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BIANCA TREVIZAN SEGOVIA DA SILVA

Estrutura e dinâmica das comunidades microbianas (bactérias e protozoários) no plâncton de ambientes tropicais de água doce: padrões espaciais, temporais e fatores intervenientes

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Tese apresentada ao Programa de Pós-Graduação em Ecologia de Ambientes Aquáticos Continentais do Departamento de Biologia, Centro de Ciências Biológicas da Universidade Estadual de Maringá, como requisito parcial para obtenção do título de Doutor em Ciências Ambientais
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Orientador: Dr. Luiz Felipe Machado Velho
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It is paradoxical, yet true, to say that the more we know, the more ignorant we become in the absolute sense, for it is only through enlightenment that we become conscious of our limitations.

Nikola Tesla

Estrutura e dinâmica das comunidades microbianas (bactérias e protozoários) no plâncton de ambientes tropicais de água doce: padrões espaciais, temporais e fatores intervenientes

RESUMO

Os microrganismos são fundamentais nos ciclos biogeoquímicos e participam ativamente do fluxo de matéria e energia através das teias alimentares planctônicas. Investigou-se os principais fatores que afetam a abundância bacteriana em ambientes de água doce tropicais, uma vez que um aparente paradoxo surgiu de estudos prévios: apesar da maior produção bacteriana encontrada em regiões tropicais, quando comparada à das regiões temperadas, menores abundâncias bacterianas eram registradas. Esse fato levantou a hipótese de que a abundância bacteriana seria controlada por flagelados heterotróficos nos trópicos. Através da análise da base de dados de diversos ambientes tropicais, constatamos que tanto a abundância bacteriana quanto a de flagelados heterotróficos é consistentemente menor em regiões tropicais quando comparada à das regiões temperadas, resultando em um acoplamento similar entre essas comunidades. Um maior controle *top-down* exercido sobre ambas as comunidades microbianas por ciliados e cladóceros, pode explicar os padrões encontrados. A abordagem experimental demonstrou que os protistas, principalmente os ciliados, são provavelmente os principais responsáveis pela perda bacteriana em uma lagoa tropical, e que a pressão de predação também influencia a estrutura de tamanho da comunidade bacteriana. Investigaram-se também, na estruturação da comunidade de ciliados planctônicos de planícies de inundação Neotropicais, as escalas temporais e espaciais, através da abordagem de metacomunidades. Estudos acerca desse tema mostraram resultados contraditórios: enquanto para alguns os microrganismos apresentavam uma natureza cosmopolita, para outros, padrões biogeográficos seriam semelhantes àqueles encontrados em macrorganismos. Análise da escala espaço-temporal indicou a influência da variação temporal na estruturação da comunidade de ciliados. Conforme a análise de uma grande extensão espacial foi possível verificar que padrões biogeográficos também ocorrem para organismos de pequeno tamanho.

Palavras-chave: Nanoflagelados heterotróficos (HNF). Interações tróficas. Latitude. Predação. Biogeografia. Múltiplas escalas espaciais. Períodos Hidrológicos.

Structure and dynamics of microbial communities (bacteria and protists) in the plankton of tropical freshwater environments: spatial and temporal patterns and intervening factors

ABSTRACT

Microorganisms play a key role in aquatic biogeochemical cycles and in the flow of matter and energy through planktonic food webs. The first two chapters of this thesis aimed to investigate the main factors affecting bacterial abundances in tropical freshwater environments, due to an apparent paradox which emerged from previous studies: despite the higher bacterial production in the tropics, the bacterial abundance found in those regions is lower, compared to temperate environments. This fact raised the hypothesis that bacterial abundance could be controlled by heterotrophic flagellates in the lower latitudes. By analyzing a large data set, we showed that both bacterial and heterotrophic flagellate abundances are consistently lower in tropical when compared to temperate regions, resulting in a similar coupling between those communities. A stronger top-down control on both microbial communities exerted by ciliates and cladocerans, may explain those patterns. The experimental approach allowed us to demonstrate that protists, mainly ciliates, are likely the main responsible for bacterial loss in a tropical lake and that predation pressure also influences bacterial community size-structure. In the third chapter, we aimed to comprehend the role of temporal and spatial scales in structuring the planktonic ciliate communities from Neotropical floodplains, through a metacommunity approach. Contrasting results have been reported in the literature: while some authors advocated that microorganisms have a cosmopolitan distribution, others argued that they displayed biogeographic patters similar to those found for larger sized organisms. Taking into account both spatial and temporal scales, our analysis allowed us to demonstrate a clear influence of the temporal variation in structuring the planktonic ciliate communities, and with the analysis of a great spatial extent, it was possible to verify that biogeographic patterns also occur for those small-sized organisms.

Keywords*:* Heterotrophic Nanoflagellates (HNF). Trophic interactions. Latitude. Predation. Biogeography. Multiple spatial scales. Hydrological periods.

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SUMÁRIO

1 INTRODUÇÃO GERAL

A concepção desses estudos surgiu devido a um aparente paradoxo: apesar da produção bacteriana tropical ser maior do que aquela encontrada em regiões temperadas (Amado et al., 2013), o registro de baixas abundâncias bacterianas em ambientes tropicais era recorrente (Roland et al., 2010; Sarmento, 2012). Assim, a hipótese de que fatores envolvendo perdas bacterianas seriam mais importantes nos trópicos era tentadora. O pressuposto de um maior controle *top-down* exercido por flagelados heterotróficos (HNF) parecia plausível, uma vez que a abundância de HNF seria teoricamente maior em ambientes tropicais devido a diferenças na estrutura da teia alimentar nas duas latitudes (Sarmento, 2012; Özen et al., 2013).

Avaliou-se o acoplamento entre as comunidades planctônicas de flagelados heterotróficos e bactérias, e as possíveis causas da baixa abundância bacteriana encontrada nos trópicos, analisando e comparando uma base de dados de diversos ambientes aquáticos continentais tropicais e temperados. A abordagem experimental desvendou importantes questões: a abundância bacteriana é controlada por mecanismos *top-down*? Quais seriam os principais predadores bacterianos em ambientes tropicais?

Investigaram-se também os principais fatores responsáveis pela estruturação da comunidade de ciliados planctônicos de planícies de inundação Neotropicais, através da abordagem de metacomunidades. Nos últimos anos, houve muita discussão acerca dos padrões espaciais encontrados em microrganismos. Enquanto alguns autores argumentavam que os microrganismos apresentavam uma natureza cosmopolita devido às altas taxas de dispersão (Finlay, 2002; Fenchel & Finlay, 2004), outros encontraram padrões biogeográficos semelhantes àqueles encontrados em macrorganismos (Green et al., 2004; Martiny et al., 2006). Atualmente, a questão a ser respondida é em qual extensão espacial os microrganismos podem exibir padrões biogeográficos (Green & Bohannan, 2006), e qual a importância da variação temporal em alterar esses padrões. Desse modo, o estudo levou em conta tanto aspectos espaciais quanto temporais, na tentativa de incorporar mais conhecimento a respeito desse assunto.

2 COUPLING BETWEEN HETEROTROPHIC NANOFLAGELLATES AND BACTERIA IN FRESH WATERS: DOES LATITUDE MAKE A DIFFERENCE?

ABSTRACT

Recent studies reported comparatively lower heterotrophic bacteria (HB) abundances in tropical regions, indicating that factors involved in bacterial losses could be more relevant in the tropics. Heterotrophic nanoflagellates (HNF) are considered the main predators of HB in aquatic ecosystems, and one should expect higher abundances in the tropics because of differences in the food web configuration (absence of large daphnids). However, there are no comprehensive studies comparing HB and HNF abundances in a latitudinal gradient. We hypothesized that HB abundance would be lower in the tropics because HNF abundance would be higher, resulting in a tighter HNF-HB coupling. To test this hypothesis, we compiled a large dataset of HB and HNF abundances from tropical and temperate freshwater environments. We found that both HB and HNF abundances were lower in the tropical region, and that HNF-HB coupling does not differ between temperate and tropical regions. The lower HNF abundance and lack of coupling may be explained by a strong top-down control on HNF and/or their herbivory preference. Besides, no relationship was found between bacterial specific growth rate and either chlorophyll-*a* and HB abundance, indicating that bacterial losses may have an important role in tropical freshwaters. Thus, we found that HNF is likely not the main controllers of HB abundance, and that grazing by ciliates and cladocerans together with the physiological effects of higher temperatures, may explain the high bacterial loss rates in the tropics.

Keywords: Bacterioplankton, Cladocera, Protist, Predation, Latitude.

2.1 INTRODUCTION

Inland aquatic ecosystems play a relevant role in the global carbon cycle (Cole et al., 2007; Tranvik et al., 2009; Raymond et al., 2013). Low latitude freshwaters, particularly wetlands, represent a large percentage of global CO_2 evasion to the atmosphere compared to colder counterparts located in temperate regions (Marotta et al., 2009; Aufdenkampe et al., 2011; Barros et al., 2011; Abril et al., 2014). The disproportionally importance of tropical fresh waters in CO_2 net diffusion would be due to the high input of organic terrestrial carbon and further microbial heterotrophic respiration (Cole et al., 1994; DelGiorgio et al., 1999). In fact, bacterial biomass and production has been related to CO_2 lake concentrations (Tadonléké et al., 2012; Fontes et al., 2013), evidencing the importance of bacterioplankton in CO_2 emission dynamics. Thus, it is essential to identify the patterns and drivers of bacterial abundance, production and respiration across latitudinal gradients.

In this way, recent studies pointed out that, although there is a higher bacterial production in lower latitudes (Amado et al., 2013), the bacterial abundance found in those regions is lower, compared to temperate environments (Roland et al., 2010; Sarmento, 2012). This points out that factors involved in bacterial loss would be more important in the tropics, since bacterial biomass does not seem to increase with increasing bacterial production in similar rates in both regions (Billen et al., 1990). The low bacteria:chlorophyll-*a* ratios found in warm waters suggest that grazing might be an important mechanism limiting bacterial abundance (Sarmento et al., 2008; Roland et al., 2010; Özen et al., 2013). These differences in bacterial abundance at different latitudes have been attributed, at least in part, to a higher top-down control of rotifers, ciliates and nanoflagellates in warmer regions (Amado et al. 2013; Roland et al. 2010; Sarmento 2012; Sarmento et al. 2010; Vázquez-Domínguez et al. 2012).

Because heterotrophic nanoflagellates (HNF) are considered the main responsible for channeling bacterial production to higher trophic levels (Berninger et al., 1991; Fenchel, 1982; Sanders et al., 1989; Sanders et al., 1992), one should expect a higher top-down control on bacteria by the HNF in the tropics. Factors known to exert an influence on the predator-prey relationship between HNF and bacteria, such as temperature, bacterial and HNF abundance (Gasol et al. 2002; Peters, 1994; Vaqué et al., 1994), vary widely with latitude. As temperature alters metabolic rates, it also influences all the other factors above cited, as predicted by the metabolic theory of ecology (MTE) (Brown et al. 2004), which might also provide some insights on differences of microbial metabolic rates and trophic interactions between tropical and temperate regions.

The cornerstone of MTE is that metabolic rates, including grazing rates (Sarmento et al. 2010) and population growth rates (Savage et al. 2004), increase exponentially with temperature (Brown et al., 2004). For instance, bacterial abundance and production is thought to increase with increasing temperatures (White et al. 1991). However, the effects of temperature are not always straightforward, as it interacts with organic matter quality and quantity. It is argued that increased temperatures may actually lead to a decrease in the abundance of the organisms, because the increased metabolic cost per individual means that a given supply of energy will support a smaller number of individuals (Brown et al. 2004; Savage et al. 2004; Sarmento et al., 2010). Yet, this assumption does not consider the effects of trophic interactions. For example, Jiang and Morin (2004) found that competition between the populations of two protists changed the outcome of temperature effects on their abundances, when compared with the isolated temperature effect on those populations. Also, Vasseur and Mccann (2005) model states that temperature alone would not affect resource

density in the absence of predators, implying that the effects of trophic interactions should also be taken into account.

Temperature has also been positively correlated with feeding rates, thereupon protist grazing rates on bacteria are expected to be higher with raised temperatures, since more food is required to fulfill their energy demand (Peters, 1994; Sarmento et al., 2010; Vaqué et al., 1994). Considering that tropical regions experience elevated temperatures all year long, bacteria might suffer a higher predation pressure, so that a larger proportion of bacterial production is taken by grazers (Sarmento et al., 2010), outbalancing bacterial growth stimulation by temperature. Indeed, in the few studies available for tropical region, HNF grazing on bacteria was found to be relatively high (Pirlot et al., 2007; Tarbe et al., 2011).

It is believed that HNF abundance in warm environments should be higher than in colder ones, owing to consistent differences in the food web structure along the latitudinal gradient (Özen et al., 2013; Sarmento, 2012). This is because in temperate environments there is a typical prevalence of large-bodied cladocerans, which are able to suppress the abundance of HNF (Gasol et al. 1995; Jürgens and Stolpe 1995; Kalinowska et al., 2015). Actually, the predation pressure of *Daphnia* on HNF was found to result in a lack of coupling between HNF-bacteria in temperate systems, highlighting zooplankton as crucial regulators of bacterial abundance (Gasol and Vaqué 1993; Jürgens et al., 1994). Meanwhile in the tropics, both temperature (Havens et al., 2015) and the high predation pressure of fish throughout the year (Fernando, 1994; Iglesias et al., 2011; Lazzaro, 1997) favor the development of small-bodied zooplankton, which would not be as efficient predators of HNF as their relatives of the temperate regions. The resulting greater HNF abundance would account for a tighter coupling between bacteria and heterotrophic nanoflagellates in tropical environments (Sarmento, 2012). Accordingly, elevated temperatures increasing microbial metabolism, along with the higher abundance of HNF and lower abundance of bacteria, all concur to the idea that HNF-bacteria coupling should differ across latitudinal gradients, being stronger in the tropics.

The aim of this study was to compare HNF and heterotrophic bacterial (HB) abundances in different latitudes (temperate vs tropical), as well as the HNF-HB coupling. Taking into account that HNF grazing pressure is thought to be the main explanation for lower bacterial abundance in tropical regions, and that the lack of HNF-bacterial coupling seems to be a widespread phenomenon in the temperate ones, we hypothesized that, in the tropics, i) HB abundance would be lower, because ii) HNF abundance would be higher, and consequently iii) HNF-HB coupling would be stronger. We also investigated the importance of other predators and resources (i.e. chlorophyll-*a*) in explaining bacterial abundance in tropical environments. In order to test these hypotheses, we compiled a large dataset of HB and HNF abundances from tropical and temperate freshwater environments and compared their abundances and the HNF-HB coupling, besides exploring other possible causes involved in bacterial losses in the tropics.

2.2 MATERIALS AND METHODS

Dataset

The dataset consists of 1047 observations of heterotrophic bacteria (HB) and heterotrophic nanoflagellates (HNF) abundances from the literature in both tropical (N_{trop}=381) and temperate (N_{temp}=666) freshwater inland aquatic ecosystems, encompassing a broad range of environment types, including shallow lakes, deep lakes and reservoirs of

various trophic status. We also used abundance data of ciliates, rotifers, cladocerans and copepods from tropical environments (Table 1).

Table 1. Database from each literature data in tropical and temperate environments used in all analysis*. *to perform the analysis of the relationship between bacterial specific growth rates, chlorophyll-*a* and bacterial abundance, we used a different dataset (see below).

References	N	HB abundance		HNF abundance	
		min	max	min	max
TROPICAL					
Domingues et al. (submitted)	46	$5.93x10^5$	$6.17x10^6$	$1.80x10^3$	$2.75x10^4$
Meira et al. (In prep.)	21	$3.03x10^5$	$2.50x10^6$	$6.52x10^0$	$2.02x10^2$
Morana et al. (2014)	21	$1.82x10^6$	$4.58x10^6$	$2.07x10^2$	$1.11x10^3$
Velho et al. (In prep.)	36	$1.46 x10^5$	$7.54x10^5$	$1.10x10^2$	$2.35x10^3$
Pereira et al. (2014)	58	$1.18x10^6$	$8.48x10^6$	$9.22x10^1$	$1.56x10^4$
Pirlot et al. (2005)	21	$1.66x10^6$	$5.63x10^6$	$2.99x10^2$	$4.08x10^3$
Segovia et al. (2014)	72	$1.46x10^5$	$1.26x10^6$	$1.09x10^2$	$1.21x10^4$
Segovia et al. (In prep.)	106	$4.18x10^4$	$2.33x10^6$	$1.78x10^1$	$1.53x10^3$
total	**381**				
TEMPERATE					
Bennett et al. (1990)	32	$2.31x10^6$	$8.73x10^6$	$1.12x10^3$	$5.61x10^3$
Berninger et al. (1993)	81	$7.33x10^6$	$2.21x10^7$	$8.07x10^3$	$7.14x10^4$
Bird and Kalff (1989)	12	$2.55x10^6$	$1.34x10^7$	$2.80x10^2$	$6.20x10^3$
Bloem and Bär-Gilissen (1989)	34	$4.00x10^6$	$1.00x10^7$	$2.00x10^2$	$3.40x10^4$
Bloem et al. (1989)	12	$5.42x10^6$	$1.45x10^7$	$5.40x10^2$	$1.05x10^4$
Christtoffersen et al. (1990)	10	$4.13x10^6$	$5.91x10^6$	$8.75x10^1$	$1.08x10^3$
Fermani et al. (2013)*	41	$2.34x10^7$	$1.08x10^8$	$9.40x10^3$	$1.12x10^5$
Fermani et al. (2015)*	36	$1.39x10^6$	$2.87x10^8$	$1.47x10^2$	$3.89 x10^5$
Finlay et al. (1988)	6	$8.70x10^6$	$2.10x10^7$	$5.00x10^4$	$1.80 x10^5$
Güde (1986)	7	$4.10x10^6$	$9.40x10^6$	$2.30x10^3$	$7.20x10^3$
Güde (1988)	9	$3.80x10^6$	$9.95x10^6$	$1.40x10^2$	$7.67x10^3$
Jürgens and Güde (1991)	19	$4.10x10^6$	$1.24x10^7$	$1.40x10^3$	$2.50x10^4$
Jürgens and Jeppesen (2000)	10	$4.76x10^6$	$1.56x10^7$	$2.29x10^3$	$1.29x10^4$
Munawar and Weisse (1989)	72	$3.90x10^5$	$3.35x10^6$	$4.40x10^2$	$5.79x10^3$
Nakano et al. (1998)	16	$1.23x10^7$	$4.87x10^7$	$3.06x10^3$	$1.42x10^5$
Pace et al. (1990)	5	$3.10x10^6$	$7.83x10^6$	$4.40x10^2$	$1.05x10^3$
Pick and Caron (1987)	22	$6.90x10^5$	$6.20x10^6$	$4.92x10^2$	$6.65x10^3$
Šimek et al. (1988)	12	$1.98x10^6$	$4.89x10^6$	$9.20 x10^1$	$1.39x10^3$
Šimek et al. (1990)	17	$1.34x10^6$	$3.99x10^6$	$8.60x10^1$	$1.29x10^3$
Šimek et al. (1997)	32	$2.05x10^6$	$4.60x10^6$	$1.35x10^3$	$4.45x10^3$
Sommaruga (1995)	36	$1.70x10^6$	$2.03x10^7$	$1.14x10^3$	$2.97x10^4$
Vaqué and Pace (1992)	64	$2.90x10^6$	$8.76x10^6$	$1.69x10^2$	$1.92x10^3$
Weisse (1990)	24	$5.69x10^5$	$6.56x10^6$	$5.40x10^2$	$8.11x10^3$
Weisse (1991)	103	$4.21x10^5$	$7.99x10^6$	$3.14x10^2$	$7.97x10^3$
Wieltschnig et al. (2001)	31	$2.91x10^6$	$6.66x10^6$	$5.59x10^2$	$2.34x10^3$
total	**743**				

* Data from those references were considered outliers and were not used in our analysis

Data analysis

HB and HNF abundance and relationship

To test whether HB and HNF abundances differ among tropical and temperate freshwater environments, we used non-parametric Mann–Whitney Rank Sum test. To examine the relationship between HNF and HB on tropical and temperate datasets, we performed model II linear regression using the major axis (MA) method, comparing the slopes and intercepts for both regions using the "ma" function of the "smatr" package, based on confidence intervals comparison. In addition, we also performed non-parametric Mann–Whitney Rank Sum test to compare median values of HB:HNF ratios between tropical and temperate environments.

