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FRANCIELI DE FÁTIMA BOMFIM

Predicting the effects of global warming and extreme climate events on zooplankton functional responses and ecological interactions

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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 Doutora em Ecologia e Limnologia.
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Orientador: Prof. Dr. Fábio Amodêo Lansac-Tôha
Coorientadora: Prof.ª Dr.ª Maria da Graça Gama Melão

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“Os que desprezam os pequenos acontecimentos nunca farão grandes descobertas. Pequenos momentos mudam grandes rotas”.

Augusto Cury

Predizendo os efeitos do aquecimento global e de eventos climáticos extremos nas respostas funcionais e interações ecológicas do zooplâncton

RESUMO

O aquecimento global pode afetar a distribuição e o desenvolvimento dos organismos indiretamente através das mudanças nos padrões climáticos e, assim, no *habitat* das espécies, e diretamente através da influência das altas temperaturas sobre o desenvolvimento fisiológico e morfológico dos organismos. Essas mudanças nos padrões de densidade, alimentação e reprodução das espécies conduzidas pelo aquecimento e mudanças ambientais induzem também alterações nas relações ecológicas em que esses organismos estão envolvidos. Desta maneira, tivemos como objetivo investigar como esses dois efeitos do aquecimento global, afetam a performance, a distribuição e as relações ecológicas das espécies zooplanctônicas, utilizando respostas funcionais dos organismos, como a produção de biomassa e traços funcionais, com o intuito de conectar essas alterações no zooplâncton com os serviços ecossistêmicos. Para responder a este objetivo, foram realizados experimentos em microcosmos controlando três temperaturas, predação e competição; e trabalho de campo com uma abordagem espacial e temporal na planície de inundação do alto rio Paraná. Nos experimentos observou-se que o aumento de temperatura de fato leva a relações mais complexas dentro das cadeias alimentares com possíveis consequências negativas para os ecossistemas aquáticos. Porém, a temperatura não foi o fator predominante em determinar a performance das espécies de cladóceros de climas temperado e subtropical, sendo que a predação foi o fator estruturante, alterando a performance e as relações de competição entre as espécies. Por meio da abordagem espacial e temporal, observou-se que os eventos climáticos extremos alteram a heterogeneidade limnológica dos lagos rasos com consequente alteração das espécies e dos traços funcionais do zooplâncton, especialmente por meio da substituição de espécies e traços entre os ambientes (*beta replacement*). Os fatores relacionados a predação, disponibilidade de alimento, variáveis limnológicas e as variações temporais influenciaram diferentemente a diversidade beta funcional do zooplâncton dependendo do evento climático e do tipo de lagoa (conectada ou isolada), mas,de fato as variáveis limnológicas foram o fator mais importante em estruturar a distribuição do zooplâncton. Tanto o aquecimento quanto as mudanças nos padrões climáticos (especialmente períodos de secas intensas) afetaram negativamente os organismos zooplanctônicos, com redução na produção de biomassa, na produção secundária, e na riqueza de traços funcionais entre os ambientes. A redução dessas variáveis funcionais do zooplâncton tem consequências negativas para os estoques de energia e para o fluxo de matéria dentro das cadeias alimentares aquáticas, com alterações nos serviços ecossistêmicos.

Palavras-chave: Predação. Competição. *Grazing*. Experimentação. Diversidade funcional. *La Niña* e *El Niño*.

Predicting the effects of global warming and extreme climate events on zooplankton functional responses and ecological interactions

ABSTRACT

Global warming can affect thedistribution and development of organisms indirectly through changes in climate patterns and thus, in the habitat of species and, directly through the influence of high temperatures on the physiological and morphological development of organisms. These changes in thepatterns of species density, feeding and reproduction driven by warming and environmental changes also induce changes in the ecological relationships in which these organisms are involved. Thus, we investigated how these two effects of global warming, affect the performance, distribution and ecological relationships of zooplankton species, using functional responses of the organisms, such as biomass production and functional traits, in order to link these changes in zooplankton with ecosystem services. This studyis divided into three approaches, microcosm experiments controlling three temperatures, predation and competition and, a spatial and temporal approach in the upper Paraná River floodplain. In our experiments, we observed that temperature increases indeed lead to more complex relationships inside food webs, with possible negative consequences for aquatic ecosystems. However, the temperature was not the predominant factor in determining the performance of subtropical and temperate cladoceran species, as predation was the mainly structuring factor, changing the performance and competition among species. The study with the spatial and temporal approach showed that extreme climate events modify the limnological heterogeneity of shallow lakes, consequentlyaltering zooplankton species and functional traits, especially by species and traits substitution among environments (beta replacement). Factors related to predation, food availability, limnological variables and, temporal variations influenced differently the zooplankton functional beta diversity depending on the climatic event and the type of lake (connected or isolated), but limnological variables are in fact the most important factor in structuring the zooplankton distribution. Both warming and changes in climate patterns (especially intense dry seasons) negatively affected zooplankton organisms, reducing biomass production, secondary production and, the richness of functional traits among environments. The reduction of these zooplankton functional variables has negative consequences for energy stocks and, the flow of matter inside aquatic food webs, changing the ecosystem services.

Keywords: Predation. Competition. Grazing. Experimentation. Functional diversity. La Niña and El Niño.

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SUMÁRIO

1 GENERAL INTRODUCTION

Theglobal temperature increase of 1.5 ºC in recent decades has caused an alarm in the international scientific community (IPCC 2014), mainly because it is a consequence of anthropic actions (Collins et al. 2013) and can have serious consequences for natural ecosystems (Loreau et al. 2001). According to thereport released in 2014 by the International Panel on Climate Change (IPCC 2014) and based on greenhouse gas emissions (Collins et al. 2013),there will bean increase between 0.3°C and 4.8°C (optimistic forecast - RCP 2.6 and pessimistic forecast - RCP 8.5, respectively) in the average global temperature by the end of this century.New reports and studies released recently show that this optimistic forecast is no longer possible to reach, as greenhouse gas emissions in the atmosphere break new records each year (WMO World Meteorological Organization, 2019).

The manifestations of warming can be seen in records of high temperaturesrecords, changes of weather patterns(such as storms, intensity and duration of floods and droughts), and rise of ocean levels because melting glaciers (Knutson et al. 2010; Callaway et al. 2012). Polar and temperate regions suffered the most changes in the average temperatures of the last century, with a warming of 4 °C (IPCC, 2014). Despite some assumptions that the negative effects are mild in tropical and subtropical regions due to naturally high temperatures (Parmesan 2007), studies show that species from the low-latitude regions will be highly influenced due to the limited adaptations generated by the smaller variations of the climate in evolutionary time (Pörtner and Knust 2007).

Aquatic ecosystems can be affected by climate variations, and are the first to undergo such changes, inducing modifications in species occurrence and ecosystem services (Beklioğlu et al. 2016). Global warming can affect the distribution and development of organisms indirectly through changes in climate patterns, and thus the species habitat, and directly through the influence of high temperatures on the physiological and morphological development of organisms (Meerhoff et al. 2012). Concerning climate patterns, the southern region of Brazil is influenced by the climatic events of El Niño and La Niña that lead to intense rains and droughts, respectively, being important to maintain the hydrological regime in Brazilian waterlands and aquatic biodiversity (Pineda et al. 2019). However, the equilibrium of these climatic events is also altered by global warming, which turned these events more frequent and intense, leading to significant physical and biological changes in aquatic ecosystems (Magrin et al. 2014; Cai et al. 2015; Cavalcantti et al. 2015).

Temperature is one of the main factors acting on the metabolism of organisms. High temperatures accelerate chemical reactions, accelerating physiological processes such as respiration rates, food assimilation rates, fertility rates, and growth rates (Kooijman 2010; Alcaraz et al. 2014), which in turn, increases metabolic demands and often reduces the performance and density of organisms because of the tolerance to high temperatures (Urban et al. 2016; Yashchenko et al. 2016). These changes in the density, feeding, and reproduction patterns of species driven by warming also induce changes in the ecological relationships in which these organisms are involved, such as interspecific and interspecific competition (Forster et al. 2011), predation (Thakur et al. 2018), and parasitism (Hall et al. 2006; Shocket et al. 2018), and can reduce stocks and availability of energy and matter in the environments, consequently affecting the ecosystems functioning (Thébault and Loreau 2003; O'Connor et al. 2012).

In aquatic ecosystems, the zooplankton community occupies a central place in the food chains, linking primary producers to secondary consumers (Hébert et al. 2017; Abo-Taleb 2019). Changes in the occurrence and distribution of these organisms can lead to a disturbance in the energy flow of aquatic environments, with consequences for ecosystem services (O'Connor et al. 2009). Based on these pronounced changes, driven by global warming, this work **aimed** to investigate how these two effects of global warming - the increase in temperature and the change in precipitation regime - affect the performance, distribution and ecological relationships of zooplankton species (temperate and subtropical), using functional responses of organisms in order to connect these changes in zooplankton with ecosystem services. To better respond to this objective, this thesis is divided into questions, the first two are an experimental approach and the third is a spatial and temporal approach:

1. How do warming alone and combined with predation alter the performance and competitive ability of cladocerans from temperate environments? What are the possible effects of these changes (in competition and predation because of warming) on ecosystem functioning?

2. How does the direct and indirect fish predation alter the performance of three subtropical cladocerans? What are the effects of warming on direct and indirect fish predation on cladocerans, and cladocerans predation on algae? Does the predation of fish on cladocerans benefit the algae population? What are the possible effects of warming on aquatic food-web interactions?

3. How do extreme climatic events of drought and rain (La Niña and El Niño), compared to neutral events, modify the limnological heterogeneity of shallow lakes (connected and isolated) in a dammed floodplain? How do these limnological changes, due to extreme climatic events, alter the distribution of species and zooplanktonic functional traits? What are the components of beta diversity (difference in richness or species/traits substitution) that most contribute to explain the variation in zooplankton community in each climatic event? What are the most important environmental variables (limnological, fish predation, food availability, and temporal factor) in structuring the variation of functional traits in each climatic event, and in each type of lake? Which zooplanktonic functional traits are related to predictors in each climatic event, and in each type of lake? And finally, how can these changes affect aquatic ecosystems?

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2 THE EFFECTS OF TEMPERATURE AND PREDATION ON PERFORMANCE AND COMPETITIVE ABILITY IN CLADOCERANS

ABSTRACT

Zooplankton body size shows a strong association with both temperature and fish predation pressure. For example, tropical zooplankton communities are dominated by small-bodied species, in line with both the higher fish predation pressure and the temperature size rule, predicting smaller body sizes at higher temperatures in ectothermic animals. Climate change not only entails higher average temperatures but is also expected to increase fish predation pressure. Here we quantified the unique and joint effect of the presence of fish predation and temperature on the performance in monoculture and competitive strength of three Daphniidae species that differ in body size: *Daphnia magna*, *D. pulex*, and *Ceriodaphnia reticulata*. We manipulated competition (monoculture, pairwise and three-species competition trials), temperature (20, 24 and, 28°C) and presence or absence of fish predation. Our results indicate that large-bodied species are more negatively affected by temperature and by predation than smaller-bodied species. Yet, in the absence of predation, *D. magna* dominates in competition experiments, even at high temperatures. *D. pulex* is more affected by predation than by high temperatures. The performance of *C. reticulata* was not affected by temperature, but this species had the lowest competitive ability under all conditions tested. Our results show that temperature strongly impacts the performance of the larger-bodied species but does not fundamentally change the competitive relationships between the three species of cladocerans that strongly differ in body size. To the extent that warming also increases fish predation pressure, however, we expect very strong shifts in zooplankton community composition, with a dominance of small-bodied species.

Keywords: Zooplankton, body size, freshwater ecosystems, global warming, ecological interactions.

2.1 Introduction

Freshwater communities are strongly affected by both temperature and predation, amongst others leading to pronounced shifts in body size distributions (Iglesias et al. 2011; Forster et al. 2012; Šorf et al. 2015). The temperature size rule predicts that ectotherms mature at smaller body sizes with increasing temperature due to greater metabolic demands (Atkinson 1994; Forster et al. 2011a; Hoefnagel et al. 2018). In addition to aquatic organisms, oxygen becomes more limiting, and smaller body sizes allow more efficient transport of oxygen to all the effector organs (Atkinson et al. 2001; Harrison et al. 2018). Size-selective predation is since long recognized as one of the key structuring processes in zooplankton (Brooks and Dodson 1965; Lampert and Sommer 1997). The prediction that both temperature and fish predation should shift the size distribution of zooplankton to smaller species and individuals is in line with the observation that in lowland tropics the zooplankton is dominated by small species (Iglesias et al. 2011), whereas at higher altitudes and latitudes larger zooplankton species become more abundant, at least during part of the growing season prior to the peaks in fish predation coinciding with the emergence of juvenile fish (Carpenter and Kitchell 1993).

Climate warming has by now affected all levels of biological organization (Scheffers et al. 2016). While aquatic ecosystems and their biota have been intensively studied in the context of climate change, it has remained a challenge to predict the effects of global warming on communities and ecosystems services (Jeppesen et al. 2014). A key reason for this is the complexity of processes that interact in determining the outcome of such a fundamental change as climate warming (Urban et al. 2016). Individuals and populations can respond through phenotypic plasticity and evolutionary trait change (Van-Doorslaer et al. 2009; Brans et al. 2017; De-Meester et al. 2018), or migrate (Norberg et al. 2012). The net response will, however, also strongly depend on species interactions and how they influence the fitness of focal species (Urban et al. 2016).

Global warming is expected to increase the strength of ecological relationships in aquatic ecosystems, such as predation (Thakur et al. 2018) and host-parasite interactions (Hall et al. 2006; Shocket et al. 2018) turning these relationships more complex, and strongly impacting population dynamics, community composition and ecosystem structure (Dossena et al. 2012). For example, Shocket et al. (2019) have observed that at high temperatures the elevate foraging rates of *Daphnia* populations can increase the exposure and transmission rate of parasites. While the increase in predation rates by fish on *Daphnia* (due metabolic demands, Meerhoff et al. 2007) can inhibit such epidemics (Hall et al. 2006) by reducing the host densities. Through the resulting increase in consumption and reproduction rates, climate warming is also expected to lead to an increase in competition for food and space (Forster et al. 2011b). These direct and indirect responses to climate warming are thus expected to result in profound shifts in the size structure of populations and communities, and the structure of food webs (Thébault and Loreau 2003).

Herbivorous cladocerans, and especially members of the Daphniidae, share similar ecological niches and compete strongly for food resources (Hu and Tessier 1995; Adamczuk 2010). In general, large-bodied Daphniidae species suppress small-bodied species because they are more efficient grazers and are more energy-efficient (Gliwicz and Kerfoot 1980; Jiang et al. 2014). However, small-bodied cladocerans can become dominant when visual predation selects against larger individuals (Brooks and Dodson 1965; Iglesias et al. 2011). Thus, under climate warming, increased metabolic demands are expected to lead to higher predation rates and more intensive competition. This leads to conflicting predictions on whether body sizes should increase or decrease under climate change. Direct effects of temperature should lead to an increased dominance of smaller-bodied species, an increase in visual predation pressure similarly should shift the body size distribution of zooplankton towards smaller individuals (Iglesias et al. 2011), but increased competition and increased predation by invertebrates should have the opposite effect.

