ECOSYSTEMS

Predation by an omnivorous fish and food availability alter zooplankton functional diversity: a microcosm approach

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Abstract: Studies considering the functional traits of organisms, populations, and communities functional indices increase the understanding of many factors on ecosystem functioning. Here, we analyze the predation effect (by fish) on zooplankton functional diversity and the effects of biomass and density of periphytic algae on zooplankton feeding type trait and body size. We expect that intense predation by fish on zooplankton leads to higher values of zooplankton functional diversity and that food resource will be positively related to the abundance of zooplankton trait and body size. For that, microcosms were established (T1- fish-absence, and T2- fish-presence, both with periphytic algae as food). We observed that fish presence decreased zooplankton functional diversity through modifications in the availability of nutrients and algae, through the middle-out effect. We also observed that body size had a negative relationship with the food resource, reaffirming that high food availability in subtropical lakes is linked to small-bodied zooplankton. The raptorial copepods covariate positively with the periphytic algae, which was an alternative food resource and, in this case, the main form of carbon input into the system. In this study, omnivorous fish reduced zooplankton functional traits, which can alter the energy stock and energy flow in aquatic ecosystems.

Key words: Experimentation, freshwater, functional traits, middle-out, periphyton, zooplankton body-size.

INTRODUCTION

To better understand the modifications in species diversity and organism abundance over time and space, recent ecological studies have investigated how environmental changes and ecological interactions influence diversity (McGill et al. 2006). Functional diversity has been an important tool to clarify the effects of environmental changes on a global scale, because through the functional diversity indices it is possible to estimate the health of ecosystems, such as the impacts of global change on biodiversity, ecosystem services, conservation and ecosystem restoration (Laliberte et al. 2010). Functional diversity measures usually estimate the differences between species traits and, consequently, they are more sensitive to communities' structural changes (Violle et al. 2007).

Functional traits can determine the species' responses to environmental disturbances and ecological interactions, revealing the effects on ecosystem processes (Mouillot et al. 2013, Arrieira et al. 2015, Santos et al. 2017, Braghin et al. 2018, Dunck et al. 2018). These organisms' traits

are reflected by how they interact among one another and with the ecosystem (Tilman 2001). The use of functional traits of the organisms, population, and communities functional indices increases the understanding of cycles of carbon and nutrients, the decomposition process, system productivity and the flux of matter and energy (Hébert et al. 2017). In this way, through the index of functional diversity, it is possible to link the organisms and the ecosystem process directly (Diaz & Cabido 2001).

Despite the recent increase in studies considering the functional approach, for zooplankton there are still some gaps to be explored. The first one is the difficulty of measuring the functional traits in small organisms (Martiny et al. 2013), which has been a barrier lasting decades and has only recently aroused the interest of ecologists once again (Hortal et al. 2015, Gomes et al. 2019). The second gap is related to the incipient knowledge of zooplankton in many unexplored ecosystems, due to the higher concentration of studies in a few regions of the world (Schwind et al. 2013). Finally, there is a gap in knowledge on the effects of zooplankton functional traits on the ecosystems, as most studies focus on the organisms response traits, which can be structured as results of ecosystem variations (Rosado et al. 2016, Gomes et al. 2019). Thus, the poor knowledge on zooplankton functional traits is a limiting factor to develop this kind of study.

Zooplankton plays important functions in aquatic environments ensuring the flow of matter and energy and linking primary producers (e.g., phytoplankton and periphyton) and secondary consumers (e.g., fish and macroinvertebrates) (Jeppensen et al. 2010). These organisms are one of the most abundant groups in aquatic environments and are consequently important in the cycling of nutrients by participating

in the trophic chain as consumers, including alternative food-webs such as microbial and debris (Balseiro et al. 1997, Segovia et al. 2016). Thus, the knowledge the structure and functional variability of the zooplankton community through functional diversity studies can assist in understanding the functioning of aquatic environments.