Bacterial specific growth rate (SGR) relationship with chlorophyll-a and bacterial abundance in the tropics

We used a dataset comprehending several tropical environments sampled in different seasons (Lobão et al., In prep.) to verify if bacterial SGR was more related to resources or predators. We performed a model II linear regression (MA) to test the relationship of SGR vs chlorophyll-*a* and SGR vs bacterial abundance. A positive correlation between SGR and chlorophyll-*a* (as a surrogate of resource availability) would be an indirect evidence that bottom-up control of bacteria is important in the tropics. We also tested the relationship between SGR and bacterial abundance, which could also give us some enlightenment on the factors controlling their abundance. The rationale is that, considering the density-dependent logistic growth of bacteria, SGR is low when bacterial abundance is reaching the carrying capacity, meaning that they are limited by resource availability. Hence, a negative relationship between SGR and abundance indicates bottom-up control. Conversely, SGR is high when bacterial abundance is far from reaching the carrying capacity. Thus, the lack of relationship between SGR and abundance indicates top-down control, so that predators could be consuming bacteria at rates equal to or higher than their production (Wright and Coffin 1984; Gasol et al. 2002).

Impact of other communities on HB and HNF abundance in the tropics

We examined the effects of potential predators and resources on bacteria and heterotrophic nanoflagellates in the tropical region. We considered the abundances of HB and HNF as response variables separately, and performed multiple regressions for each one. For HB, we used the abundance of the predators HNF, ciliates, rotifers and cladocerans as explanatory variables, excluding copepods, which have a very low capture efficiency of picoplankton (Wilson, 1973; Finlay and Roff, 2004; Sommer and Sommer, 2006). For HNF, we used the abundance of the predators known to exploit them as food, such as ciliates, rotifers, cladocerans and copepods.

Data was log-transformed and all analyses were performed in R (R Development Core Team 2013) using the libraries "vegan" (Oksanen et al., 2015), "lmodel2" (Legendre, 2014) and "smatr" (Warton et al., 2012). Figures were made on SigmaPlot v.12 software (Systat Softare Inc.).

2.3 RESULTS

At first, we considered all data we gathered from the literature in our analyses. However, some of the studies performed in highly eutrophic environments have found

extreme values of HB and HNF abundance, never before reported on the literature (i. e. Fermani et al., 2013 and Fermani et al., 2014). Therefore, since we noticed that those studies were outliers and were given us biased results, we decided to disregard those values from all our analyses. In this way, we maintained a similar distribution of points among trophic state classes in the temperate (oligotrophic:16%, mesotrophic: 50%, eutrophic: 34%) and tropical (oligotrophic:19%, mesotrophic: 45%, eutrophic: 36%) regions. Nevertheless, we show them in the regression figure (Fig. 3) for comparison purposes.

HB and HNF abundance

Comparing HB and HNF abundance in tropical and temperate freshwater environments, we found higher values in the temperate region for both HB (logHB: $p<0.001$, Mann–Whitney U Statistic=218964.5; Fig. 1A) and HNF (logHNF: $p<0.001$, Mann–Whitney U Statistic=189579; Fig. 1B) communities.

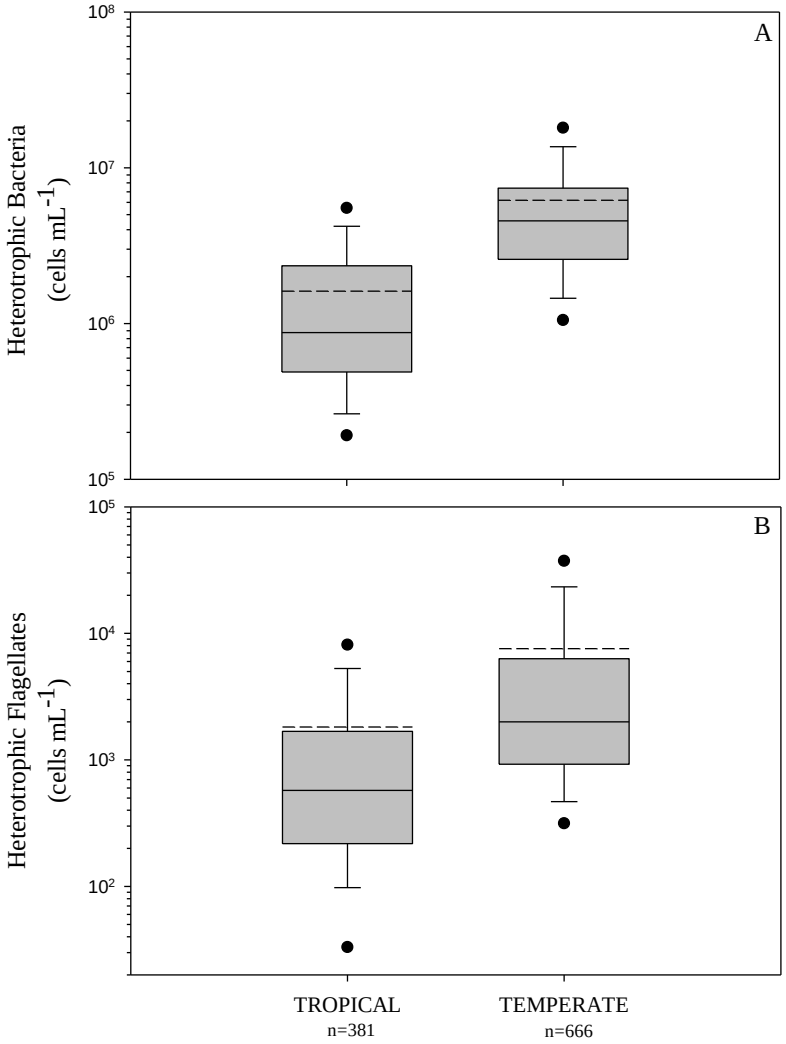

Fig. 1 Comparison of heterotrophic bacteria (A) and heterotrophic flagellates (B) among tropical and temperate freshwater environments. The central full line indicates the median value, the dotted line indicates the arithmetic mean value, the boxes indicate the lower and upper quartiles, the vertical lines indicate the 10th and 90th percentiles, and the dots represent the 5th and 95th percentiles. Tropical and temperate data were significantly different (non-parametric Mann-Whitney Rank Sum test) in the two variables (HB and HNF with $p < 0.001$, see text for details).

HB-HNF relationship

HB:HNF ratios were not significantly different between tropical and temperate environments (HB:HNF: p=0.3049, Mann–Whitney U Statistic=131703; Fig. 2).

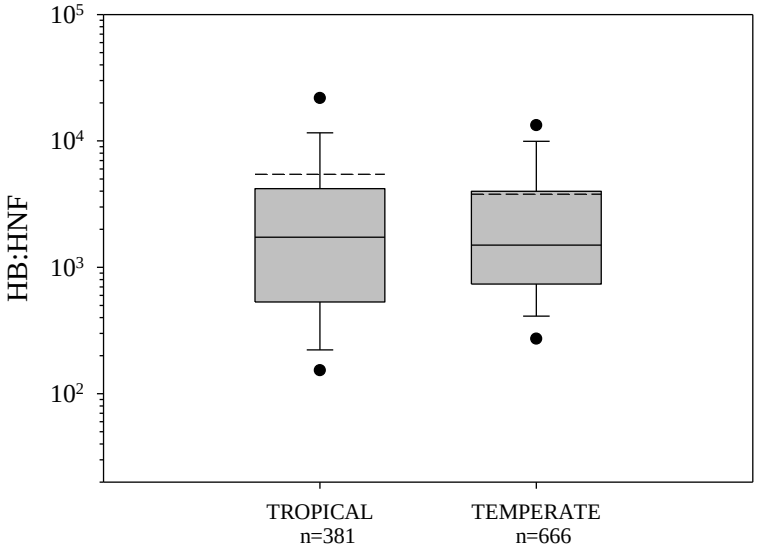

Fig. 2 Comparison of HB:HNF ratios among tropical and temperate freshwater environments. The central full line indicates the median value, the dotted line indicates the arithmetic mean value, the boxes indicate the lower and upper quartiles, the vertical lines indicate the 10th and 90th percentiles, and the dots represent the 5th and 95th percentiles. HB:HNF ratios were not significantly different between tropical and temperate environments (see text).

We found a significant positive relationship between HNF and HB for tropical and temperate regions. Comparing the regression models from both regions, we found no significant differences between the slopes, besides no differences in the confidence intervals for the intercepts (Table 2; Fig. 3).

Table 2. Model II Linear Regression parameters between HNF and HB for tropical and temperate regions.

LogHNF vs. LogHB	**Slope**	**95% (c. i.)**	**Intercept**	**95% (c. i.)**	**n**	**r^2**	**p**
Tropical	2.49	(1.98 : 3.29)	-12.12	(-16.92 : -9.04)	381	0.14	<0.0001
Temperate	2.48	(2.22 : 2.81)	-13.13	(-15.28 : -11.38)	666	0.3	<0.0001

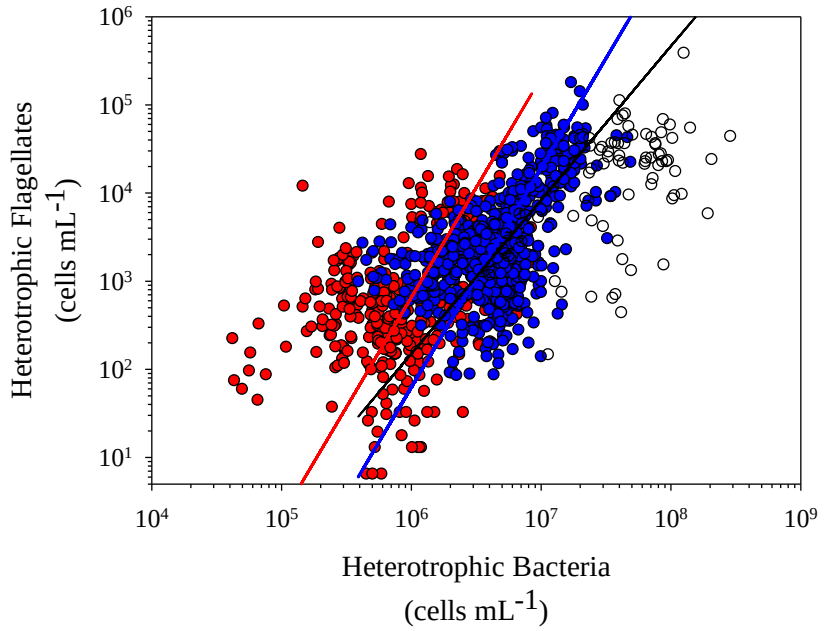

Fig. 3 Model II linear regressions between HNF and HB for tropical (red dots and red line; r^2=0.14;p<0.0001) and temperate (blue dots and blue line; r^2=0.30;p<0.0001) freshwater environments (see Table 2 for confidence intervals). Outliers disregarded from our analyses (blank dots and black line) are also shown (see Materials and Methods section).

Factors controlling HB and HNF abundances

The relationship between bacterial specific growth rates (SGR) and chlorophyll-*a* (Fig. 4A) and SGR and bacterial abundance (Fig. 4B) were both non-significant, pointing to a regulation of bacterial numbers by predation.

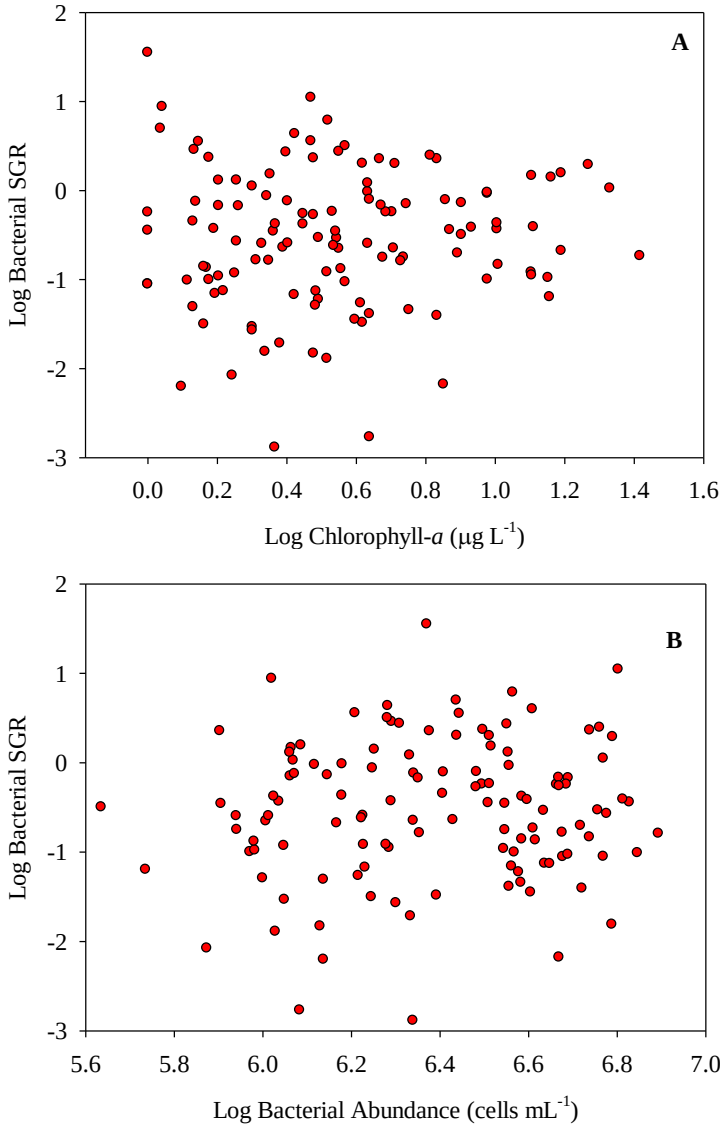

Fig. 4 Scatterplot of the relationship between bacterial SGR and chlorophyll-*a* (A) and bacterial SGR and bacterial abundance (B) for tropical freshwater environments. Model II linear regressions were non-significant.

We performed multiple regressions to evaluate the effects of potential predators on HB abundance. The regression model explained 28% of the variation in HB abundance of the tropical data and included the abundances of HNF, ciliates, rotifers and cladocerans (Table 3). The standardized regression coefficients of both HNF and rotifers were positive, thus an increase in HNF and rotifer abundance was associated with an increase in HB abundance, suggesting a bottom-up effect. As for the ciliates and cladocerans, we found a negative relationship, indicating a top down effect, since an increase in ciliate and cladoceran abundance was associated with a reduction in bacterial abundance.

The best multiple regression model for HNF abundance included only ciliates and cladocerans and explained 32% of the HNF abundance variation. The standard regression coefficient of ciliates was positive, indicating a simultaneously increase in both variables. As for the cladocerans, we found a negative relationship, indicating a top down effect of this group on HNF (Table 3).

Table 3. Regression statistics for HB and HNF abundance of the tropical region. β=standard regression coefficient; HB= heterotrophic bacteria; HNF=heterotrophic nanoflagellates; Cili=ciliates; Rot=rotifers; Clad=cladocerans; Cop=copepods. Bold values are the negative β values.

	Models	r^2	β (±SE)				
			HNF	Cili	Rot	Clad	Cop
HB	HNF, Cili, Rot, Clad	0.28	0.55 (±0.05)	**-0.36** (±0.05)	0.31 (±0.05)	**-0.13** (±0.06)	
HNF	Cili, Clad	0.32		0.60 (±0.05)		**-0.17** (±0.06)	

2.4 DISCUSSION

We compiled for the first time a consistent HNF and HB abundance database for tropical freshwaters, and compared the abundances of those communities with the ones from the temperate environments, as well as explored probable causes why bacterial abundance seem to be lower in the tropics. We found that both HNF and HB abundances are lower in the tropics and that there is no difference in the HNF-HB coupling between those regions. Besides, bacteria are apparently more regulated by predation, especially from ciliates and cladocerans.

No evidence of resource limitation

The lack of relationship between SGR and chlorophyll-*a* in our dataset (Brazilian lakes and reservoirs), suggests that bacterial growth dependence on phytoplankton derived dissolved organic carbon (DOC) supply might not always be that relevant in low latitudes. Accordingly, although there is evidence that phytoplankton derived DOC would be important for the bacterioplankton of large African tropical lakes (Stenuite et al., 2009; Morana et al., 2014), low bacteria:phytoplankton biomass ratios have been found (Sarmento et al., 2008). In a comparative analysis using different types of Brazilian freshwater ecosystems, Roland et al., (2010) found a much weaker bacteria:chlorophyll-*a* correlation in tropical when compared to the non-tropical environments. In this way, the bacteria-phytoplankton uncoupling seems to be a recurrent situation in south-American lowland lakes (e.g. Carvalho et al., 2003; Gocke et al., 2004; Rejas et al., 2005; Petrucio et al., 2006; Teixeira et al., 2011; Almeida et al., 2015), which are smaller and shallower, comparing to the east-African great lakes. White et al., (1991) also reported a rather weak correlation between SGR and chlorophyll-*a* in freshwaters, and suggested that variations in the importance of grazing pressure may have contributed to this finding.

