Assim, os resultados do presente estudo não corroboraram a hipótese inicialmente proposta e futuros estudos são necessários para elucidar a complexidade da influencia de gradientes tróficos em NFH, incluindo outras variáveis preditoras relacionadas às interações abióticas como morfometria, hidrologia, prioridade de uso e condições climáticas.

Palavras-chave: plâncton, protozoários, disponibilidade de alimento, efeito cascata, ecossistemas de água doce.

Introduction

The purpose and conceptualisation of the microbial loop paradigm recognised the important contribution of bacteria and protozoans to biomass, energy and carbon flow and nutrient cycling in ocean systems (AZAM et al., 1983; POMEROY, 1974). This paradigm introduced the idea that the traditional view of the phytoplankton-zooplankton food web should take into account a series of direct and indirect interactions between the components of both food webs (ZÖLLNER et al., 2003).

As a result of this new perspective, an increasing number of studies continue to elucidate the factors and interactions that affect heterotrophic nanoflagellates (HNF) in aquatic ecosystems. These protozoa are important consumers of bacterial production (DOMAIZON et al., 2003), viruses (GASOL et al., 1995), cyanobacteria (PERNTHALER et al., 1996) and phytoplankton (SHERR; SHERR, 1992). They also assimilate dissolved organic carbon (DOC) (GASOL et al., 1995). Thus, HNF actively participate in the transfer of carbon and energy to higher trophic levels since they are consumed by ciliates (BERNINGER et al., 1993; HWANG; HEATH, 1997) and certain mesoplankton (TANDOLÉKÉ et al., 2004; VARGAS et al., 2008).

Ecological studies of HNF have shown that, among biotic control factors, bottom-up effects caused by changes in food quantity and quality (SAMUELSSON et al., 2006), as well as top-down effects (BURNS; SCHALLENBERGER, 1998) are most relevant to the structure of HNF communities. Specifically, studies of pelagic food webs have shown that an increase in the availability of nutrients in the environment results in an increase in algal production, which leads to a subsequent increase in the number of bacteria, heterotrophic flagellates and microplankton (ciliates, rotifers and bosminids) (AUER et al., 2004; PEREIRA et al., 2005; SIPURA et al., 2005; SAMUELSSON et al., 2006). Moreover, predation by ciliates and metazoans can also control HNF abundance (WEISSE, 1990) and produce cascading interactions (CARPENTER, 1988; NISHIMURA et al., 2011), in which prey species are reciprocally affected by changes in predator density (BETTEZ et al., 2002).

Both control mechanisms mentioned above can act together, but under certain conditions one can prevail (WEISSE, 1991). Current views acknowledge that bottom-up mechanisms primarily control components of the planktonic food web when food sources are readily available (ANDERSSON et al., 2006) or along a trophic gradient (AUER et al., 2004). However, top-down control prevails in environments where nutrients are limited (MCQUEEN et al., 1986).

The aim of the current study was to assess the variability in HNF abundance that could be attributed to variables responsible for the influence of bottom-up and top-down mechanisms in the plankton food web along a trophic gradient in 29 subtropical reservoirs during dry and rainy seasons. In particular, we hypothesised that food resources are the main determinant factor of HNF abundance along a trophic gradient.

Material and methods

Study site

The 29 reservoirs included in the study are located in six hydrographic basins in Paraná State, Brazil (Figure 1), covering environments with different characteristics. Trophic status of the reservoirs which vary according to the hydrological period and morphological variables are shown in Table 1. A detailed description of the study sites can be found in Julio Júnior et al. (2004), while limnological characterisation was previously described by Pagioro et al. (2004). Usage priorities for these reservoirs vary, but include hydropower production, water supply and public recreation. Energy production is the most common use.