The effect via trophic cascade (bottom-up/top-down) is a classic approach in food-web studies, with the purpose of evaluating the mechanisms acting on the communities. When the predation effect is stronger (direct effect), there is a "top-down" regulation; and when the regulation is via the availability of food resources, there is a "bottom-up" regulation (Carpenter et al. 1985, Heath et al. 2014). Some studies that analyzed the predator-prey relationship in freshwater shallow lakes observed changes in the functional structure of the planktonic community by the effect of small fishes that feed on plankton. Examples of such changes are the differences in the body size of organisms or in the average size of communities where predation pressure is higher (Iglesias et al. 2008, 2011, Mazzeo et al. 2010, Chaparro et al. 2014).

A predator can influence other communities by indirect effects, leading to positive or negative consequences (DeVries & Stein 1992). In this way, trophic relationships create complex and unpredictable effects, and the reactions form a hybrid of top-down and bottom-up effects (Allen & Fulton 2010). The middle-out effect is a different approach to understanding food webs and illuminating patterns and processes that escape traditional trophic cascade knowledge and form hybrid effects (DeVries & Stein 1992, Stein et al. 1995). Thus, a fish can affect its prey by direct consumption, then reduces interspecific competition between prey individuals, subsequently increase nutrient cycling and

primary system productivity (Kaemingk et al. 2017).

In an approach of biomanipulation and functional diversity, Setubal & Riccardi (2020) showed that the removal of planktivorous fish from the food web, to control eutrophication in a shallow lake, changed the functional structure of zooplankton. An increase in the body size of zooplanktonic organisms was expected, due to the low predation pressure, increasing the abundance of large herbivores, which did not occur. Instead, there was a transitional process between the predominance of the pelagic food web to the coastal food web, with raptorial feeding mode, carnivorous preference and smaller body size.

Scenarios with nutrient enrichment and consequent increase in primary productivity may favor consumers at all trophic levels, due to the increase in biomass production (Attayde & Hansson 2001). The enrichment of nutrients alters the structure of the zooplankton community and their interaction with phytoplankton. More nutrient availability increases the development of primary producers, sometimes favoring non-edible algae, such as filamentous or colonial. These algae directly affect the zooplankton's secondary productivity and species dominance (Melo et al. 2019). Zooplankton body size is also affected by nutrient enrichment and phytoplankton composition, as large-sized algae might favor larger-bodied zooplankton organisms (Bomfim et al. 2018).

In the littoral region, periphytic algae can be an alternative and important food resource for the zooplankton community, especially the zooplankton adapted to living in this region (Rautio & Vincent 2006). The increase of diatom biomass in the epiphyton biofilm leads to a significant increase in the abundance of copepods and cladocerans in the littoral region (Cardoso & Marques 2004, Rosa et al. 2016). This

might occur when the phytoplankton quality energy decrease (e.g. bloom of cyanobacteria) or in events of scarcity (Faria et al. 2017). Zooplankton herbivory relationships with periphyton seem to favor larger-bodied organisms such as macrozooplankton; consequently, the periphyton becomes an important carbon source into the aquatic food webs (Faria et al. 2017).

In this study we had two aims: the first was to analyze the predation effect by an omnivorous fish (top-down effect) on the functional diversity of the zooplankton. The second was to analyze the possible herbivorous relationship of zooplankton on periphytic algae, as the patterns linking zooplankton functional diversity to periphytic algae are poorly known. Related to our first objective, we expect that intense predation on zooplankton will cause, (a) higher values in functional diversity indices attributed by regulation of dominance in the zooplankton community; for the second objective, we predict that the greater quantity of biomass and density of the periphytic algae will lead to (b) correlation with the feeding type trait, reinforcing the link with primary productivity of adhered algae, (c) greater raptorial copepods and scrapers cladoceran abundance (individuals with these traits), as they have specific food apparatus to get feed from the periphyton and (d) greater zooplankton body size (as a result of the prevalence of cladocerans and copepods than rotifers).

MATERIALS AND METHODS

Field sampling and establishment of microcosms

This study was set up in microcosms (40 L aquariums), located in an open area in the Advanced Research Base of the State University of Maringá/NUPELIA, on the Paraná River margins, in Porto Rico, Paraná, Brazil. The experiment

treatments were set up in fish-absence (T1-Zoo) and in omnivorous fish-presence (T2-Zoo+fish); each treatment had three replicates, resulting in six sample units.