However, as allochthonous DOC may also constitute a significant food source for bacteria (Tranvik, 1992), a regulation of bacteria by those carbon sources could also explain the weak dependency of bacteria on phytoplankton. Unfortunately, we do not have data concerning those variables, which would allow us to elucidate this point. Nonetheless, our results of a non-significant relationship between bacterial SGR and HB abundance reinforce the idea that predation might be more relevant than resource limitation, whatever that resource could be. If HB abundance and SGR were not related, grazing is likely consuming HB at such a rate that it is limited by a small range of possible growth rates (Wright and Coffin, 1984; Gasol et al., 2002). Thus, we could infer that resource limitation is not likely to restrain HB abundance in tropical freshwater environments, and that a top-down control might prevail in these systems.

HNF abundance is also lower in tropical environments

The assumption of a higher abundance of HNF in tropical, relative to temperate environments, was not corroborated in our study. Although large-bodied cladocerans are relatively low abundant in the tropics, the typical small bodied cladocerans, seem to exert a strong predation pressure on HNF, as evidenced by the negative standard coefficient multiple regression model (Table 3).

The impact of small-bodied cladocerans on HNF is somewhat unexpected, since in the tropics there is usually a smaller proportion of Daphniidae, which is replaced by Bosminids, Sidids and Moinids (Dumont, 1994; Elmoor-Loureiro, 2000). However, the influence of cladocerans on the abundance of HNF was already verified in the bottom layer a tropical floodplain lake where those predators were more abundant, specially represented by *Bosmina hagmanni* and *Ceriodaphnia cornuta* (Segovia et al., 2014). In fact, the small-bodied cladocerans *Bosmina*, *Ceriodaphnia* and *Diaphanosoma* were found to achieve higher weight-specific clearance rates on HNF than that of *Daphnia* species (Jürgens et al., 1996). Specifically, *Bosmina* have a particular foraging mode, different from filter-feeding, which allows certain selectivity and consequently more efficient removal of small flagellates compared to *Daphnia* (DeMott and Kerfoot 1982), even at low food concentrations (DeMott, 1982). Thus, even though Daphnids are recognized as the main responsible for hampering the development of HNF in temperate ecosystems (Pace and Vaqué, 1994; Gasol et al., 1995; Jürgens and Stolpe, 1995), their low abundance in the tropics would not result in a weaker predation pressure of cladocerans on HNF, since other small-bodied cladocerans such as the Bosminids may be their "equivalents", in the sense that they would also be able to suppress HNF effectively. As for the ciliates, we found a positive relationship with HNF, indicating that both variables are increasing. It is possible that this could be the result of the control of both HNF and ciliates by variables related to their shared resources and predators (Auer et al., 2004; Segovia et al., 2014).

HNF-HB coupling in the tropics do not seem to differ from that of the temperate regions. A top-down control by cladocerans on HNF may be keeping them from reaching the high abundances they presumably would have in the tropics, blurring their effects on bacteria (Gasol and Vaqué, 1993; Gasol, 1994; Wieltschnig et a., 2001; Segovia et al., 2014; Kalinowska et al., 2015). Another possible cause for the lack of HNF-HB coupling is the use of an alternative food resource by the HNF, such as the picophytoplankton (PPP). Herbivory preference by nanoflagellates, rather than bacterivory, was verified in the large tropical Lake Tanganyika (Tarbe et al., 2011). The preference of HNF for PPP was also found in shallow floodplain lakes in the tropical region (Meira et al., In prep). To sum up, the lower HNF abundance found, together with the similar HNF-HB coupling, suggests that HNF is probably not related to the lower HB abundance in the tropics.

Grazing by ciliates and cladocerans may explain the lower HB abundance in the tropics

The variables associated with HB abundance in the tropics were HNF, ciliates, rotifers and cladocerans. HNF and rotifers were positively related with HB abundance, which means that they are likely feeding on bacteria but are not able to suppress their abundance. On the contrary, both ciliates and cladocerans showed a negative relationship, suggesting a top-down control on bacterial abundance. As stated before, resource limitation or predation by HNF are unlikely to be the reason why bacterial abundance is lower in the tropics. Thus, the negative effect of both ciliates and cladocerans could be part of the explanation for such a pattern.

Although there is a vast literature relating the prevalence of HNF as the major bacterivores (Berninger et al., 1991; Fenchel, 1982; Sanders et al., 1992; Sanders et al., 1989), the relatively higher importance of ciliates as predators of bacteria was also documented. The dominance of ciliates as grazers of bacteria has been reported in occasions where HNF abundance is rather low (Kisand and Zingel, 2000; Tadonleké et al., 2005; Zingel et al., 2007). Also, ciliate community structure in the tropics may differ from that of the temperate regions. It is known that bacterivory is predominant among the small oligotrich ciliates (Stabell, 1996; Šimek et al., 2000), thus perhaps features such as ciliate community composition might be playing a role on the impact of ciliates in tropical environments, where there could be a larger proportion of those bacterivorous taxa. However more studies are necessary to draw such a conclusion. Another overlooked aspect would be the influence of temperature on the ciliate feeding rates. It has been shown that ciliate feeding rates increase considerably with the raise of temperature (Sherr et al., 1988; Rychert, 2011). Therefore, it is plausible to infer that the higher temperatures of the tropics may be a relevant factor.

Similarly to the impact on HNF, weight-specific filtering rates on bacteria were found to be higher for *Ceriodaphnia* and *Bosmina* than for the large *Daphnia magna* (Porter et al., 1983). Vaqué and Pace (1992) found that lakes dominated by large populations of *Bosmina longirostris* showed even slightly higher maximum values of grazing on bacteria (35×10^6 bacteria L^{-1} h^{-1}) than *Daphnia pulex* (30×10^6 bacteria L^{-1} h^{-1}), and concluded that, when in large numbers, populations of small cladocerans compensate for the lack of large Daphnids. Thus, if tropical environments are dominated by those small-bodied cladocerans, then the impact on bacterioplankton could be higher than in temperate environments. In addition, a positive relationship has been found between cladoceran filtering rate and temperature (Burns, 1969). For example, Mourelatos and Lacroix (1990) found that at a temperature of 20ºC, a *Daphnia* of 0.5 mm size filtered as much as one twice its size but at a 10ºC temperature, suggesting that at higher temperatures those small-bodied cladocerans should have an even greater impact. Moreover, a recent study found that pelagic cladocerans significantly explained the variation in bacterial community composition in tropical South American shallow lakes (Souffreau et al., 2015), demonstrating that the predation pressure of those microcrustaceans might also be responsible for changes in bacterial diversity.

Thus, ciliates and small cladocerans seem to have a central role in the pelagic food webs of tropical freshwater environments, and the fundamental differences in the food web structure of freshwater environments in temperate and tropical environments, together with the higher temperatures of the tropical ones, likely dictate the fate of bacterial production (Fig. 5).

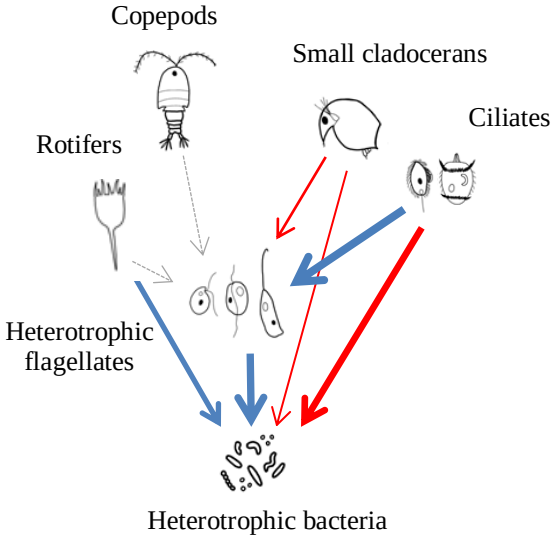

Fig. 5 Schematic representation showing possible impacts of predators and resources on HB and HNF in tropical regions. Dashed arrows indicate no relationship, blue arrows indicate positive and red arrows indicate negative relationships. The thickness of the arrows is proportionally to the strength of the interaction.

It is worth noting that virus lysis is also recognized as a major source of bacterial losses (Fuhrman and Noble, 1995), however few studies concerning this topic were performed in the tropics. Low virus-to-bacterium ratios and frequency of visible infected cells were found in Amazonian floodplain lakes (Barros et al., 2010; Almeida et al., 2015) and African lakes (Bettarel et al., 2006). Barros et al. (2010) suggested that these low values could be related to the registered low bacterial abundances, which restrain the rates of encounter between the virus and the bacterial host cell, resulting in a low level of viral predation. As a corollary for this explanation, the comparable lower abundances of bacteria in the tropics should result in lower loss rates by viral attack than in the temperate systems. Nonetheless, relatively high values of virus-to-bacterium ratios were found in tropical reservoirs (Peduzzi and Schiemer, 2004) and in a tropical lake (Araújo and Godinho, 2009). Thus, bacterial mortality caused by virus should be taken into account when studying mechanisms controlling bacterial abundance in tropical freshwaters in the future to elucidate this issue.

2.5 CONCLUSION

Comparing tropical against temperate data reinforced the previous findings that bacterial abundance is lower in the tropics. Moreover, bacterial specific growth rate was not related to either chlorophyll-*a* and bacterial abundance, pointing to an important role of bacterial losses in the tropics. Besides, we found that HNF abundance is also lower in the tropics and that HNF-HB coupling is not different across latitudes. A top-down control on HNF and their herbivory preference may help explain the lack of HNF-HB coupling, and suggests that HNF is likely not the main cause for bacterial loss. It is possible that grazing by ciliates and cladocerans play a large role in controlling bacterial abundance in the warmer regions.

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3 CONTROLE *TOP-DOWN* EM BACTÉRIAS HETEROTRÓFICAS POR DIFERENTES COMUNIDADES DE PREDADORES NA REGIÃO NEOTROPICAL

RESUMO

Baixas abundâncias bacterianas registradas em ambientes tropicais de água doce, quando comparadas aos ambientes temperados, levantaram a questão de um possível maior controle *top-down* em sistemas trópicais. Porém, esse controle não seria exercido pelos tradicionais predadores bacterianos, os flagelados heterotróficos, que também possuem uma menor abundância na região tropical. Assim, nosso objetivo foi investigar qual predador seria o responsável pela maior perda bacteriana na região tropical. Nós realizamos um experimento de predação separando diferentes frações de tamanho do zooplâncton, a fim de verificar os efeitos de predação de diferentes grupos de predadores sobre as bactérias totais e os grupos HNA (*high-nucleic acid*) e LNA (*low-nucleic acid*). Nossos resultados indicaram que as perdas bacterianas por predação são cruciais para o controle das abundâncias bacterianas em ambientes tropicais de água doce, e que o protozooplâncton, principalmente ciliados, foram os principais responsáveis pela maior parte dessa perda. Embora o controle exercido pelos cladóceros não tenha sido tão efetivo quanto o dos protistas sobre a abundância bacteriana, seu impacto de predação sugere uma rota mais eficiente para o carbono nas teias alimentares planctônicas, sem passar por tantos níveis intermediários. Não só a abundância, mas também a estrutura da comunidade bacteriana foi afetada pela predação, com uma mudança nas proporções relativas de células HNA e LNA em função de diferentes graus de pressão de predação.

Palavras-chave: Citometria de fluxo; Seletividade; Teia alimentar microbiana; Taxa de remoção.

ABSTRACT

Lower bacterial abundances registered in tropical freshwater environments, when compared to the temperate systems, raised the issue of a possible stronger top-down control in the tropical systems. However, this top-down control would not be exerted by the traditional bacterial predators, the heterotrophic flagellates, which also have lower abundances in the tropics. Thus, our aim was to establish which predator would be responsible for most bacterial loss in a tropical lake. We performed a predation experiment with different zooplankton size-fractions to verify the predation effects of the different predator groups on total bacteria and on HNA (high-nucleic acid) and LNA (low-nucleic acid) bacteria. Our results indicate that bacterial loss by predation really is a crucial factor controlling bacterial abundances in tropical

freshwater environments, and that protozooplankton (mainly ciliates) were likely the main responsible for most of this loss. Although the control exerted by the cladocerans on bacterial abundances were not as effective as that of protists, its predation impact suggests a more efficient carbon route through the planktonic food webs, not involving so many intermediate levels. Not only the abundance, but also the bacterial community structure was probably affected by predation, with a change in the relative proportion of HNA and LNA cells as a function of different degrees of predation pressure.

Keywords: Clearance rates; Flow cytometry; Microbial food web; Selectivity.

3.1 INTRODUÇÃO

Baixas abundâncias bacterianas têm sido frequentemente reportadas em ecossistemas aquáticos continentais tropicais (Barros et al. 2010; Roland et al. 2010; Sarmento 2012), apesar da produção bacteriana ser relativamente maior do que aquela encontrada em ecossistemas temperados (Amado et al. 2013). Um estudo compilando uma grande base de dados de ambientes tropicais de água doce sugeriu que as taxas de perdas bacterianas por predação seriam mais importantes nos trópicos (em vez de limitação de recursos), o que poderia explicar as menores abundâncias encontradas, quando comparadas aos ambientes temperados (Segovia et al. In prep.). Também foi proposto que, em vez de flagelados heterotróficos (HNF), os maiores predadores bacterianos nas águas mais quentes seriam os ciliados e pequenos cladóceros (Segovia et al. In prep.).

Contudo, o conhecimento que temos hoje sobre os mecanismos controladores da abundância bacteriana em ambientes aquáticos vem principalmente de estudos realizados em regiões temperadas. Nessas regiões, os protistas são tradicionalmente considerados os maiores responsáveis pelas perdas bacterianas por predação, especialmente os HNF (Berninger et al. 1991; Fenchel 1982; Sanders et al. 1992; Sanders et al. 1989). Os ciliados podem ser importantes estruturadores da comunidade bacteriana quando a abundância de HNF é muito baixa (Kisand & Zingel 2000; Zingel et al. 2007). Por sua vez, vários experimentos abordando a influência dos microcrustáceos sugeriram um efeito *top-down* insignificante na abundância bacteriana (Pace & Funk 1991; Pace & Vaqué 1994; Adrian et al. 2001; Riccardi 2002; Agasild & Nõges 2005; Zingel et al. 2007), incluindo estudos que mostraram um consumo relativamente alto da biomassa bacteriana pelo mesozooplâncton, mas uma regulação ineficiente (Pedrós-Alió & Brock 1983; Kim et al. 2000).

Porém, quando encontrados em grande abundância, grandes cladóceros podem exercer um impacto significativo em bactérias pelágicas (Pace et al. 1990; Vaqué & Pace 1992; Jeppesen et al. 1996; Cottingham 1997; Wickham 1998; Hwang & Heath 1999; Langenheder & Jürgens 2001). Por outro lado, evidências consistentes mostram que os copépodes têm efeitos mínimos nas comunidades bacterianas (Burns & Schallenberg 1996; Hwang & Heath 1999; Kim et al. 2000). Rotíferos também podem se alimentar de bactérias, mas geralmente em taxas muito pequenas e, portanto, não devem ser capazes de afetar as abundâncias bacterianas (Sanders et al. 1989; Pace et al. 1990; Arndt 1993; Vadstein et al. 1993).

Vale a pena lembrar que, além da predação, as abundâncias bacterianas também estão sujeitas aos efeitos indiretos de cascatas tróficas exercidos pelo zooplâncton (Jürgens et al. 1994; Kalinowska et al. 2015), que podem resultar em um impacto positivo no número de bactérias, uma vez que o consumo de protistas pelo zooplâncton livram as bactérias da pressão de predação por esses organismos. Outro efeito positivo seria o crescimento compensatório, no qual as abundâncias bacterianas aumentariam em resposta à liberação de nutrientes pelo zooplâncton (i. e. excreção e defecação), ou à liberação do carbono resultante da predação do zooplâncton sobre as algas (*sloppy feeding*; Güde 1988; Peduzzi & Herndl 1992; Reche et al. 1997).

Modificações na estrutura de tamanho das bactérias também são frequentemente descritas como uma resposta ao aumento da pressão de predação, já que células bacterianas maiores e mais ativas são geralmente selecionadas pelos predadores (Andersson et al. 1986; Gonzalez et al. 1990; Langenheder & Jürgens 2001; Corno et al. 2008). Por essa razão, a abundância bacteriana também pode permanecer inalterada em face da predação, porque as células bacterianas que são menos predadas podem ser capazes de crescer e compensar as perdas de bactérias edíveis, simplesmente realocando sua biomassa (Jürgens & Güde 1994; Pernthaler et al. 1996), assim como as estratégias de defesa utilizadas pelo fitoplâncton (Sommer 2008).

Desde os primeiros estudos ecológicos usando a citometria de fluxo para rápida contagem bacteriana, dois grupos citométricos foram identificados (Li et al. 1995, Marie et al. 1997): HNA (*high nucleic acid*) and LNA (*low nucleic acid*), e que são persistentes independente do ambiente de estudo (Bouvier et al. 2007). HNA eram inicialmente considerados como o grupo bacteriano mais dinâmico e ativo, enquanto que as LNA eram consideradas como inativas ou células mortas (Gasol et al. 1999; Lebaron et al. 2001; Lebaron et al. 2002), apesar de outros estudos demonstrarem que as células LNA também seriam capazes de crescer (Zubkov et al. 2001, Jochem et al. 2004; Longnecker et al. 2005; Williams

et al. 2008; Wang et al. 2009; Huete-Stauffer & Morán 2012). Recentemente, Vila-Costa et al. (2012), utilizou pirosequenciamento para analisar ambas as frações, e descobriu que a maioria dos taxa estão na verdade relacionados a apenas um dos grupos com pouca sobreposição, sugerindo que na realidade essas frações possuem diferentes composições.

As bactérias HNA são geralmente correlacionadas com a clorofila-*a* e o carbono derivado do fitoplâncton (Li et al. 1995; Bouvier et al. 2007; Morán et al. 2007; Sarmento et al. 2008; Morán et al. 2011), demonstrando que esse grupo está sob forte controle *bottom-up*. Concomitantemente, devido ao maior tamanho e maior crescimento das células HNA relativo às LNA, predadores geralmente exibem uma preferência por esses componentes (Gasol et al. 1999; Vaqué et al. 2001; Tadonléké et al. 2005; Garzio et al. 2013; Sintes & Del Giorgio 2014; Baltar et al. 2015). Portanto, a importância relativa de mecanismos de controle *bottom-up* e *top-down* influencia principalmente essa fração da comunidade bacteriana.