Here we carried out a laboratory microcosm experiment directly testing for the unique and joint effects of temperature, fish predation pressure and competition on population biomass and species composition using three cladocerans of the Daphniidae family that differ in body size: *Daphnia magna* Straus, 1820, *D. pulex*Leydig, 1860, and *Ceriodaphnia reticulata*(Jurine, 1820). We expect that 1) warming impacts the species performance and has a stronger effect on large-bodied than on small-bodied species thus, 2) the competitive dominance of large-bodied species over small-bodied species is reduced at higher temperatures. In addition, we assessed 3) whether and to what extent the direct effect of temperature alone can switch the relative dominance of large- versus small-bodied cladocerans, or whether fish predation pressure is needed to enforce such a switch.

2.2 Materials and Methods

All the experiments were conducted at the Laboratory of Aquatic Ecology, Evolution, and Conservation, KU Leuven/ Belgium in the first semester of 2019. We used three experimental temperatures for cultivation, life-history and competition/predation experiments. The experimental temperatures were 20°C, 24°C, and 28°C. The two first, 20°C and 24°C reflect the average in summer temperature in Belgium rural and urban population (respectively) (Brans et al. 2018), the third one (28°C) was chosen based on the pessimistic forecast to climate change from Intergovernmental Panel on Climate Change (IPCC 2014).

2.2.1 Study organisms, cultivation and life-history

The three species selected for our experiment vary widely in body size, arewidespread and frequently found in Belgian ponds and shallow lakes (Louette et al. 2007). *Daphnia magna* (mean ± SD = 3.2 ± 0.36 mm), *Daphnia pulex* (mean ± SD 1.87 ± 0.23 mm) and

Ceriodaphnia reticulata (mean ± SD 0.87 ± 0.15 mm) are classified in this study by size as large, medium and small-bodied species, respectively. All species were collected from communities sampled in ponds and shallow lakes in Belgium, then isolated and cultured in laboratory. The experimental species were cultured for almost three months (multiple generations by cyclical parthenogenesis) under standardized conditions (in 1 L glass jars, dechlorinated tap water, 14 h light/10 h dark photoperiod and at the three experimental temperatures). Half of the culture medium (dechlorinated tap water) was renewed twice a week. To ensure no limitation by food availability, the species were fed with a mixture (1:1 ratio/carbon) of two different algae species, *Chlorella vulgaris* (Beyerinck) and *Scenedesmus obliquus* (Turpin-Kützing). Both algae species were cultured in isolation with the addition of trace elements.

To analyze the effect of fish predation on cladocerans, we used pumpkinseed sunfish (*Lepomis gibbosus* Linnaeus 1758), with body-size varying between 8 and 10 cm. This species was chosen because it occurs in Belgian lakes, accepts the experimental temperatures (Power and Todd 1976) and feeds on the three cladoceran species (observed in a previous experiment performed before the final experiment). The fishes were maintained at the three experimental temperatures with air pump, the water was renewed three times a week, they were fed with Chironomidae larvae every day (the usual procedure for feeding), including the days after the predation on the experimental replicates. This experiment has been approved by the Ethical Commission from the Katholieke Universiteit Leuven under the registration: P006/2019 (LA1210204).

We carried out life history experiments for *D. magna*, *D. pulex* and *C. reticulata* at 20°C, 24°C and 28°C (constant temperatures), 14 h light/10 h dark photoperiod. Neonates (< 24h old) were placed in 100 ml jars with dechlorinated tap water and food (1:1 ratio/carbon) (10 replicates, with 1 neonate each), the experimental medium and food were renewed every day. The bionomic parameters such as body length for all life stages (neonates, juveniles, and adults), the presence of exuviae(cladocerans' exoskeleton remaining from molt), posture, and the number of eggs were daily observed in a stereomicroscope until the second clutch. We also calculated the dry weight (using drying oven at 70°C, over 48 h) for each species in each development stage (neonates, juveniles, adults and ovate adults), with five replicate each. The data from life-history parameters and dry weight are in the Appendix A. With our length and weight data, we build a length-weight regression to calculate the biomass ($\mathrm{LnW} = \mathrm{Ln}a + b\,\mathrm{LnL}$) (Bottrell et al. 1976). The equation includes the weight logarithmic transformation (W) of dry weight µg (DW) and the length (L in mm), and, a = intercept estimation and, b = slope estimation.

2.2.2Experimental set-up

In order to analyze the effect of temperature on the competitive ability of the species and, the combined effect of temperature and predation on cladocerans competition, it was set up a randomized three-way factorial design manipulating: competition (absence/presence with different species combinations); temperature (20, 24 and, 28°C) and predation (absent/present). The treatments were divided as follow, *monoculture* treatments: consisting of the species alone (as a control, i.e. in absence of interspecific competition); *competition* treatments: with the three species combined; and *predation* treatments with the same combination from competition but under fish predation (Table 1). Ninety-nine microcosms of 5 L (four competition levels x two levels of predation + three levels of monoculture x three temperature levels x three replicates) were placed in "Bain Marie" with controlled temperature (20, 24 and 28°C ± 1°C) and 14 h light/10 h dark photoperiod. All microcosms contained artificial plants as a refuge for zooplankton. Half of the experimental medium (dechlorinated tap water) was renewed twice a week. The species were fed with a mixture (1:1 ratio/carbon) of *C. vulgaris* and *S. obliquus* every two days.

The experiment began with the individual numbers standardized by biomass, the biomass used for each species (adult females) were as follows, *D. magna* = 28.6 µg DW ind^{-1}; *D. pulex* = 8.1 µg DW ind^{-1} and *C. reticulata* = 3.2 µg DW ind^{-1}. The initial biomass in each microcosm was calculated to be 286 µg DW i.e. equivalent to ten *D. magna* adults. When there were two species, each one should sum approximately 143 µg DW (286÷2) and when there were three species, each one should sum approximately 95.33 µg DW (286÷3) (Table 1).

Table 1. The number of individuals at the beginning of the experiment standardized by biomass in each microcosm, showed by species, by combination in the competition/predation treatments and the acronym for each combination. *Body size.

Species combination	**Treatments**	**Acronym**	**Individual numbers**
D. magna	Monoculture	Mono	10
D. pulex	Monoculture	Mono	36
C. reticulata	Monoculture	Mono	90
D. magna X *D. pulex*	Competition (large x medium*)	Mxp	5+18
D. magna X *C. reticulata*	Competition (large x small*)	Mxc	5+45
C. reticulata X *D. pulex*	Competition (small x medium*)	Cxp	45+18
C. reticulata X *D. pulex* X *D. magna*	Competition (small x medium x large*)	Cxpxm	30+12 +3
D. magna X *D. pulex*	Predation (large x medium*)	mxp.F	5+18
D. magna X *C. reticulata*	Predation (large x small*)	mxc.F	5+45
C. reticulata X *D. pulex*	Predation (small x medium*)	cxp.F	45+18
C. reticulata X *D. pulex* X *D. magna*	Predation (small x medium x large*)	cxpxm.F	30+12+3

In the predation treatments, based on previous pilot experiments, we randomly and carefully put one fish per aquarium on the 10th,15th and 20th day for 10 minutes (using a chronometer). Each fish was used just once a day, the fishes used in each temperature correspond to the temperature where they were maintained. All the microcosms were sampled in the middle of the experiment (10th day, 20% of the water volume = 1L) and, in the end (20th day, 100% of the water volume = 5L). The predation microcosms were sampled at these same days before and after the fish predation, totalizing 270 samples (the data over time can be seen in the Appendix B). The samples were fixed with formalin (4%) plus sugar to better conserve the animals. At the end of the experiments, each sample (previously concentrated in 100 mL) was fully quantified by size class (neonates, juvenile, adults, and ovate adults). The density (ind. L^{-1}) was transformed in biomass (µg DW L^{-1}) following the regression already cited.

2.2.3Statistical analyses

With the purpose of investigating whether the temperature and competition had an effect on each species (*D. magna, D. pulex,* and *C. reticulata*) biomass, we performed two-way analyses of variance (ANOVA). The two categorical independent variables were temperature (20, 24, 28°C) and competition combinations (monoculture, mxp, mxc, cxp, cxpxm), the dependent variable was biomass. Post-hoc analyses (Tukey HSD) were performed to verify which treatments differ from each other (these analyses respond to the two first predictions). To analyze whether the temperature and predation had an effect on the competition of each species we performed three-way analyses of variance (ANOVA). In these analyses the three categorical independent variables were temperature (20, 24, 28°C), competition combinations (mxp, mxc, cxp, cxpxm) and fish predation (presence and absence), the dependent variable was biomass. Post-hoc analyses (Tukey HSD) were performed to verify which treatments differ from each other (these analyses respond to the third prediction). To performed ANOVA and post-hoc analyses we used the packages “car” (Fox et al. 2019), “lattice” (Sarkar 2008) and “lmtest” (Hothorn et al. 2019) in R.

To better visualize the species distribution in each treatment and thus, the species performance, we performed an NMDS (non-metric multidimensional scaling).The distances were tested by a PERMANOVA (Permutational analysis of variance). Through the PERMANOVA was possible to access the significance of the species distribution under influence of temperature (a), predation (b) and under different combination of competition (c).

For these analyses were used a, b, c as independent variables and, a matrix with species biomass as a dependent variable. For PERMANOVA we used the "Bray-Curtis" distance and 999 permutations. The packages used were "vegan" (Oksanen et al. 2019) and "MASS" (Ripley et al. 2019) in R.

Finally, to analyze the relative contribution (%) of each factor on the species biomass, we performed a partial redundancy analysis (pRDA). Four matrices were used: a biological matrix (dependent variable, biomass)and other three representing the explanatory variables (competition, temperature and, predation). For pRDA, we used the package "vegan" (Oksanen et al. 2019) in R. For all analyses that we performed (described above) the assumptions were verified, and the biomass was transformed using square root in order to achieve the assumption of normality. The significance level adopted was $p \leq 0.05$. All the graphics were performed using the package "ggplot2" (Wickham et al. 2019) in R. All the analyses were performed using the program R Core team (2019).

2.3 Results

2.3.1 Temperature and competition effects

Temperature, competition and the interaction between them had a significant negative effect on *D. magna* and *D. pulex* biomass ($p < 0.001$, Fig. 1, Table 2). Only competition treatments had a significant negative effect on *C. reticulata* biomass ($p < 0.001$, Fig. 1, Table 2). Post-hoc analyses indicated that the increase from 20 °C to 24 °C and 28 °C significantly reduced biomass production of *D. magna* ($p=0.003$, $p=0.001$ respectively) and of *D. pulex* ($p=0.004$, $p=0.001$ respectively). *D. magna* and *D. pulex* had higher biomass production in the monoculture treatments followed by the combination with *C. reticulata* and, lower biomass in the treatment with the three species together (Fig. 1). *D. magna* and *D. pulex* affected each other, but *D. pulex* was the most affected. *C. reticulata* had higher biomass production in monoculture treatment and was affected by competition in all species combinations (Fig. 1, p< 0.001).

Table 2. Two-way ANOVA results, tested for competition (C) and temperature (T) effects on each species biomass. d.f.: degrees of freedom. Significant results are shown in bold.

Species biomass under competition and temperature treatments									
	D. magna			***D. pulex***			***C. reticulata***		
Factor	**d.f.**	**F-value**	***p-value***	**d.f.**	**F-value**	***p-value***	**d.f.**	**F-value**	***p-value***
C	3	8.97	< **0.001**	3	9.38	< **0.001**	3	147.8	< **0.001**
T	2	15.98	< **0.001**	2	9.81	< **0.001**	2	1.97	0.161
C:T	6	5.34	**0.030**	6	2.72	**0.037**	6	1.04	0.423

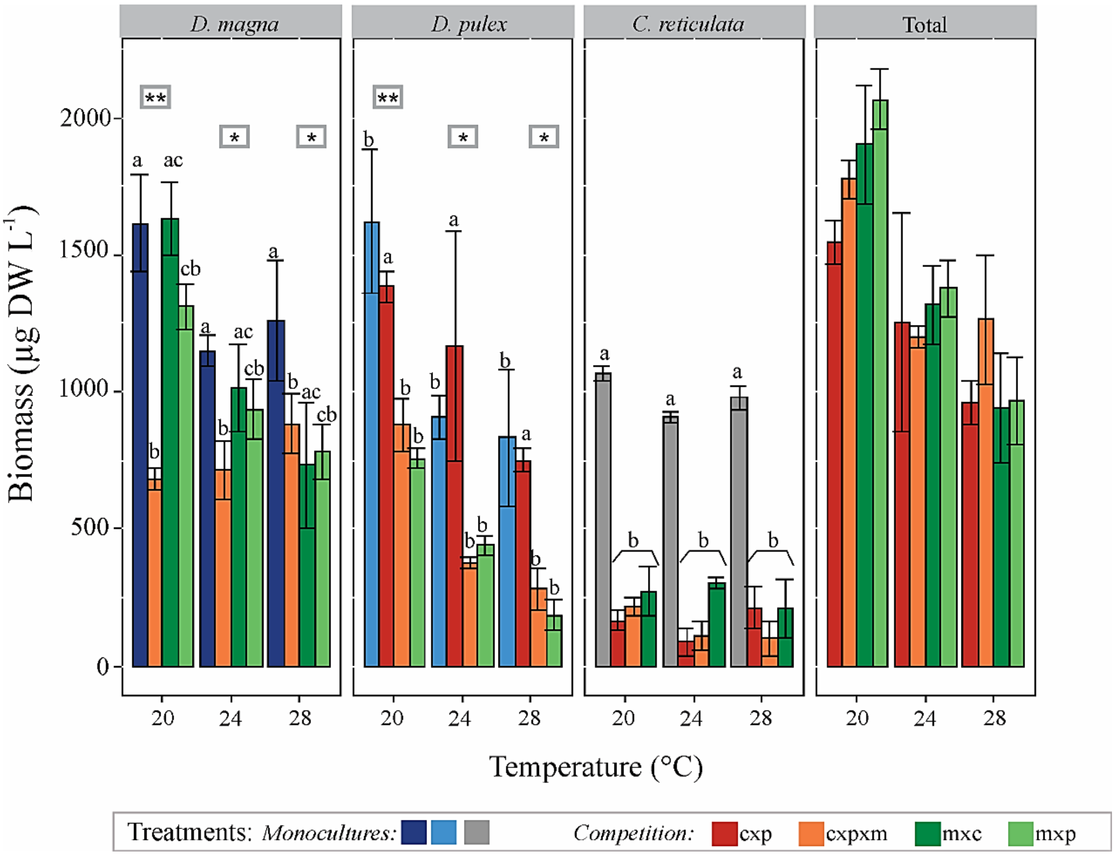

Fig. 1 Box plots depict biomass production by temperature, by speciesand by total biomass (the species summed) in each competition treatment. Monocultures, cxp: *C. reticulata* x *D. pulex*; cxpxm: *C. reticulata* x *D. pulex* x *D. magna*; mxc: *D. magna* x *C. reticulata*, mxp: *D. magna* x *D. pulex*. The central lines denote the mean value, whiskers represent ± standard error. Letters above

the columns indicate significant differences in post-hoc analyses between competition treatments - treatments that share a letter do not differ significantly. The asterisk indicates differences in post-hoc analyses between temperature treatments - treatments that share the asterisk amount do not differ significantly.