Zooplankton – The microcosms were inoculated with a pool of zooplankton species (for the abundance of inoculated organisms see Supplementary Material - Table SI) sampled by vertical dragging with a plankton net of 45 and 68 micrometers, approximately 500 liters of water was filtered. The sample was performed in the littoral region of lakes in the upper Paraná River floodplain, Brazil (22°54'30.3 "S, 53°38'24.3 "W), close to macrophyte banks (*Eichhornia azurea* Kunth). *Fish* - Individuals of *Moenkhausia* aff. *sanctaefilomenae* Steindachner 1907 were sampled by acrylic traps (plexiglass-type minnow traps (Dibble & Pelicice 2010)), and acclimatized in an aquarium (for one week), with the assistance of air compressors. The selected fish were adults only, including randomly males and females, with mean length of 29.6 cm (min= 21 cm and max= 39 cm). They were fed (commercial fish feed) during acclimatization. Later they were inserted in the experimental aquariums with fish treatments.

In each microcosm were placed 35 liters of water from the Paraná River filtered in a plankton net (45 μm of mesh opening) and, in the fish-absence (Zoo, treatment T1) 350 ml of zooplankton mix were added to the filtered water; in the fish-presence (Zoo+fish, treatment T2), 350 ml of zooplankton mix plus seven individuals of *Moenkhausia* aff. *sanctaefilomenae* Steindachner was added to the filtered water.

The microcosms were randomly established in the open-air area, keeping the light/dark cycle; the experiment lasted 7 days. In each microcosm were placed 21 artificial substrates, which were built with glass slides and polystyrene. The artificial substrates had periphytic algae, which

had been previously colonized for 28 days, comprising the food resource for zooplankton and fish. Details of the experimental design, such as the colonization by algae in the substrates and calculations of algae biomass and density (used as predictor variables in this study) can be found in Dunck et al. (2018).

Ethical standards

This experimental research was approved by the Instituto Chico Mendes de Conservação da Biodiversidade from Brazil (SISBIO / ICMBIO; License nº 22442-1) and the Ethics Board for the use of experimental animals from the Universidade Estadual de Maringá (CEUA; Technical Advice nº 123/2010).

Aquarium sampling

Zooplankton samples were taken after a few hours of the experiment (time 0), and on the 7th day (final time). The samples from time 0 were considered as control of the zooplankton community, confirming the homogeneity of the mix placed in each sample unit. The samples from the final time were used to calculate the functional diversity. A total of 350 ml of water was filtered in a plankton net (45 micrometers) and subsequently preserved in 4% formaldehyde. At the end of the experiment, the fish were euthanized, placed in bottles and fixed in 10% formaldehyde for four days; after this period, they were preserved in 70% alcohol. The individuals were dissected, and the stomachs were removed and fixed in 70% alcohol. The stomach content was analyzed under a stereoscope, and then each food item was quantified and identified at the lowest taxonomic level possible, following specialized literature (Hyslop 1980).

Limnological variables were measured in all sample units with the multiparameter probe Horiba U-50, including water temperature (°C), pH, electrical conductivity (mS cm^{-1}), dissolved

oxygen (mg L^{-1}) and total dissolved solids (mg L^{-1}). Water samples were filtered in Whatman GF/F filters at low pressure (<0.5 atm) and stored at -20 °C for subsequent determination of total phosphorus (PT, µg L^{-1}) and total nitrogen (NT, µg L^{-1}), following the methodology described by Bergamin et al. (1978). The analyses of the environmental variables were carried out according to Roberto et al. (2009).

Laboratory analysis

The rotifers, cladocerans, and copepods were identified at the species level, following specialized literature (Koste 1978, Sendacz & Kubo 1982, Reid 1985, Elmoor-Loureiro 1997, 2010). The effort for the species identification continued until the accumulation curve stabilization. Zooplankton quantification (ind L^{-1}) was performed under an optical microscope in a Sedgewick-Rafter chamber. At least 50 adult individuals were counted from each species belonging to each zooplankton group (rotifers, cladocerans, and copepods). The counting was through three sub-samples with a Hensen-Stempell pipette, totaling 10% of the sample (methodology modified from Bottrell et al. 1976).Samples with fewer than 50 individuals were counted in full.

