The Role of Microorganisms in a Planktonic Food Web of a Floodplain Lake

Bianca Trevizan Segovia · Danielle Goeldner Pereira · Luis Mauricio Bini ·
Bianca Ramos de Meira · Verônica Sayuri Nishida ·
Fabio Amodêo Lansac-Tôha · Luiz Felipe Machado Velho

Abstract Food webs include complex ecological interactions
that define the flow of matter and energy, and are fundamental
in understanding the functioning of an ecosystem. Temporal
variations in the densities of communities belonging to the
planktonic food web (i.e., microbial: bacteria, flagellate, and
ciliate; and grazing: zooplankton and phytoplankton) were
investigated, aiming to clarify the interactions between these
organisms and the dynamics of the planktonic food web in a
floodplain lake. We hypothesized that hydrological pulse de-
termines the path of matter and energy flow through the
planktonic food web of this floodplain lake. Data were col-
lected monthly from March 2007 to February 2008 at three
different sites in Guaraná Lake (Mato Grosso do Sul State,
Brazil). The path analysis provided evidence that the dynam-
ics of the planktonic food web was strongly influenced by the
hydrological pulse. The high-water period favored interac-
tions among the organisms of the microbial loop, rather than
their relationships with zooplankton and phytoplankton.
Therefore, in this period, the strong interaction among the
organisms of the grazing food chain suggests that the micro-
bial loop functions as a sink of matter and energy. In turn, in
the low-water period, higher primary productivity appeared to
favor different interactions between the components of the
grazing food chain and microorganisms, which would
function as a link to the higher trophic levels.

B. T. Segovia (✉) · D. G. Pereira · B. R. de Meira · V. S. Nishida ·
F. A. Lansac-Tôha · L. F. M. Velho
Universidade Estadual de Maringá, Nupelia, Av. Colombo,
5790-Bloco G90, CEP 87020-900 Maringá, PR, Brazil
e-mail: biatsegovia@gmail.com

L. M. Bini
Universidade Federal de Goiás, ICB, DE, CEP 74001-970 Goiânia,
GO, Brazil

Introduction

Understanding the flow of matter and energy through an
ecosystem, as described in a food web, is of paramount
importance [1]. Until a few decades ago, only the grazing
food chain (involving the trophic relationships among phyto-
plankton, zooplankton, and fish) [2] was recognized. Howev-
er, the use of other techniques (e.g., DAPI) [3] indicated high
bacterial densities in aquatic ecosystems and formerly
neglected communities (i.e., bacteria, flagellates, and ciliates)
were recognized as key players in aquatic food webs.

Several studies have highlighted the important roles of
bacteria, flagellates, and ciliates in food webs. Pomeroy [1]
stressed the role of microorganisms in the production of
marine pelagic ecosystems, and Azam et al. [4] developed
the "microbial loop" concept. These investigators highlighted
that microorganisms have a much greater role than was pre-
viously described [5]. According to the microbial loop con-
cept, the dissolved organic carbon released by phytoplankton
is used by bacteria, which are then preyed upon by protozoa
that are subsequently consumed by zooplankton. Sherr and
Sherr [6] also emphasized the existence of direct links be-
tween small-sized algae and heterotrophic protozoa. These
protozoans are then responsible for recovering and
reintroducing the fixed carbon of bacteria and algae, making
it available for larger organisms of higher trophic levels,
forming a more complex microbial food web.

Depending on certain characteristics of the aquatic envi-
ronments, the microbial food web predominates in the flow of
matter and energy in pelagic regions, when compared to the
grazing food chain. It is believed that in oligotrophic oceanic
or coastal waters, where most algal photosynthesis is

accomplished by algae that are too small to be effectively consumed by metazoans, the main trophic link is via heterotrophic flagellates and ciliates [6]. Therefore, the size of phytoplankton is considered a determining factor for the different energy paths through the planktonic food web [7]. Thus, seasonal variability affecting phytoplankton growth can also modify the relative importance of the microbial food web in the transfer of matter and energy to higher trophic levels [8–10].

River floodplain areas are subject to strong hydrological, physical, chemical, and biological variability [11,12]. During the low-water period, or limnophase, primary production predominates, since lakes are shallower and the wind action induces water circulation and destratification, providing nutrients in the whole water column, thus supporting high phytoplankton abundance [13], especially microphytoplankton (algae over 100 μm are markedly predominant in the limnophase) [14]. During the high-water period, or potamophase, there is an input of allochthonous organic matter (e.g., dissolved organic matter) from decaying plant material, flooded soil, and overland runoff [15]. Thus, during this period, decomposition processes predominate, and increased heterotrophic bacterial density in floodplain lakes is likely to occur [16,17], given the increase in carbon sources [18].

Our hypothesis was that the hydrological pulse determines the strength of interactions in planktonic food webs. During the potamophase, we expected to find a strong relationship between the components of the microbial loop, due to the input of allochthonous organic matter, which leads to an increased abundance of these microorganisms. The expected relationships would include the predation of flagellates on bacteria and the predation of ciliates on flagellates. Ciliates in turn would account for the transfer of matter and energy to the zooplankton, as is proposed in the traditional microbial loop of marine environments [4]. During the limnophase, the strongest relationship would be found between phytoplankton and zooplankton, and the grazing food chain would be favored by the phytoplankton development in this hydrological period (Fig. 1).