Nosso objetivo foi verificar, através de uma abordagem experimental, qual fração de tamanho da comunidade zooplanctônica é responsável pela maior perda bacteriana por predação na região tropical. Nós esperamos que a fração de tamanho contendo tanto ciliados quanto pequenos cladóceros contribua mais para o impacto total da predação. Ainda, esperamos que as frações LNA e HNA sejam diferentemente influenciadas pela predação, uma vez que as HNA seriam provavelmente mais afetadas devido ao maior tamanho.

3.2 MATERIAIS E MÉTODOS

A coleta de água para o experimento foi realizada na superfície da lagoa das Garças (22º43'27.18"S; 53º13'4.56"W), localizada na planície de inundação do alto rio Paraná. Essa lagoa é rasa (profundidade média: 2,0m), com uma área de 14,1 ha e conectada ao rio por um canal estreito. A zona litorânea é composta por várias espécies de macrófitas aquáticas. No dia da amostragem, a temperatura da água era de 26,3ºC, o pH de 6,32, a turbidez 25,4 (NTU) e o oxigênio dissolvido 6,32 mg/L. A profundidade total era de 1,2 metros e a profundidade do Secchi 0,55 metros. A água foi coletada em garrafas de polietileno pretas de 20 litros, transportadas para o laboratório no escuro.

Experimento em laboratório

O experimento de predação foi realizado em laboratório por 24 horas, com temperaturas *in situ* (26ºC). Nós rodamos o experimento em condições baixas de luz para evitar o crescimento excessivo e competição pelo fitoplâncton (Calbet & Landry 1999). Para testar diferenças na bacterivoria atribuída às diferentes frações de tamanho do zooplâncton, parte da água coletada foi filtrada e fracionada por tamanho através de malhas com diferentes

tamanhos de abertura de poro. Nós designamos três diferentes tratamentos de predação, com: i) água não filtrada, contendo todos os bacterívoros (microcrustáceos + rotíferos + ciliados + flagelados); ii) água filtrada através de uma malha de 100 µm (rotíferos + ciliados + flagelados), excluindo os microcrustáceos e iii) água filtrada através de uma malha de 45µm, composta principalmente por protistas (ciliados + flagelados). Essas frações de tamanho serão denominadas como: Zoo Total (água não filtrada: Microcrustáceos + Microzoo), Microzoo (<100 µm: rotíferos + Protozoo) e Protozoo (<45µm: protistas ciliados e flagelados) ao longo do texto (Fig. 1). Embora o microzooplâncton seja geralmente considerado como os organismos na faixa de tamanho 20-200 µm (Sieburth et al., 1978), aqui nós consideramos todos os organismos com tamanho menor que 100 µm. Para o tratamento controle, nós filtramos água através de filtros de fibra de vidro GF/C (Whatman), que retém partículas maiores que 1,2 µm, para que apenas as bactérias pudessem ser capazes de crescer, sem a interferência de predadores (filtros GF/C possuem uma baixa eficiência de retenção de bactérias; Gasol & Morán 1999).

Garrafas de polietileno de um litro foram cheias com 800 mL de água, com um total de 12 réplicas para cada tratamento e o controle. Nós homogeneizamos gentilmente as garrafas a cada duas horas e amostramos água de cada tratamento para a análise bacteriana no começo (0h) e no fim do experimento (24h). Amostras de água adicionais foram tomadas em 12 horas para acompanhar a abundância bacteriana durante o experimento. As amostras foram imediatamente fixadas com formol tamponado com borato (1% concentração final) e armazenadas em nitrogênio líquido até a contagem. Amostras de água para estimar a abundância de predadores foram tomadas no início e no fim do experimento. Amostras fixadas com formol tamponado com borato (4% concentração final), lugol e tiossulfato foram usadas para a contagem de ciliados e zooplâncton. Amostras de flagelados foram fixadas com glutaraldeído (2% concentração final).

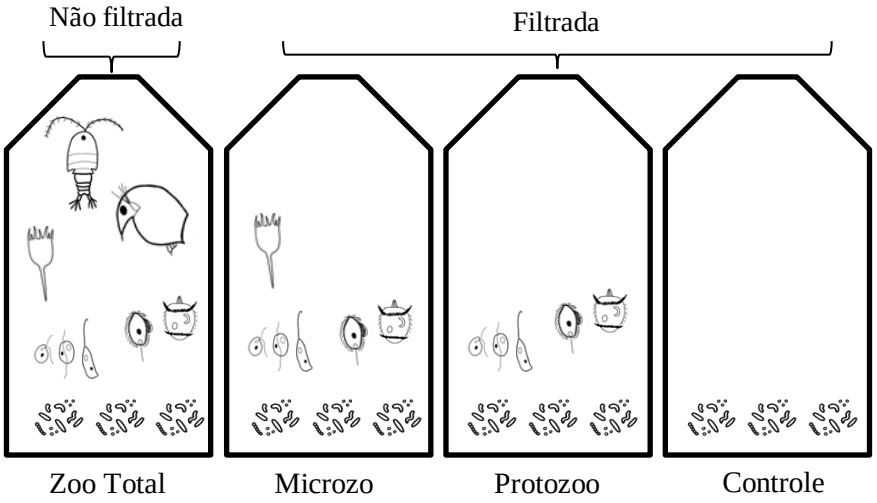

Figura. 1 Esquema da montagem do experimento mostrando as diferentes frações de tamanho e o controle. No tratamento Zoo Total, microcrustáceos, rotíferos, protistas e bactérias estavam presentes. No tratamento Microzoo, os microcrustáceos foram removidos e apenas os rotíferos, protistas e bactérias estavam presentes. O tratamento Protozoo foi composto principalmente por protistas e bactérias. Finalmente, o tratamento controle não continha predadores.

Contagem de bactérias e predadores

Nós estimamos a abundância bacteriana em um citômetro de fluxo FACSCalibur, corando 200µl de amostra com SYTO-13 (Molecular Probes; 2.5 µmol L^{-1} concentração final) no escuro. Nós detectamos as bactérias plotando o *side scatter* (SSC) contra FL1 (fluorescência verde), e identificamos dois grupos de bactérias: LNA (*low-nucleic acid*) e HNA (*high-nucleic acid*), de acordo com Gasol & Del Giorgio (2000). Os dados foram processados com o programa FlowJo. As amostras de HNF foram filtradas através de um filtro de policarbonato preto de 0,8µm, coradas com DAPI (Porter & Feig 1980), e a abundância estimada em um microscópio de epifluorescência (Olympus BX51). Ciliados foram contados sob um microscópio invertido (Olympus CK40) e identificados no menor nível taxonômico possível (Foissner & Berger 1996; Foissner et al. 1999). O zooplâncton foi contado sob um microscópio óptico (Olympus CX31) e identificados em nível de espécie (Koste 1978; Reid 1985; Elmoor-Loureiro 1997).

Análise de dados

As taxas líquidas de crescimento bacteriano (*net growth rates*: NGR) foram calculadas para as bactérias totais, HNA e LNA de acordo com a seguinte equação, assumindo um crescimento exponencial: $\mu = (\ln N_t - \ln N_0)/t$, onde *t* é o tempo de incubação, N_t é a abundância bacteriana após 24 horas, N_0 é a abundância bacteriana no começo do experimento (0h). Nós usamos análises de variância unifatoriais (*one-way* ANOVAs) para testar diferenças nas NGR nos tratamentos de predação e controle e usamos um teste de

Tukey para comparar as médias. Os efeitos de predação sobre a NGR foram calculados considerando a remoção sucessiva dos predadores nos tratamentos, da seguinte forma:

$$Efeito_{microcrustáceos} = NGR_{ZooTotal} - NGR_{Microzoo}$$

$$Efeito_{rotíferos} = NGR_{Microzoo} - NGR_{Protozoo}$$

$$Efeito_{protistas} = NGR_{Protozoo} - NGR_{Controle}$$

Nós também calculamos a razão HNA/LNA da abundância bacteriana nas condições iniciais (0h) e em cada tratamento ao fim do experimento (24h), e testamos as diferenças utilizando ANOVA unifatorial. Diferenças nas abundâncias de predadores também foram testadas através de análises de variância. As análises estatísticas foram realizadas utilizando os pacotes "multcomp" (Hothorn et al. 2008) e vegan (Oksanen et al. 2015) no *software* R 3.1.3 (R Core Team 2013).

3.3 RESULTADOS

Abundância bacteriana e taxas de crescimento líquidas

As bactérias totais apresentaram uma maior abundância nos tratamentos controle através do tempo, onde todos os predadores foram excluídos, enquanto que a abundância em todos os tratamentos de predação foi quase uma ordem de magnitude menor (Fig. 2).

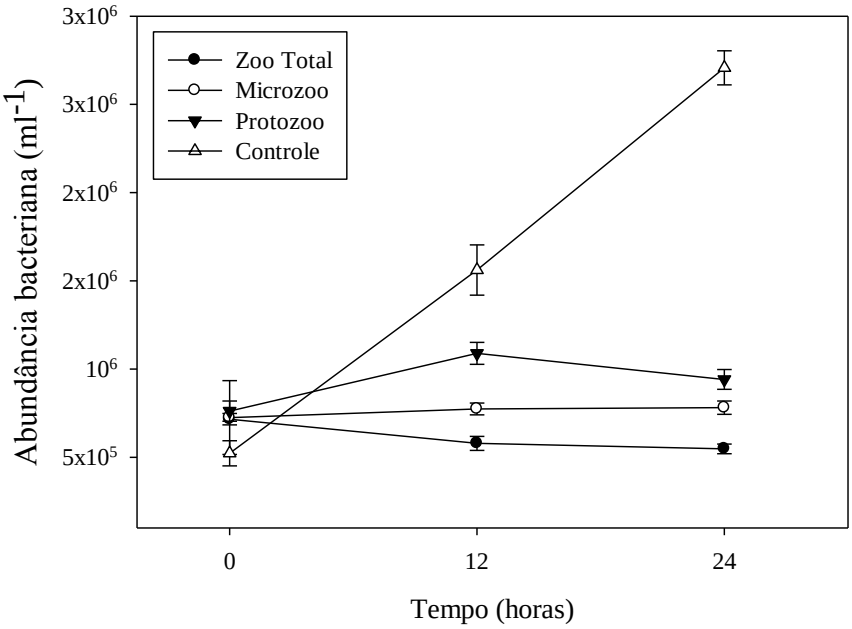

Figura 2. Abundância de bactérias totais nos três tratamentos de predação e nos tratamentos controle através do tempo. O ponto representa o valor médio e a barra representa o erro padrão.

Por conseguinte, as taxas líquidas de crescimento bacterianas (NGR) calculadas foram significativamente maiores no controle do que em todos os tratamentos de predação. A

comparação par-a-par dos tratamentos de predação mostrou que os tratamentos Zoo Total–Microzoo e Microzoo-Protozoo foram similares entre eles, enquanto que os tratamentos Zoo Total–Protozoo foram significativamente diferentes (Fig. 3; Tabela S1). Os valores de NGR observados nos tratamentos contendo todos os predadores (Zoo Total) foram os únicos a apresentarem valores negativos, enquanto que nos tratamentos filtrados (Microzoo e Protozoo) os NGR permaneceram positivos, mas mostraram baixos valores. Os tratamentos controle mostraram valores positivos e expressivos de NGR, oito vezes maiores do que nos tratamentos de predação (Fig. 3A).

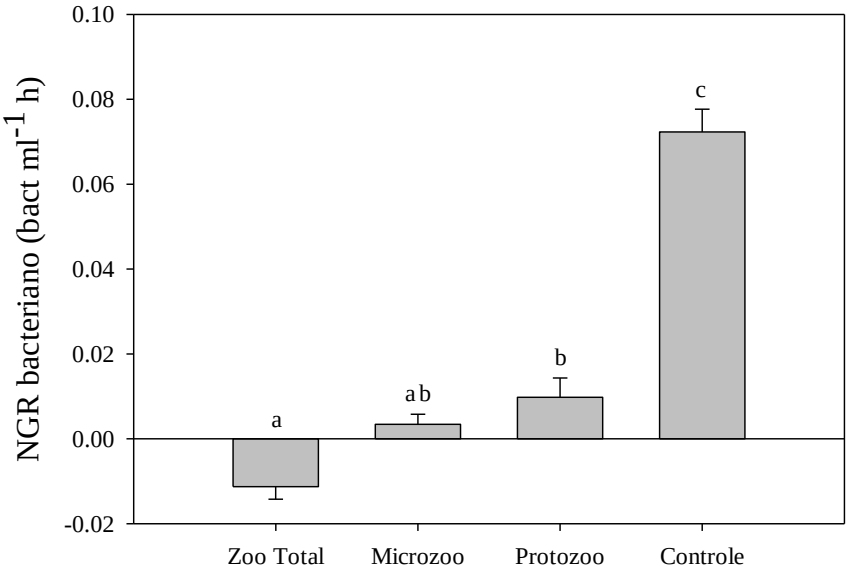

Figura 3. Valores médios das taxas líquidas de crescimento (NGR) bacterianas nos três tratamentos de predação e no tratamento controle. As barras representam o erro padrão. As letras nas colunas indicam a significância estatística- tratamentos que não compartilham uma letra diferem significativamente $P<0.05$ (Tukey's HSD).

Os efeitos de predação mostram que os protistas foram responsáveis pelo maior impacto de predação nas comunidades bacterianas, com um efeito seis vezes maior que o dos microcrustáceos. Os rotíferos apresentaram um efeito ínfimo (Fig. 4).

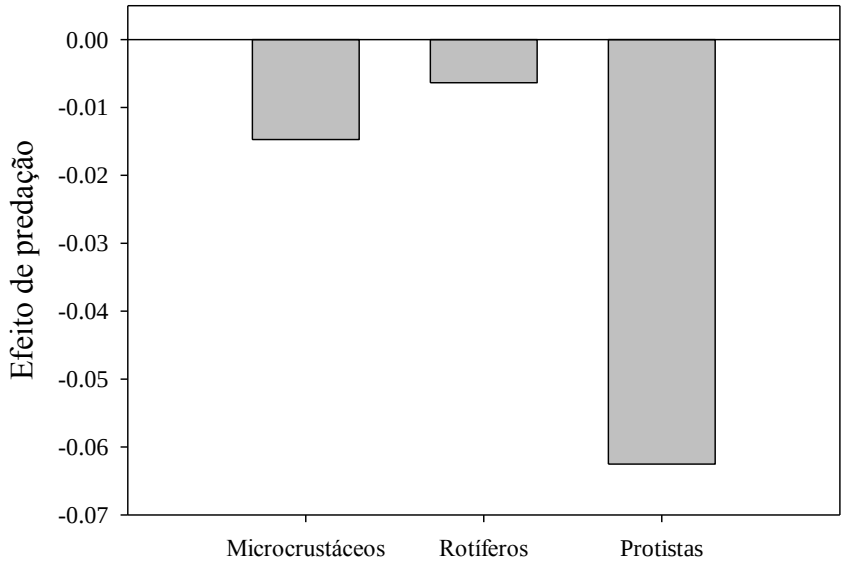

Figura 4. Efeito de predação sobre as taxas liquidas de crescimento bacteriano pelos três grupos de predadores.

Grupos HNA e LNA

Tanto as bactérias HNA quanto as LNA mostraram os mesmos padrões observados para as bactérias totais, entretanto a abundância das HNA (Fig. 5A) foi maior que a abundância observada para as LNA nos tratamentos controle através do tempo (Fig. 5B).

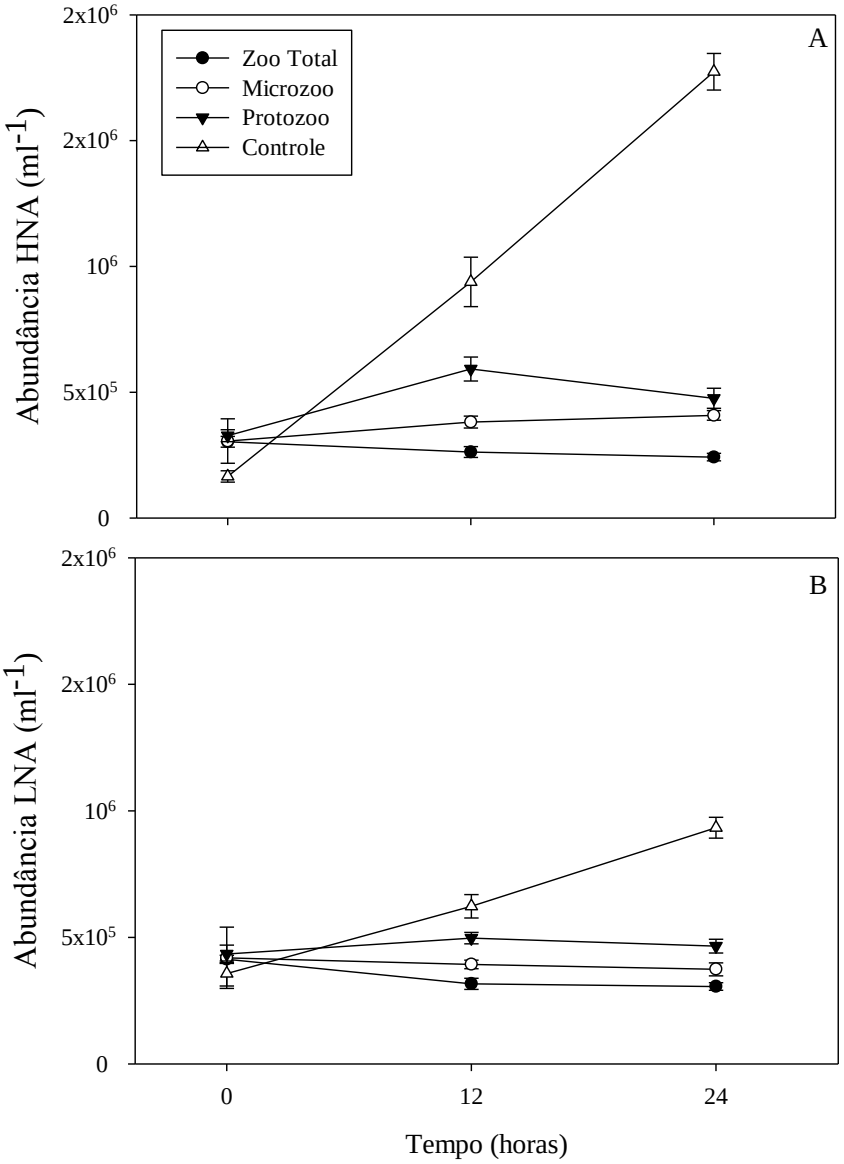

Figura 5. Abundância das bactérias HNA (A) e LNA (B) nos três tratamentos de predação e nos tratamentos controle através do tempo. O ponto representa o valor médio e a barra representa o erro padrão.