2.3.2 Temperature, competition andpredation effects

D. magna biomass was significantly affected by the interaction between temperature, competition, and predation (p= 0.024, Table 3, Fig. 2). *D. magna* was extinguished at the end of the experiment at 24 and 28°C under predation pressure. At 20°C, *D. magna* had higher biomass in combination with *C. reticulata* than when were the three species together (Fig. 2, p= 0.021). We also observed interaction between competition and predation on *D. magna* and *D. pulex* biomass (Table 3). Under predation pressure, in the combination with *C. reticulata, D. pulex* had higher biomass than in other combinations (Table 3, mxp p=0.000, cxpxm p=0.005). Both competition and predation independently influenced*C. reticulata*performance, that increasedthe biomass in combination with *D. magna*under predation pressure (cxp p=0.029, cxpxm p= 0.006, Fig. 2).

Table 3. Three-way ANOVA results, tested for temperature (T), competition (C) and predation (P) effects on the biomass of each species. d.f.: degrees of freedom. Significant results are shown in bold.

	Species biomass under competition and predation treatment								
	D. magna			***D. pulex***			***C. reticulata***		
Factor	**d.f.**	**F-value**	***p-value***	**d.f.**	**F-value**	***p-value***	**d.f.**	**F-value**	***p-value***
T	2	19.80	**0.000**	2	31.39	**0.000**	2	0.04	0.836
C	3	3.95	**0.027**	3	10.47	**0.000**	3	5.99	**0.005**
P	1	931.55	**0.000**	1	98.80	**0.000**	1	7.49	**0.009**
T:C	6	5.74	**0.006**	6	0.12	0.879	6	0.02	0.981
T:P	2	0.80	0.374	2	2.37	0.131	2	2.84	0.099
C:P	3	2.20	0.123	3	5.44	**0.008**	3	0.06	0.938
C:T:P	6	4.089	**0.024**	6	1.31	0.281	6	1.41	0.253

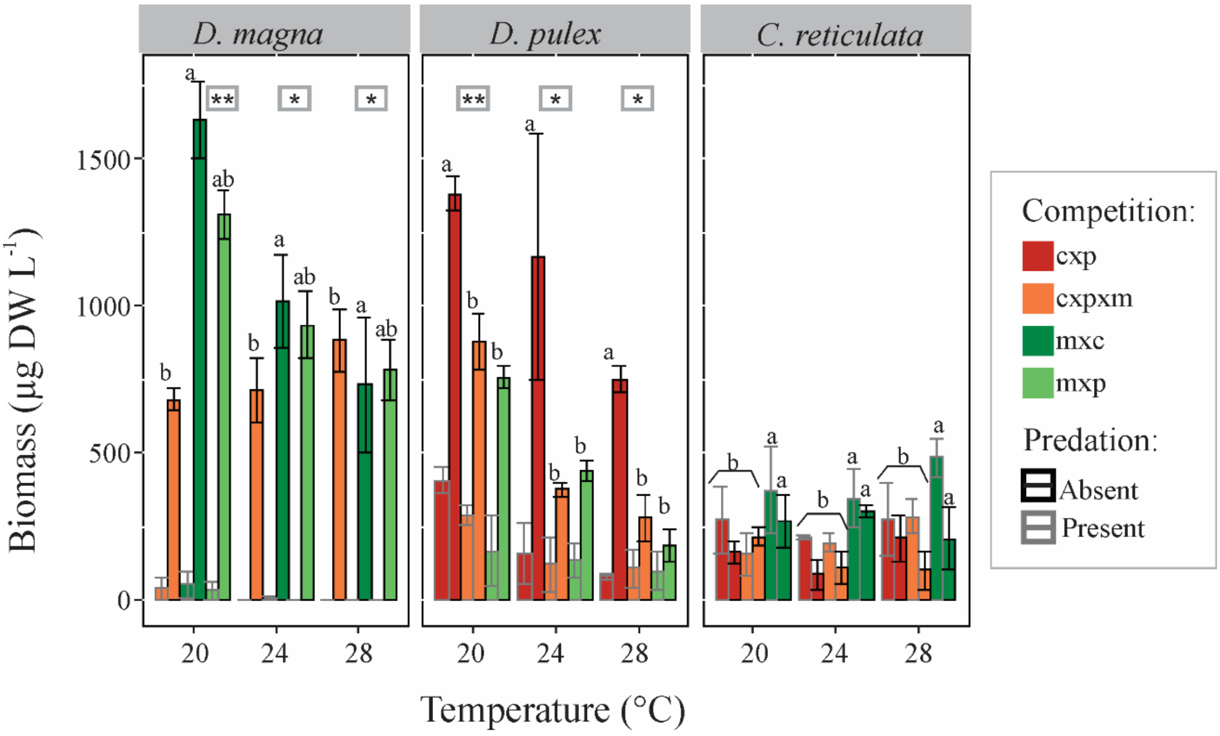

Fig. 2 Box plots depict biomass production by temperature and by species in each treatment of competition and predation. (cxp: *C. reticulata* x *D. pulex*; cxpxm: *C. reticulata* x *D. pulex* x *D. magna*; mxc: *D. magna* x *C. reticulata*, mxp: *D. magna* x *D. pulex*). The central lines denote the mean value, whiskers represent ± standard error. Letters above the columns indicate significant differences in post-hoc analyses between competition treatments - treatments that share a letter do not differ significantly. The asterisk indicates differences in post-hoc analyses between temperature treatments - treatments that share the asterisk amount do not differ significantly.

Predation had an overarching effect mediating the influence of both competition (Pseudo-F = 8.68, p = 0.001) and temperature (Pseudo-F = 8.79, p = 0.002) on biomass distribution among treatments (Table 4, Fig. 3) (post-hoc results are shown in the Appendix C). The distances represented through the NMDS show that the treatments under predation pressure presented a more similar distribution of biomass (homogenization),being especially associated with a higher biomass of *C. reticulata* when this species was present (Fig. 3). Without predation pressure, the competition treatments separated the biomass composition following the species combination used (Fig. 3). In the treatments that there was the presence of *D. magna*, the biomass distribution was most associated with this species, especially in both the two-species treatment (*D. magna* with *C. reticulata* and *D. magna* with *D. pulex*)

(Fig. 3, $p > 0.05$). The competition between *D. pulex* and *C. reticulata* was most associated with *D. pulex* without predation pressure. In the absence of predation, biomass composition tended to differ among temperatures, with increased contribution of *C. reticulata* in warmer treatments (Fig. 3).

Table 4 Permutational analysis of variance (PERMANOVA) results tested for temperature (T), competition (C) and predation (P). d.f.: degrees of freedom, SS: the sum of the squares. Significant results are showed in bold.

	Df	**SS**	**R^2**	**F-values**	**p-values**
T	2	0.197	0.008	9.00	**0.001**
P	1	3.783	0.170	172.72	**0.001**
C	3	15.596	0.701	118.68	**0.001**
T:P	2	0.192	0.008	8.79	**0.002**
T:C	6	0.068	0.003	0.52	0.916
P:C	3	0.570	0.025	8.68	**0.001**
T:P:C	6	0.136	0.006	2.08	0.053

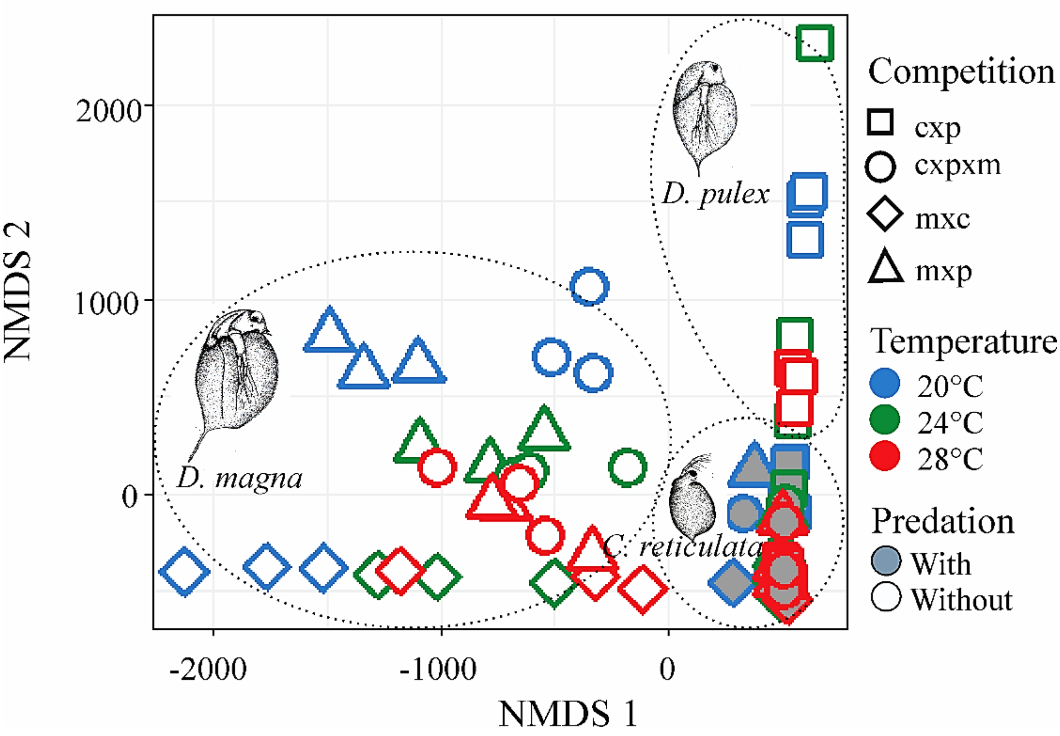

Fig. 3 Non-metric multidimensional scaling showing the distances among the species in the competition treatments (cxp: *C. reticulata* x *D. pulex*; cxpxm: *C. reticulata* x *D. pulex* x *D. magna*; mxc: *D. magna* x *C. reticulata*, mxp: *D. magna* x *D. pulex*) temperature and, predation.

The partial RDA confirmed that competition had a huge effect in explaining the variation of*C. reticulata* (R^2=0.80 p =0.000) and, temperature did not explain the biomass variation of this species (Fig. 4). The biomass variation of *Daphnia pulex* was more explained by predation (R^2=0.24 p =0.001), followed by temperature (R^2=0.14, p=0.004). The component predation was the one that most explained the variation of *D. magna* biomass (R^2=0.59, p = 0.001), followed by competition (R^2=0.05%, p = 0.001) and temperature (R^2=0.03, p = 0.002) (Fig. 4).

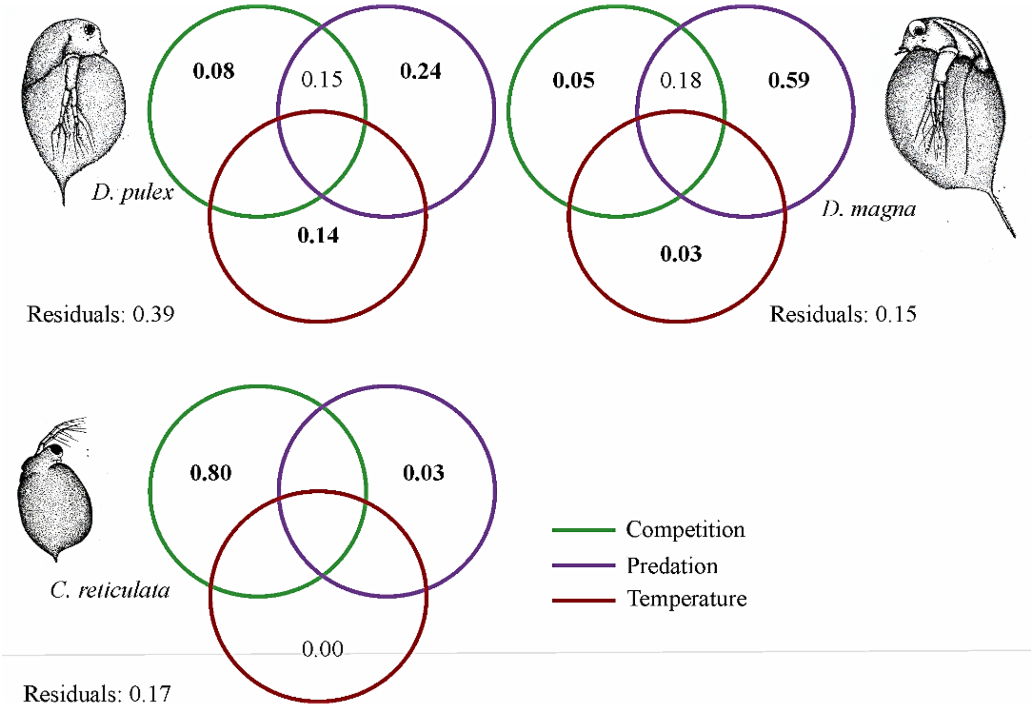

Fig. 4 Venn diagrams of partial redundancy analysis (pRDA) results. Relative contribution (adjusted R^2) of competition, predation, temperature, the shared component and residuals that explain the *D. pulex*, *D. magna*, and *C. reticulata* biomass variation. Bold numbers indicate significant values (p<0.05).

2.4 Discussion

The climate warming has affected many ecological levels, leading to changesin the species occurrence. But, the decrease or even loss of some species considered key-species can have serious consequences to ecosystems' functionality (Loreau et al. 2001; Antiqueira et al. 2018). Our results reaffirm important ecological issues. Fish predation had a strong negative effect on the largest species and a positive effect on the smallest, switching the dominance of large-bodied on small-bodied species. The combined effect of temperature and predation,as well, the combination of temperature and competition affected negatively the large-bodied species (both *Daphnia*). In this way, the combined effect of competition and predation at higher temperatures may favor small-bodied species at the expense of large-bodied, causing a homogenization in the composition of aquatic communities, which implies in a disruption in the energy flow, affecting other levels inside food-webs and, the ecosystems functioning.