Material and Methods

Study Area and Sampling

This study was conducted in the Guaraná Lake, located in the Upper Paraná River floodplain, on the north bank of the Baía River (Mato Grosso do Sul State, 22°43′S and 53°18′W), to which it is permanently connected. It is a humic lake, with a mean dissolved organic carbon concentration of 10 mg/L [15]. Guaraná Lake is round, shallow (mean depth=2.1 m), and small (length=386.5 m, perimeter=1058.3 m and area= 4.2 ha). The littoral zone is colonized by several species of

aquatic macrophytes, including *Eichhornia azurea* and *Eichhornia crassipes* [19,20].

Samples were collected monthly from March 2007 to February 2008, at three different sites in the lake, from three depths (subsurface, middle, and bottom), with a Van Dorn bottle. For the analysis of the microbial community, samples were transported to the field station laboratory in 2-L polyethylene vials in an insulated container. Subsequently, 100-mL aliquots were treated with a fixative solution (alkaline Lugol solution, formaldehyde, and thiosulfate) in sterilized glass vials for analysis of bacteria and flagellates [21]. For analysis of ciliates, samples were concentrated using a plankton net (10 μm) coupled to 500-mL polyethylene flasks. For zooplankton, 25 L of water was collected with a Van Dorn bottle and filtered through a plankton net (68 μm). Samples were concentrated to 150 mL and fixed in a buffered formalin solution (4 %). Phytoplankton samples were taken directly below the water surface, using glass vials, and fixed in situ with acetic Lugol's solution. Samples for physical, chemical, and chlorophyll *a* analyses were stored in 500-mL plastic flasks.

The following abiotic variables were determined in the field: water temperature (°C), dissolved oxygen (mg/L) (YSI 55 handheld dissolved oxygen equipment), conductivity (μS/cm), pH (Digimed digital potentiometers), depth (m), and water transparency (m) (Secchi disk). Concentrations of suspended organic matter (mg C/L), chlorophyll *a* (μg/L), total phosphorus (total-P) (μg/L), and total nitrogen (total-N) (μg/L) were determined in the laboratory, according to Teixeira et al. [22], Golterman et al. [23], and Mackereth et al. [24], respectively.

Laboratory Analysis

Microbial Communities

The density of heterotrophic flagellates (cells/mL) was estimated by first filtering subsamples (5–15 mL) through black Nuclepore filters, with a pore size of 0.8 μm and a diameter of 25 mm, and staining with fluorochrome DAPI (4,6-diamidino-2-phenylindole) at 0.1 % for 15 min in the dark [3]. The filters were mounted on slides and stored in the freezer. The flagellates were then counted with an epifluorescence microscope (Olympus BX51) and classified simultaneously, using as a criterion the reddish color of the autotrophic flagellates, when exposed to blue light, in contrast to the green color of heterotrophic flagellates. To determine flagellate density on each slide, at least 300 cells or 100 fields were counted under UV light, at ×400 and ×1,000 magnification.

Bacterioplankton density (cells/mL) was determined using the same protocol for filtration, assembly, and storage of the slides as for the flagellates. Water subsamples (0.1 mL) were

filtered through black Nuclepore membranes, with a 0.2-μm pore size and 25-mm diameter, and stained with fluorochrome DAPI at 0.1 % for 5 min in the dark [3]. Bacteria were quantified under ×1,000 magnification with an epifluorescence microscope (Olympus BX51).

The density of ciliates (cells/mL) was estimated in vivo, within a maximum of 6 h after sampling, using an Olympus CX41 optical microscope, under magnifications of ×100 and ×400 [25].

Plankton

Three subsamples were taken per zooplankton sample, using a Hensen-Stempel pipette. Approximately 50 individuals were counted per subsample [26] with an Olympus CX41 optical microscope using ×100 magnification. The density was expressed as individuals/liter. Phytoplankton density (individuals/mL) was calculated with the use of an inverted microscope [27]. The phytoplankton was classified by size (nanophytoplankton 2–60 μm, microphytoplankton 60–500 μm, and mesophytoplankton 0.5–1 mm) according to Reynolds [28]. We used the terms "edible" and "less-edible" considering the degree of edibility commonly used by other investigators [29], which takes into account several characteristics including size and shape. Thus, edible phytoplankton would be in general fast-growing unicellular nanoplankton, while filamentous algae would be considered less edible.

Data Analysis

A principal components analysis (PCA) [30] was used to reduce the dimensionality of the abiotic data, along with the broken-stick criterion to determine the number of interpretable axes [31]. All data, except for pH, were previously log-transformed in order to reduce the influence of outliers and to linearize the relationships.

The magnitudes of the differences in densities between the phases were assessed using the Hedges' g statistic [32]. For instance, following a rule of thumb, these magnitudes can be considered small, medium, and large when the values of g (in module) are approximately 0.2, 0.5 and 0.8, respectively.