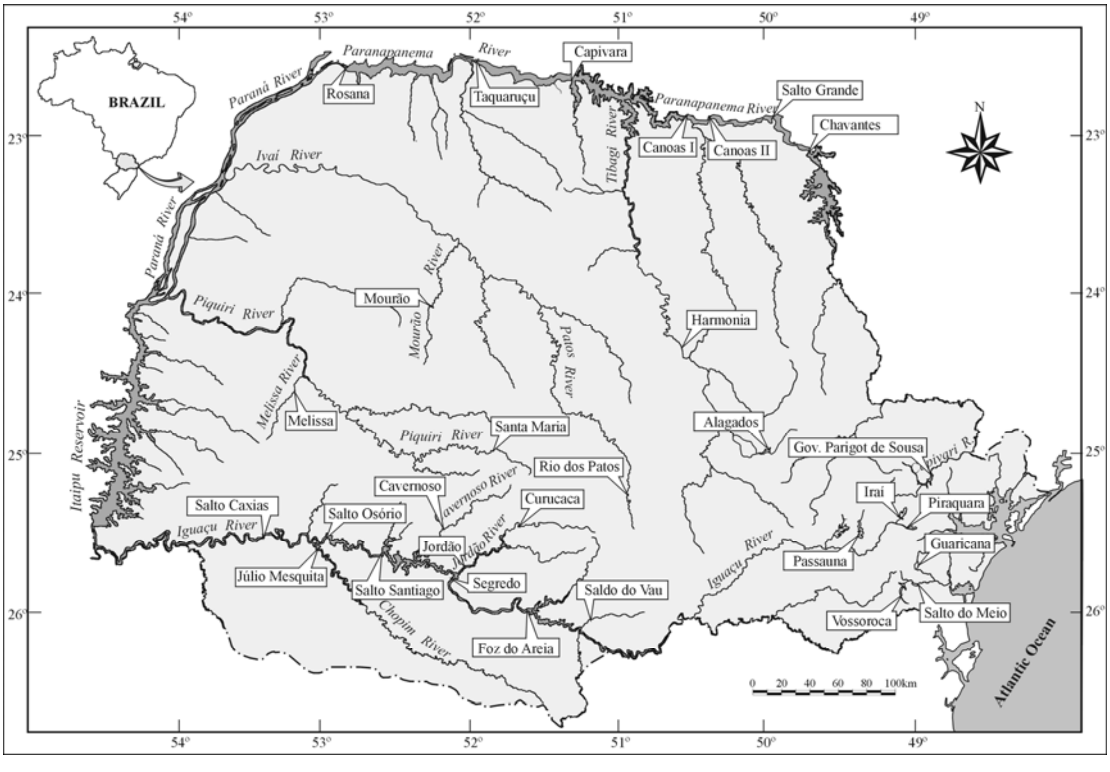

Figure 1. Location of the 29 reservoirs sampled in Paraná state, Brazil.

Table 1. Trophic status index (TSI) as well as morphological and trophic state characterisation of the 29 studied subtropical reservoirs during dry (d) and rainy (r) season.