We were expecting a negative progressive relation between temperature and biomass production, especially for the large-bodied species, since the energy demands increase with body size (Goulden et al. 1982) and can be more intense at elevated temperatures, causing high stress and metabolic losses (Kooijman 2010), which reduce the biomass production. The increase in temperature had a stronger negative effect only on *D. pulex.* That gradually decreased biomass production with the increase in temperature, being even more affected by competition and predation at higher temperatures. Other studies have shown that *D. pulex* is more sensitive to other stressors (such as predation and, contaminations) when they need to deal also with higher temperatures (Jaikumar et al. 2018).*D. magna* was also negatively affected by temperature, while this variable had no influence on *C. reticulata*. Although the temperature had an effect on *D.magna*, this variable wasn’t the most important factor to explain *D. magna* performance. As shown in other studies, *D. magna* might be responding to higher temperatures through phenotypic plasticity and/or adaptive evolutionary trait, by genetic changes in body size and increasing hemoglobin concentrations (Van-Doorslaer et al. 2009; Geerts et al. 2015; Brans et al. 2017). *D. pulex* also showed in previous studies that can adjust hemoglobin production above 20 °C expressing phenotypic plasticity to warming

(Gerke et al. 2011). The adjustments in hemoglobin quantity and quality in response to temperature is an essential mechanism for thermal acclimation as the stress came from oxygen supply and demand at high temperature (Pörtner and Knust 2007; Gerke et al. 2011), especially in aquatic ecosystems where the oxygen may become limiting with the warming (Forster et al. 2012). This temperature pattern concerning the three species may also be related to inherent temperature tolerance, linked to geographic distribution in natural habitats. *D. magna* and *D. pulex* are predominant in temperate environments (Sarma et al. 2005), whereas *C. reticulata* also occurs in tropical and subtropical regions (Smirnov et al. 1995).

Concerning competition, energy allocation is positively related to body size and, as *D. magna* is one of the biggest species in the plankton, normally, it has also bigger lipid accumulation (Goulden et al. 1982), this species has also great efficiency on feeding. These characteristics gave *D. magna* greater competitive advantage on *D. pulex* and on *C. reticulata*, while *D. pulex* had an advantage on *C. reticulata* probably due to the same reasons cited above (body size related, as the larger species are normally the best competitors (Hart and Bychek 2011)). Thus, both *Daphnia* species have a bigger potential to compete with *Ceriodaphnia* but not in all scenarios tested, as predation might favor small-bodied species. These potential shifts in species composition, due to the interaction between warming and competition, might reflect in gaps in the aquatic environments related to the different ecological functions that each species plays and, the connection that they have inside food-webs (O'Connor et al. 2009). Moreover, we were expecting higher *C. reticulata* biomass production in the monoculture and predation treatments but *Ceriodaphnia* persisted in low numbers, this could be connected to the natural (annual) oscillation of *Daphnia* populations in temperate ponds and, the differences in energy allocation (Goulden et al. 1982), as these two genera, have different tactics that may lead to coexistence in natural environments, even in the condition that *Ceriodaphnia* could be dominant.

Regarding predation, there is an old discussion why large Daphniidae as *D. magna* aren’t in the tropics, is that because of energy demands or because of predation? Our study suggests once again that it is by the predation effect. *D. magna* is always highly preyed by visual predators, including *D. magna* neonates by predators that select small-bodied prey, such as *Chaoborus*(Viaene et al. 2015). The high densities, large body size, and energy content make *D. magna* the first choice for predators in temperate ponds (Goulden et al. 1982; Lazzaro 1987). *D. pulex* was also affected by predation but survived in lower densities even at high temperatures. We observed that *D. magna* only survived from predation at the lowest temperature (even at low densities), presenting also better recovering capacity at this temperature (Appendix B). Therefore, the combined effect of temperature and predation reduced drastically both *Daphnia* species, which caused a strong homogenization in the treatments, overpowering the competition of *D. magna* and *D. pulex* on *C. reticulata*. The reduction in biomass production by the large-bodied species such as *Daphnia* species could lead to consequent cascading changes in the natural ecosystems. *D. magna* and *D. pulex*are key-species on temperate ponds, with great efficiency of food acquisition, been important in the phytoplankton control and in energy cycling (Dodson 1974; Scheffer et al. 1993). Changes in the predominance of these species lead by the increased predation and warming, reflect directly in the energy stocks inside aquatic food-webs, altering primary and secondary productivity and, the matter cycling (O’Connor et al. 2009; Kratina et al. 2012; Thakur et al. 2018).

Many studies showed that predation reduces cladocerans' body size distribution in two ways, instantly by selecting large-bodied prey and by inducing the reduction on body size over time (as preys respond to predation producing small individuals) (Iglesias et al. 2011; Šorf et al. 2015). In this way, fish predation might reduce the efficiency of energy transfer by reducing cladocerans' body-size, as the dominance of small-bodied species is linked to a lower partition of resources among the consumers (Ersoy et al. 2017). Consequently, the reduction in *Daphnia* biomass could result in a weak control on phytoplankton bloom, a less efficient nutrient cycling and, lower quality of food supply for invertebrates and fishes (Barnett et al. 2007; Obertegger and Manca 2011).

Our study indicates that large-bodied species are more affected by temperature and predation than small-bodied species. Also, large-bodied species are better competitors than small-bodied even at high temperatures, being outnumbered only in the presence of predation. In this study, we analyzed only the competition among three species and it was possible to observe warming and predation changing the connection among them, with possible negative consequences for the ecosystem functioning, however, in natural ecosystems, there are many other species and factors interacting. Thus, we reaffirm the importance to analyze as many levels as possible to try to predict how climate warming will change food-web interactions, that are very vulnerable to warming and, which reflects in many ecosystems functions (Petchey et al. 2010; Antiqueira et al. 2018).

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APPENDIX A - Life history parameters for each species in each experimental temperature. TDE=time of embryonic development, TDP I = time of post-embryonic development I - neonate to juvenile, TDP II = time of post-embryonic development II -juvenile to adult, Primiparous = time from neonate to the first clutch. Fecundity = average number of eggs per female in the first three clutches. Each measurement from the life history parameters had at least 10 replicates, except for dry weight that had five replicates.

	Daphnia magna			***Daphnia pulex***			***Ceriodaphnia reticulata***		
Temperature (°C)	**20**	**24**	**28**	**20**	**24**	**28**	**20**	**24**	**28**
Body size (mm)									
Neonate	1.09	0.95	1.07	0.79	0.78	0.84	0.45	0.39	0.44
Juvenile	1.85	1.74	2.14	1.21	1.20	1.60	0.65	0.65	0.74
Adult	2.85	2.99	3.45	1.74	1.88	1.98	0.83	0.83	0.92
Dry weight (µg DW)									
Neonate		8.80			4.75			2.93	
Juvenile		9.43			5.50			3.10	
Adult		28.6			8.1			3.20	
Development time (hours)									
TDE	72	48	48	50.6	48	37	52	30.8	36
TDP I	59	23	23	71	47	59	75	54.8	23
TDP II	86.4	98.6	74.7	77.3	62.4	61.3	42	58.2	26.4
Primiparous	145.4	121.6	97.7	148.3	109.4	120.3	117	113	49.4
Fecundity	18.1	13.4	18.5	11	11	7	6.1	4.3	5.3

APPENDIX B - Graphic representation.

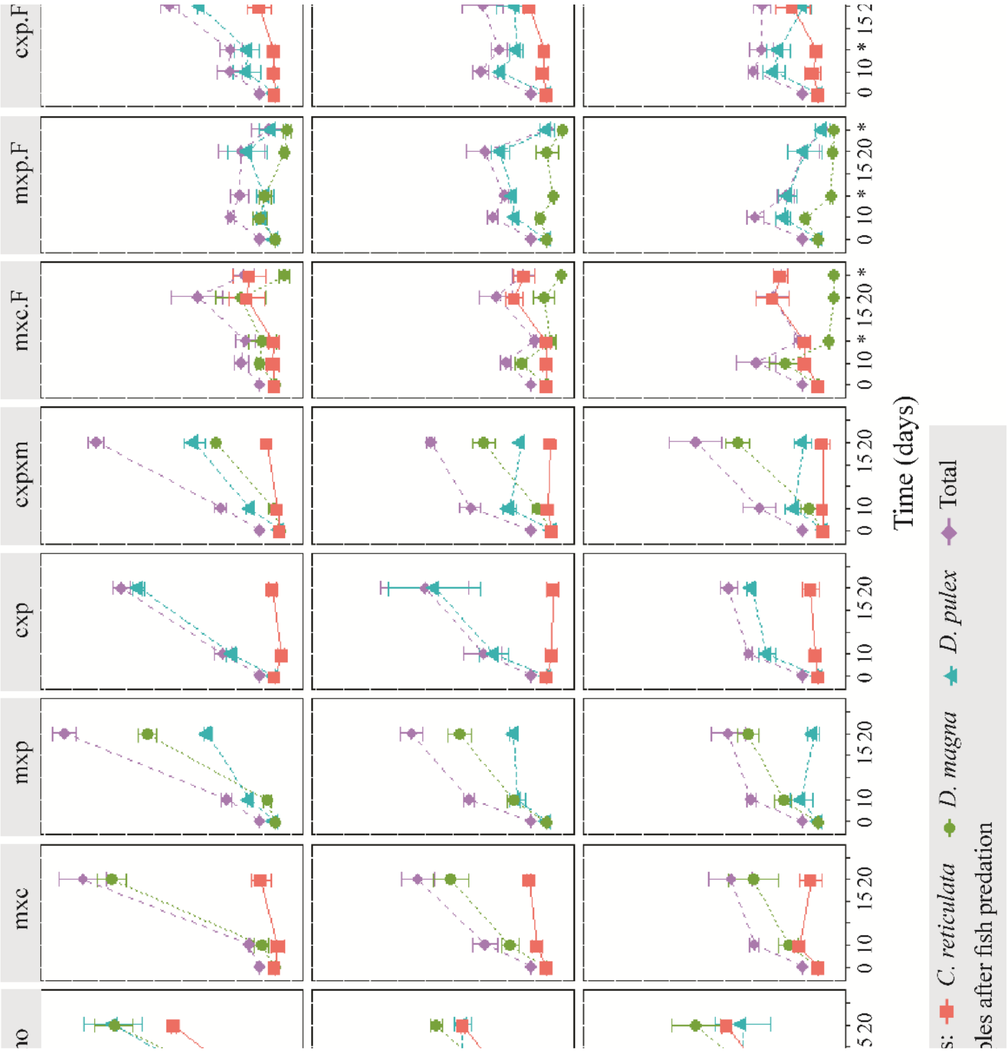

Fig. Box plots showing biomass production of the three species at 20, 24 and 28°C by competition and predation (.F) treatments over time. Mono: monoculture; cxp: *C. reticulata* x *D. pulex*; cxpxm: *C. reticulata* x *D. pulex* x *D. magna*; mxc: *D. magna* x *C. reticulata*, mxp: *D. magna* x *D. pulex.*

APPENDIX C - Post-hoc analysis with the adjusted p-value for the Permutational analysis of variance (PERMANOVA) resultstested for temperature (T), competition (C) and predation (P). Significant results are shown in bold. (cxp: *C. reticulata* x *D. pulex*; cxpxm: *C. reticulata* x *D. pulex* x *D. magna*; mxc: *D. magna* x *C. reticulata*, mxp: *D. magna* x *D. pulex*, combination with ‘F’ in the end means fish predation treatments).

Post-hoc by Pairs	F. Model	R2	p. adjusted
mxc vs mxp	12.06257	0.429846	**<0.05**
mxc vs cxp	74.70141	0.823597	**<0.05**
mxc vs cxpxm	12.42661	0.437147	**<0.05**
mxc vs D.magna	3.285127	0.162866	1
mxc vs D.pulex	82.60261	0.837733	**<0.05**
mxc vs C.reticulata	103.3551	0.865946	**<0.05**
mxc vs mxc.F	27.00062	0.627912	**<0.05**
mxc vs mxp.F	27.87286	0.63531	**<0.05**
mxc vs cxp.F	32.17851	0.667902	**<0.05**
mxc vs cxpxm.F	29.9674	0.651927	**<0.05**
mxp vs cxp	50.97371	0.7611	**<0.05**
mxp vs cxpxm	3.270288	0.169706	1
mxp vs D.magna	11.74135	0.423244	**<0.05**
mxp vs D.pulex	41.90959	0.723707	**<0.05**
mxp vs C.reticulata	245.4617	0.938806	**<0.05**
mxp vs mxc.F	61.618	0.793862	**<0.05**
mxp vs mxp.F	20.39953	0.560434	**<0.05**
mxp vs cxp.F	39.52095	0.711821	**<0.05**
mxp vs cxpxm.F	38.8994	0.708558	**<0.05**
cxp vs cxpxm	38.2809	0.705237	**<0.05**
cxp vs D.magna	155.8896	0.906917	**<0.05**
cxp vs D.pulex	2.418432	0.131305	1
cxp vs C.reticulata	152.1696	0.904858	**<0.05**
cxp vs mxc.F	40.2007	0.715306	**<0.05**
cxp vs mxp.F	17.95518	0.528791	**<0.05**
cxp vs cxp.F	18.20059	0.532172	**<0.05**
cxp vs cxpxm.F	19.72581	0.552145	**<0.05**
cxpxm vs D.magna	34.19935	0.681271	**<0.05**
cxpxm vs D.pulex	38.47449	0.706285	**<0.05**
cxpxm vs C.reticulata	216.276	0.931116	**<0.05**
cxpxm vs mxc.F	45.75565	0.740914	**<0.05**
cxpxm vs mxp.F	20.46886	0.56127	**<0.05**
cxpxm vs cxp.F	26.53214	0.623814	**<0.05**
cxpxm vs cxpxm.F	25.95734	0.61866	**<0.05**
D.magna vs D.pulex	127.8113	0.888743	**<0.05**
D.magna vs C.reticulata	529.1321	0.970649	**<0.05**
D.magna vs mxc.F	73.98591	0.822194	**<0.05**
D.magna vs mxp.F	33.47784	0.676623	**<0.05**
D.magna vs cxp.F	72.35076	0.818904	**<0.05**

D.magna vs cxpxm.F	68.32124	0.81025	<**0.05**
D.pulex vs C.reticulata	161.325	0.90977	<**0.05**
D.pulex vs mxc.F	55.87465	0.77739	<**0.05**
D.pulex vs mxp.F	15.43736	0.491052	<**0.05**
D.pulex vs cxp.F	26.80547	0.626216	<**0.05**
D.pulex vs cxpxm.F	28.70678	0.642113	<**0.05**
C.reticulata vs mxc.F	15.59959	0.493664	<**0.05**
C.reticulata vs mxp.F	37.24832	0.699521	<**0.05**
C.reticulata vs cxp.F	34.03308	0.680212	0.11
C.reticulata vs cxpxm.F	36.12054	0.693019	<**0.05**
mxc.F vs mxp.F	23.1577	0.591396	<**0.05**
mxc.F vs cxp.F	7.485879	0.31874	<**0.05**
mxc.F vs cxpxm.F	7.66014	0.323757	0.11
mxp.F vs cxp.F	9.437454	0.371006	<**0.05**
mxp.F vs cxpxm.F	10.10153	0.387009	<**0.05**
cxp.F vs cxpxm.F	0.370018	0.022603	1

3 WARMING AND PREDATOR DRIVE FUNCTIONAL RESPONSES OF THREE SUBTROPICAL CLADOCERANS

Abstract The shifts in species performance and ecological interactions related to climate warming might result in fluctuations in the energy stocks, negatively affecting the aquatic ecosystems. In this study, microcosms functioning at three temperatures were set to analyze the functional response of three subtropical cladocerans (*Ceriodaphnia silvestrii*, *Daphnia laevis* and, *Simocephalus serrulatus*) to warming in different predation scenarios: fish-larvae presence, fish-larvae absence and, fish chemical sign. We assume that the temperature increase will induce an increase in growth, reproduction and feeding rates, which will lead to an increase in predation of fish on zooplankton, and of zooplankton on phytoplankton. The cladocerans' functional responses (secondary productivity, turnover and grazing rates) to warming (control, +2°C and +4°C) and predation, were tested through general linear models (GLMs) and by a Structural Equation Model (SEM). The functional responses of the cladocerans were primarily dictated by predation and chemical sign, but, the grazing relationship of cladocerans on phytoplankton also depended on temperature. The unique and combined effect of temperature and predation influenced differently each species, *C. silvestrii* was negatively affected by the combination of the predictors, *D. laevis* only by predation and, *S. serrulatus* only by temperature. The three species increased the secondary productivity in response to the fish chemical sign. Through the cladocerans' functional responses were possible to clarify the potential effect of temperature and predation on the energy availability into the food-web levels, as temperature and predation might reduce secondary productivity and increase primary productivity, altering the energy stocks in freshwater ecosystems.