Path analyses were used to test models describing the relationships among the aquatic communities. According to Legendre and Legendre [33], path analysis "is an extension of multiple linear regression that allows the decomposition and interpretation of linear relationships among a (small) number of descriptors." One major advantage of path analysis is that it allows us to formally state and test a priori hypotheses concerning the causal relationships among variables. Alternatively, the use of automated methods to select a minimum adequate model (e.g., stepwise multiple regression) has been heavily criticized (e.g., Whittingham et al. [34] and references therein). In path analysis, a specific hypothesis can be depicted

as an input-path diagram, such as the one shown in Fig. 1. Specifically, during the potamophase, we assumed that bacteria directly affect the growth of flagellates, which in turn affect the abundance of ciliates, as expected in a marine microbial food web [4]. An increase in the abundance of ciliates would account for an increase in the zooplankton abundance (Fig. 1). During the limnophase, we expected that the strongest relationship would be found between phytoplankton and zooplankton (Fig. 1). This would be because of the increase in primary production, as conjectured above (see "Introduction"). The strength of the interactions between variables is measured by path coefficients (i.e., the standard partial regression coefficients). Usually, in the output-path diagram, thicker lines connecting the variables represent the strongest interactions between the variables. Further details on path analysis estimation and testing can be found in Sokal and Rohlf [35] and Shipley [36]. We used SYSTAT statistical software [37] for path analysis.

The abundance data for all communities were used in the path analysis. Although biomass may be a way of documenting energy flow, taking differences in cell sizes into account, we have no data on zooplankton biomass. Nevertheless, we found strong correlations between abundance and biomass for all microbial communities (bacteria, 0.71; and protozoans, 0.63), which, we believe, allows us to make inferences from the abundance data as well.

Results

The months of March, April, and December 2007 and January and February 2008 were typical of the potamophase with respect to water depth, while the other months (May to November 2007) characterized the limnophase (Fig. 2). Only the first PCA axis applied to the limnological data was retained for interpretation according to the broken-stick criterion. This axis explained 37.4 % of the total variance in the data and their scores showed a clear differentiation between hydrological periods (Fig. 3). Thus, during the potamophase, the temperatures and total phosphorus concentrations were higher (variables positively correlated with the first axis). On the other hand, water transparency was higher during the limnophase than during the potamophase. Also, we recorded higher values of dissolved oxygen, chlorophyll a, pH, and suspended organic matter (variables negatively correlated with the first principal component).

According to the Hedges' g statistics (Table 1), bacteria showed a slightly higher density during the limnophase, while the flagellate density during the potamophase was more than twice as high as that recorded in the limnophase. Ciliate abundance was similar in both periods. Nanophytoplankton predominated in both periods, but a greater participation of

Fig. 1 Representation of path analysis models in two hydrological periods in Guaraná Lake (*thick arrows* indicate the strengths of the relationships expected)

Potamophase   **Limnophase**
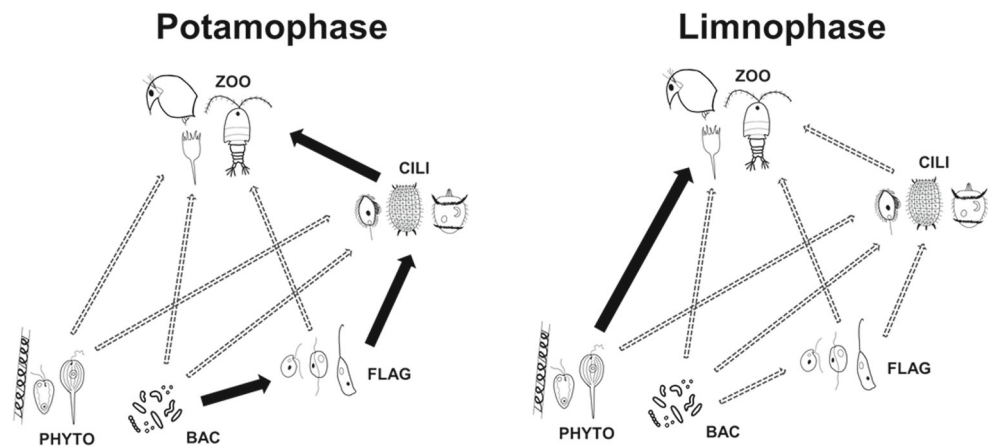
microphytoplankton was recorded during the limnophase. The density of mesophytoplankton was very low in both phases. Both cladocerans and copepods showed higher densities during the potamophase. For rotifers, the highest density was recorded during the limnophase.

During the potamophase, high negative path coefficients were estimated between bacteria and flagellates (−0.78), between bacteria and ciliates (−0.82), and between phytoplankton and zooplankton (−0.46) (Fig. 4a). During the limnophase, we recorded negative path coefficients between flagellates and zooplankton (−0.41) and between ciliates and phytoplankton (−0.46). A positive coefficient was recorded between phytoplankton and zooplankton (0.58) (Fig. 4b).

Discussion

Abiotic and biotic factors are important predictors of bacterioplankton growth [38]. Considering the high

temperatures and phosphorus concentrations during the potamophase and the negative relationship between bacteria and protozoans, it can be inferred that the lowest bacterial density in this period was mainly due to predation by flagellates and ciliates, as observed in other studies conducted in humic lakes [39–42].

The assumed high predation rates of flagellates on bacteria can be explained by the large amount of small-sized nanoflagellates in the lake (92.1 % of the total density between 2 and 7 μm), which may be responsible for most of the bacterial loss [43], including in humic lakes [44]. Ciliates were especially represented by *Balanion planctonicum*, which feeds on bacteria and algae [45] and was twofold more abundant during the potamophase than during the limnophase. *Cyclidium glaucoma* and *Halteria grandinella*, which are mainly bacterivorous [45], also had a high abundance and were at least threefold more abundant in the potamophase [Appendix A].