Reservoir	Basin	Depht (m)	Area (km^2)	Age (years)	TSI		Trophic State	
					d	r	d	r
Rio dos Patos	Patos/Ivaí	5.7	1.3	55	34.62	45.96	oligotrophic	mesotrophic
Jordão	Jordão	60	3.3	8	35.94	34.98	oligotrophic	oligotrophic
Cavernoso	Cavernoso	8.3	0.05	39	36.97	31.37	oligotrophic	oligotrophic
Salto do Vau	Palmital/Iguaçu	3.7	0.4	45	37.19	40.01	oligotrophic	mesotrophic
Curucaca	Jordão	10.5	0.8	22	38.73	39.08	oligotrophic	oligotrophic
Chavantes	Paranapanema	87	242	34	39.05	42.26	oligotrophic	mesotrophic
Salto Santiago	Iguaçu	78	208	24	41.34	53.95	mesotrophic	eutrophic
Salto Grande	Paranapanema	9.2	8.4	46	42.03	39.67	mesotrophic	oligotrophic
Salto Caxias	Iguaçu	53	141	6	42.17	49.21	mesotrophic	mesotrophic
Santa Maria	Piquiri	4.3	0.05	30	42.49	34.55	mesotrophic	oligotrophic
JMF	Chopim	6	0.4	34	42.85	39.6	mesotrophic	oligotrophic
Salto Osório	Iguaçu	43	62.9	29	43.59	47.58	mesotrophic	mesotrophic
Parigot de Souza	Capivari	43	13	34	43.86	49.32	mesotrophic	mesotrophic
Foz do Areia	Iguaçu	135	139	24	44.04	51.59	mesotrophic	eutrophic
Voçoroca	Cubatão	12.5	5.1	55	44.85	50.73	mesotrophic	eutrophic
Taquaruçu	Paranapanema	26.5	40.8	12	44.92	45.86	mesotrophic	mesotrophic
Canoas II	Paranapanema	16.5	22.5	4	45.01	48.77	mesotrophic	mesotrophic
Rosana	Paranapanema	26	220	18	46.34	48.02	mesotrophic	mesotrophic
Piraquara	Rio Piraquara	18	3.3	25	46.63	42.91	mesotrophic	mesotrophic
Mourão	Mourão	12.7	11.2	40	46.75	46.93	mesotrophic	mesotrophic
Canoas I	Paranapanema	26	30.85	5	46.78	44.87	mesotrophic	mesotrophic
Capivara	Paranapanema	52.5	515	29	47.32	43.86	mesotrophic	mesotrophic
Alagados	São Jorge	9.25	7.31	85	48.34	53.87	mesotrophic	eutrophic
Salto Segredo	Iguaçu	101	80.4	12	48.64	42.85	mesotrophic	mesotrophic
Salto do Meio	Cubatão	6.2	0.12	73	49.04	43.86	mesotrophic	mesotrophic
Melissa	Melissa/Piquiri	5.3	0.05	42	49.44	42.22	mesotrophic	mesotrophic
Harmonia	Harmonia	12	0.64	62	51.63	53.55	eutrophic	eutrophic
Guaricana	Arraial	17	0.86	47	53.8	49.25	eutrophic	mesotrophic
Iraí	Iraí	8.5	14.4	5	65.54	64.54	eutrophic	eutrophic

Data from Julio Júnior et al. (2004) and Pagioro et al. (2004).

Sampling schedule and methods

Subsurface samples (0.5 m) were collected in the lacustrine zone of the reservoirs during the dry (July) and rainy (November) seasons of 2001. Water samples for chemical and biological analyses were taken using a Van Dorn bottle (5 L). Water aliquots were stored in separate sample bottles for analyses of HNF (100 mL), phytoplankton (100 mL), bacteria (50 mL) and ciliates (500 mL). Zooplankton was collected using a motorized pump and plankton net (68 μm).

Phytoplankton and bacteria were preserved in acetic lugol and formaldehyde (4%), respectively. HNF were preserved in fixative solution containing formaldehyde, alkaline lugol and thiosulfate (SHERR; SHERR, 1992). Ciliates were preserved in glutaraldehyde (1%) according to methodoly utilized by Urrutxurtua et al. (2003). Zooplankton were preserved in formaldehyde (4%) buffered with calcium carbonate. All samples were stored in styrofoam boxes until further analysis.

Measurement of physical and chemical variables

In the field, pH was determined using a portable pH meter (Digimed), while water temperature (ºC) and dissolved oxygen (mg L^{-1}) were measured using a multiparameter probe (YSI 550A, USA). Dissolved organic carbon (DOC) concentration (ppm) (Schimadzu-TOC 5000), chlorophyll a (mg L^{-1}) (GOLTERMAN et al., 1978), turbidity (NTU) (turbidimeter, Digimed), total phosphorus (total-P, μg L^{-1}) and total nitrogen (total-N, μg L^{-1}) (MACKERET, 1978) were measured in the laboratory.