Nós também observamos nos citogramas que as bactérias nas condições iniciais (Fig. 6A) e nos tratamentos de predação (Fig. 6B) apresentaram uma maior proporção do grupo LNA. Um aumento no grupo HNA, que se tornou mais abundante que as LNA, foi evidente no tratamento controle, mas não nos tratamentos com predadores (Fig. 6C).

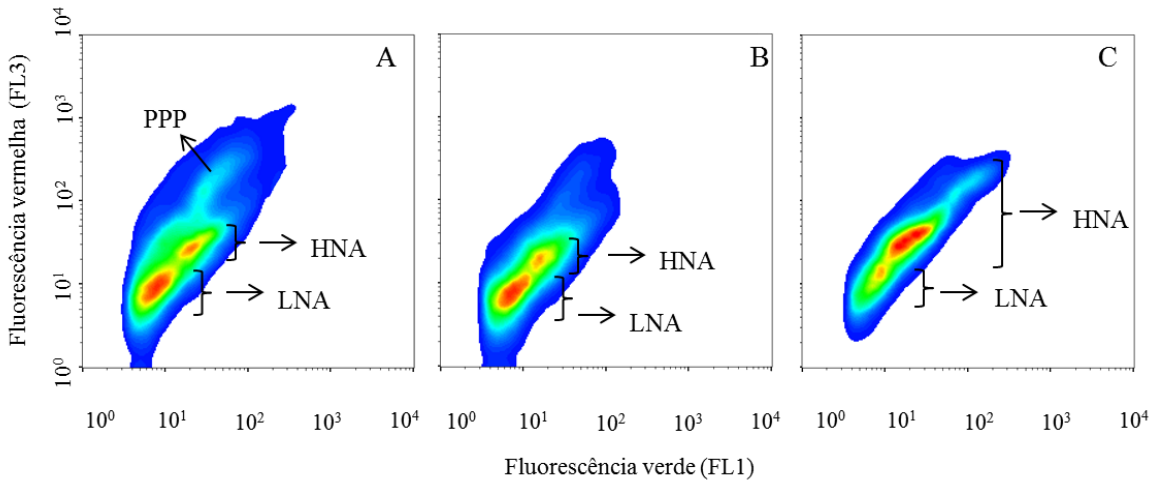

Figura 6. Exemplos de citogramas de bactérias coradas com SYTO-13 de amostras de água das condições iniciais (A), um tratamento de predação (B) e um controle (C), obtidos por citometria de fluxo. Identificação dos grupos LNA e HNA. Em todos os tratamentos controle, foi possível observar uma segunda população de HNA após 12h. PPP = picofitoplâncton.

As taxas líquidas de crescimento bacterianas tanto de HNA quanto de LNA foram significativamente maiores nos tratamentos controle. Para HNA, a NGR no tratamento Zoo Total foi negativo e significativamente menor do que a dos outros tratamentos de predação, enquanto que os tratamentos de predação fracionados por tamanho não diferiram entre eles. Para as LNA, valores negativos de NGR foram encontrados tanto no tratamento Zoo Total quanto no Microzoo, que foram similares entre si, enquanto que a NGR no Protozoo foi positiva mas não diferente do tratamento Microzoo (Fig. 7; Tabela S2).

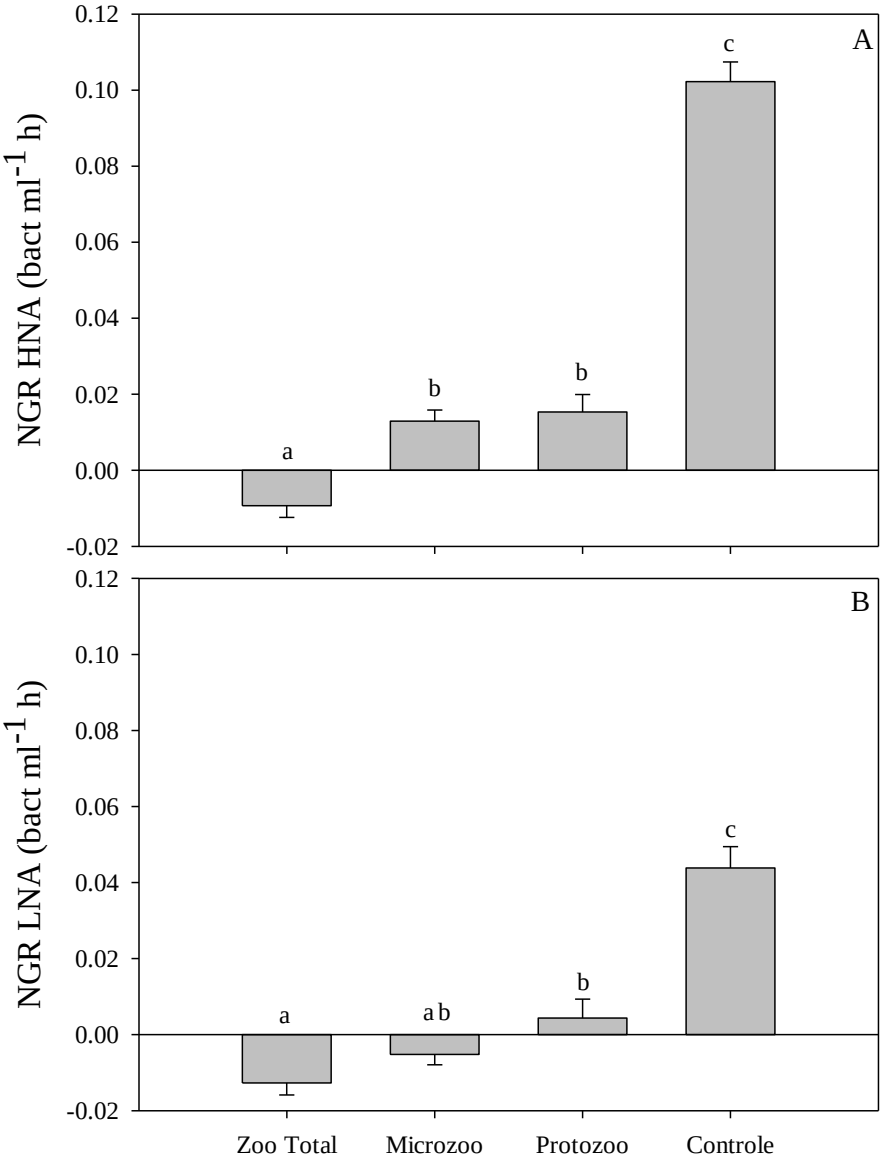

Figura 7. Valores médios das taxas líquidas de crescimento (NGR) de HNA (A) e LNA (B) nos três tratamentos de predação e no tratamento controle. As barras representam o erro padrão. As letras nas colunas indicam a significância estatística- tratamentos que não compartilham uma letra diferem significativamente $P<0.05$ (Tukey's HSD).

De fato, nós encontramos diferenças significativas na razão HNA/LNA entre os tratamentos ao fim do experimento. Testes de Tukey revelaram que os tratamentos contendo todos os predadores em 24h permaneceram similares às condições iniciais. Além disso, sua razão HNA/LNA foi menor que a dos tratamentos Microzoo e Protozoo, que por sua vez foram similares entre eles. A razão HNA/LNA foi significativamente maior que no tratamento controle (Fig. 8; Tabela S3).

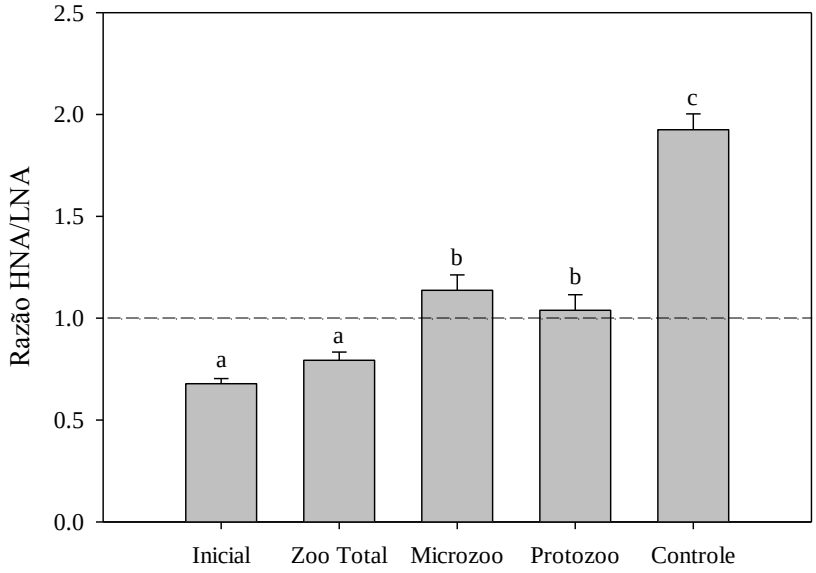

Figura 8. Razão HNA/LNA media da abundância bacteriana em condições iniciais e ao fim do experimento (24h) nos tratamentos de predação e no controle. As barras representam o erro padrão. As letras nas colunas indicam a significância estatística- tratamentos que não compartilham uma letra diferem significativamente P<0.05 (Tukey's HSD).

Abundância de predadores

A abundância de flagelados heterotróficos (HNF) não diferiu entre os tratamentos ao fim do experimento. A abundância de ciliados foi significativamente maior nos tratamentos sem microcrustáceos (Microzoo e Protozoo; Fig. 9; Tabela S4).

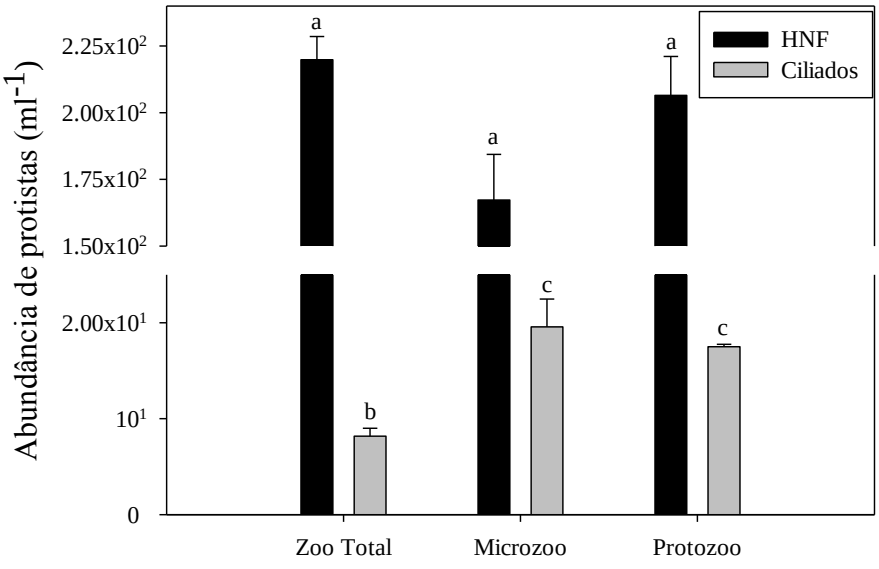

Figura 9. Valores médios da abundância de protistas nos três tratamentos de predação em 24h. As barras representam o erro padrão. As letras nas colunas indicam a significância estatística- tratamentos que não compartilham uma letra diferem significativamente P<0.05 (Tukey's HSD).

As abundâncias dos grupos zooplanctônicos variaram marcadamente entre os tratamentos. Nós intencionalmente removemos os microcrustáceos nos tratamentos filtrados, de forma que eles estavam presentes somente no tratamento Zoo Total. Rotíferos estavam presentes nos tratamentos Zoo Total e Microzoo, mas suas abundâncias foram significativamente maiores nos tratamentos Microzoo (Fig. 10; Tabela S4).

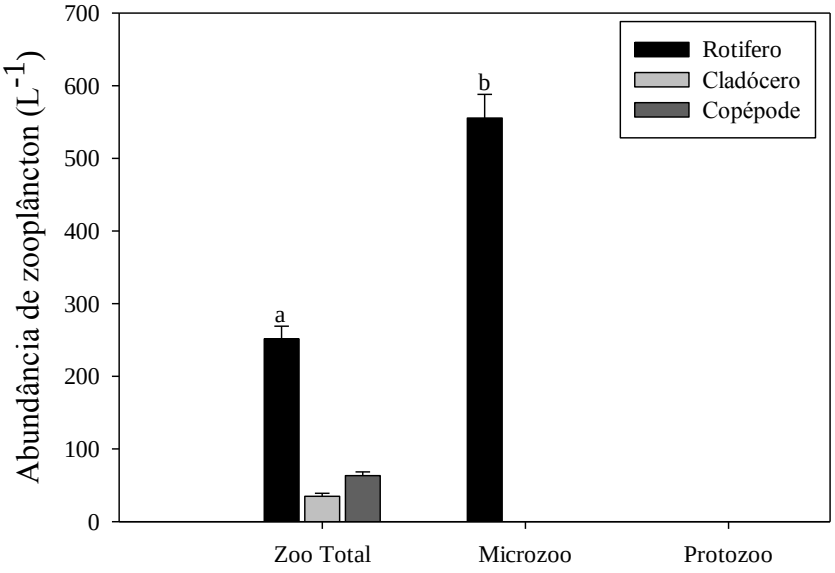

Figure 10. Valores médios da abundância de zooplâncton nos três tratamentos de predação em 24h. As barras representam o erro padrão. As letras nas colunas indicam a significância estatística- tratamentos que não compartilham uma letra diferem significativamente P<0.05 (Tukey's HSD). Microcrustáceos estavam presentes somente nos tratamentos Zoo Total, e os rotíferos presentes em Zoo Total e Microzoo (veja a seção Materiais e Métodos).

3.4 DISCUSSÃO

As 12 réplicas utilizadas para cada tratamento foram extremamente robustas, o que confere consistência aos resultados encontrados nesse experimento. Nós observamos que as taxas de crescimento bacteriano foram significativamente maiores nos tratamentos sem predadores, muito menores nos tratamentos Microzoo e Protozoo e negativas somente no tratamento contendo todos os predadores. Seguindo o mesmo raciocínio usado por Langenheder & Jürgens (2001), considerando que o tratamento controle pode representar a capacidade de suporte do sistema, logo o consumo em todos os tratamentos manteve as abundâncias bacterianas abaixo da abundância máxima que elas poderiam alcançar. Especialmente no tratamento contendo todos os predadores, que são similares às condições ambientais da lagoa, a abundância bacteriana pareceu sofrer um forte controle *top-down* (i. e. taxas de crescimento negativas, Fig. 3).

Comparando as abundâncias bacterianas em ambientes de água doce de regiões tropicais e temperadas encontramos abundâncias bacterianas muito menores nos trópicos, o que sugere que as perdas bacterianas por predação seriam a provável causa para esse padrão (Segovia et al. In prep.). De fato, a maior produção bacteriana encontrada em ecossistemas de regiões tropicais (Amado et al. 2013), e que parecem não se traduzir em maiores abundâncias (Roland et al. 2010; Sarmento 2012), sugerem que as perdas bacterianas estão provavelmente restringindo o seu desenvolvimento. Dessa maneira, nossos resultados acrescentam evidências para a ideia de que a predação é um importante fator que mantém as bactérias em baixas abundâncias em ambientes tropicais de água doce. Ademais, esses resultados indicam que a biomassa bacteriana pode ser uma fonte de carbono efetiva para a teia alimentar, como postulado por Azam et al. (1983).

Calculando o efeito de predação, foi possível observar que o maior impacto sobre a comunidade bacteriana foi exercido pelos protistas. Entre eles, os HNF são conhecidos por serem os maiores predadores bacterianos das águas mais frias (Berninger et al. 1991; Fenchel 1982; Sanders et al. 1989; Sanders et al. 1992). Porém, encontramos uma abundância muito baixa desses organismos em todos os tratamentos de predação (máx~$2x10^2$ cels HNF ml^{-1}), e, de fato, menores abundâncias de HNF são encontradas em ambientes tropicais, enquanto que em ambientes temperados as abundâncias registradas chegam a ser uma ou duas ordens de magnitude maiores. Estudos demonstraram que os ciliados podem exercer os maiores impactos sobre a abundância bacteriana quando a densidade de HNF é relativamente baixa (Kisand & Zingel 2000; Zingel et al. 2007). Experimentos similares ao nosso, realizados em um reservatório, mostraram uma maior contribuição de scuticuciliados na bacterivoria total (*Cyclidium glaucoma*; Tadonleké et al. 2005). Porém, pequenos oligotriquideos são reconhecidamente os maiores bacterívoros dentro da comunidade de ciliados (Stabell 1996; Šimek et al. 2000), e em nosso experimento os oligotriquideos dominaram a comunidade (~97% da abundância). Comparando as taxas de remoção de bactérias de HNF já reportadas na literatura (5-31 bact HNF^{-1} h^{-1} em Šimek et al. 2000 e 4-15,4 bact HNF^{-1} h^{-1} em Unrein et al., 2007) com as taxas de remoção de ciliados oligotriquideos (62 bact $cili^{-1}$ h^{-1} em Kisand & Zingel 2000 e até 1782-3220 bact $cili^{-1}$ h^{-1} em Šimek et al. 2000), vemos que os ciliados podem exceder em muito o impacto sobre as bactérias, quando comparados aos HNF. Diante das taxas de consumo extremamente altas exibidas pelos ciliados, juntamente com as baixas abundâncias registradas para os HNF, sugerimos que os ciliados foram provavelmente os principais responsáveis pelo efeito de predação das bactérias nessa lagoa tropical.

O efeito de predação exercido pelos microcrustáceos é provavelmente o reflexo do consumo de cladóceros sobre as bactérias. Isso porque os copépodes não são predadores eficientes do picoplâncton, mostrando um impacto mínimo sobre a comunidade de bactérias (Burns & Schallenberg 1996; Hwang & Heath 1999; Kim et al. 2000). Por outro lado, os cladóceros são capazes de se alimentar de uma ampla gama de tamanhos de partículas, incluindo protistas e bactérias (Geller & Müller 1981; Knoechel & Holtby 1986). Nosso experimento foi dominado pelas espécies de cladóceros *Bosmina hagmann*i e *Bosminopsis deitersi*, que apresentam pequeno tamanho corporal (~ 0.2-0.3 mm; Maia-Barbosa & Bozelli 2005). Porém, mesmo os pequenos cladóceros são capazes de exercer pressões de predação sobre bactérias comparáveis àquelas exercidas pelos Dafinídeos (Vaqué & Pace 1992). Assim, mesmo considerando que os cladóceros não foram tão efetivos quanto os protistas no controle as bactérias, eles também podem ser considerados importantes predadores bacterianos nesse ambiente tropical. Ademais, a predação direta sobre as bactérias corta o caminho da passagem de carbono e energia, evitando as perdas por ingestão e respiração associadas à passagem por níveis intermediários (Lindeman 1942) do elo microbiano (Ducklow et al. 1986), aumentando assim a eficiência na transferência de carbono através das teias alimentares planctônicas.