Fig. 2 Mean depths recorded in the Guaraná Lake (data were obtained in three sites); the *dashed line* represents the mean value of all months
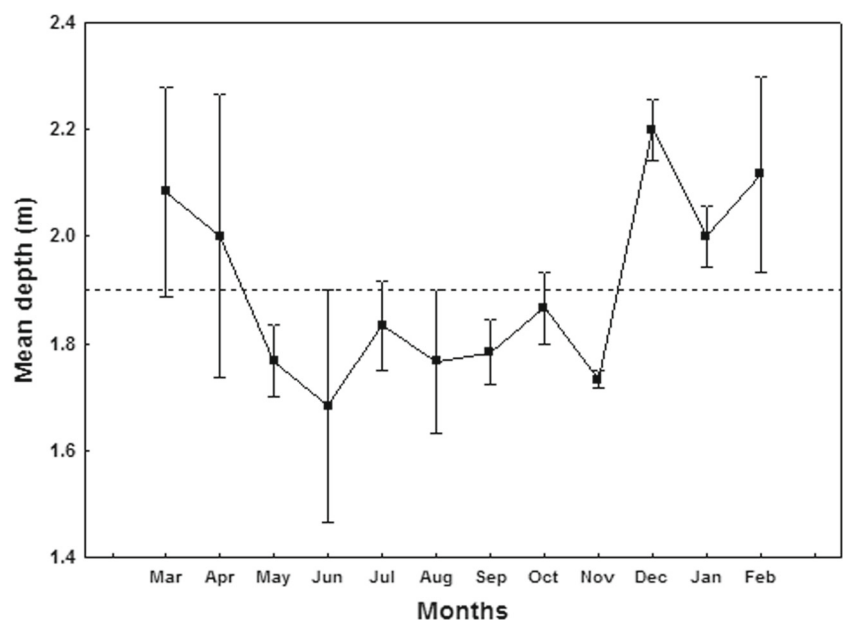
Fig. 3 Distribution of scores for samples along the PCA axis, defined by the variables: *Temp* = temperature, *TP* = total phosphorus, *DO* = dissolved oxygen, *Chlor* = chlorophyll *a*, *SOM* = suspended organic matter, Secchi and pH
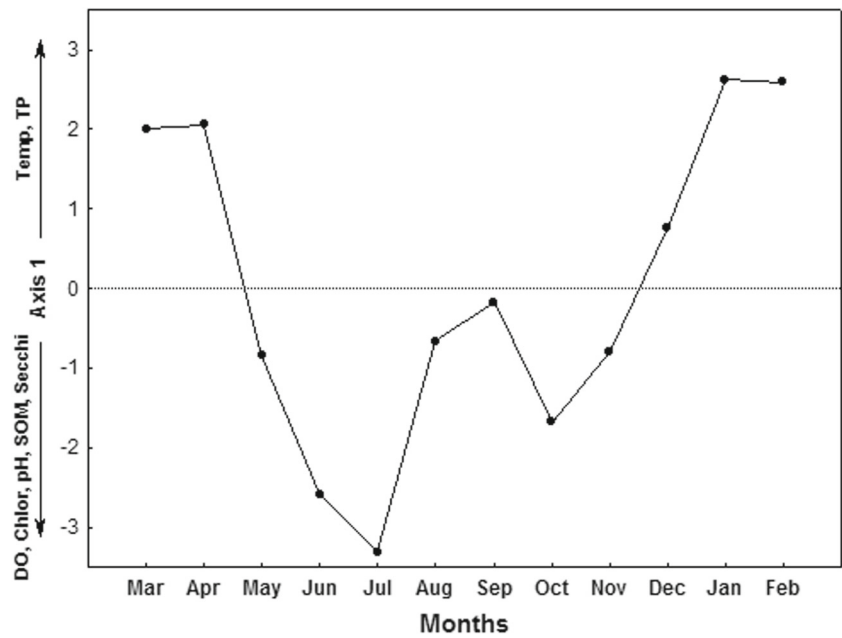
After the flood, the suspended sediment in lakes increases considerably. There seems to be a consensus that protozoans are less affected by the input of sediment than are larger organisms [46,47]. A reduced feeding efficiency is among the effects of suspended sediment on planktonic communities, especially that of filter-feeding organisms. Studies on the effect of the flood on the composition of the zooplankton community found similar results, in which rotifers were negatively affected, while the densities of flagellates and ciliates increased [48]. Nevertheless, some genera of cladocerans, such as *Moina*, present in Guaraná Lake, proved to be tolerant to suspended sediment [49], which could explain the higher density of these microcrustaceans during the potamophase.

Also, during the potamophase the input of humic substances and reduction of water transparency likely have a direct impact on phytoplankton [50]. One of these impacts is the increased dominance of phytoflagellates, due to their competitive advantages over other phytoplankton groups [51]. Accordingly, nanophytoplankton reached high densities in Guaraná Lake and comprised the majority of phytoplankton during the potamophase (Table 1). *Cryptophyceae* was the most abundant class during this period (42.7 % of total phytoplankton); this group of flagellated (and potentially mixotrophic) algae is important in several humic lakes [52,53]. *Cryptomonas marssonii*, the species with the highest density in Guaraná Lake [APPENDIX B], is capable of extensive migration in the water column [54], which results in a more efficient use of PAR (in the epilimnion) and nutrients (in the hypolimnion), besides being able to prey on bacteria [55].

The relationship between zooplankton and phytoplankton during the potamophase can be explained by several factors, including, as mentioned above, the dominance of flagellated algae, which are more palatable than *Cyanobacteria* [56]. Another plausible explanation is that the high density of microcrustaceans in this period increased the predation pressure on phytoplankton.