Keywords: Grazing. Global warming. Zooplankton. Productivity. Experiment. *Daphniidae.*

3.1 Introduction

Predict the responses of ecosystems to ongoing and accelerating global change remains a challenge in ecology. Temperature and precipitation have changed dramatically over the world and are expected to change even more (IPCC 2014; Hoegh-Guldberg et al. 2018). But, despite the many fields and experimental works approaching the variation in temperature and precipitation (Settele et al. 2014), it is still difficult to predict the species responses to these variables due to many reasons. One reason is the evolutionary responses of the species to these changes, as some of them have presented adaptation to the increase in temperature while others will possibly be extinct (Loreau et al. 2001; Brans et al. 2017; De-Meester et al. 2018). Another reason refers to changes in local environmental conditions that are conducted by simultaneously environmental fluctuations in global geochemical cycles (Shurin et al. 2012). Finally, direct and indirect effects on food web interactions leading to responses in the physiology and demography of organisms (Meerhoff et al. 2012; Šorf et al. 2015).

Food web interactions, as predation and competition became more intense with the rise in temperature, once high temperatures increase metabolic rates (such as intake rates, growth and, reproduction) (Brown et al. 2004), inducing greater demand for food and, consequently, greater predation pressure, where the predators could extinguish their prey (Meerhoff et al. 2007; Jeppesen et al. 2010). In the same way when the competition for food rises, the most competitive species are able to survive (Shurin et al. 2012), turning dominant and extinguishing others. But at certain levels, predation can reduce the effect of competition by controlling the numerical density of the species (Chase et al. 2002). Freshwater ecosystems are very susceptible to temperature variation, especially shallow lakes, turning these environments a good tool to test predictions related to global warming (Adrian et al. 2009; Jeppesen et al. 2014).

In freshwater food-webs zooplankton play an important role in energy transference, with strong interaction with the primary producers, controlling phytoplankton bloom, and grazing on microbial food-web (Jeppesen et al. 2000; Auer et al. 2004). Zooplankton species are affected by higher temperatures depending on their ontogeny and physiology (Huntley and Lopez 1992), changing their diversity, density and intake rates (Savage et al. 2004; West and Post 2016). These alterations can impact a single or multiple ecosystem functions (ecosystem multifunctionality), depending on the connectivity of these groups and species with others inside the aquatic food-webs (Yvon-Durocher et al. 2015; Antiqueira et al. 2018).

Moreover, zooplankton organisms are affected by the increased top-down regulation from fish due to warming climate (Jeppesen et al. 2010; Shurin et al. 2012) and, are also affected by changes in the density, size and nutrient quality of the phytoplankton (van de Waal et al. 2010). In this way, at high temperatures, it is expected that the increased fish predation pressure on zooplankton will reduce the secondary productivity of these organisms, allowing higher primary productivity by the indirect effect of fish on phytoplankton community (Gyllstrom et al. 2005). Consequently, the combined effect of warming and predation on zooplankton could lead to a mismatch in the relations that they play and, in their functional responses. However, the direction and magnitude of the top-down effects might depend on the intrinsic characteristics of the consumer organisms (such as body size and, foraging type) and on the environmental characteristics (Scherer and Smee 2016; Santangelo et al. 2018).

In subtropical freshwaters, there is a prevalence of small planktivorous fish and fish larvae predating on zooplankton (Moore and Folt 1993; Jeppesen et al. 2010; Meerhoff et al. 2012; Picapedra et al. 2018), which induce higher frequency of occurrence of small-sizes forms of zooplankton by the replacement of large-bodied species (that are preferred by visual predator) by the small-bodied (Daufresne et al. 2009). But, even with the recognized importance of the predation effect by fish larvae on zooplankton, there is still a lack of studies exploring this relation, especially under temperature variations. The subtropical freshwater environments are also dominated by small-bodied genera of cladocerans (Sarma et al. 2005), and despite the preference of the visual hunting predators by large-bodied cladocerans, the small-bodied forms are also found in the fish gut contents (Elmoor-Loureiro and Soares 2010). On the other hand, several aquatic preys such as cladocerans are able to detect the presence and identify predators through the chemical signs released by them (Scherer and Smee 2016). If the preys recognize the info chemicals early they are capable of changing their behavior, morphology and life-history characteristics (accelerating growth, reproduction, and increasing fecundity), which makes them less susceptible to predation (Ferrari et al. 2010; Santangelo et al. 2018).

Secondary productivity and population turnover rates are a direct way to measure the effects of many factors in the cycling of matter and energy and thus, on ecosystem functioning (Dias et al. 2017). Secondary productivity (P) is the amount of new biomass (B) that is produced by animals in a given period of time and the turnover rates (P/B) considers the renewal rate of the population or community (Benke 2010). Thus, these variables measure the functional role of the heterotrophic organisms, transforming their numerical density and biomass into a functional variable capable of quantity the flow of matter and energy that is available for the higher trophic levels (Lemke and Benke 2009; Dias et al. 2017). In the same way that through zooplankton grazing rates is also possible to quantify the amount of energy that it is flowing through the trophic levels (Persson et al. 2007). For that reason,the grazing rates, secondary productivity and population turnover are described here as the cladocerans' functional responses.

In this study, microcosms functioning at three temperatures were set to analyze the unique response of three subtropical cladocerans(*Ceriodaphnia silvestrii* Daday, 1902, *Daphnia laevis* Birge, 1878 and, *Simocephalus serrulatus* (Koch, 1841))and, as a community to warming in different predation scenarios: fish-larvae presence (direct effect), fish-larvae absence, and fish chemical sign (indirect effect of predator), for that we measured cladocerans secondary productivity, population turnover and grazing rates. We assume that, the temperature increase will induce an increased growth, reproduction and feeding rates (due to metabolic demands), which will lead to an increased predation of fish-larvae on zooplankton, and of zooplankton on phytoplankton; also the chemical sign will induce faster growth and reproduction. For this premise we have three predictions: (1) in the treatments of fish-absent and fish chemical sign, the cladocerans species would respond to warming by presenting higher secondary productivity, population turnover and grazing rates (due to the increase in metabolic demands), as fish chemical sign accelerate growth, reproduction and increase fecundity; (2) fish-larvae would increase the predation at higher temperatures reducing the cladocerans secondary productivity reflecting in (3) lower grazing rates by the cladocerans and a positive relation between fish-larvae (predation) and phytoplankton population.

3.2 Methods

The experiments were conducted at the Plankton Laboratory of the Federal University of São Carlos (SP, Brazil) during the spring of 2017. We chose three experimental temperatures for cultivation, life history, and predation experiments. The experimental temperatures were 22°C, 24°C, and 26°C. The first one, 22°C was set based on the average to the water temperature in natural subtropical environments in dry periods (the period that it is found higher individual abundances(Dias et al. 2017)), allowing further comparisons with natural environments. The temperatures 24 °C (heated 2°C) and 26 °C (heated 4°C) were chosen based on IPCC (2014) climate changes prediction, which estimates the increase of approximately 1.6 ºC (optimistic scenario) and 4.8 ºC (pessimistic scenario) in natural waters temperatures.

3.2.1 Study organisms, cultivation and life history

The cladoceran species selected for our experiment, *Ceriodaphnia silvestrii* (average of body size: 0.75 mm, habit: pelagic), *Daphnia laevis* (average of body size: 1.47 mm, habit: pelagic) and *Simocephalus serrulatus* (average of body size 1.76 mm, habit: littoral), are all from Daphniidae family. They were chosen because they vary in body size, they are widespread in subtropical environments and, present two different habitat preferences (Orlova-Bienkoswskaja 2001; Fuentes-Reines and Elmoor-Loureiro 2015). The stock cultures of the cladocerans were kept in incubator chambers under controlled conditions of temperature (22, 24 and 26 °C,± 0.5°C) and photoperiod (12:12 h light: dark cycle). The cladocerans were kept in 2L beakers filled with reconstituted water as culture medium, with hardness between 40-48 mg $CaCO_3$ L^{-1}, pH between 7.0-7.6 and conductivity around 160 µS cm^{-1}. The culture medium was renewed twice a week. The cladocerans were fed every two days with 1 x 10^5 cells mL^{-1} of *Raphidocelis subcapitata* (Korshikov) Nygaard, Komárek, J.Kristiansen et Skulberg. This Chlorophyceae was cultivated in Erlenmeyer flasks of 2 L filled with 1 L of CHU-12 medium (Müller 1972). The algae were initially inoculated at 1 x 10^5 cells mL^{-1} and maintained at 25 ± 2 °C, under a 12:12 h (light/dark) photoperiod until reach the exponential growth stage. After that, the algal cultures were centrifuged in order to remove the CHU-12 medium (which can eventually become toxic to the zooplankton) and were subsequently stored at 4 ºC for up to one week.

To analyze the effect of fish predation on cladocerans, we used young - larvae - guppies (*Poecilia reticulata* Peters, 1860), with body-size varying between 7 and 11 mm. This species was chosen because it occurs in natural environments, accepts the experimental temperatures and feeds on the zooplankton species (observational previously experiment before setting up the final experiment). The fishes were maintained at the three experimental temperatures with an air pump; the water was renewed once a week, and they were fed with fish food every day (before the experiment start). The procedures using fish in this experiment are in agreement with the Ethics Commission on the Use of Animals (CEUA) from the Federal University of São Carlos/SP under protocol number: 7683021017 (ID 000815).

The experiments to study the life history parameters of *C. silvestrii, D. laevis* and *S. serrulatus* were conducted at the three experimental temperatures. For these experiments, neonates (< 24h old) were placed in 50 ml beakers (10 replicates, with 1 neonate each) filled with reconstituted water plus 1 x 10^5 cells mL^{-1} of *R. subcapitata* (as food), renewed every day. The bionomic parameters such as body length for all life stages (neonates, juveniles, and adults), the presence of exuviae (cladocerans' exoskeleton remaining from molt), posture, and the number of eggs were daily observed under a stereomicroscope (Leica MZ6, Germany) until the third clutch. From these observations were obtained the embryonic development time (the time from egg-laying to hatching), post-embryonic development time (neonate to juvenile, juvenile to adult, and neonate to the first clutch), and the average of body size of each development stage. We also calculated the cladocerans dry-weight, five replicates for each species for each development stage (neonates, juveniles, adults and ovate adults), totalizing 60 model units, the 60 units were placed in a drying oven at 70°C, over 48 h. The data from life-history parameters and dry weight are presented in the Appendix D. With these data, we build our own length-weight regression to calculate the biomass ($\text{LnW} = \text{Ln}a + b\ \text{LnL}$) (Bottrell et al. 1976). The equation includes the weight logarithmic transformation (W) of dry weight µg (DW) and the length (L in mm), and, *a* = intercept estimation and, *b* = slope estimation.

3.2.2 Experimental design to secondary productivity and grazing rates

In order to analyze (1) the effect of temperature increase on cladocerans secondary productivity, turnover and grazing rates, and (2) the combined effect of temperature and predation on these same attributes, it was set up a randomized two-way factorial experiment manipulating three levels of temperature (22, +2°C and, +4°C) and, four levels of predation: **(i)** fish-absence (control - **C**); **(ii)** fish-presence 1(the fish remained in the aquarium during the first halftime of the experiment - **P1**) or **(iii)** fish-presence 2(the fish remainedin the aquarium during the second halftime of the experiment – **P2**);and finally, **(iv)** fish chemical sign (**CS**). These levels of predation were set up in order to analyze only the predator chemical sign; the effect of the predation on a lower cladocerans density and on a 'pre-established' community (higher individuals' density). The 36 microcosms of 5 L (four treatments x three temperature x three replicates) were placed in incubator chambers with controlled temperature (22, 24 and 26°C ± 0.5°C) and 12 h light/12 h dark photoperiod for 10 days. All microcosms contained the same amount of artificial plants as a refuge for zooplankton. The experimental medium (reconstituted water) was renewed twice a week; the aquaria were randomized every day inside of the chambers. The cladoceran species were fed every two days with *R. subcapitata*. The experiment started with the individual numbers standardized by the approximate biomass, considering adult females (*C. silvestrii* = 100 individuals, *D. laevis* = 50 individuals and *S. serrulatus* = 50 individuals).

The grazing rates experiments were conducted in the 36 aquaria, following the procedure described in Rodgher et al. (2008). The experimental medium, 5L of reconstituted water plus 1 x 10^5 cells mL^{-1} of *R. subcapitata*was fully renewed, three subsamples of each replicate were taken at 0 and 2 h in order to quantify the initial and final algae concentrations. Moreover, two controls (1°: no animals added, only algae and, 2°: fish with algae) were incubated under the same experimental conditions, in order to evaluate only the algal growth and if there was fish consumption over algae, after 2 h. All subsamples were fixed with 1% formaldehyde buffered with sodium borate, frozen in liquid nitrogen and stored (-20 °C) until analysis. Defrosted samples (500 µL) were analyzed in a FACSCalibur cytometer (Becton and Dickinson Franklin Lakes, NJ, U.S.A.) equipped with a 15 mW Argon-ion laser (488 nm emission) using the FL3-H (red fluorescence) and the SSC-H (lateral dispersion) channels, following Sarmento et al. (2008) procedures. It was added fluorescent beads (6 µm, Fluoresbrite® carboxylate microspheres, Polysciences Inc., Warrington, PA, U.S.A.) into the samples, as an internal standard. The cytometry data were analyzed using the FlowJo software, version 10.0 (Treestar.com, USA).