Abundance estimation

Bacteria (0.2-2 μm) and nanoflagellates (2-20 μm) were stained with DAPI (4,6'-diamidino-2-phenylindole; 0.001% final concentration), collected onto 0.2 μm (bacteria) and 0.8 μm (nanoflagellates) Nucleopore polycarbonate black membranes under gentle vacuum filtration (<15 cm Hg) (PORTER; FEIG, 1980). The volume of water filtered ranged from 0.1-1.5 mL for bacteria and 5-15 mL for flagellates depending on the trophic state of the environment. For bacteria, we highlighted that DAPI was added on membranes before the water sample. Thus a minimum of 2 mL (DAPI + water sample) ensured homogenous bacteria distribution on membranes. Approximately 400 bacteria (or 50 fields) and 100-300 nanoflagellates (or 100 fields) per sample were counted randomly using an Olympus epifluorescence microscope at 1000× magnification. Total bacteria and nanoflagellate abundance was assessed under UV light (365 nm), resulting in blue-fluorescence.

Pigmented (PNF) and heterotrophic (HNF) nanoflagellates were distinguished by chlorophyll autofluorescence according to methodoly utilized by

Adrian and Schneider-Olt (1999). Specifically, they were differentiated by a filter set that provided blue excitation (495 nm), which resulted in red autofluorescence by chlorophyllous organisms and green fluorescence by heterotrophic organisms. HNF abundance was defined as the difference between total nanoflagellate abundance and PNF.

Using an inverted microscope, quantitative analysis of ciliate protozoa was performed as previously described by Utermöhl (1958). The settled volume was defined based on the concentration of ciliates and/or detritus present in the sample. Settling time was calculated based on the height of sedimentation in the chamber, where each cm of height was estimated to take at least three hours (MARGALEF, 1983). A minimum of 50 ciliate specimens were counted in 10 random transects.

Densities of bacteria, flagellate and ciliate communities were expressed in cells. mL^{-1}. The density of phytoplankton was estimated using an inverted microscope after sedimentation in Utermöhl chambers (UTERMÖHL, 1958). Calculations were made according to APHA (1985). Counting of phytoplankton was performed until 100 specimens of the most abundant species were obtained. The results were expressed as specimens per ml.

Zooplankton abundance was determined using an adapted Sedgewick-Rafter counting chamber (2.5 mL) under an optical microscope (Olympus CX41). At least 80 specimens were counted (BOTTREL et al., 1976) in each of 3 sequential subsamples, which were collected using a Hensen-Stempell pipette (2.5 mL). Abundance was expressed as specimens per m^3.

Statistical analyses

In order to select models predicting variability in the nanoflagellate community attributed to the influence of both bottom-up and top-down control mechanisms, we calculated an Akaike's Information Criterion (AICc, second-order AIC, which is necessary for a small sample) using Spatial Analysis in Macroecology (SAM) software (RANGEL et al., 2006). HNF density was considered a dependent variable, while density of bacteria, small and large phytoplankton, ciliates and small and large zooplankton were considered predictor variables. Models including combinations of all variables were tested. Specifically, we were interested in comparing the importance of bottom-up (bacteria plus small and large phytoplankton) versus top-down models (ciliates plus small and large zooplankton) during each season.

The best models for predicting variability in the nanoflagellate community were those with AICc differences (Δ_i AICc) ≤ 2 (BURNHAM; ANDERSON, 2002). Akaike's weight of each model (wi) was also calculated, denoting the likelihood that a particular model is the most reasonable one among the set of models evaluated. These values of wi are usually standardized by their sum across all models evaluated, ranging from 0 to 1. Higher weights signify better explanatory power.

The type of the relationship between response and main predictor variables was determined by the standard partial regression coefficient (*beta*) for each predictor variable from the Akaike's Information Criterion analysis.

To normalise the variance in all statistical analyses, variables were log(x+1) transformed.

Results

Heterotrophic Nanoflagellates

Density values ranged from 0.092-8.19 x 10^3 and 0.118-15.5 x 10^3 cells mL^{-1} in the dry and rainy season, respectively. In general, higher values were recorded in the rainy season. Although higher peaks were recorded in eutrophic reservoirs, a clear pattern in density values along the trophic gradient was not observed (Figure 2).