Além do impacto direto sobre as bactérias, os microcrustáceos também parecem exercer um impacto direto sobre ciliados e rotíferos, uma vez que maiores abundâncias de ambos os organismos foram encontradas depois da remoção de microcrustáceos dos tratamentos filtrados (Fig. 9; Fig. 10). Esse impacto pode ser resultado de um controle *top-down*, que parece ser exercido principalmente por copépodes sobre rotíferos (Brandl 2005; Miracle et al. 20007) e ciliados (Whickham 1998; Adrian & Schneider-Olt 1999; Burns & Schallenberg 2001). Porém, esse controle não resultou em um efeito de cascata trófica em nossos experimentos, uma vez que o crescimento bacteriano foi na realidade menor nos tratamentos não filtrados do que no tratamento em que os microcrustáceos foram removidos. Em contraste, Jürgens et al. (1994) encontrou que a remoção de microcrustáceos resultou em um aumento tão grande na abundância de protistas que esses foram capazes de ter um impacto maior nas abundâncias bacterianas do que na presença de microcrustáceos, sugerindo um efeito em cascata. Porém, esse experimento teve duração de 120 horas, enquanto que em nosso experimento de curta duração (24h), os ciliados e rotíferos não foram capazes de incrementar sua abundância a ponto de exercer tal impacto sobre as bactérias. Incubações mais curtas são, em geral, mais realistas, de modo que os efeitos diretos e indiretos (cascata trófica) observados no nosso estudo podem ser computados de forma aditiva, com menor impacto de “efeito confinamento” ou “efeito garrafa”.

Nós encontramos um impacto muito baixo exercido pelos rotíferos nos tratamentos de predação. As taxas de predação dos rotíferos são muito baixas quanto comparadas às taxas de crescimento bacteriano, fazendo com que eles não sejam capazes de controlar as abundâncias bacterianas (Arndt 1993), mesmo quando dominam a comunidade zooplanctônica (Sommaruga 1995). Assim, podemos inferir que os rotíferos são provavelmente predadores pouco efetivos da produção bacteriana (Sanders et al. 1989; Pace et al. 1990; Vadstein et al. 1993) também nessa lagoa tropical. De fato, a produção secundária de rotíferos foi altamente correlacionada com a clorofila-*a* em lagoas dessa mesma planície de inundação (Dias et al. submitted), o que também pode indicar uma preferência desses organismos pela herbivoria.

Os valores positivos da NGR encontrados nos tratamentos filtrados (Fig. 3) mostram que a pressão de predação nesses tratamentos não foi suficiente para controlar a abundância bacteriana. Isso porque as células HNA foram capazes de crescer rápido o suficiente para compensar por perdas da predação (Fig. 7A), consequentemente mudando a proporção HNA/LNA (Fig. 8). Nossos resultados diferem dos encontrados por Pernthaler et al. (1996), no qual o crescimento compensatório apenas equilibrou os efeitos da predação, mantendo níveis similares de abundância e biomassa bacteriana. Sommer (2008) postulou que efeitos *top-down* somente afetariam a estrutura de tamanho e não abundância dos organismos, caso a capacidade de suporte permanecesse inalterada. Em vez disso, nossos resultados indicam que as células HNA foram capazes de crescer e exceder as perdas por predação nos tratamentos filtrados. Além disso, observamos que as células LNA também exibiram crescimento (Fig. 7B), assim como reportado por outros estudos (Zubkov et al. 2001, Jochem et al. 2004; Longnecker et al. 2005; Williams et al. 2008; Huete-Stauffer & Morán 2012), porém somente nos tratamentos Protozoo e Controle, demonstrando que o lento crescimento dessa fração só supera as taxas de perda em tratamentos com menor quantidade ou com nenhum predador.

A preferência de protistas por células HNA tem sido observada em vários estudos (Gasol et al. 1999; Vaqué et al. 2001; Tadonléké et al. 2005; Garzio et al. 2013; Sintes & Del Giorgio 2014; Baltar et al. 2015). A explicação para essa seletividade é possivelmente o maior tamanho exibido pelas células HNA (Gasol & Morán 1999; Baltar et al. 2015), característica essa há muito tempo conhecida por ser importante na seletividade de protistas (González et al. 1990). De maneira passiva, os cladóceros também exercem maior impacto sobre células bacterianas maiores, uma vez que possuem maior eficiência de retenção sobre essa fração da comunidade (Güde 1988; Brendelberger 1991; Burns & Schallenberger 1996). Embora as células LNA não sejam selecionadas, suas menores taxas de crescimento impediram seu desenvolvimento, consequentemente inibindo um crescimento compensatório da fração

menos predada, como proposto por Jürgens & Güde (1994). Assim, apesar de sofrer maior predação, maiores taxas de crescimento foram observadas para as células HNA (Fig. 7A), que foram responsáveis pela maior parte do crescimento observado nas bactérias totais (Fig. 3). Não obstante, a maior proporção de células LNA no tratamento contendo todos os predadores demonstra que seu pequeno tamanho evitou a forte predação sobre essa fração, e contribuiu para a sua persistência e dominância, apesar de menor crescimento, em situações de grande pressão de predação.

Desse modo, nós encontramos uma mudança gradual na proporção relativa de LNA e HNA com a remoção de predadores, com uma mudança na dominância de HNA no tratamento controle, sob nenhuma pressão de predação (Fig. 8). Tal inversão na razão HNA/LNA também foi encontrada por Gasol et al. (1999), reportando os resultados de experimentos com e sem predadores. Nossos resultados confirmam que a proporção relativa de HNA/LNA encontrada nos ambientes é uma função de diferentes graus de pressão de predação presentes nos ecossistemas (Tadonleké et al. 2005). Também, sugerem uma modificação na estrutura de tamanho da comunidade bacteriana em face da predação (Andersson et al. 1986; Gonzalez et al. 1990; Langenheder & Jürgens 2001; Corno et al. 2008). Finalmente, considerando que as frações HNA e LNA são compostas por comunidades com distintas OTUs (*Operational Taxonomic Unit*; Vila-Costa et al. 2012), é provável que também a composição bacteriana seja afetada pela predação nessa lagoa tropical.

3.5 CONCLUSÃO

De maneira geral, nossos resultados indicaram que as perdas bacterianas por predação são cruciais para o controle das abundâncias bacterianas em ambientes tropicais de água doce. Além disso, os ciliados seriam os principais responsáveis pela maior parte da perda bacteriana em lagoas da região tropical, como a que sustentou nosso experimento. Assim, a biomassa bacteriana deve ser uma fonte de carbono importante para as teias alimentares microbianas. Embora os cladóceros não tenham exercido um controle tão efetivo quanto o dos protistas sobre a abundância bacteriana, seu impacto de predação sugere uma rota mais eficiente para o carbono, sem passar por níveis intermediários. Ademais, não só as abundâncias bacterianas, mas também a estrutura de tamanho é afetada pela predação, com uma resultante mudança nas proporções relativas de células HNA e LNA em função de diferentes graus de pressão de predação. Considerando que ambas as frações representam taxa distintos, possivelmente a composição também pode ser afetada pela predação.

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APÊNDICE A – Tabelas com resultados da ANOVA

Tabela S1. Resultados da ANOVA unifatorial e teste de Tukey para as taxas líquidas de crescimento bacteriano (NGR) entre os tratamentos de predação (Zoo Total, Microzoo, Protozoo) e o controle. Valores de P em negrito são significativos em P<0.05. df = graus de liberdade, SS = soma dos quadrados, MS= quadrados médios, SE = erro padrão.

ANOVA	**df**	**SS**	**MS**	**F**	**Pr(>F)**
Tratamentos	3	0.0490	0.0163	86	< **0.001**
Resíduos	44	0.0084	0.0002		
Tukey HSD	**SE**	**t**	**Pr(>|t|)**		
Zoo Total-Microzoo	0.0056	2.62	0.0563		
Zoo Total-Protozoo	0.0056	3.75	**0.0029**		
Zoo Total-Controle	0.0056	14.87	< **0.001**		
Microzoo-Protozoo	0.0056	1.13	0.6725		
Microzoo-Controle	0.0056	12.25	< **0.001**		
Protozoo-Controle	0.0056	11.12	< **0.001**		

Tabela S2. Resultados da ANOVA unifatorial e teste de Tukey para as taxas líquidas de crescimento de HNA e LNA entre os tratamentos de predação (Zoo Total, Microzoo, Protozoo) e o controle. Valores de P em negrito são significativos em P<0.05. df = graus de liberdade, SS = soma dos quadrados, MS= quadrados médios, SE = erro padrão.

ANOVA	**df**	**SS**	**MS**	**F**	**Pr(>F)**
HNA					
Tratamentos	3	0.0873	0.0291	147	< **0.001**
Resíduos	44	0.0087	0.0002		
LNA					
Tratamentos	3	0.0228	0.0076	34.26	< **0.001**
Resíduos	44	0.0098	0.0002		
Tukey HSD	**SE**	**t-value**	**Pr(>|t|)**		
HNA					
Zoo Total-Microzoo	0.0057	3.88	**0.0021**		
Zoo Total-Protozoo	0.0057	4.29	< **0.001**		
Zoo Total-Controle	0.0057	19.43	< **0.001**		
Microzoo-Protozoo	0.0057	0.42	0.9755		
Microzoo-Controle	0.0057	15.56	< **0.001**		
Protozoo-Controle	0.0057	15.14	< **0.001**		
LNA					
Zoo Total-Microzoo	0.006	1.24	0.6075		
Zoo Total-Protozoo	0.006	2.81	**0.0356**		
Zoo Total-Controle	0.006	9.30	<**0.001**		
Microzoo-Protozoo	0.006	1.58	0.4029		
Microzoo-Controle	0.006	8.06	<**0.001**		
Protozoo-Controle	0.006	6.49	<**0.001**		

Tabela S3. Resultados da ANOVA unifatorial e teste de Tukey para as razões HNA/LNA entre as condições iniciais, os tratamentos de predação (Zoo Total, Microzoo, Protozoo) e o controle. Valores de P em negrito são significativos em P<0.05. df = graus de liberdade, SS = soma dos quadrados, MS= quadrados médios, SE = erro padrão.

ANOVA	**df**	**SS**	**MS**	**F**	**Pr(>F)**
Tratamentos	4	15.761	3.94	89.84	< **0.001**
Resíduos	91	3.991	0.044		
Tukey HSD	**SE**	**t**	**Pr(>|t|)**		
Zoo Total-Microzoo	0.0855	4.02	**0.0010**		
Zoo Total-Protozoo	0.0855	2.87	**0.0383**		
Zoo Total-Controle	0.0855	13.24	< **0.001**		
Zoo Total-Inicial	0.0676	-1.70	0.4307		
Microzoo-Protozoo	0.0855	-1.15	0.7719		
Microzoo-Controle	0.0855	9.22	< **0.001**		
Microzoo-Inicial	0.0676	-6.79	< **0.001**		
Protozoo-Controle	0.0855	10.37	< **0.001**		
Protozoo-Inicial	0.0676	-5.33	< **0.001**		
Controle-Inicial	0.0676	-18.45	< **0.001**		

Tabela S4. Resultados da ANOVA unifatorial e teste de Tukey para as abundâncias de predadores entre os tratamentos de predação (Zoo Total, Microzoo, Protozoo) no fim do experimento (24h). Valores de P em negrito são significativos em P<0.05. df = graus de liberdade, SS = soma dos quadrados, MS= quadrados médios, SE = erro padrão. Microcrustáceos não são mostrados pois estiveram presents apenas no tratamento Zoo Total.

ANOVA	**df**	**SS**	**MS**	**F**	**Pr(>F)**
FLAG					
Tratamentos	2	4438	2218.8	3.83	0.0848
Resíduos	6	3478	579.7		
CILI					
Tratamentos	2	220.65	110.33	11.89	**0.0082**
Resíduos	6	55.65	9.28		
ROT					
Tratamentos	2	463980	231990	171.6	< **0.001**
Resíduos	6	8113	1352		
Tukey HSD	**SE**	**t-value**	**Pr(>|t|)**		
FLAG					
Zoo Total-Microzoo	19.66	-2.66	0.0829		
Zoo Total-Protozoo	19.66	-0.68	0.7840		
Microzoo-Protozoo	19.66	1.98	0.1968		
CILI					
Zoo Total-Microzoo	2.487	4.58	**0.0091**		
Zoo Total-Protozoo	2.487	3.75	**0.0224**		
Microzoo-Protozoo	2.487	-0.83	0.6991		
ROT					
Zoo Total-Microzoo	30.02	10.13	**<0.001**		
Zoo Total-Protozoo	30.02	-8.37	**<0.001**		
Microzoo-Protozoo	30.02	-18.50	**<0.001**		

4 COMMON AND RARE TAXA OF PLANKTONIC CILIATES: INFLUENCE OF FLOOD EVENTS AND BIOGEOGRAPHIC PATTERNS IN NEOTROPICAL FLOODPLAINS

Summary

After much discussion about the cosmopolitan nature of microbes, the great issue nowadays is to identify at which spatial extent microorganisms may display biogeographic patterns, and if temporal variation is important in altering those patterns. Planktonic ciliates were sampled from shallow lakes of four Neotropical floodplains in the high and low water period, along with several abiotic and biotic variables potentially affecting the ciliate community. We found that common ciliate species were more associated with the environmental gradients and rare species were more related to spatial variables, however, this pattern seemed to change depending on the temporal and spatial scales considered. Environmental gradients were more important in the high waters for both common and rare species. In low waters, common species continued to be mainly governed by the environmental conditions, but rare species were more associated with the spatial component, suggesting dispersal limitation likely because of differences in dispersal ability and ecological tolerance of species. We also found that common and rare species were related to different environmental variables, suggesting different ecological niches. At the largest spatial extents, both common and rare species showed biogeographic patterns.

4.1 INTRODUCTION

The differences in the ecological requirements between species stand out as the basis of the niche theory (Hutchinson, 1957), implying that community composition variation would be dependent on the environmental variation. Thus, under the niche theory, different habitats would favour different species to coexist (Chase and Leibold, 2003). Environmental gradients are indeed recognized as playing a pivotal role in structuring microbial communities in diverse habitats (Kuang *et al.*, 2013; Zhao and Xu, 2013; Ortmann and Ortell, 2014) and across all spatial scales (Pinel-Alloul and Ghadouani, 2007; Souffreau *et al.*, 2015). The importance of the environmental conditions in shaping microbial communities is the basis of Baas Becking's (1934) hypothesis: "Everything is everywhere, but, the environment selects" (EiE; De Wit and Bouvier, 2006).

The rationale behind the EiE would be that due to their small size, high abundance and the ability to encyst or produce spores, microorganisms would have such high dispersal rates that they would not be restricted by geographical barriers (Finlay, 2002; Fenchel and Finlay, 2004). In fact, microbial dispersal limitation seems to be much lower than that of macroorganisms (Beisner *et al.*, 2006; De Bie *et al.*, 2012). Nonetheless, a decrease in the similarity with increasing geographical distance have also been found for microbial communities (Whitaker *et al.*, 2003; Eisenlord *et al.*, 2012; Lepère *et al.*, 2013). These results suggest that biogeographic patterns, as found for macroorganisms, could also hold for microorganisms (Green *et al.*, 2004; Green and Bohannan, 2006; Martiny *et al.*, 2006).

Rather than questioning whether microorganisms display patterns that resemble that of macroorganisms, now we should focus on answering questions such as: at what spatial extent the biogeographical patterns of microbes are comparable to those of macroorganisms? (Green and Bohannan, 2006). When considering multiple spatial scales, it is possible to investigate the relative importance of niche processes (Chase and Myers, 2011; Martiny *et al.*, 2011), taking into account that dispersal limitation becomes more evident at large spatial extents (Heino *et al.*, 2015). Yet, not only spatial, but also temporal scales should be taken into account (Langenheder *et al.*, 2012), especially in ecosystems where environmental conditions are known to change seasonally (Bulit *et al.*, 2009). In floodplains, periodic flood events can be considered as a disturbance, potentially changing the relative importance of deterministic and stochastic processes in community assembly (Chase, 2007). Particularly, floods are expected to homogenize the environments (Thomaz *et al.*, 2007), reducing beta diversity (Bozelli *et al.*, 2015).

Another relevant issue is that different parts of a community appear to be assembled by different mechanisms (Lindström and Langenheder, 2012). Through a deconstructive approach (Marquet *et al.*, 2004), it is possible to disentangle the responses of groups of species with similar traits, such as dispersal ability, dispersal modes, body size or habitat specialization (Pandit *et al.*, 2009; Grönroos *et al.*, 2013; Algarte *et al.*, 2014; Székely and Langenheder, 2014; Kärnä *et al.*, 2015). For instance, dividing the community between common and rare species may better elucidate the main assembly mechanisms and processes accounting for the distribution patterns of species (Magurran and Henderson, 2003; Dolan *et al.*, 2009; but see Siqueira *et al.*, 2012). Chase et al. (2005) suggested that common and rare species should be under different prevailing assembly processes, and similarly to what is found in population genetics, rare species may be more subject to ecological drift (due to a smaller population size), while common species may be more subject to competition. In a metacommunity perspective, rare species should be more related to spatial processes, while common species are more likely to fit the environmental processes perspectives (Chase et al., 2005).

Thus, the four main perspectives of the metacommunity framework (Leibold *et al.*, 2004) can be used to enlighten the main processes structuring common and rare species in a community. In the species-sorting paradigm, species occurrence is subject to biotic interactions and environmental filtering (Chase and Leibold, 2003), implying enough dispersal for those species to be able to track the variation of the environmental conditions (Soininen *et al.*, 2014). Alternatively, the neutral perspective assumes no ecological difference in trophically similar species, such that dispersal, speciation and extinction are the main drivers of community structure (Hubbel, 2001). The two other perspectives, mass effects and patch dynamics, could be considered as special cases of species sorting (Heino *et al.*, 2015). In this way, Winegardner *et al.* (2012) encourage a novel view of metacommunities as neutral or governed by the species sorting with i) limiting dispersal (patch dynamics); ii) efficient dispersal (species sorting); or iii) high dispersal (mass effects), because those terms better apprehend the causal mechanisms behind each perspective (Winegardner *et al.*, 2012). This alternative way of approaching metacommunities targets the relative roles of dispersal and environmental signals, since differences in dispersal rates are pivotal in determining the extent to which species sorting influences the metacommunity.