Table 1 Mean densities (±S.D.) of aquatic communities recorded during two hydrological periods in the Guaraná Lake. The magnitudes of the differences between the phases are given by the Hedges' g statistic

	Potamophase		Limnophase			
	Mean	S.D.	Mean	S.D.	g	Var(g)
Bacteria (×10^5 cells/mL)	5.30	1.78	6.22	2.81	−0.35	0.30
Heterotrophic flagellates (×10^3 cells/mL)	1.71	2.19	0.73	0.44	0.63	0.31
Ciliates (ind/mL)	12.83	2.98	12.72	4.84	0.03	0.29
Nanophytoplankton (ind/mL)	1,107.80	433.65	1,188.71	569.74	−0.14	0.29
Microphytoplankton (ind/mL)	120.20	119.69	454.86	262.39	−1.42	0.38
Mesophytoplankton (ind/mL)	0.20	0.45	0.00	0.00	0.65	0.31
Rotifers (ind/L)	49.84	34.82	67.48	34.40	−0.47	0.30
Cladocerans (ind/L)	33.67	29.72	21.75	12.70	0.52	0.30
Copepods (ind/L)	26.59	9.47	16.16	10.79	0.94	0.33
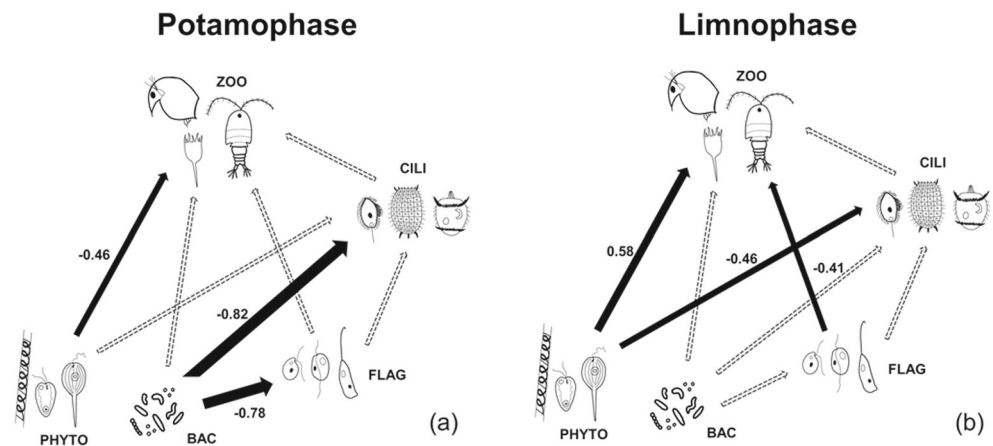
We found a larger contribution of microphytoplankton in the limnophase (22 % of total phytoplankton; Table 1), compared to the potamophase (7.5 % of total phytoplankton). This group of algae was dominated by *Bacillariophyceae*, especially *Urosolenia eriensis* and *Aulacoseira granulata* var. *granulata* [Appendix B], which are filamentous algae, and thus are considered less edible for zooplankton. In general, in tropical environments, large microcrustaceans (such as *Daphnia*) that could consume filamentous algae are less abundant, or absent, which hinders the control of phytoplankton by zooplankton [57]. However, *Cryptophyceae* also reached high abundance during the limnophase (35.5 % of total phytoplankton). Therefore, as edible algae also constituted a large part of the phytoplankton community, this favored phytoplankton–zooplankton relationships, although they were not negative.

Negative relationships between phytoplankton and ciliates were also observed during the limnophase. Predation on phytoplankton by protozoans has been found in several studies; in addition to efficiently consuming smaller phytoplankton, protozoans are also able to consume larger organisms, competing with the meso- and macrozooplankton for all phytoplankton size classes [58–61]. Some ciliates even feed on filamentous algae larger than themselves [62], hindering the understanding of trophic relationships based on a simple differentiation of organisms into size classes [63]. The most important species during the limnophase, *Tintinnidium pusillum* and *Codonella cratera* [APPENDIX A], are potentially algivorous. *T. pusillum* feeds on flagellated algae, diatoms, and bacteria [45], and were twofold more abundant during the limnophase, while *C. cratera* prefers diatoms, green, and flagellated algae [45], and were almost absent during the potamophase.

If the influence of herbivory is predominant, phytoplankton should be dominated by size classes that are less edible because small algae are selectively removed [62]. When this occurs, larger phytoplankton has greater competitive advantage in terms of nutrient uptake (which under other conditions would favor smaller cells) [64]. Thus, the high density of

microphytoplankton during the limnophase, compared to the potamophase (Table 1), can be explained, at least in part by the predation pressure on other phytoplankton size classes by zooplankton and ciliates (Fig. 4b).

In environments or periods dominated by the less-edible phytoplankton, metazooplankton can consume the elements of the microbial food web more intensely. For example, flagellates may be consumed in the absence of edible phytoplankton [65], which explains the negative relationship between zooplankton and flagellates during the limnophase, when the less-edible phytoplankton showed higher densities. A higher predation pressure on flagellates could then explain the very low abundance found during this period (Table 1). In some humic lakes, it has been reported that heterotrophic flagellates transfer allochthonous carbon from bacterioplankton to zooplankton [66,67]. In general, protozoans are key organisms in several humic lakes, thus representing an "important link in the energy flow from bacteria to metazoans" [68].