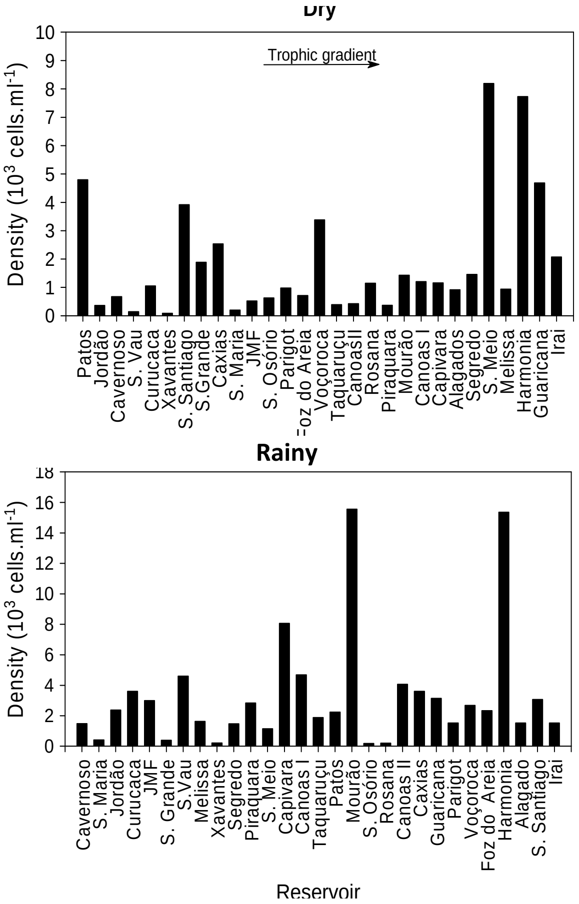

Figure 2. Density of heterotrophic nanoflagellates (cells. mL^{-1}) during the dry and rainy season along a trophic gradient in 29 reservoirs in the Paraná State, Brazil.

Food resource

Bacteria and Phytoplankton

Density of bacteria, small phytoplankton and large phytoplankton ranged from 11.8-65.4 x 10^5, 4-2653 and 0-25783 cells mL^{-1} in the dry season, respectively, and 14.84-84.8 x 10^5, 11.3-617 x 10^{-1} and 0-2053 cells mL^{-1} in the rainy season, respectively. In general, higher values were recorded in the rainy season for bacteria, while higher densities of small and large phytoplankton were noted in the dry season, but a clear seasonal pattern not observed for large phytoplankton. Similar to flagellates, absence of a clear density pattern along the trophic gradient was observed for bacteria and phytoplankton communities (Figure 3).

Predators

Ciliates and Zooplankton

Flagellate predator density ranged from 0.2-47 cells mL^{-1}, 0.1-785 x 10^3 and 0-70 x 10^2 ind. m^{-3} for ciliate, small zooplankton and large zooplankton, respectively, during the dry season, and from 0.16-12.2 cells mL^{-1}, 0.3-1029 x 10^3 and 0-165 x 10^2 ind. m^{-3} for ciliate, small zooplankton and large zooplankton, respectively, during the rainy season. A clear seasonal pattern was not observed for any of the communities. A spatial pattern along the trophic gradient was noted only for small zooplankton, with generally higher density values in eutrophic reservoirs (Figure 4).