The grazing rates (G) (µgDW^{-1}µL h^{-1}) were calculated according to the modified equation of Gauld (1951), with a correction factor (*A*):

$$G= \frac{V}{n} * [\frac{(lnC0-lnCt)}{t} - A]$$

$$A = lnC0 - \frac{lnC't}{t}$$

where '*C0*' and '*Ct*' are respectively the initial and final algae concentration (cells µL^{-1}), '*t*' is the experimental time (hours) and '*n*' is the biomass of individualsin the volume '*V*' (µL). '*A*' refers to a correction factor for changes in the control with algae final concentration '*C't*' after the time '*t*'.

The secondary productivity (DW L^{-1} day $^{-1}$) was calculated according to Winberg et al. (1965) equation:

$$P = [(NI \times \Delta WI)\ TI^{-1}] + [(NII \times \Delta WII)\ TII^{-1}] + [(NIII \times \Delta WIII)\ TIII^{-1}]$$

where: I = neonates; II = juvenile; III = adults; NI, NII, and NIII are density data (Cladocerans L^{-1}); ΔWI = (average of juvenile dry weight) – (average ofneonates dry weight); ΔWII = (average ofadults dry weight) – (average ofjuvenile dry weight); ΔWIII = (average ofegg dry weight x average ofeggs number per female); TI = embryonic development time, TII = development time from neonate to juvenile, TIII = development time from juvenile to adult. The turnover rates (T) (T=secondary productivity/Biomass) were also calculated for each treatment.

3.2.3 Statistical analyses

In order to analyze the effect of temperature and the combined effect of temperature and predation on the cladocerans secondary productivity and turnover taking account the time effect, as each aquarium was sampled twice (at 5th day and 10th day), six GLMMs (generalized linear mixed model) were performed, one for each response variables and for each species (2x3), the predictor were temperature and predation and the random effect was the time. For that, it was used the function “lme” of the package “nlme” in R (Pinheiro et al. 2020). To attend the assumptions, the response variables were log-transformed. A GLM (generalized linear model) was also performed to analyze the effect of temperature and the combined effect of temperature and predation on the cladocerans grazing rates using the function “lm” of package “stats” (Bolar 2019) in R. Grazing rates were also log-transformed to attend the assumptions.

Finally, to respond the third prediction were performed a Structural Equation Model (SEM - Path analysis) to summarize the community relations and to investigate (i) the direct effects of temperature and fish predation on the cladocerans density, (ii) the direct effect of temperature and cladocerans on phytoplankton density and, (iii) the indirect effect of fish predation on phytoplankton density. The data matrix was composed by two categorical variables (temperature and predation) and by two numeric variables (density of cladocerans and algae). A model was created and fitted through a Confirmatory Factor Analysis (CFA) using the functions “cfa” and “semPaths” of the packages “lavaan” (Rosseel 2012) and, “semPlot” in R (Epskamp et al. 2019). All the graphics were performed using the package “ggplot2” in R (Wickham et al. 2019). All analysis was performed in the program R Core Team (2019).

3.3 Results

3.3.1 Populations functional responses

The species analyzed responded differently to each predictor factor. The secondary productivity of *C. silvestrii* was negatively influenced by predation (F= 45.72, p<0.001), temperature (F= 4.83, p= 0.011) and by the interaction of these two factors (F= 2.61, p=0.026). The secondary productivity of *D. laevis* was only negatively affected by predation (F= 23.93, p<0.001) and of *S. serrulatus* only negatively by temperature (F= 8.121, p<0.001) (Fig. 1). For *D. laevis*, the treatment P1 (predation in the first halftime of the experiment) differed from the treatments: C (control, p<0.001), CS (chemical sign, p<0.001) and P2 (predation in the second halftime of the experiment, p<0.001) (Fig. 1). For *S. serrulatus*, the temperature control differed from +2°C (p=0.046) and from +4°C (p=0.002) (Fig. 1). The differences between treatments for *C. silvestrii* were not interpreted as it was observed interaction between the two predictors (based on statistics rules). The turnover rates of *C. silvestrii* were influenced by the interaction between temperature and predation (F= 2.30, p=0.045), whereas, the turnover rates of *S. serrulatus* were affected by temperature (F= 3.94, p=0.024) and the turnover of *D. laevis* were not significantly affected by the predictors (Fig. 2).

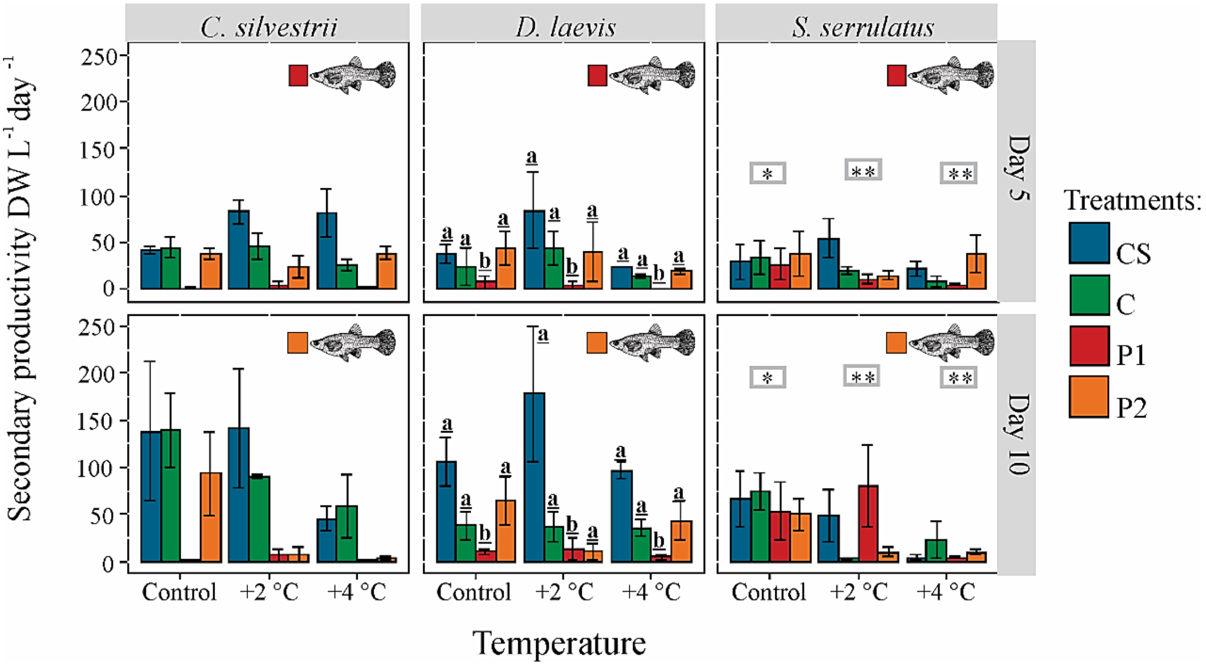

Fig. 1 Bars plots showing the secondary productivity by predation treatments (CS: chemical sign, C: control, P1: predation in the first halftime and P2: in the second halftime of the experiment), by temperature treatments (control, +2°C and, +4°C) and, by species in the middle (5th day) and in the end (10th day) of the experiment. The central lines denote the mean value, whiskers represent ± standard error. Letters above the columns indicate significant differences in posthoc analyses between predation treatments - treatments that share a letter do not differ significantly. The asterisk indicates differences in posthoc analyses between temperature treatments - treatments that share the asterisk amount do not differ significantly.

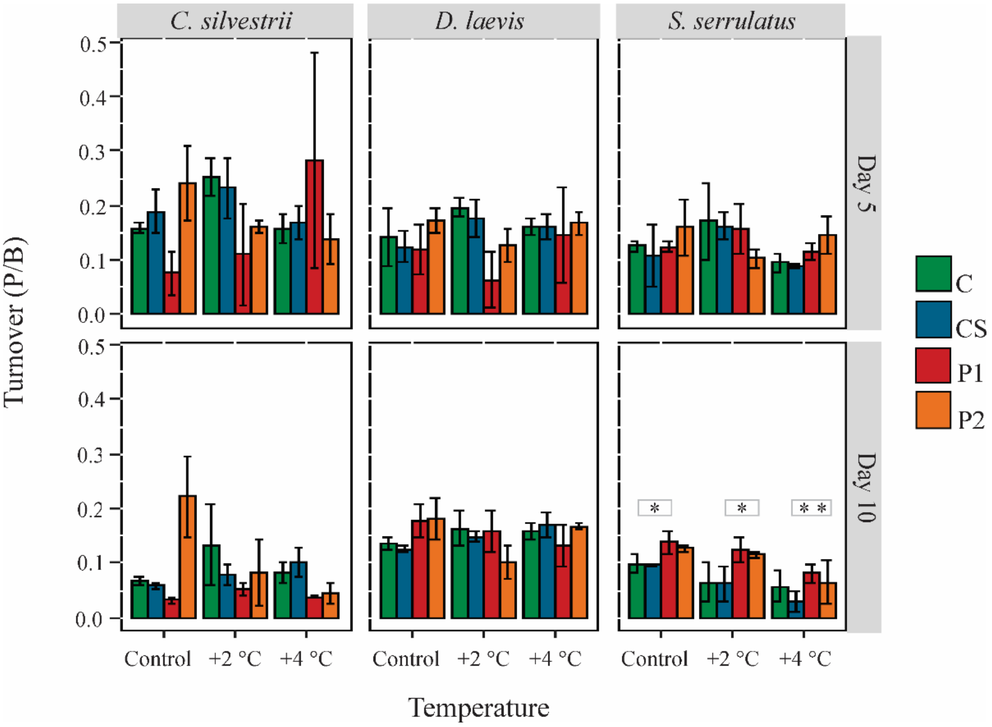

Fig. 2 Bars plots displaying the turnover rates by predation treatments (CS: chemical sign, C: control, P1: predation in the first halftime and P2: in the second halftime of the experiment), by temperature treatments (control, +2°C and, +4°C) and, by species in the middle (5th day) and in the end (10th day) of the experiment. The central lines denote the mean value, whiskers represent ± standard error. The asterisk indicates differences in posthoc analyses between temperature treatments - treatments that share the asterisk amount do not differ significantly.

3.3.2 Community functional responses

The grazing rates of the cladocerans were influenced by temperature (F= 3.566, p=0.044) and by predation treatments (F= 8.363, p<0.001). The temperature control differed from +2°C (p=0.007) and from +4°C (p=0.008), and the predation treatment CS differed from P1 (p=0.021) and from P2 (p=0.040) (Fig. 3). The regression models from the Structural Equation Model showed a strong negative effect of predation on the cladocerans density (-0.66) and a negative effect of cladocerans on phytoplankton density (-0.59); temperature also has a negative effect on cladocerans density (-0.37); fish predation had a positive effect on phytoplankton density (+0.21) (Fig. 4). Temperature and predation explained together 44% (R^2) of the cladocerans variation,also, predation, temperature and, cladocerans density explained together 58% (R^2) of the variation on phytoplankton density (Table 1).

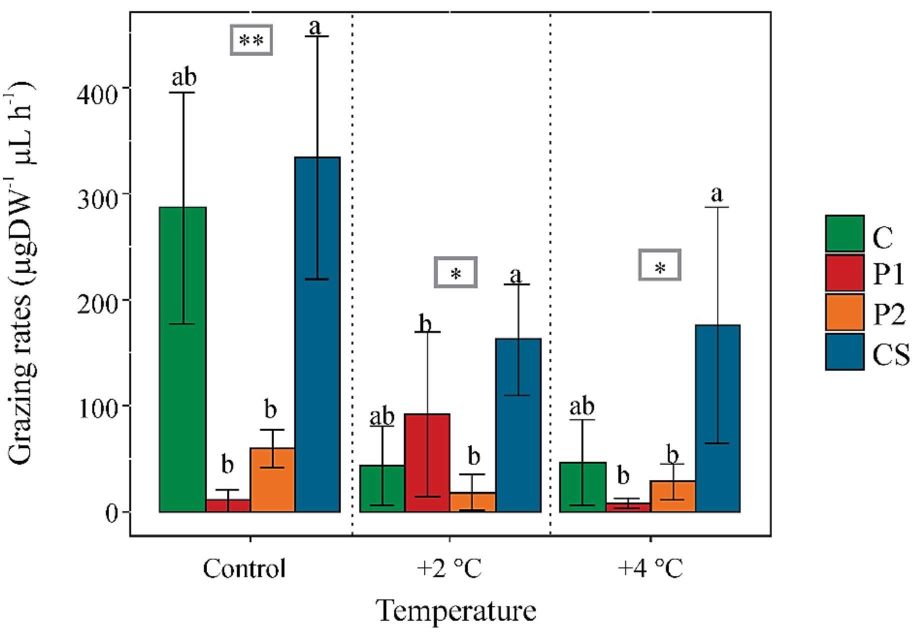

Fig. 3 Bars plots showing the cladocerans grazing rates by predation treatments (CS: chemical sign, C: control, P1: predation in the first halftime and P2: in the second halftime of the experiment) and by temperature treatments (control, +2°C and, +4°C). The central lines denote the mean value, whiskers represent ± standard error. Letters above the columns indicate significant differences in posthoc analyses between predation treatments (treatments that share a letter do not differ significantly). The asterisk indicates differences in posthoc analyses between temperature treatments (treatments that share the asterisk amount do not differ significantly).

Table 1 Regression results from the Structural Equation Model. Significant results are shown in bold. The z values are the statistics, R^2 is the total explanation for each response variable.

Regressions	z-value	p-value	R^2
Cladocerans			
~ Predation	-4.405	**0.000**	**0.44**
~ Temperature	-2.948	**0.003**	
Phytoplankton			
~ Cladocerans	-4.145	**0.000**	**0.58**
~ Predation	2.588	**0.003**	
~ Temperature	-0.727	0.467	
Predation			
~ Temperature	-0.000	1.000	

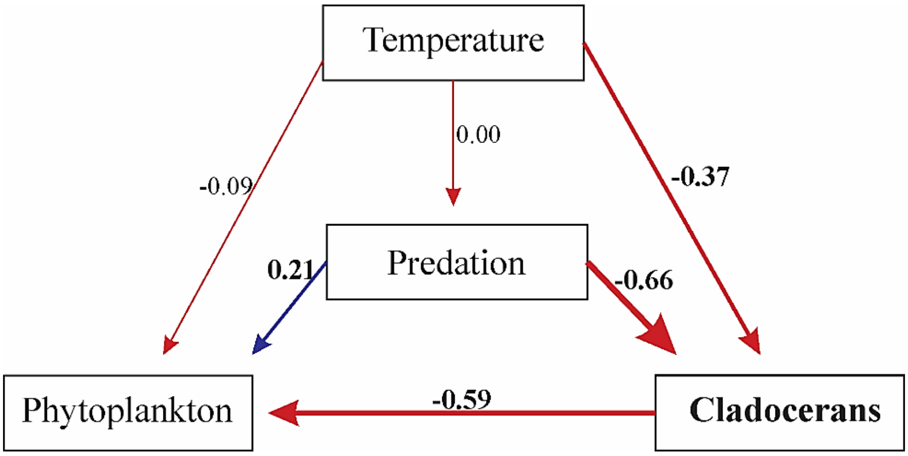

Fig. 4 Structural Equation Model (SEM) plot displaying the results from the Path analysis model (path coefficients). Red lines indicate negative relations and the blue line indicates positive relation, the thickness is proportional to the strength of the interaction. The cladocerans and phytoplankton here are the final density per mL from each aquarium.