Bacterial density showed weak relationships with the other organisms studied, suggesting that they were nearly free from major predation pressure, which, together with other abiotic factors, allowed the bacteria to reach a higher density during the limnophase. A trophic-cascade effect seems to have occurred, in which the bacteria were favored by the predation pressure of zooplankton on flagellates. The reduction in predation pressure on bacteria by flagellates, resulting from zooplankton predation on flagellates, was also suggested in other studies from humic lakes [50,69]. Dissociation between bacteria and flagellates was also reported by Gasol and Vaqué [70], who inferred that flagellates cannot achieve the density allowed by resources (in this case bacteria) due to the downward control exerted by zooplankton.

During the potamophase, the relationship between phytoplankton and zooplankton was stronger than the relationship between organisms of the microbial loop and zooplankton, supporting a grazing food chain model. In an experiment conducted in a humic lake, with addition of nutrients (humic

and nutrient-rich conditions, similar to those observed in Guaraná Lake), and input of terrestrial DOC (such as in the potamophase), zooplankton was mainly sustained by phytoplankton. Under these conditions, although bacteria use large amounts of terrestrial DOC, very little seems to reach the higher trophic levels by this pathway [71].

Although bacteria are able to use allochthonous humic substances rapidly, increasing the activity of the microbial link [39], there may be a loss of matter and energy through the ingestion and respiration of bacterial carbon by small bacterivores, which leads to the loss of significant fractions of the total carbon fixed by these organisms from the trophic system [72]. Thus, the input of allochthonous carbon and its utilization by bacteria during the potamophase appear to support a microbial loop that does not interact with the grazing food chain, with microorganisms representing a sink for carbon and acting mainly in the regeneration of inorganic nutrients [72,73].

Flagellates appear to have been an alternative prey for zooplankton during the limnophase. Ciliates also actively participated in the planktonic food web, and alternated their resource, from bacteria in the potamophase to phytoplankton in the limnophase, probably being able to feed on both edible and less-edible phytoplankton. These facts emphasize the potential of flagellates and ciliates as stabilizing groups in the food chain, making both bacterial and phytoplankton production available to higher trophic levels [74]. In short, the interactions among the components of the microbial loop were weaker during the limnophase than during the potamophase, but with links between protozoans and organisms of the higher trophic levels.

Conclusion

The hydrological variability influenced the strength of the interactions among the components of the food web. During the potamophase, the interactions among organisms of the microbial loop, at the expense of their relationships with components of the grazing food chain, suggest that the microbial loop may act as a sink of matter and energy on this lake. On the other hand, during the limnophase, the higher primary production favored different interactions, allowing for the inflow of matter and energy through both traditional and alternative trophic pathways (including microbial components) to the higher trophic levels. We believe that our results are likely to apply to other tropical humic lakes from floodplain systems. However, further studies are required to test if the relationships we observed here exist in floodplain lakes that are not humic or those found in other latitudes.

References

1. Pomeroy LR (1974) The ocean's food web, a changing paradigm. Bioscience 24:499–504
2. Lindeman R (1942) The tropho-dynamic aspect of ecology. Ecology 23:399–418
3. Porter KG, FEIG YS (1980) The use of DAPI for identifying and counting aquatic microflora. Limnol Oceanogr 25:943–948
4. Azam F, Fenchel T, Field JG, Gray JS, Meyer-Reil LA, Thingstad F (1983) The ecological role of water-column microbes in the sea. Mar Ecol Prog Ser 10:257–263
5. Fenchel T (2008) The microbial loop—25 years later. J Exp Mar Biol Ecol 366:99–103
6. Sherr EB, Sherr BF (1988) Role of microbes in pelagic food webs: a revised concept. Limnol Oceanogr 33:1225–1227
7. Legendre L, Rassoulzadegan F (1995) Plankton and nutrient dynamics in coastal waters. Ophelia 41:153–172
8. Nielsen TG, Richardson K (1989) Food chain structure of the North Sea plankton communities: seasonal variations of the role of the microbial loop. Mar Ecol Prog Ser 56:75–87
9. De Wever A (2006) Spatio-temporal dynamics in the microbial food web in Lake Tanganyika. PhD Thesis, University of Ghent.
10. Shinada A, Ban S, Ikeda T (2008) Seasonal changes in the planktonic food web off Cape Esan, southwestern Hokkaido, Japan. Plankon Benthos Res 3:18–26
11. Junk WJ, Bayley PB, Sparks RE (1989) The flood pulse concept in river-floodplain systems. Can Spec Public Fish Aquat Sci 106:110–127
12. Neiff JJ (1990) Ideas para la interpretación ecológica del Paraná. Interciencia 15:424–441
13. Thomaz SM, Pagioro TA, Bini LM, Roberto MC, Rocha RRA (2004) Limnology of the Upper Paraná Floodplain habitats: patterns of spatio-temporal variations and influence of the water levels. In: Agostinho AA, Rodrigues L, Gomes LC, Thomaz SM, Miranda LE (eds) Structure and functioning of the Paraná River and its floodplain. Maringá, EDUEM, pp 37–42
14. Train S, Rodrigues LC (2004) Phytoplankton assemblages. In: Thomaz SM, Agostinho AA, Hahn NS (eds) The upper Paraná river and its floodplain. Backhuys, Leiden, The Netherlands, pp 103–124
15. Teixeira MC, Santana NF, Azevedo JCR, Pagioro TA (2008) Padrões de variação do carbono orgânico na planície de inundação do alto rio Paraná. Oecologia Bras 12:57–65
16. Thomaz SM, Roberto MC, Bini LM (1997) Caracterização limnológica dos ambientes aquáticos e influência dos níveis fluviométricos. In: Vazzoler AEAM, Agostinho AA, Hahn NS (eds) A planície de inundação do alto Rio Paraná: aspectos físicos, biológicos e socioeconômicos. Maringá, EDUEM, pp 73–102
17. Carvalho P, Thomaz SM, Bini LM (2003) Effects of water level, abiotic and biotic factors on bacterioplankton abundance in lagoons of a tropical floodplain (Paraná River, Brazil). Hydrobiologia 510:67–74