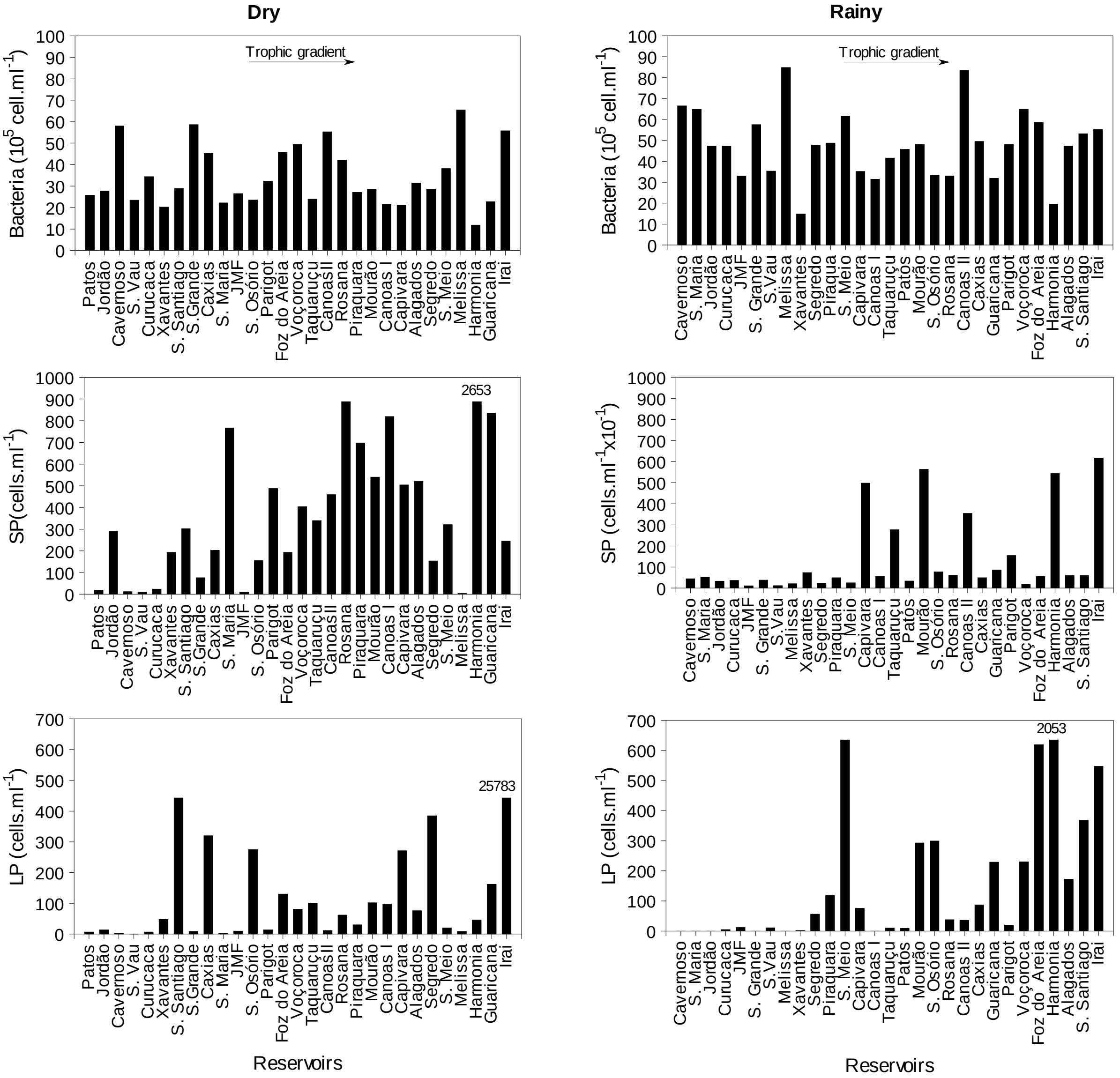

Figure 3. Density of bacteria, small phytoplankton (SP) and large phytoplankton (LP) during the dry and rainy season along a trophic gradient in 29 reservoirs in the Parana State, Brazil.