The 57 zooplankton species recorded in our experiment (32 rotifers, 15 cladocerans, and 10 copepods, Table SI) were classified into three functional response traits (Barnett et al. 2007): body size, escape response to predator and feeding type (Table I). The body size was obtained concomitantly with the quantification of the zooplankton by measuring the length (µm) of up to 20 individuals of each species, using the body size average in the analysis. For rotifers and cladocerans, measurements were taken according to the shape of the body, and measured between the upper head and the end of the carapace, without the helmet or spines,

when present (Ruttner-Kolisko 1977, Hardy 1989). For copepods, body length measurements were taken between the head and the last abdominal segment, except for the spines of the caudal branch (Azevedo et al. 2012). The trait of escape response to a predator was classified as low, medium, high and maximum. The feeding type trait was classified as filter, sucker, predator, raptorial and scraper, following Braghin et al. (2018).

Statistical analyses

To describe the variation in the limnological variables between treatments at the end of the experiment, a Principal Components Analysis (PCA; Pearson 1901) was performed. The variables were previously transformed into log10x+1, except the pH. Only the first axis was retained for interpretation, according to the Broken-Stick criterion (Jackson 1993).

To achieve the first objective and subsequent predictions, the indices of functional diversity were calculated. For that, a community matrix was made, containing individuals' abundance (transformed by the square root) per species in the columns and microcosms in the rows, and a functional matrix was made with the functional traits (traits in the columns and species in the rows); then these were transformed through a Principal Coordinates Analysis (PCoA; Laliberté & Legendre 2010) using Gower dissimilarity (Gower 1971), ordering the species in a multivariate functional space. Both qualitative and quantitative traits were applied to characterize the species. The first 22 axes of this ordination (PCoA) were used as a new functional matrix of the species for the estimation of functional diversity. The functional diversity of the zooplankton was calculated through four indices: functional richness (Fric), functional equitability (Feve), functional divergence (Fdiv) and functional dispersion (Fdis) (Mason et al.

Table I. Zooplankton functional traits by type of the trait, the categories of subdivision of each trait and its ecosystemic relevance.

Traits	Type	Category	Link with ecosystem functions
Body size	Continuum	Mean of body size (µm)	Body size is related to many body properties and physiological rates, such as: total body content in energy and elements, diet, excretion and basal metabolism, which directly affect the stocks or ecosystem flows (Litchman et al. 2013, Hébert et al. 2017). Another important function determined by body size is the cycling of nitrogen and phosphorus, as structured communities with larger organisms have greater influence on the recycling rate, assuming that organisms eat more and excrete more fecal pellets (Hébert et al. 2016).
Escape response to predator	Categorical	Low Medium High Maximum	Reducing the risk of predation increases the longevity of organisms, and one of the strategies of zooplankton is capture evasion (Litchman et al. 2013). This trait is related to the energy expenditure of organisms with the anti-predator strategy and with the allocation of biomass in the zooplankton and biogeochemical cycles (carbon sedimentation and vertical nutrient export (Hébert et al. 2017).
Feeding type	Categorical	Filter-R Sucker-R Predator-R Filter-Clad Scraper-Clad Filter-Cop Raptorial-Cop	This trait was chosen because it represents the efficiency of organisms in obtaining food and the type of food ingested, because each group requires different rates of nutrients and requirements (Andersen & Hessen 1991), contributing to the cycling of nutrients, primary productivity, algae composition through top-down regulation and productivity of heterotrophic bacteria, depending on the interaction or position of consumers in the food chain (Segovia et al. 2015, Hébert et al. 2017).

2005, Villéger et al. 2008, Laliberté & Legendre 2010). Fric is the potential niche volume occupied by all species of the community within the functional space (Schleuter et al. 2010). Feve is the uniformity of the distribution of the species' relative abundance in the functional space, in which high values indicate a regular distribution and low values are interpreted as an over-representation by certain traits (Mason et al. 2005, Schleuter et al. 2010). Fdiv is the heterogeneity of traits values in the community, representing the probability that two randomly chosen species have the same trait value (Lavorel et al. 2008), so high values are found by grouping species and/or abundance at the edges of the traits space (Schleuter et al. 2010). Fdis, on the other hand, is the dispersion of species in the multidimensional space: traits are weighted by their relative abundance, and high values are found when many species have similar abundance (Laliberté & Legendre 2010). To test the difference in each index of functional diversity between treatments, a t-test (Student) was performed. The assumptions of normality and homoscedasticity were previously verified through the Shapiro-Wilk and Levene tests, respectively.