18. Arvola L, Salonen K, Rask M (1999) Trophic interactions. In: Keskitalo J, Eloranta P (eds) Limnology of humic waters. Backhuys, Leiden, pp 265–279

19. Thomaz SM, Lansac-Tôha FA, Roberto MC, Esteves FA, Lima AF (1992) Seasonal variation of some limnological factors of lagoa do Guaraná, a várzea lake of the high Paraná river, State of Mato Grosso do Sul, Brazil. Rev Hydrobiol Trop 25:269–276

20. Souza-Filho E, Comunello E, Petry AC, Russo MR, Santos AM, Rocha RRA, Leimig RA (2000) Descrição dos locais de amostragem: a planície de inundação do Alto Rio Paraná. Programa PELD/CNPq sítio 6 – Relatório Anual. Available on:< http://www.peld.uem.br/ Relat2000/2_2_CompBioticoDesLocAmost.PDF>. Accessed 17 July 2013.

21. Sherr EB, Sherr BF (1993) Preservation and storage of samples for enumeration of heterotrophic protists. In: Kemp P, Sherr B, Sherr E, Cole J (eds) Current methods in aquatic microbial ecology. Lewis, New York, pp 207–212

22. Teixeira C, Tundisi JG, Kutner MB (1965) Plankton studies in a mangrove. II: the standing-stock and some ecological factors. Bol Inst Oceanogr 24:23–41

23. Goltermam HL, Clymo RS, Ohnstad MAM (1978) Methods for physical and chemical analysis of freshwater. Blackwell Scientia, London

24. Mackereth JFH, Heron J, Talling JF (1978) Water analysis: some revised methods for limnologists. Freshw Biol Assoc Sci Publ 36:121

25. Weisse T (1991) The annual cycle of heterotrophic freshwater nanoflagellates: role of bottom-up versus top-down control. J Plankton Res 13:167–185

26. Bottrell HH, Duncan A, Gliwicz ZM, Grygierek E, Herzig A, Hillbricht-Ilkowska A, Kurosawa H, Larsson P, Weglenska T (1976) A review of some problems in zooplankton production studies. Norw J Zool 24:419–456

27. American Public Health Association (1995) Standard methods for the examination of water and wastewater. APHA/WEF/AWWA, Washington

28. Reynolds CS (2006) The ecology of phytoplankton. Cambridge University Press, Cambridge

29. Vasseur DA, Gaedke U, McCann KS (2005) A seasonal alternation of coherent and compensatory dynamics occurs in phytoplankton. Oikos 110:507–514

30. Manly BFJ (1994) Multivariate statistical methods: a primer. Chapman & Hall, London

31. Jackson DA (1993) Stopping rules in principal component analyses: a comparison of heuristical and statistical approaches. Ecology 74: 2204–2214

32. Borenstein M, Hedges LV, Higgins JPT, Rothstein HR (2009) Introduction to meta-analysis. Wiley, West Sussex, UK

33. Legendre P, Legendre L (2012) Numerical ecology. Elsevier, Amsterdam

34. Whittingham MJ, Stephens PA, Bradbury RB, Freckleton RP (2006) Why do we still use stepwise modelling in ecology and behaviour? J Anim Ecol 75:1182–1189

35. Sokal RR, Rohlf FJ (1995) Biometry: the principles and practice of statistics in biological research. W. H. Freeman, New York

36. Shipley B (2002) Cause and correlation in biology. A user's guide to path analysis, structural equations and causal inference. Cambridge University Press, Cambridge, UK

37. Wilkinson L (2010) SYSTAT. Wiley Interdisciplinary Reviews. Comput Stat 2:256–257

38. Vrede K (2005) Nutrient and temperature limitation of bacterioplankton growth in temperate lakes. Microb Ecol 49:245–56

39. Carlsson P, Granéli E, Tester P, Boni L (1995) Influences of riverine humic substances on bacteria, protozoa, phytoplankton, and copepods in a coastal plankton community. Mar Ecol Prog Ser 127:213–321

40. Sarvala J, Kankaala P, Zingel P, Arvola L (1999) Zooplankton. In: Keskitalo J, Eloranta P (eds) Limnology of humic waters. Backhuys, Leiden, pp 173–188

41. Kalinowska K (2004) Bacteria, nanoflagellates and ciliates as components of the microbial loop in three lakes of different trophic status. Pol J Ecol 52:19–34

42. Kent AD, Jones SE, Yannarell AC, Graham JM, Lauster GH, Kratz TK, Triplett EW (2004) Annual patterns in bacterioplankton community variability in a humic lake. Microb Ecol 48:550–560

43. Sleigh MA (2000) Trophic strategies. In: Leadbeater BSC, Green J (eds) The flagellates. Taylor & Francis, London, pp 147–166

44. Salonen K, Jokinen S (1988) Flagellate grazing on bacteria in a small dystrophic lake. Hydrobiologia 161:203–209

45. Foissner W, Berger H, Schaumburg J (1999) Identification and ecology of limnetic plankton ciliates. Informationsberichte des Bayerischen Landesamtes für Wasserwirtschaft.