Considering the species sorting perspective, both biotic and abiotic variables are important in determining the community compositions. Although most studies so far consider only abiotic variables in the environmental model, it has been shown that the inclusion of

biotic variables considerably improves the explanatory power of the analyses (Boulangeat *et al.*, 2012; Göthe *et al.*, 2013; Soininen *et al.*, 2013; Souffreau *et al.*, 2015; Viana *et al.*, in press). Because of direct and indirect effects, biotic interactions should be considered in order to better understand the influence of dispersal on the community assembly at various spatial extents (Verreydt *et al.*, 2012; Berga *et al.*, 2015).

Here, we aimed to investigate the temporal variation of the ciliate community structure at different spatial scales. We hypothesize that common species would be mainly related to environmental variables and rare species to spatial variables. We also expected a temporal variation on the contribution of the spatial and environmental components, such that during high water periods, we predicted a negligible importance of spatial variables within floodplains because, at this spatial extent, floods increase the connectivity between the floodplain habitats. When compared to low water periods, we also expected a lower contribution of environmental variables during high water periods, because the increase in connectivity would mask the effects of species-sorting processes (a pattern consistent with the mass effect perspective). Alternatively, this result would derive from the homogenization effects of floods that reduce the range of environmental gradients. During low water periods, lakes are more isolated and environmentally heterogeneous and, therefore, we expected an increase in the importance of both environmental and spatial variables. Among floodplains, dispersal limitation is expected to prevail, regardless of the period (Fig. 1). We further explore the relative contribution of abiotic and biotic factors in common and rare species.

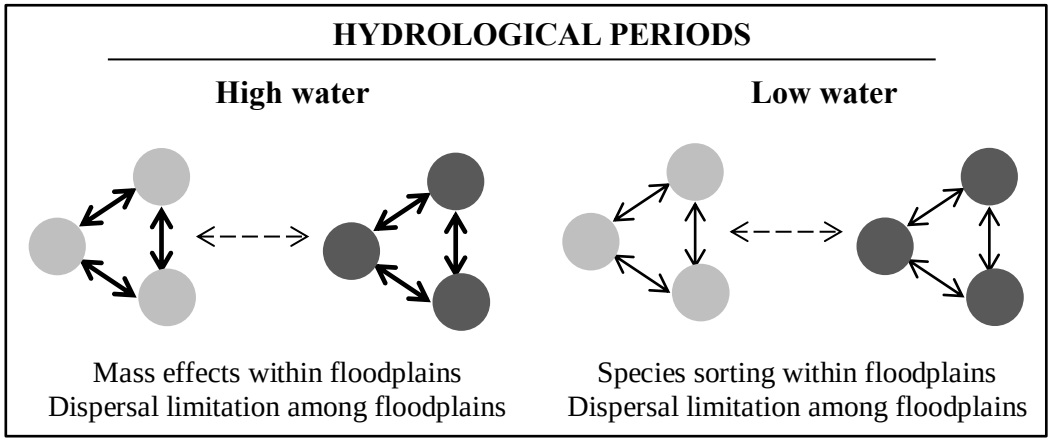

Fig. 1. Illustration of the importance of spatial scales for the ciliate community in each hydrological period. Grey circles represent the lakes within the floodplains, thicker, thin and dashed arrows represent higher, intermediate and limited dispersal, respectively.

4.2 RESULTS

Abiotic and biotic variables

Of the several abiotic variables measured, the most conspicuous variation between the hydrological periods was registered for depth: during the high water period, Amazonian lakes reached more than 15 m, with a mean value of 6 m considering all floodplains; on the other hand, during the low water period, mean values were around 2 meters, with a maximum depth of 7.2 m. Some biotic variables also showed great variation. For example, much higher values of chlorophyll-*a* and zooplankton abundance (rotifers, cladocerans and copepods) were registered during the low water period (Table 1).

Table 1. Mean, minimum (min) and maximum (max) values of abiotic and biotic variables registered in lakes from all floodplains during two hydrological periods.

	HIGH WATER (*n* = 57)			**LOW WATER** (*n* = 55)		
	Mean	Min	Max	Mean	Min	Max
Temperature (ºC)	27.5	18.1	33.3	28.3	22.8	34.4
Dissolved Oxygen (mg/L)	3.2	0.1	7.3	5.9	0.8	11.3
pH	6.9	5.7	9.6	6.7	5.2	9.0
Conductivity (µS/cm)	53.5	18.3	162.1	46.8	6.9	165.8
Inorganic nutrients (µg/L)	43.0	7.7	323.8	69.4	8.7	387.1
Turbidity (NTU)	9.3	1.6	44.9	28.0	0.7	162.0
Depth (m)	6.0	0.8	15.7	2.0	0.3	7.2
Chlorophyll-*a* (µg/L)	6.5	0.2	31.9	20.4	1.4	139.3
Bacteria (ind/ml)	$7.76x10^5$	$1.07x10^5$	$1.97x10^6$	$7.75x10^5$	$4.29x10^4$	$2.40x10^6$
Flagellates (ind/ml)	$2.98x10^2$	$0.31x10^2$	$9.62x10^2$	$2.29x10^2$	$0.18x10^2$	$1.53x10^3$
Rotifers (ind/m^3)	3404	31	38083	34691	44	218134
Cladocerans (ind/ m^3)	8812	1	104654	25661	6	135467
Copepods (ind/ m^3)	259	0	3051	2524	0	26561

Common and rare ciliate species

Out of the 22 species considered as common in the study, most taxa were shared between the two hydrological periods (Fig. S1). Oligotrichous ciliates were by far the most representative, with seven species being considered common. Among them, *Halteria grandinella*, *Tintinnidium* sp. and *Tintinnopsis* sp. showed particularly high abundances in both periods (Table S1). As for the rare species, exclusive taxa in each period slightly exceeded the shared part (Fig. S1).

Variation partitioning

Most of our predictions were supported by the data (Table 2). First, common species were mainly related to abiotic (A) and biotic (B) variables, but not significantly related to the spatial component within floodplains (S). For rare species, at least during the low water period, we detected a significant adjusted coefficient of determination (adj. R^2) associated to the spatial component S (within-floodplains) and non-significant coefficients associated to A or B. During the high water period, rare species were significantly related to B. Second, the prediction of higher adj. R^2 (pure components A+B+S) during the low water period than during the high water period was also supported. For rare species and for the whole community, our prediction of a high importance of S during the low water period, but a negligible importance during the high water period, was also supported. Finally, we found that the spatial component among floodplains (F) was the component with the highest adjusted coefficient of determination independently of the response matrix and hydrological period (Table 2).

Common and rare species were influenced by distinct environmental variables. Abiotic variables were important to explain the variation of common species, in both hydrological periods. Temperature and turbidity were selected for both periods, while depth was included in the abiotic model only in the high water period and both dissolved oxygen and conductivity were exclusive of the low water period. The biotic component was important for rare species during the high water period (copepods abundance) and for common species in the low water period (bacteria and chlorophyll-*a* and rotifers abundance). Total species matrix was always explained by the same variables as the common or rare species, when the same component was concomitantly significant.

Table 2. Variation partitioning of ciliate community data in relation to pure abiotic (A), biotic (B) and spatial variables (S and F) during two hydrological periods. Shown are the adjusted coefficients (*adj.* R^2, %) and *P*-values (*P*) derived from partial Redundancy analyses applied to the whole community and matrices composed by common and rare species. S: spatial variables derived from MEM (see Declerck et al., 2011). F: dummy variable differentiating the floodplains.

	Common species		**Rare species**		**Total species**	
	adj. R^2(%)	P	adj. R^2(%)	P	adj. R^2(%)	P
HIGH WATER						
A|(B+S+F)	**4.6**[a]	**0.001**	*	*	**3.7**[a]	**0.010**
B|(A+S+F)	*	*	**2.4**[b]	**0.004**	**1.5**[b]	**0.025**
S|(A+B+F)	3.3	0.089	0	0.514	3.4	0.076
F|(A+B+S)	**8.7**	**0.001**	**4.5**	**0.001**	**5.2**	**0.010**
Residual	74.5		94.3		77.4	
LOW WATER						
A|(B+S+F)	**4.4**[c]	**0.008**	0.7	0.205	**3.8**[c]	**0.005**
B|(A+S+F)	**3.2**[d]	**0.010**	0	0.459	**2.3**[d]	**0.015**
S|(A+B+F)	3.1	0.067	**2.8**	**0.032**	**3.6**	**0.017**
F|(A+B+S)	**12.5**	**0.001**	**5.0**	**0.001**	**11.2**	**0.005**
Residual	69.9		88.7		73.8	

*no variables of this component were selected in the forward procedure.

[a] abiotic model constructed from the environmental variables temperature, depth and turbidity.

[b] biotic model constructed from the environmental variable copepod.

[c] abiotic model constructed from the environmental variables temperature, dissolved oxygen, turbidity, conductivity.

[d] biotic model constructed from the environmental variables bacteria, chlorophyll-*a* and rotifer.

4.3 DISCUSSION

The patterns of the limnological variables describe a very typical situation found in floodplain lakes: first and more obvious, depth was much higher in the high water period, demonstrating that flood events were really conspicuous within floodplains. The lower dissolved oxygen found in the higher waters is also a common feature of floodplains (Carvalho *et al.*, 2001), as a reflection of the organic matter input and consequent increased decomposition rates (Thomaz *et al.*, 2004). The higher values of nutrients and chlorophyll-*a* found in the low waters can be explained by the nutrients provided by water circulation and destratification in the shallower environments, which in turn supports the development of phytoplankton (Thomaz *et al.*, 2004) and zooplankton (Lansac-Tôha *et al.*, 2004).

Most of the common species were found in both periods, demonstrating the persistent high abundance and wide range of those species over time. On the other hand, the majority of rare species were inherent of each hydrological period. This indicates a more ubiquitous

distribution of a few, more abundant taxa (Doherty *et al.*, 2010), however, our further analyses were able to better explore this issue.

The relative importance of the assembly processes of the planktonic ciliate communities seemed to be dependent on the hydrological period and the spatial extent considered. In the high water period, the spatial component was not significant for any of the ciliate groups (common, rare and total); on the other hand, environmental components (either abiotic or biotic) were always significant. Therefore, we could verify a predominant role of species sorting in structuring the ciliate communities in flood events, when dispersal is enhanced. A similar result was found for the phytoplankton community of the highly connected habitats of "De Maten", where only the environmental component was found to be significant (Vanormelingen *et al.*, 2008; Dias et al., submitted). Indeed, higher dispersal rates can decrease the relevance of stochastic processes such as priority effects (Chase, 2003), in which species arriving first have time to adapt to the habitat conditions decreasing the successful establishment of those species arriving later (Urban and De Meester, 2009). Also, random extinctions, which would affect mainly the rare taxa, are diminished due to recolonizations and rescue effects (Leibold *et al.*, 2004) at situations of increased dispersal. These processes may promote a higher degree of determinism in the face of increased dispersal, which was also found to be true in experimental bacterial metacommunities (Berga *et al.*, 2015).

The fact that we did not find significance in the spatial components in high waters suggests that dispersal rates were not high enough to mask the effects of species sorting (mass effects). Mass effects were not detected in bacteria living in physically connected shallow lakes either, in which species sorting also predominated (Van der Gucht *et al.*, 2007). As the enhanced dispersal was not enough to overcome the effects of species sorting in the ciliate community, it is possible that the flood event actually mitigated some degree of dispersal limitation that the ciliates were suffering in the absence of flood, which prevented perfect species sorting to occur. This seems to be true in the case of rare species, because we could compare the spatial signals temporally, and considering it was not significant in the high waters (higher dispersal rates promoted by the flood: Thomaz *et al.*, 2007), but it was significant in low waters when lakes are more isolated (decreasing the rates of dispersal: Thomaz *et al.*, 2007), we can infer that this suggests dispersal limitation of the rare species within floodplains in the absence of the flood event. This explanation was inspired by Declerck *et al.* (2013), which found that the experimental results of a dispersal treatment in bacterial and viruses communities promoted the association between community composition

and environmental conditions, likely because it alleviated dispersal limitation. Total species also showed the same pattern, however that was probably the reflection of the rare species in the matrix.

Therefore, we showed that the patterns of rare species were influenced by hydrology. Floods also seem to affect the relative importance of environmental and spatial processes for common and rare taxa of benthic invertebrates and zooplankton groups (Dias et al., submitted) in the Upper Parana River floodplain (Petsch *et al.*, 2015). Temporal variations were likewise found to be meaningful for driving dissimilar patterns of rare and abundant protists (Nolte *et al.*, 2010) and bacteria (Kim *et al.*, 2013). Thus, our results were partially in line with our expectations that rare species seem to be mainly driven by ecological drift (Chase et al., 2005), because we found that temporal disturbances (i. e. flood) can alter this pattern. Nevertheless, in the absence of disturbance, this pattern seems to apply to rare ciliates in shallow lakes of floodplains, as well as for marine ciliates (Dolan *et al.*, 2009; Doherty *et al.*, 2010), marine fishes (Magurran and Henderson, 2003), bacteria (Galand *et al.*, 2009) and microbial eukaryotes (Logares *et al.*, 2014).

For common species, however, the environmental component was significant in the high waters and in the low waters as well, indicating that species sorting truly prevailed (Van der Gutch *et al.*, 2007; Soffreau *et al.*, 2015) regardless of the hydrological period, but only at the smallest spatial extent. When considering the largest spatial extent (among-floodplains), we found that common ciliates were influenced by both the environmental and spatial components. Although the concomitant significance of those components could be viewed as the mass effects (Cottenie, 2005), we can infer that it is more likely reflecting a species sorting partially limited by dispersal (Ng et al., 2009). This is because we could compare the spatial signal at two spatial scales, and it was only significant at the largest spatial scale, suggesting a more limiting dispersal with increasing spatial extent (Soininen *et al.*, 2011). Thus, the degree to which the spatial component was important depended on the spatial extent considered (Heino *et al.*, 2015).

In addition, the spatial signal was also always stronger in the largest spatial scale for rare species. Consequently, our results suggest that ciliates show biogeographic patterns in shallow lakes of Neotropical floodplains. Although microorganisms are known to exhibit very high dispersal rates due to small size and high abundance (Finlay, 2002; Fenchel and Finlay, 2004), and the ability to encyst results in a potential dispersal by atmospheric transport over thousands of kilometres (Griffin *et al.*, 2002; Wilkinson *et al.*, 2012), not all microbial species have the same probability to passively disperse. For example, in the atmosphere, most

microorganisms are constrained by many factors such as UV radiation (Smith *et al.*, 2011), so that air microbes are usually formed by the many taxa adapted to have prolonged viability during the transportation (Smith *et al.*, 2013). Size also seems important, since airborne microbes of <20µm were found to have a wider range of reach than the ones with <40µm (Wilkinson *et al.*, 2012). Considering that planktonic ciliates range from ~15µm -200 µm in size (Pace, 1982), only a subset of the species would be easily dispersed at great distances.

Moreover, after the dispersal and arrival of the microorganisms in the habitat, those species need to encounter favourable conditions to reproduce, and are subject to the competition exerted by the indigenous microbes. In this way, Weisse (2008) called *effective dispersal* the "successful establishment of immigrants" as the result of the dispersal itself plus the abiotic and biotic constrains determining the observed distribution of species over space and time. Accordingly, Jones and McMahon (2009) found that taxa coming from the atmosphere had a weak influence on lake bacterial communities, likely because of the imposed environmental filtering of the lakes, suggesting that the role of species sorting was still dominant. This is in line with our findings, which suggests that even though ciliates may have a high *potential dispersal* (sensu Weisse, 2008) that does not seem to be translated in an *effective dispersal* (sensu Weisse, 2008), especially at larger spatial extents.

The divergent patterns found for common and rare species could be explained due to differences in their dispersal ability, with the rare having poorer dispersal ability than the most abundant taxa (Gaston and Kunin, 1997). In other words, dispersal rates tend to decrease from common to rare species (Weisse, 2014). In the case of the ciliates, differences in the rates of dispersal may be mainly attributable to the abundance of the populations (Finlay *et al.*, 2002) such that rare species, with much smaller population sizes, have lower dispersal rates compared to the common ones. Thus, if we expect bad dispersers to be more affected by spatial distance (Thompson and Townsend, 2006; Astorga *et al.*, 2012; Padial *et al.*, 2014), the same should hold true for rare species. As a corollary to the model proposed by Orrock and Watling (2010), in which the role of stochasticity is larger in smaller communities, rare species (as a smaller subset of the community) should also be more subject to neutral dynamics.

Yet, considering common species as being generalists and rare species as specialists (Finlay *et al.*, 2002; Székely and Langenheder, 2014), habitat generalists are usually the ones who thrive in the habitats where they arrive, since they have a broad ecological tolerance (Comte *et al.*, 2014). Thus, strong dispersal limitation is much less likely to occur in the common species, and being stronger dispersers, they should be more subject to environmental

control (Heino, 2013). Accordingly, common species were also more strongly driven by the environmental gradient in bacterial communities from rock pools (Székely and Langenheder, 2014). Thus, likely because of the greater dispersal ability and broad ecological tolerance, common species were less affected by dispersal limitation than the rare species. However, we can say that our results only partially confirm our expectations that common species would be mainly affected by environmental variables, because this pattern seems to change at larger spatial scales.

Another interesting aspect was that common and rare species were differently affected by the abiotic (physical and chemical variables) and biotic factors (resources and predators). Common and rare species were indeed shown to have different ecological niches (Magurran and Henderson, 2003; Lennon *et al.*, 2011; Kim *et al.*, 2013; Liu *et al.*, 2015). We found that rare species were more influenced by biotic interactions in the high waters, namely copepods, which are considered efficient predators of ciliates (Stoecker and Capuzzo, 1990; Burns and Gilbert, 1993; Sommer *et al.*, 2003). The fact that rare species appeared to be more influenced by predation may seem at odds with the view that the rare microbial taxa, by presenting very low abundances, should be protected from loss rates (i. e. viral lysis and predation); however, this view is based on the bacterial community and it assumes that most predators are selective and preferentially prey on active bacteria (Pedrós-Alió, 2006). Dunthorn *et al.* (2014), in their review concerning the ciliate rare biosphere, states that predation is assumed to be important only for the most abundant taxa because of a higher probability of finding them, but opened a parenthesis calling attention to the fact that "…species-specific predation may not be that common in nature". Pedrós-Alió (2006) also affirmed that when predators are not selective, both common and rare species would be equally affected, and that it would not be an advantage to be a rare species in those cases.