3.4 Discussion

The aquatic species are responding differently to global warming around the world,this is expected, as each species has its own ontogeny, thermal tolerance and ability to present adaptation to climate warming (De-Meester et al. 2018). The rise in temperature has also strongly impacted the species interaction changing the flow of energy through food webs and the stability of the ecosystems (O'Connor et al. 2012). Here, we show how three subtropical cladocerans *C. silvestrii*, *D. laevis*and, *S. serrulatus* with different body sizes, respond functionally to the increase in temperature, to fish chemical signs and, to the combined effect of temperature and predation by fish-larvae.

The positive responses of the three cladoceran species to the fish chemical sign by changing their physiology, reflects their strategies to survive from predation (Ferrari et al. 2010). In presence of chemical sign, cladocerans can increase body size to escape from small-bodied predators, or grow faster to invest in reproduction, producing bigger clutches and anticipate the release of neonates, all these to try to save the neonates and juvenile from predation (Scherer and Smee 2016; Santangelo et al. 2018). In our study, the increase in secondary productivity, in the chemical signs treatments was mainly related to the clutch-specific size (number of neonates produced) in agreement with another study (Santangelo et al. 2018). Also, the effect of the chemical sign had an interaction with temperature on *C. silvestrii* performance inducing a slight increase in the turnover rates. These findings are important as most of these strategies to survive from predation, are reported for cladocerans from the temperate region, that have large body size and also large-bodied predators (Sarma et al. 2005; Santangelo et al. 2018), the opposite that is being analyzed in our study that presents prey and predator species with subtropical characteristics (small-bodied cladocerans and, small-bodied predator).

The huge effect of predation on cladocerans is well recognized as much as the combined effect of predation and temperature (Meerhoff et al. 2007; Shurin et al. 2012). But, as we observed, this interaction can follow different ways depending on the species involved, the environmental conditions (in this case, temperature) (Scherer and Smee 2016) and, the density of prey, altering the energy availability inside freshwater food-webs. Numerical traits such as zooplankton fecundity, body size, and growth summarized here by secondary productivity, are considered a direct way to link organisms to stocks and fluxes of materialand energy inside ecosystems(Hébert et al. 2017). Further, the central trophic position that zooplankton plays in aquatic food-webs connect them to multiple ecosystem functions (multifunctionality), as their biomass production affect directly the biomass stock and the community structure of their prey and predators (O’Connor et al. 2009; Abo-Taleb 2019) and, indirectly other trophic levels, consequently, changing the energy stock and fishing stock size in aquatic environments (Abo-Taleb 2019). The undergoing warming has the potential to reinforce the consumer-resource interactions, increasing zooplankton top-down control on primary producers (O’Connor et al. 2009; West and Post 2016) and fish top-down control on zooplankton (Jeppesen et al. 2010; Shurin et al. 2012) (as noted in our study), which might intensify even more these alterations on energy stock in aquatic environments (above-cited).

In our study, the cladocerans' grazing rates decrease with the increase in temperature and, the negative effect of fish predation on cladocerans had a positive effect on phytoplankton. The effect of predation in reducing the zooplankton density allowing greater primary productivity is commonly reported in temperate environments (Brooks and Dodson 1965). But, such relation is turning more sensitive with the rise in temperature creating unpleasant consequences for the ecosystems and even for human well-being (Brooks et al. 2016). The mismatch between cladocerans and phytoplankton interaction has the potential to modify the timing and magnitude of the phytoplankton bloom and cladocerans growth affecting the energy availability in freshwater environments(Kratina et al. 2012). Also, at high temperatures the composition of phytoplankton community changes, prevailing sometimes non-edible algae (Visser et al. 2016), even if some cladocerans have the potential to graze on this non-edible algae, the combined effect of temperature and predation reducing the cladocerans potential grazing could allow huge algae bloom in natural environments, causing even eutrophication characteristics in shallow lakes. One of the unpleasant impacts of algal blooms is the oxygen dynamics in freshwater environments. Despite algae produce oxygen through photosynthesis they are also consumers (Hallegraeff 1993), so algal bloom can result in greater drawdowns of oxygen, especially at higher temperatures (Lopez-Urrutia et al. 2006). Another impact is on the quality of the water resource of human uses (drinking), as the increase in non-edible algae could also increase the noxious or toxic solutes and clogging of filters by mucilage, and/or a health hazard to recreational users (Brooks et al. 2016).

Analyze the transfer of carbon and energy across trophic levels in food webs has been a central purpose in ecology since the consumer-resource interactions are important to maintaining the ecosystem's function and stability (Persson et al. 2007). But as it appears, such interactions are very vulnerable due to the temperature fluctuations (thermal means and extremes) resulting from global warming (Petchey et al. 2010; Antiqueira et al. 2018). Here, we observed that the ecological interactions (fish with zooplankton and, zooplankton with phytoplankton) were stronger in determining the species density, followed by temperature. At high temperatures the cladoceran species need to handle with the physiologic stress due to metabolic demands (Savage et al. 2004), reflecting in a decrease in the carrying capacity of the species (Allen et al. 2002), possibly, as observed here for *C. silvestrii* and *S. serrulatus* that decrease secondary productivity in response to an increase of 2°C. Despite some suppositions that the negative effects of warming will be milder in the tropical and subtropical regions due to naturally high temperatures in these regions (Parmesan 2007), other studies show that species from the low-latitude regions will be highly influenced due to the limited adaptations generated by the minor variations of the climate in the evolutionary time (Pörtner and Knust 2007). The increase in 2°C could be considered mild warming for subtropical environments, but it was plenty to change the performance and the interactions of these species.

Despite the three species belong to the same family, they have two different habitat preferences, *Simocephalus* is more related to the littoral region, being always found attached to macrophytes and other structures (Orlova-bienkowskaja 2001; Verbitsky and Verbitskaya 2011). Whereas *Daphnia* and *Ceriodaphnia* are predominant planktonic and, easily preyed. The fish larvae in our study presented feeding preference for *C. silvestrii* and *D. laevis* both planktonic and with reduced body size, compared to *S. serrulatus*. *Daphnia* and *Simocephalus*, in our data, differed approximately 300 µm, the neonates and juveniles of *S. serrulatus* could be easily eaten by the fish larvae, which means that probably the predation effect here was controlled by the cladocerans habitat preferences, as *Simocephalus* could hide in the artificial macrophytes. This preference by the predator for some species, combined with the increase in predation at high temperatures and the reduction of the prey densities, might also change the competition among species (Gurevitch et al. 2000; Chase et al. 2002). This could be related to high values of *Simocephalus* secondary productivity in the treatment that the other two species were suppressed by predation, since in other treatments this species maintained low productivity. Still, in regard to *Simocephalus*, other studies have shown that despite the species of this genus be well spread in tropical and subtropical environments presenting high thermal plasticity, they have a preference for mild temperatures (Verbitsky and Verbitskaya 2011), confirming our finds.

The way that each species responds to predators and temperature fluctuations depends on the species strategy and the turnover rates can be a good way to analyze these strategies (Shuter and Ing 1997; Adrian et al. 2009). Even with *D. laevis* presenting low secondary productivity, this species maintained constant turnover rates over time in all treatments, in natural environments this could allow *D. laevis* to maintain itself and survive from predation even that at low densities. *C. silvestrii* on the contrary, presented high turnover rates in the first half of the experiment and a decrease in the end, showing possibly the relation between population turnover and body size. As it expected smaller species to have a faster initial growing (Shuter and Ing 1997), but probably the environmental conditions were not suitable for maintaining high turnover rates in the course of the experiment.

Our study reinforces the idea that the impact of global warming on the species interactions will be complex and difficult to predict. The functional responses of the cladocerans were primarily dictated by predation and chemical sign, but, the grazing relationship of cladocerans on phytoplankton also depended on temperature. The unique and combined effect of temperature and predation influenced differently each species, *C. silvestrii* was negatively affected by the combination of the factors, *D. laevis* only by predation and, S. *serrulatus* only by temperature. The three species increased the secondary productivity in response to the fish chemical sign. Finally, through the functional responses of the cladocerans were possible to clarify the potential effect of temperature and predation on the energy availability inside the levels offreshwater food-webs, as temperature and predation might reduce secondary productivity and increase primary productivity, which could lead to unpleasant consequences for the aquatic ecosystems.

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APPENDIX D - A Life history parameters for each species in each experimental temperature. TDE=time of embryonic development, TDP I = time of post-embryonic development I - neonate to juvenile, TDP II = time of post-embryonic development II – juvenile to adult, Primiparous = time from neonate to the first clutch. Fecundity = average number of eggs per female for the first three clutches. Each measurement from the life history parameters had at least 10 replicates, except for dry weight that had five replicates.

	Ceriodaphnia silvestrii			***Daphnia laevis***			***Simocephalus serrulatus***		
Temperature (°C)	**22**	**24**	**26**	**22**	**24**	**26**	**22**	**24**	**26**
Body size (mm)									
Neonate	0.37	0.40	0.36	0.72	0.70	0.70	0.65	0.64	0.60
Juvenile	0.49	0.55	0.50	0.86	0.85	0.86	1.06	1.01	1.03
Adult	0.74	0.75	0.76	1470	1400	1450	1.76	1.75	1.70
Dry weight (µg DW)									
Neonate	2			1.95			3.69		
Juvenile	2.15			2.08			4.29		
Adult	2.3			4.5			5.69		
Development time (hours)									
TDE	39.1	35.5	27.6	48	46.5	47.5	40.5	41.3	43.5
TDP I	40.3	35.7	29.3	27.8	31.0	29.5	36.2	37.4	39.0
TDP II	54.0	32.6	39.8	87.84	86.0	87	80.3	81.4	92.0
Primiparous	94.3	68.3	69.1	115.7	117	116.5	116	119	131
Fecundity	2.5	3.2	2.5	6.6	5.8	5.1	8.34	6.7	4.5

4 EXTREME CLIMATE EVENTS DRIVE THE DISTRIBUTION OF ZOOPLANKTON FUNCTIONAL TRAITS VIA LIMNOLOGICAL CHANGES AND ECOLOGICAL INTERACTIONS

ABSTRACT

In this study, we show how neutral and, extreme climate events (under influence of El Niño and, La Niña) affect the limnological heterogeneity and, the distribution of zooplankton species and functional traits over 19 years, in a floodplain that suffer by dam constructions. We also show the importance of different factors (temporal, limnological, fish predation and, food resource) in structuring beta functional diversity in shallow lakes with distinct connections to the main rivers, and which functional traits were related to these predictors. Our findings showed that the magnitudes of zooplankton beta diversity varied depending on the climate event and the hardness of such events. The limnological heterogeneity and beta diversity followed a different pattern than expected with higher beta diversity and heterogeneity observed in El Niño (extreme rainy), and lowest in La Niña (extreme dry). Also, limnological variables, food availability, fish predation and, the temporal predictor can have distinct importance to structure zooplankton beta diversity and functional traits depending on the climate season and type of lake. The potential of species and traits homogenization observed during the extreme dry season could lead to losses in ecosystem functions and services if these events continue to be more frequent and prolonged as a result of dam regulation and/or global warming. Thus, we reaffirm that the natural flood caused by the rainy seasons (neutral and extreme) became extremely important to maintain the high limnological heterogeneity and biodiversity, allowing the coexistence of more functional traits and ecosystem functions.

Keywords: Beta diversity. El Niño Southern Oscillation. Climate warming. Temporal approach. Dams effect. Shallow-lakes.

4.1 Introduction

The ongoing global changes have threatened the diversity of populations and species (Loreau et al. 2001; Thomas et al. 2004), keeping up the investigationsof which factors causethe spatial and temporal variations in biodiversity (Heino et al. 2019). Different facets of diversity may be used to access the effects of global change and natural environmental variations. These facets comprise alpha, beta, and gamma components (Whittaker 1960). The differences in the distribution of communities' composition among sites and/or among gradients (temporal and spatial) are described by beta diversity (Legendre et al. 2005), which can include different components such as replacement and richness difference (Podani and Schmera 2011).

Beta diversity has been highly used to evaluate how assemblage responds to environmental changes (Braghin et al. 2018; Heino et al. 2019; Lansac-Tôha et al. 2019). But, there are only a few studies with a functional approach taking account of the global changes, especially, the alteration in precipitation related to climate phenomena. The *El Niño South Oscillation* (ENSO) is a global climate phenomenon that influences the discharge of rivers from all over the world (Kane 2002; Wilby et al. 2008). El Niño and La Niña are characterized by the heating and cooling (respectively) of the surface waters of the Central and Eastern Tropical Pacific Ocean and, influence in different ways the Brazilian geographic regions (Marengo 2006; Marengo et al. 2012). In the South, Southeast and Midwest regions of Brazil there are intense droughts during La Niña and, excessive precipitation leading to record floods during El Niño, there are also neutral seasons when these phenomena are not so intense (Marengo et al. 2012; Magrin et al. 2014).

The change in global temperature is strongly connected to ENSO, as the temperature increase is sensed largely on the oceans affecting the variation in heating and cooling of El

Niño and La Niña (Cai et al. 2014). The increase in extreme rainfall and dry events linked to ENSO is reported in some studies (Berbery et al. 2006; Re and Barros 2009; Marengo et al. 2012), which demonstrate that in the recent decades these phenomena are stronger as a result of the rise in the global average temperature (Collins et al. 2013). While future forecasts show that these extreme events will get worse (Cai et al. 2014, 2015; Cavalcantti et al. 2015), letting South America more exposed to intense and frequent dry, rains and, heatwaves (Magrin et al. 2014).

The interannual variations in precipitation lead to changes in biotic and abiotic characteristics of aquatic environments (Simões et al. 2013), reflecting in alterations on food web dynamics and ecosystem functioning (Scheffer et al. 1993; Mormul et al. 2012). In floodplains, during intense rains, the rivers and lakes get connected, causing homogenization of species and abiotic characteristics, such as the turbidity, pH, nutrients and organic matter (Thomaz et al. 2007; Bozelli et al. 2015). These alterations influence the establishment of many communities, such as aquatic macrophytes (Maltchik et al. 2004), zooplankton (Lansac-Toha et al. 2004; 2009) and, fish (Pelicice et al. 2005). Thus, in floodplains during the rainy season, the community dynamics are conducted by regional factors (Rodriguez and Lewis-Jr 1997; Bozelli et al. 2015). The opposite occurs during dry seasons, the environments get isolated, the species become more restricted and again the environmental conditions are altered, as a result, the local factors act more strongly in the communities (Thomaz et al. 2007; Simões et al. 2013). The stress generated by these environmental fluctuations acts as filters selecting the species and traits, inducing population fluctuations and, alterations in the functional structure of the communities (Sparks and Spink 1998); turning also ecological interactions stronger (Sousa 1984; Obertegger and Flaim 2015).