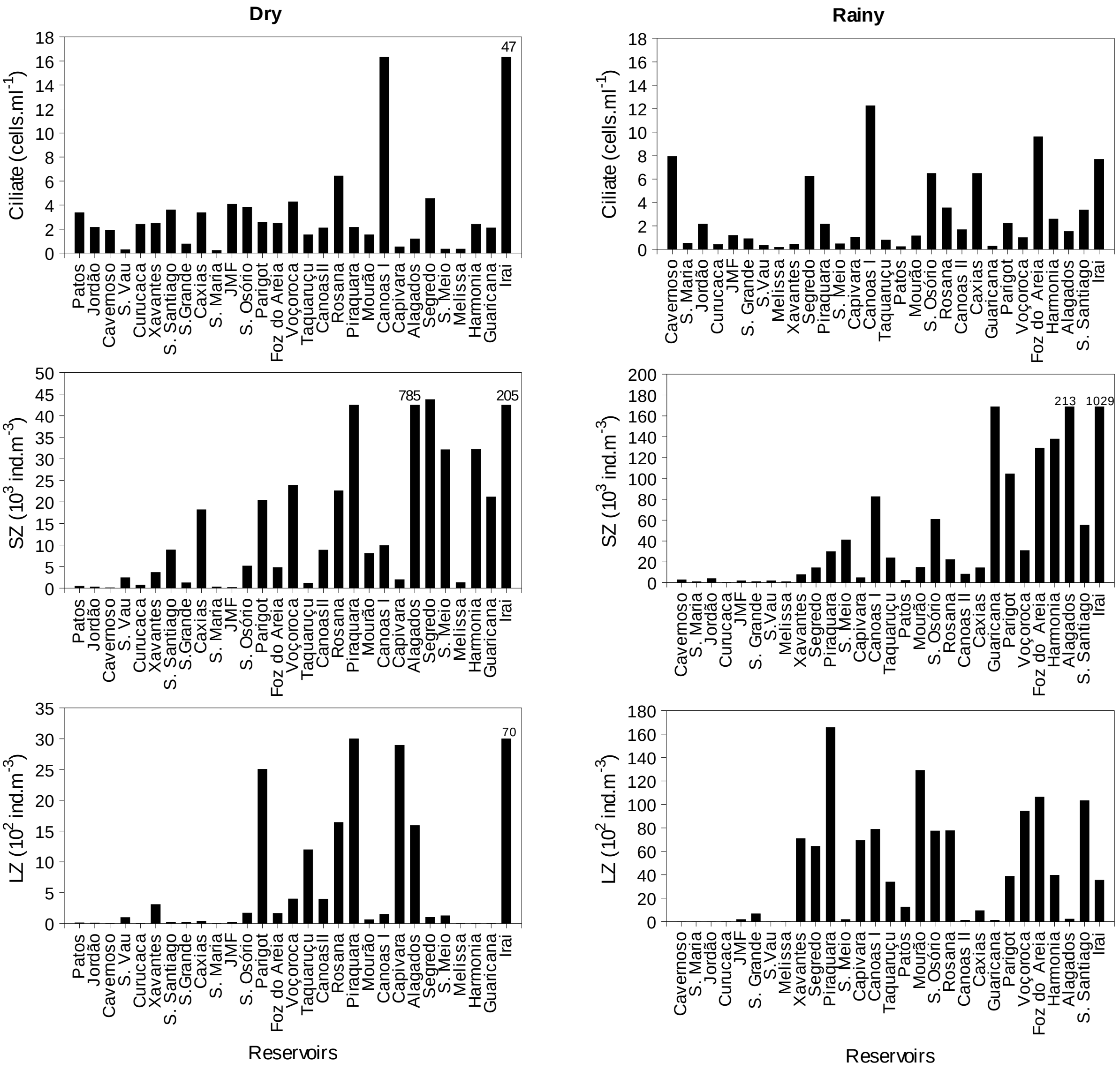

Figure 4. Density of ciliate, small zooplankton (SZ) and large zooplankton (LZ) during the dry and rainy season along a trophic gradient in 29 reservoirs in the Parana State, Brazil.

Bottom-up and top-down factors driving changes in HNF

The best model for our data as determinants of HNF spatial structure along the trophic gradient of the reservoirs included bacteria, large phytoplankton and large zooplankton ($R^2 = 0.313$) in the dry season, but only large phytoplankton and small zooplankton ($R^2 = 0.195$) in the rainy season (Table 2). However, for the rainy season, the AICc differences ($\Delta_i < 2$) calculated over all candidate models in the set indicated that the first model was not the only one considered to have substantial empirical support. In this way, the next three models were also selected as plausible for inferences, thus, in addition to large phytoplankton and small zooplankton, large zooplankton were considered as important determinants of HNF assemblage structure during this period (Table 2).