In order to attend the second objective and respective predictions, a Fourth-Corner analysis (Legendre et al. 1997) was performed to

evaluate the relationship between the predictor variables (limnological) and the functional traits of the species in each treatment. The predictor variables were previously summarized as follows: NT and PT were grouped as *nutrients*, biomass and periphytic algae density as a *food resource*, pH and electrical conductivity as *chemical variables*. In this way, the number of variables was reduced and a better representation of the data was obtained. We also summarized these data through a Principal Coordinates Analysis (PCoA), transforming the three predictors into Euclidean distances, and used the first axis of each predictor for subsequent Fourth-Corner analysis. Thus, three matrices were used to perform the Fourth-Corner, one including the species distribution with the respective logarithmized abundance of organisms (L), another with the functional traits (Q), and the third containing the environmental variables (R - which included total dissolved solids and the first axis of PCoA for nutrients, food resource and chemical variables). Model 2 was used, which permutes the lines of the L matrix, with 999 permutations and a correction p-value significance, taking into consideration the correction for multiple comparisons, following the Benjamini & Hochberg (1995) method.

Furthermore, to achieve the second objective and predictions, Pearson's correlations coefficient was performed to investigate which categories of food types (numerical density of Filter-R, Sucker-R, Predator-R, Filter-Clad, Scraper-Clad, Filter-Cop and, Raptorial-Cop) covariates with the density and biomass of periphytic algae. We also investigated the relationship between zooplankton body-size and periphytic algae. For that, the values of body-size weight weighted by the abundance of species were used (CWM, Lavorel et al. 2008). The CWM is obtained through the default of the dbFD function, which is calculated together with the diversity indices. The CWM was correlated with the biomass and density of the periphyton, also through Pearson's correlation coefficient. All analyses and graphics were performed using the statistical software R (R Development Core Team 2019).

RESULTS

Description of limnological variables and fish diet

The first axis from PCA explained 53% of the total variability of the limnological data (Fig. 1). This axis was positively related to total phosphorus (0.96), total dissolved solids (0.95), total nitrogen (0.92), pH (0.86) and electrical conductivity (0.73). The ordination demonstrated the increase in nutrients, total dissolved solids, pH and electrical conductivity in microcosms with fish-presence (Fig. 1).

The analysis of the fish stomach contents revealed intense herbivory. The periphytic algae were the food item preferred by the fish (83%), and the ingestion of zooplankton contributed only 0.4% of items found in the stomachs (Table II).

Zooplankton functional diversity

The indices of zooplankton functional diversity differed between treatments. The Fdis mean was 0.15 in the T1 treatment (fish-absence) and 0.10 in T2 (fish-presence). The Fdiv mean value was 0.67 in T1 and 0.61 in T2; Feve presented mean values of 0.28 in T1 and 0.29 in T2. The Fric mean was 5.88 in T1 and 3.79 in T2. In general, the presence of fish reduced the functional dispersion (Fdis: t = 4.42, p = 0.01), the functional divergence (Fdiv: t = 3.16, p = 0.03) and the functional richness (FRic: t = 2.84, p = 0.04). Only the functional equitability was not affected by the fish-presence treatment (Feve; t = -8.21, p =1) (Fig. 2).
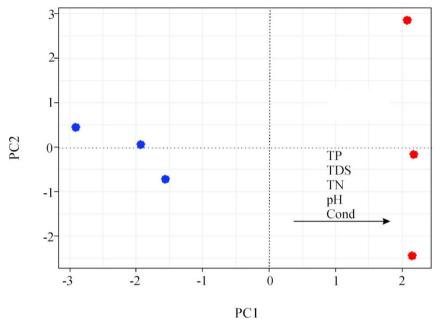
Figure 1. Graphic representation of the first two axes of the PCA (Principal Component Analysis). The points represent the treatments, where left is non-predation (T1) and right is the presence of predation (T2). Only the scores of the first axis were interpreted according to the Broken-Stick criterion. TP = total phosphorus, TDS = total dissolved solids, TN = total nitrogen, Cond = conductivity.