46. Jack JD, Gilbert JJ (1993) The effect of suspended clay on ciliate population growth rates. Freshw Biol 29:385–394

47. Boenigk J, Novarino G (2004) Effect of suspended clay on the feeding and growth of bacterivorous flagellates and ciliates. Aquat Microb Ecol 34:181–192

48. Muylaert K, Vyverman W (2006) Impact of a flood event on the planktonic food web of the Schelde estuary (Belgium) in spring 1998. Hydrobiologia 559:385–394

49. Cuker BE, Hudson L (1992) Type of suspended clay influences zooplankton response to phosphorus loading. Limnol Oceanogr 37: 566–576

50. Jones RI (1992) The influence of humic substances on lacustrine planktonic food chains. Hydrobiologia 229:73–91

51. Arvola L, Eloranta P, Järvinen M, Keskitalo J, Holopainen AL (1999) Phytoplankton. In: Keskitalo J, Eloranta P (eds) Limnology of humic waters. Backhuys, Leiden, pp 137–171

52. Smolander U, Arvola L (1988) Seasonal variation in the diel vertical distribution of the migratory alga *Cryptomonas marssonii* (Cryptophyceae) in a small, highly humic lake. Hydrobiologia 161: 89–98

53. Rodriguéz P, Pizarro H (2007) Phytoplankton productivity in a highly colored shallow lake of a South American floodplain. Wetlands 27: 1153–1160

54. Jones RI (1988) Vertical distribution and diel migration of flagellated phytoplankton in a small humic lake. Hydrobiologia 161:75–87

55. Joniak T (2007) Seasonal variations of dominant phytoplankton in humic forest lakes. Oceanol Hydrobiol Stud 36:49–59

56. Gliwicz M. Zooplankton. In: O´Sullivan PE, Reynolds CS (eds.) The lakes handbook: limnology and limnetic ecology. Oxford: Blackwell; 2004. pp. 461–516.

57. Lazzaro X (1997) Do the trophic cascade hypothesis and classical biomanipulation approaches apply to tropical lakes and reservoirs? Verh Internat Verein Limnol 26:719–730

58. Sherr EB, Sherr BF (1994) Bacterivory and herbivory: key roles of phagotrophic protists in pelagic food webs. Microb Ecol 28:223–235

59. Sherr EB, Sherr BF (2002) Significant of predation by protists in aquatic microbial food webs. Anton Leeuw Int J G 81:293–308

60. Tillmann U (2004) Interactions between planktonic microalgae and protozoan grazers. J Eukaryot Microbiol 51:156–168

61. Putland JN, Iverson RL (2007) Microzooplankton: major herbivores in an estuarine planktonic food web. Mar Ecol Prog Ser 345:63–73

62. Sommer U, Stibor H (2002) Copepoda-Cladocera-Tunicata: the role of three major mesozooplankton groups in pelagic food webs. Ecol Res 17:161–174

63. Berman T, Stone L (1994) Musings on the microbial loop: twenty years after. Microb Ecol 28:251–253

64. Samuelsson K, Berglund J, Haecky P, Andersson A (2002) Structural changes in an aquatic microbial food web caused by inorganic nutrient addition. Aquat Microb Ecol 29:29–38

65. Hart D, Stone L, Berman T (2000) Seasonal dynamics of the Lake Kinneret food web: the importance of the microbial loop. Limnol Oceanogr 45:350–361

66. Laybourn-Parry JEM, Parry JD (2000) Flagellates and the microbial loop. In: Leadbeater BSC, Green J (eds) The flagellates. Taylor & Francis, London, pp 216–239

67. Järvinen M (2002) Control of plankton and nutrient limitation in small boreal brown-water lakes: evidence from small- and large-scale manipulation experiments. Dissertation, University of Helsinki, Finland.

68. Hessen DO (1998) Food webs and carbon cycling in humic lakes. In: Hessen DO, Tranvik LJ (eds) Aquatic humic substances. Springer, New York, pp 285–315

69. Segovia BT, Pereira DG, Bini LM, Velho LFM (2014) Effects of bottom-up and top-down controls on the temporal distribution of planktonic heterotrophic nanoflagellates are dependent on water depth. Hydrobiologia 736:155–164

70. Gasol JM, Vaqué D (1993) Lack of coupling between heterotrophic nanoflagellates and bacteria: a general phenomenon across aquatic systems? Limnol Oceanogr 38:665–670

71. Cole JJ, Carpenter SR, Pace ML, Bogert MCV, Kitchell JL, Hodgson JR (2006) Differential support of lake food webs by three types of terrestrial organic carbon. Ecol Lett 9:558–568

72. Ducklow HW, Purdie DA, Williams PJL, Davies JM (1996) Bacterioplankton: a sink for carbon in a coastal plankton community. Science 232:865–867

73. Cole JJ, Carpenter SR, Kitchell JL, Pace ML (2002) Pathways of organic carbon utilization in small lakes: results from a whole-lake ^{13}C addition and coupled model. Limnol Oceanogr 47:1664–1675

74. McManus GB (1991) Flow analysis of a planktonic microbial food web model. Mar Microb Food Webs 5:145–160