Table 2. Summary of parsimonious models to explain HNF density along a trophic gradient of 29 subtropical reservoirs during dry and rainy seasons. As a selection criterion, models with AICc≤2 were considered the best approximated models. Akaike's weight (wi) indicates the probability that a certain model is the most parsimonious among all the models tested.

Model	Code	AIC_C	ΔAICc	wi
Dry season				
LP, LZ, B	1	87.94	0	0.17
LP, LZ	2	88.11	0.173	0.15
Rainy season				
LP, SZ	1	91.37	0	0.13
LP, LZ	2	91.85	0.47	0.10
LP	3	92.29	0.92	0.08

Bacteria (B), Large Phytoplankton (LP), Small Zooplankton (SZ), Large Zooplankton (LZ).

The standard partial regression coefficient (*beta*) revealed that, in the dry season, HNF was positively correlated with bacteria (0.24 ± 0.21) and large phytoplankton (0.65 ± 0.14) but negatively correlated with large zooplankton (-0.51 ± 0.07). During the rainy season, HNF was positively correlated with large phytoplankton (0.6 ± 0.22) and negatively correlated with small (-0.44 ± 0.09) and large zooplankton (-0.29 ± 0.03).

Discussion

Bottom-up effects on HNF abundance, as a response to the increase in nutrient availability (AUER; ARNDT 2001; AUER et al., 2004; SAMUELSSON et al., 2002, 2006; SIMEK et al., 2003) and along a trophic gradient (AUER et al., 2004, PEREIRA et al., 2005) are frequently reported in the literature. In this way, the weak effects of the bottom-up mechanism on the HNF community during dry and rainy seasons, as supported by the analysis in the present study (R^2 values), cannot be regarded as a highly expected result. Furthermore, a sign of top-down control by zooplankton assemblage is evidence that bottom-up is not the main control mechanism regulating HNF abundance along a trophic gradient in the studied reservoirs.

An experiment developed in a Mediterranean stream showed that flagellates did not respond significantly to fertilization and suggested that the increase of nanoflagellate abundance to high food resource availability is not a general pattern for different ecosystems (DOMÈNECH et al., 2006). Therefore, our results and those from Domènech et al. (2006) highlight the importance of weak/negative results in addressing the generality of the effect of bottom-up mechanism on the HNF community.

The environmental variables used in our study are recognized as fundamental in determining the effect of bottom-up and top-down mechanism control on HNF abundance along a trophic gradient. In this way, a lack of key environmental variables sampled cannot be related to the results of the present study. A source of unpredictability that can be ruled out is that "snapshot" assessments may not take into account important processes that can be structuring nanoflagellate communities.

Considering the bottom-up effects registered when only three of the reservoirs investigated in the present study were analyzed (PEREIRA et al., 2005), another important issue is that our study covered high variability of water chemistries and physical properties as well as morphometric (i.e., length, area, depth) and water characteristics (i.e., stratification, flow, residence time), priorities uses and climatic conditions. Thus, complex relationships between HNF and physical and chemical variables, especially during the rainy season, could have produced the weak bottom up effects observed when the 29 reservoirs were analyzed.

We do not exclude the possibility that a bottom-up mechanism along a trophic gradient is structuring the HNF community in the studied subtropical reservoirs. In fact, considering that heterotrophic flagellates are important consumers of bacteria (SIMEK et al., 1999) and phytoplankton (SHERR; SHERR 1992) assemblages, we highlight that important predator-prey relationships probably existed among them. However, we have to consider the weak bottom-up effect and the evidence of a top-down control mechanism.

Conclusion

The results of the present study did not corroborate the hypothesis that food resources are the main determinant of HNF abundance along a trophic gradient. Further studies are needed to elucidate the complex outcomes of trophic gradients on planktonic heterotrophic nanoflagellate communities, including other predictor variables such as morphometrics, hydrology, priorities uses and climate conditions.

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