Table II. Diet of the fish during the experiment. Gross values and relative frequency of stomach items.

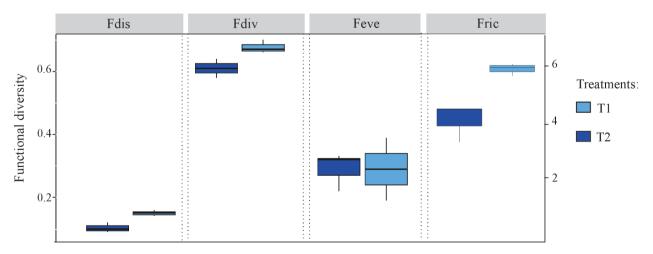| Stomach items	Quantity of the items	Relative frequency (%)
Typically periphytic algae	2490	83.45
Typically planktonic algae	83	2.78
Algae from both compartments	392	13.14
Zooplankton	13	0.44
Insects	6	0.20

Figure 2. Results of the indices of zooplankton functional diversity between treatments (non-predation - T1 - box of light color and predation by an omnivorous fish - T2 - box of dark color). The indexes of Fdis (functional dispersion), Fdiv (functional divergence), Feve (functional eveness) are represented on the y-left axis of the graph and, Fric (functional richness) on the y-right axis.

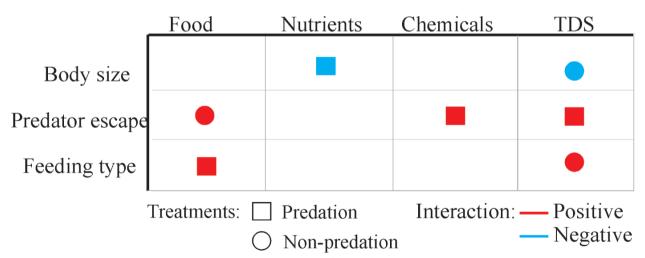## Correlations of predictor variables and functional traits

 Body-size was negatively related to nutrients in fish-presence treatment and to TDS in fish-absence, i.e., the higher the amount of nutrient or TDS, the smaller the body size of zooplankton organisms (Fig. 3). The escape response to predator was positively related to the food resource in fish-absence treatment, to the chemical variables and the TDS in fish-presence treatment. That is, the higher the value of the predictor variables, the greater the abundance of zooplankton organisms in each trait (Fig. 3). On the other hand, the type of feeding was positively correlated with the food resource in fish-presence treatment and with the TDS in fish-absence. Thus, the higher the concentration of the food resource and TDS in the fish-presence, the more traits of feeding type were present in the community (Fig. 3). Pearson's correlation revealed that only the feeding type of raptorial copepods was positively correlated with the biomass of periphytic algae (Table III).

DISCUSSION

The results reveal a mechanism that can be important in structuring the zooplankton community, the middle-out effect. This effect was produced by the presence of the omnivorous fish, which caused a reduction in zooplankton functional diversity. These results are supported by the fact that in our study, the intense predation of the omnivorous fish on the periphytic algae (direct effect, based on stomach analysis, Fernandes et al. 2020) started the process of nutrients enrichment in the microcosms. Thus, primary productivity increased and altered the variability of zooplankton traits (indirect effect).

The fish excrement increases the amount of nitrogen and total phosphorus in the water column (Attayde & Hansson 2001) and these have important indirect effects on

Figure 3. Graphic representation of significant correlations (p<0.05) of the Fourth-Corner analysis. The zooplankton functional traits are shown on the left side of the graph (rows) and the environmental variables (predictor variables) at the top of the graph (columns). Food represents the biomass of periphytic algae; nutrients are total nitrogen and total phosphorus; chemicals are pH and conductivity, and TDS is total dissolved solids.

zooplankton functional structure by food web (Setubal & Riccardi 2020). We attributed the environmental changes in our microcosms in fish-presence treatment (first axis of the PCA) to the intense predation by fish on algae. This process accelerated the cycling of nitrogen and phosphorus through the excrement released by the fish, consequently altering nutrients and food availability, which decreased the zooplankton functional diversity, reinforcing the middle-out effect (DeVries & Stein 1992, Kaemingk et al. 2017).