Therefore, because ciliate predators such as the copepods are composed of both filter-feeding (non-selective/generalists - calanoids) and raptorial (selective/specialists - cyclopoids) taxa (Gliwicz, 2004), it is more plausible to consider what is usually found for macroorganisms: rare species may be more affected by biotic interactions (Siqueira *et al.*, 2012) such as predation (Chase *et al.*, 2009) than common species, so that they are actually more prone to extinctions (Davies *et al.*, 2004; Sodhi *et al.*, 2009). Indeed, experimental evidence of predation by a generalist copepod on ciliates showed that species with slow growth and low relative abundance were quickly led to extinction, while the fast growth and high abundances of the other species prevented, or at least delayed, the same fate (Limberger and Wickham, 2011). Interestingly, that circumstance was only found when the predator

accompanied the dispersal of the prey, resulting in a negative effect of the connectivity, because rescue effects were overcome by extinctions through predation (Limberger and Wickham, 2011). Thus, as in our study rare taxa were influenced by predators only in the high waters, it is likely that predation on rare ciliates was promoted by a concomitant higher dispersal of both predator (i. e. copepod) and the prey (i. e. rare ciliates) in flood events.

Some biotic factors also seemed important for common species in the low waters, especially resources such as bacteria and chlorophyll-*a*. It is in the low water period, when floodplain lakes are more isolated and shallower, that the phytoplankton community develops and reaches high abundances (Thomaz *et al.*, 2004). Indeed, ciliates were found to have a negative impact on the phytoplankton community in a humic floodplain lake in the low waters (Segovia *et al.*, 2015), demonstrating that ciliates should be efficient grazers of phytoplankton (Sherr and Sherr, 2002; Tillmann, 2004; Yasindi and Taylor, 2006) in floodplains as well. Ciliates are also long been known as sources of mortality for bacteria (Pace, 1982; Šimek *et al.*, 2000; Zingel *et al.*, 2007), although they were found to have a greater impact on the bacterioplankton in a floodplain lake in the high waters (Segovia *et al.*, 2015), contrary to our findings. Rotifers were also found to be important in explaining the variation of the ciliate community, which could mean that they were exploiting the ciliates as food (Arndt, 1993) or rather they were correlated because they share the same resources and predators (Auer *et al.*, 2004).

Nonetheless, abiotic factors prevailed in explaining the variation of common ciliate species, regardless of the hydrological period. Temperature and turbidity were always important. Temperature is a key variable, because it is directly linked to population growth rates (Savage *et al.*, 2004) and also to protist feeding rates (Peters, 1994; Vaqué *et al.*, 1994; Sarmento *et al.*, 2010). In situations of increased turbidity some species seem unaffected while others are negatively affected (Jack and Gilbert, 1993; Boenigk and Novarino, 2004), such that this differential response of some populations results in changes in the abundance and composition of ciliates. Depth was important in the high waters, which is not surprising considering the wide water level disparity among the floodplains (i. e. the depth of a Pantanal lake was 0.8 meters, while some Amazonian lakes reached a depth of more than 15 meters). Exclusively in the low waters, both dissolved oxygen and conductivity were important. Although protists may range from fully aerobics to obligate anaerobes, most of them depend on aerobic metabolism (Fenchel, 2014), and would benefit in circumstances of higher dissolved oxygen availability. Conductivity also seems to affect the composition of ciliates,

since some species seem to prefer medium to high values of conductivity, while other appear not to tolerate very high values of this parameter (Küppers and Claps, 2012).

In conclusion, we can view ciliate metacommunities as constituted by subcommunities of common and rare species which differ in their dispersal ability, ecological niches and, consequently, in their main assembly mechanisms. Our results partially agree with the perspective that common species are more close to be considered cosmopolitan while rare species display restricted distributions (Pither, 2007; Galand *et al.*, 2009; Liu *et al.*, 2015), because temporal and spatial scales may affect those patterns. All in all, microbial biogeography should not really differ from that of macroorganisms, considering that both common and rare taxa showed biogeographic patterns at the largest spatial extent (Green *et al.*, 2004; Martiny *et al.*, 2006).

It is interesting to note that the overall predictive power of our models was not very high (ranging from ~ 6% - 30% of explained community variation). This is unlikely to reflect the lack of important contemporary variables sampled in our study, which incorporated several abiotic and biotic variables known to exert a strong influence on the ciliate community (see Methods). Instead, it seems to be an intrinsically characteristic of the small sized organisms which are usually less predictable than their larger counterparts (Farjalla *et al.*, 2012; Soininen *et al.*, 2013). However, as the majority of studies so far, ours was performed with snapshot samplings, so we suggest that future studies take into account the past environmental conditions, which can better predict microbial spatial differences (Andersson *et al.*, 2014).

4.4 EXPERIMENTAL PROCEDURES

Study Area

We sampled from 15 to 20 lakes in each of the following Neotropical floodplains: Pantanal (18°46´-19°34′S and 56°58´-57°46′W), Paraná (22°40′–22°50′S and 53°10′–53°30′W), Amazon (3°02´-3°34′S and 59°38´-60°50´ W) and Araguaia (12°49´- 13°25´S and 50°28´-50°43´W), in both low and high water periods. Those floodplains are widely distributed in the Brazilian territory, comprehending a wide latitudinal gradient (~ 3,000 km; Fig. 2).

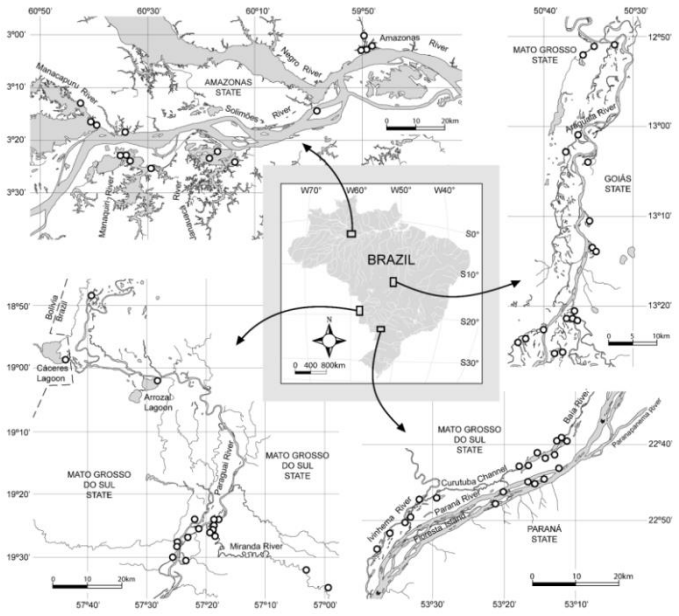

Figure 2. Study area showing all sampling sites within each floodplain.

Sampling and Laboratory Analysis

Environmental variables that are key drivers for the ciliate community were measured, including both abiotic (physical and chemical) and biotic (resources and predators) variables. The following abiotic variables were measured in the field (pelagic zones of the lakes): dissolved oxygen (YSI portable oximeter), temperature (thermometer), water depth (portable depth sounder), pH, conductivity (Digimed portable potentiometers) and turbidity (LaMotte2008© portable turbidimeter). Water for inorganic nutrients (Orthophosphate, nitrate and ammonia) was also sampled and further analysed in the laboratory (Mackerett et al., 1978; Giné et al., 1980). We recorded the geographic coordinates of the sampling sites using a GPS.

Water samples for the analyses of the biotic variables, including all microbial communities (bacteria, flagellates and ciliates abundances) and chlorophyll-*a* concentrations, were taken in the pelagic zone from each lake using polyethylene flasks. For zooplankton analyses, we filtered 600 litres using a pump and plankton net (68µm).

We analysed the ciliates *in vivo* (Madoni, 1984) by concentrating 2L of water with the help of a plankton net (10 µm). Ciliates were identified to the lowest taxonomic level possible (morphospecies), using an optical microscope (Olympus CX-41) at a magnification of 100-400×, based mainly on the work of Foissner and Berger (1996) and Foissner et al. (1999).

Bacteria and heterotrophic flagellates were analysed from water samples treated with a fixative solution (Sherr and Sherr, 1993) filtered through black Nuclepore filters (0.2 µm and

0.8 µm, respectively) and stained with fluorochrome DAPI (4,6-diamidino-2-phenylindole; Porter and Feig, 1980). Quantification was done with an epifluorescence microscope at a magnification of 1000× (Olympus BX51). Chlorophyll-*a* concentrations were determined in the laboratory (Golterman et al., 1978). Zooplankton total abundance was estimated by counting at least three subsamples under an optical microscope at a magnification of 40-400X depending on the taxonomic group (Olympus CX41), according to Bottrell (1976).

Data analysis

We partitioned the variance of three response matrices: whole community (all species), and the groups of common and rare ciliate species. We defined common and rare species using an adaptation of the quartile criterion (Gaston, 1994; Magurran, 2004), in which the species are ranked from the most to the least abundant, but defining 25% of the most abundant as the common and 75% of the least abundant as the rare species, which seems to give similar information as other criteria (i. e. inflection point; Siqueira et al., 2012). We tested through a partial Redundancy Analysis (pRDA; Legendre and Legendre, 2012) the relative importance of three groups of explanatory variables (spatial, abiotic or biotic) in explaining ciliates community structure (for each period and group of species). The species matrices were Hellinger-transformed to minimize the effects of many zeros in the data (Legendre and Gallagher, 2001). We also tested for multicollinearity among explanatory variables, removing those with a variance inflation factor (VIF) value higher than ten (Borcard et al., 2011).

We applied the forward selection procedure (Blanchet et al., 2008) to the abiotic (dissolved oxygen, temperature, water depth, pH, conductivity, turbidity and inorganic nutrients) and biotic (bacteria, chlorophyll-*a*, flagellates and zooplankton) datasets to construct the models for each of those components. To evaluate the role of the spatial component, we built different models for the two spatial extents. The fine scale spatial model (S) was based on the spatial variables produced by Moran's Eigenvector Maps (MEM, Dray et al. 2006), derived from the geographical coordinates of the lakes (previously called PCNM - principal coordinates of neighbour matrices; Borcard et al., 2004); because we wanted this component to reflect the within-floodplain variation, we constructed the S-matrix based on MEM variables arranged in blocks, such that each block represented one floodplain, and lakes from other floodplains received a 0 value. We followed the R function proposed in Appendix 1 of the supplementary material in Declerck et al. (2011) to construct the S component. To construct the broad scale spatial component (F), we used a dummy variable representing floodplain identity (among-floodplains; Declerck et al., 2011).

We then evaluated the relative importance of the abiotic (A), biotic (B) and spatial components (S and F) in each hydrological period. The significance of each component was tested with 999 Monte Carlo permutations (Peres-Neto et al. 2006).

All the statistical procedures were performed using the libraries "vegan" (Oksanen et al., 2015) and "PCNM" (Legendre et al., 2013) of software R 3.1.3 (R Core Team 2013).

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SUPPORTING INFORMATION

Fig. S1 Venn diagram of the common and rare species found in the study. Shown in each circle are the numbers of exclusive species in each hydrological period while the intersection shows the shared species.

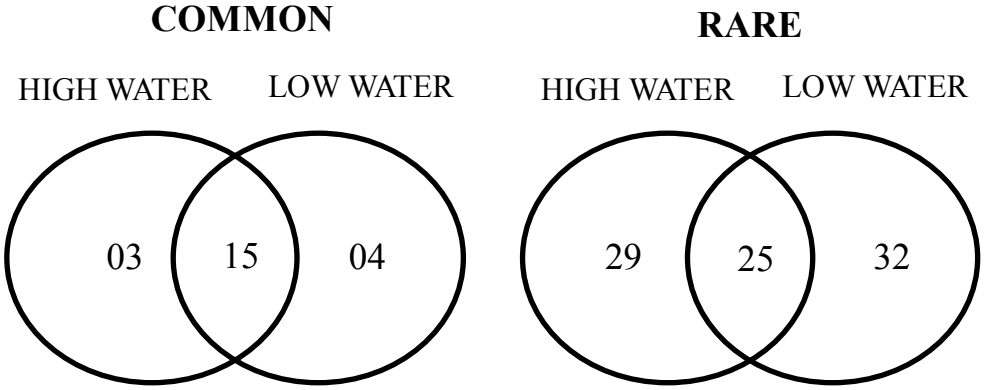

Table S1. Mean abundance (ind/L) of ciliates per lake in both hydrological periods. Species in bold and with an asterisk were considered common species (see Methods for detail).

	HIGH WATER	**LOW WATER**
Actinobolina radians	1.75	0
Actinobolina smalli	0	1.8
Actinobolina vorax	2.19	20.5
Askenasia volvox	1.32	0.9
Aspidisca cicada	0.88	0.9
Aspidisca lynceus	0.44	0.5
Balanion planctonicum	**95.6***	**251.4***
Blepharisma coeruleum	0	0.9
Bursaria truncatella	1.32	4.1
Bursaridium sp.	0.88	0
Bursellopsis sp.	3.51	26.4
Charchesium polypinum	**12.3***	**218.2***
Cinetochilum margaritaceum	3.51	9.1
Climacostomum virens	0	1.8
Codonella cratera	**13.6***	**73.2***
Coleps hirtus	**86.8***	**64.1***
Coleps spetai	0.88	14.5
Colpidium kleini	4.82	20.5
Colpoda magna	1.32	4.5
Colpoda steinii	1.75	4.5
Ctedoctema acanthocryptum	5.26	0
Cyclidium glaucoma	**40.8***	**35.0***
Cyclidium heptatrichum	0.88	0
Cyclotrichium sp.	0	9.1

Cyrtolophosis mucicola	0	7.3
Didinium nasutum	**62.3***	**30.9***
Discomorphella pectinata	0	0.9
Disematostoma tetraedricum	0	3.6
Enchelyodon elegans	0.88	0
Enchelys gasterosteus	3.95	25.0
Epystilis plicatilis	1.75	0
Epistylis procumbens	0	5.5
Epistylis pygmaeum	0	1.4
Euplotes aediculatus	0	2.7
Euplotes crassus	0.44	0
Euplotes eurystomus	0.88	0
Euplotes moebiusi	0	0.9
Euplotes sp.	0.88	**55.9***
Frontonia acuminata	0	0.9
Frontonia atra	3.95	0
Frontonia leucas	6.58	**33.6***
Glaucoma reniforme	0.88	0
Glaucoma scintillans	0.44	0
GYMNOSTOMATIDA	0	4.5
Halteria grandinella	**83.3***	**123.6***
HYPOTRICHIDA	10.96	0
Hypotrichidium conicum	0	21.8
Kerona pediculus	0	2.7
Lacrymaria olor	0.88	0
Lagynophrya acuminata	1.32	0.9
Lembadion bullinum	3.51	0
Lembadion lucens	**14.9***	1.8
Limnostrombidium viride	**22.8***	**31.4***
Limnostrombidium sp.	10.96	14.5
Litonotus alpestris	0.88	0
Litonotus lamella	0	2.7
Loxophyllum meleagris	0.88	0
Mesodinium pulex	**17.5***	**38.2***
Mesodinium sp.	0	3.2
Metopus spp.	4.39	4.5
Microthorax pusillus	3.51	0
Nassula picta	1.75	0
Opercularia sp.	0	4.5
Ophryoglena spp.	5.26	0.9
Opisthonecta sp.	0	4.5
Oxytricha similis	0.44	0
Paracolpidium truncatum	0.88	0
Paradileptus elephantinus	**22.5***	**71.4***
Paradileptus sp.	0.44	0
Paramecium aurelia	7.89	0.9

Paramecium caudatum	0.88	0
Pelagohalteria sp.	0.44	0
Phialina spp.	3.51	17.3
Platyophrya vorax	0	1.8
Rimostrombidium humile	**30.3***	**40.9***
Rimostrombidium lacustris	**24.1***	24.5
Spathidium spp.	0.88	0.9
Spirostomum caudatum	0	0.9
Stentor muelleri	1.75	6.4
Stichotricha aculeata	0.44	5.5
Stichotricha secunda	0.88	1.8
Stichotricha sp.	0	2.7
Stokesia vernalis	4.82	5.5
Stylonychia mytilus	3.95	0
Stylonychia pustulata	0	0.5
Stylonychia putrina	0	0.9
Stylonychia vorax	0	3.6
Tetrahymena pyriformis	**39.9***	**49.5***
Tintinnidium sp.	**236.8***	**184.1***
Tintinnopsis sp.	**44.7***	**265.0***
Urocentrum turbo	4.82	7.7
Uroleptus piscis	0	3.2
Uroleptus rattulus	0	0.9
Uronema nigricans	2.63	10.9
Urostyla grandis	0	0.5
Urotricha farcta	**41.7***	**39.1***
Vorticella aquadulcis	3.95	**49.1***
Vorticella campanula	1.75	0.5
Vorticella natans	7.89	**31.4***
Vorticella sp.	**11.4***	9.1

5 CONSIDERAÇÕES FINAIS

Demonstramos que a abundância bacteriana é consistentemente menor em regiões tropicais, quando comparada à das regiões temperadas. Porém, uma menor abundância de HNF (nanoflagelados heterotróficos) também foi encontrada, resultando em um acoplamento similar entre as comunidades de bactérias e HNF, independente da latitude. A explicação para tais padrões poderia ser um maior controle *top-down* das comunidades bacterianas e de HNF por ciliados e cladóceros em ambientes tropicais de água doce. A abordagem experimental demonstrou que os protistas, principalmente os ciliados, são os principais responsáveis pela maior parte da perda bacteriana em uma lagoa tropical. Além disso, os predadores influenciam não só a abundância bacteriana, como também a estrutura de tamanho da comunidade, mudando a proporção relativa de células HNA (high-nucleic acid) e LNA (low-nucleic acid), sendo que uma maior pressão de predação resulta na dominância de células menores.

Ainda, utilizando uma abordagem de metacomunidades, constatamos que a estruturação dos ciliados planctônicos de planícies de inundação Neotropicais é fortemente influenciada pelo aspecto temporal (períodos hidrológicos), que é capaz de modificar a força relativa com que as variáveis abióticas, bióticas e espaciais atuam. Ademais, a desconstrução da comunidade em espécies comuns e raras demonstrou que diferentes variáveis ambientais estão associadas a cada grupo analisado, sugerindo diferentes nichos ecológicos. Porém, quando consideramos uma grande extensão espacial, as variáveis espaciais tornam-se dominantes na estruturação da comunidade, sugerindo que os padrões biogeográficos encontrados para esses microrganismos assemelham-se àqueles encontrados para organismos de maior tamanho.