In addition, the Fourth-Corner showed that the zooplankton functional traits were related to specific environmental variables, suggesting that this was the main factor acting on the zooplankton functional structure, considering also the low predation by the fish. Environmental changes can alter a total species richness at a location, and cause also a shift in functional space occupation by removing species with traits that are poorly adapted to the new environment allowing colonization by better-adapted species (Mouillot et al. 2013, Violle et al. 2007). These environmental changes act as filters, mechanisms that allow the establishment of only species with traits favorable to that new condition, and the occurrence of similar trait among species (Dunck et al. 2016, Rosado et al. 2016).

It was also observed that the raptorial copepods covaried positively with the periphytic algae, suggesting an interaction in the trophic chain via the bottom-up, i.e., periphytic algae were an alternative path for food resource and, in this case, the main carbon input into the system. Predation of periphyton by fish may have promoted changes in the successional rates, as well as affected the abundance of the primary producers' size classes (Colina et al. 2016), allowing increase of palatable and non-palatable algae biomass (Dunck et al. 2018). In this way, the complex feeding apparatus in raptorial copepods (Huys & Boxshall 1991), allow them to select good quality food on periphyton (Cardoso & Marques 2004, Faria et al. 2017), as strongly adhered to the substrate and large algae, algal characteristics which do not represent a limiting factor for these zooplankton group.

In shallow lakes of the Neotropical region, small fish are abundant in the margins of the

Table III. Result of Pearson's correlations of feeding type categories (abundance) and body size of zooplankton (CWM) with biomass and periphytic algae density.

	Biomass		Density	
Category	*r*-Pearson	*p*	*r*-Pearson	*p*
Filter-R	0.14	0.78	-0.58	0.22
Sucker-R	0.17	0.73	-0.45	0.36
Predator-R	-0.51	0.29	-0.43	0.36
Filter-Clad	0.29	0.57	0.53	0.27
Scraper-Clad	-0.33	0.51	-0.34	0.50
Filter-Cop	0.20	0.69	-0.08	0.86
Raptorial-Cop	**0.94**	**0.004**	0.14	0.78
Body size	0.23	0.65	0.01	0.97

lakes and are associated with macrophytes, which they use for refuge and foraging, having an omnivorous diet (Agostinho et al. 2007, Chaparro et al. 2014). Thus, they occupy an intermediate level in the aquatic food chains, between primary producers, primary consumers and top predators (Mazzeo et al. 2010). In environments modified by human activities, such as the Paraná River which has a series of dams upstream, the abundance of these intermediate fish underwent major changes over time, attributed mainly to the construction of dams (Agostinho et al. 2008). This fact might affect the environments associated with the rivers (such as lakes and channels), generating a chain-response, considering that these fish reduced the functional variability of the zooplankton, and also increased the primary productivity the periphytic community, consequently increased the abundance of raptorial copepods, as shown in our results.

Theoretically, lower values in functional richness occur when the evaluated resources are available but are not being used, opening gaps in the functional space (Mason et al. 2005). Similarly, the species have more similar functional attributes when lower values are found in the functional divergence (Mason et al. 2005). Ecologically, in our study, it can be considered that the environmental changes provided by the fish caused a reduction in the functional space and favored the use of resources by the opportunistic species of zooplankton, which are mainly rotifer species that have a high fecundity rate, direct development, parthenogenic reproduction and fast growth rates (Pourriot et al. 1997). Furthermore, the group composed of the most abundant species in T2 (fish-presence) was functionally more similar than in T1 (fish-absence), as observed by the values of Fdiv. This could lead to greater competitiveness among the organisms and lower efficiency in the use of

resources, altering the ecosystem functioning, by reducing the decomposition rates, nutrients and carbon sequestration and causing a decrease in secondary productivity. All these changes could lead to less energy in the food chain, could affect fish recruitment and reduce water quality for human use, as a result of the "bloom" of algae with toxins (Laureto et al. 2015, Hébert et al. 2017, Jiang et al. 2017).

In a long-term study using the functional diversity of phytoplankton (Abonyi et al. 2018), on the Danube River, Hungary, the authors demonstrated that oligotrophication altered the functional composition of phytoplankton over time and provided greater functional dispersion by the gradual change of the community, where the species that presented high biomass was replaced by functionally distinct organisms. In our results, the decrease in functional dispersion in T2 (fish-presence) could be an inverse pattern to that observed by Abonyi et al. (2018). The environmental filters provided by the presence of the predator and by the increase in nutrients generated a replacement in the abundance of common zooplanktonic organisms, selecting traits that allowed the persistence of the organism in the microcosm (Ribera et al. 2001), leading to a loss in rare traits.

Body size is related to several ecosystem processes, and it is an important descriptor for zooplankton communities, providing information for an estimate of traits that are difficult to measure (Hébert et al. 2017). In our study, the trait of body size was negative influenced by the increase in nutrient concentration in fish-presence treatment. Normally, in these cases, the main food resource is small algae that are preferential prey for rotifers (Bomfim et al. 2018, Silva et al. 2019) which have fast reproduction and smaller body size (Havens 1998). In contrast, when the food resource is scarce, larger-bodied zooplankton have a competitive advantage, as

they can feed on alternative resources, such as bacteria, ciliates, heterotrophic flagellates (Segovia et al. 2015) and periphytic algae of different sizes (Dunck et al. 2018). Thus, even though it was not possible to analyze these other types of zooplankton food resources, we observed the increase in primary productivity by the influence of fish excrements on nutrient availability, favoring the abundance of smaller-bodied organisms, possibly due to the type of food resource available (nanoplankton and microplankton algae).

In addition, the trait of escape response to the predator was related to the increase in food concentration in the fish-absence treatment. Even though the relationship was positive, it is certainly an indirect relationship. This is because the heterogeneity in food resource is related to the increase in traits variability, and nutrients for that could come from zooplankton excretion, from the rupture of algae cells by the zooplankton during predation on algae and from the senescence of organisms (Andersen & Hessen 1991). Moreover, in this treatment (fish-absence), another analysis relationship was found between the trait feeding type and the concentration of total dissolved solids, indicating that the food resource is possibly phytoplankton too.

It is important to note that, despite the small number of replicates ("n"), our study demonstrated strong and significant relationship among the evaluated variables. Possibly, if the "n" were greater, the significance would also be. From these results, we believe that our study will help further studies with experimental approaches, that could evaluate the influence of other factors (such as different sources of productivity-phytoplankton, bacterioplankton, heterotrophic nanoflagellates; and/or with other predator- insect larvae) on the functional

structure of the food web, incorporating higher number of replicates.

In summary, this study demonstrated that small fish with an omnivorous diet can control the functional variability of aquatic communities not only by traditional trophic-chain (top-down and bottom-up) but also by the middle-out mechanism (DeVries & Stein 1992), acting directly on nutrient concentration and indirectly on zooplankton, when they are preying on phytoplankton and periphyton. Thus, our predictions were partially corroborated, and the middle-out effect caused by omnivorous fish was more important in zooplankton functional structuring than top-down effect and the consequence was a reduction in functional diversity, and the increase in biomass of periphytic algae increases the abundance of individuals with raptorial copepods traits (mainly copepods), but the interaction between zooplankton and periphyton did not change the body size of the zooplankton community.

In trophic chain studies, biotic interactions are commonly linked with a unidirectional sequence, where a trophic level directly influences the next trophic level (primary producer ↔ secondary producer ↔ predator). In this study, we demonstrated these interactions linked in a different way (predator → primary producer ↔ positive feedback via nutrient cycle → secondary producer), patterns analyzed with indices of diversity and functional traits as response variables, a modern approach to the trophic-chain. Also, we contribute to clarify ecosystem processes in freshwater environments, as well as the action of small fish accelerating nutrient cycling and primary productivity. However, it does not mean efficiency in the flow of matter and energy, since the functional diversity of zooplankton has decreased.

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DB and AD designed the project; DB, AD and FU contributed to the data collection and processing of biological samples; AD analysed the data and led the writing of the manuscript. DB, BL, BF, BC and LF contributed to the paper writing.