Functional traits are species-specific characteristics capable of defining their performance in the environment and their ecologic niche (Mayfield and Levine 2010), ensuring also their capacity to compete with other species and to escape from predators (Violle et al. 2007). Functional traits are related to the organism's morphology, physiology or phenology and can describe the effect of environmental filters and/or responses to environmental conditions, reflecting the potential effects on ecosystems process (Hébert et al. 2017). The zooplankton community is very sensitive to environmental variations, being used as a tool to access variation in ecosystem processes, as changes in the structure of zooplankton often proceed to shifts in the energy stocks, altering other levels inside aquatic food webs (Havens 1998; Hébert et al. 2017).

Paraná River basin is the second largest in South America, its hydrologic extremes events are intensively related to ENSO (Bovo-Scomparin and Train 2008; Pineda et al. 2019). Evaluate the impacts caused by intense climate alterations in this ecosystem, can be a way to better understand the climate change effects on the structure of aquatic ecosystems. Especially using beta diversity and functional approach that reveals the changes in the ecosystem process (Hébert et al. 2017). Thus, this study was conducted to analyze how extreme dry and rainy seasons caused by ENSO affect the zooplankton functional and taxonomic beta diversity in isolated and connected shallow lakes in a Neotropical floodplain; and, which are the variables causing this structuration.

We assume that the extreme climate events will promote major changes in the limnological characteristics of the lakes, consequently, affecting the distribution of zooplankton species and functional traits, as well, the density of their predators and food resource. For this hypothesis, we have four predictions, 1) in extremely dry seasons, the environmental filters are stronger in each lake selecting different functional traits and species, reflecting in a higher total-beta and, in a higher replacement; 2) in extremely rainy seasons the opposite occurs, there is homogenization in the functional traits and species leading to a lower total-beta; 3) in neutral seasons these effects are milder causing lower beta; 4) finally, during extremely dry seasons the limnological variables, food availability and predation will explain more the functional total-beta diversity than in other seasons, as a result of stronger local factors acting during this period.

4.2 Methods

4.2.1Selection of the years under ENSO influence

The data for limnological and community variables was obtained from the long-term ecological research (LTER) in the Paraná River floodplain (Brazil), from 2000 to 2018 (19 years). Based on the “Oceanic Niño Index” (ONI - Golden Gate Weather Services, 2019) and, considering our data, we chose three years that reflect El Niño events (2002, 2009 and 2015, extreme rainy seasons) and three years that reflect La Niña events (2007, 2010 and 2011, extreme dry seasons) (Fig. 1). The neutral events were chosen based on the same years considering the natural seasonality, rainy during February and dry during August (Table 1).

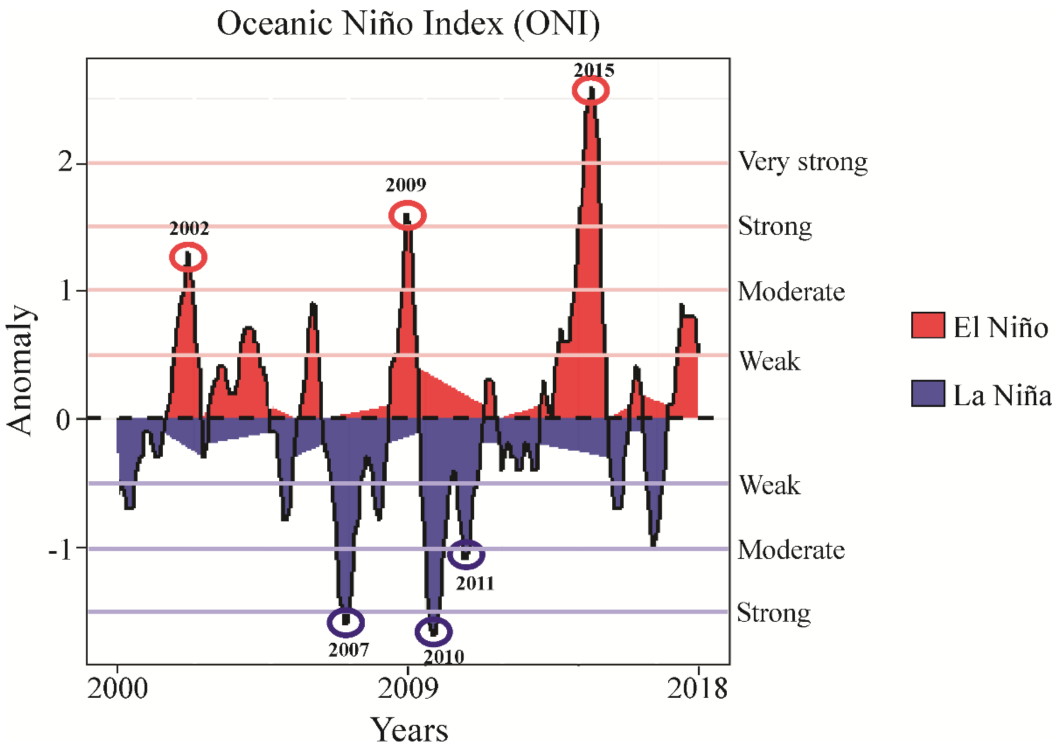

Fig. 1 Graphic displaying the Oceanic Niño Index (ONI) over the years under study (2000-2018) classified by the extent of heating (El Niño) and cooling (La Niña) of the tropical Pacific. This graphic was performed with data from ONI - Golden Gate Weather Services, (2019). Circles display the years chosen for the analysis.

Table 1 The chosen years under influence of ENSO following Oceanic Niño Index (ONI), in the dry seasons (August) and rainy seasons (February) at the Upper Paraná River floodplain. In bold are the more intense seasons, other than not bold are mild seasons.

Year	**Month**	**Season**	**Climate event**	**Classification**
2002	**February**	**Rainy**	**El Niño (moderated)**	**El Niño**
2002	August	Dry	El Nino (moderated)	Neutral dry
2007	February	Rainy	La Nina (strong)	Neutral rain
2007	**August**	**Dry**	**La Niña (strong)**	**La Niña**
2009	**February**	**Rainy**	**El Niño (moderated)**	**El Niño**
2009	August	Dry	El Nino (moderated)	Neutral dry
2010	February	Rainy	La Nina (strong)	Neutral rain
2010	**August**	**Dry**	**La Niña (strong)**	**La Niña**
2011	February	Rainy	La Nina (moderated)	Neutral rain
2011	**August**	**Dry**	**La Niña (moderated)**	**La Niña**
2015	**February**	**Rainy**	**El Niño (very strong)**	**El Niño**
2015	August	Dry	El Nino (very strong)	Neutral dry

4.2.2 Study area and sampling

The upper Paraná River floodplain (22º40′ - 22º53′S; 53º10′ - 53º38′W) belongs to two Brazilian States (Paraná - PR and, Mato Grosso do Sul - MS). The Paraná River is the main river of this ecosystem, the principal tributaries on the right of the riverside are Baia and Ivinhema (Fig. 2). This floodplain is characterized by high biodiversity and, diverse aquatic environments with high heterogeneity (backwaters, connected and isolated lakes, rivers and channels) controlled by the inundation pulse, which is a consequence of the hydrologic regime (floods and droughts) (Agostinho et al. 2004). Expecting more differences in the environmental heterogeneity over the seasons, we analyzed data from three *isolated lakes* (one of each sub-basin – Paraná, Baia, and Ivinhema), which turn connected to the main riversonly during the extremely rainy events (Fig. 2). Whereas expecting lower changes in heterogeneity over the seasons, we also analyzed data from three lakes that are always connected to the main rivers (Paraná, Baia, and Ivinhema) (Fig. 2). The short description of each lake is in the Appendix E.

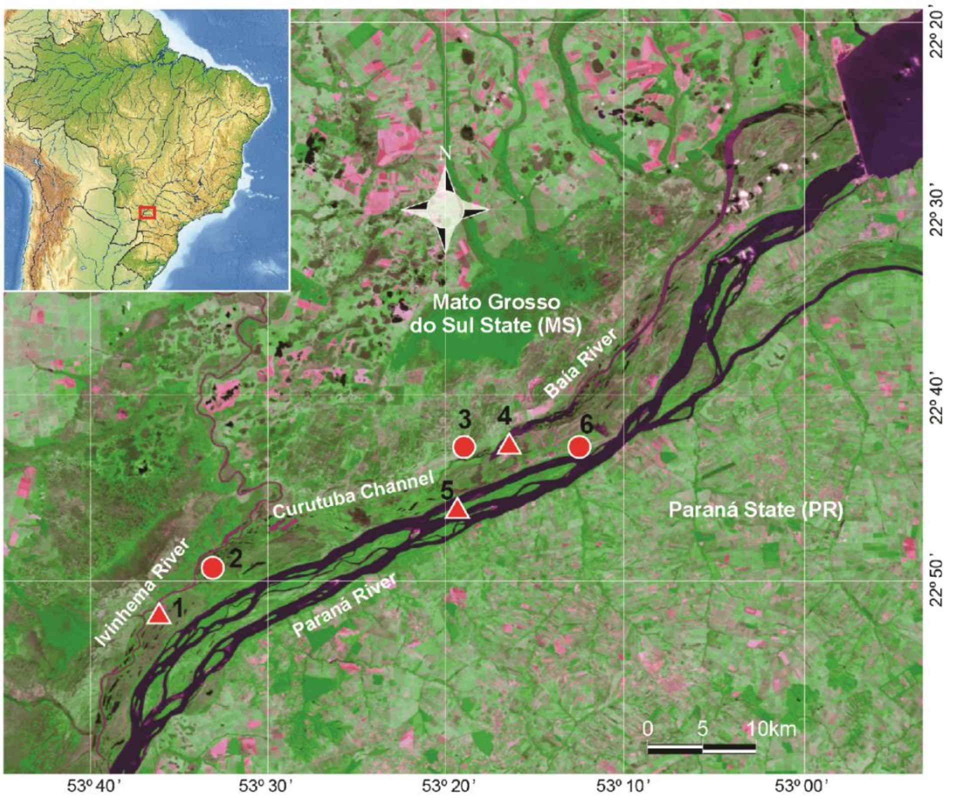

Fig. 2 Upper Paraná River floodplain (PR/MS) in Brazilian territory. Sampled isolated lakes are represented by red triangles and connected lakes are represented by red circles.

Sampling occurred quarterly, covering the annual hydrological regime. The zooplankton was sampled on the sub-surface of the pelagic region in each lake. It was used a motorized pump and plankton net (68 µm mesh) to filter 600 L of water per sample. The samples were kept in flasks with formalin 4% tamponed with calcium carbonate. The species identification and abundance estimative followed the methodology described by Lansac-Tôha *et al.* (2009). The sampling of limnological variables (Secchi transparency (m), depth (m), wind velocity (m s^{-1}), water temperature (°C), dissolved oxygen (mg L^{-1}), pH, total nitrogen (µg L^{-1}), nitrite (µg L^{-1}), nitrate (µg L^{-1}), total phosphorus (µg L^{-1}), phosphate (µg L^{-1}), conductivity (µS cm^{-1}), alkalinity (mEq L^{-1}), turbidity (NTU), inorganic (mg L^{-1}) and organic suspended matter (mg L^{-1})) occurred concomitantly with the sampling of the community and followed the methodology described by Roberto et al. (2009).

Sampling methodology and counting of planktonic ciliates were based on the methods described by Negreiros *et al.* (2017). Phytoplankton sampling and biovolume determination were based on Bortolini *et al.* (2017) and the phytoplankton size classification (picoplankton, nanoplankton and, microplankton) was based on the methodology described by Kruk et al. (2010). Fish were sampled using gillnets of different mesh sizes (ranging from 3 to 16 cm opposite knots) and trammel nets (internal mesh of 6, 7 and 8 cm opposite knots). The species abundances in every sample were indexed by the capture per unit effort (CPUE; the number of individuals 1000 m^2 of gill nets in 24 hours) (Fernandes et al. 2009). Only species classified as potential zooplanktivorous (Graça and Pavanelli 2007) were used in the analysis.

4.2.3 Functional traits

It was categorized 255 zooplanktonic species (148 rotifers, 76 cladocerans and, 31 copepods) into six functional traits, based on data from specialized literature (the categorization of each species in each trait is presented in the Appendix F). The first trait was *habitat*, classified in pelagic and littoral; the second was *feeding type*, classified in filter, sucker, predator, raptorial and scraper; the third trait was *time of life*, classified in short and long; the fourth was the *type of predominant reproduction* (asexual and sexual); the fifth trait was the *ability to escape from predator*, classified in low, medium, high and, maximum; the last one was *body size* (body length average in µm). Body length values were obtained from local literature sources, related to the species from the Upper Paraná River floodplain. These traits were chosen because they describe the responses and effects of organisms on the ecosystem process (Barnett et al. 2007), for more details about these functional traits see Braghin *et al.* (2018). The traits that most contributed to the functional structure of the zooplankton community in each lake and season (rainy and dry) are described in Appendix G.

4.2.4 Preparing the predictor variables

The limnological matrix (preditor *a*) for all analyses was composed of the standardized limnological variables described above. We checked the multicollinearity among environmental variables using variance inflation factors (VIF) and removed variables that were strongly correlated with other variables (VIF >10) before statistical analyses (Oksanen et al. 2019). The second step was to prepare the other predictor variables, food availability(*b*), predation(*c*), and temporal component(*d*), for the distance-based redundancy analysis (db-RDA). For that, we performed three Principal Coordinates Analysis (PCoA) using the packages “vegan” (Oksanen et al. 2019) and “ape” (Paradis and Schliep 2018) in R, to transform into distances phytoplankton biovolume (picoplankton, Nanoplankton, and microplankton), plus ciliate abundance that represents the food availability (*b*) and, the abundance of zooplanktivorous fish as predation variable (*c*). This transformation was used to provide more accuracy to the data, the first two axes (axis 1 and 2) were used from each variable (phytoplankton, ciliates, and fish abundances).

The temporal variables (predictor *d*) for db-RDA were derived from asymmetric eigenvector maps (AEM) (Blanchet et al. 2008), in which the eigenvectors are proxies for temporal processes (Legendre and Gauthier, 2014). As the distances between the years under the influence of El Niño are different from the years under La Niña, we constructed two different AEM maps. The process followed three steps: **1-** random deviates were generated (for each difference in time - La Niña, El Niño) taking account how many years we have (three for each season), also, the maximum and minimum distances between the years, for that we used the function “runif” in R; **2-** considering the values from the random deviates, it was calculated the vector of weights for the AEM (representing the ease of communication among years), the weights were the difference between the distances divided by the maximum distance (distances/max(distances)) and finally, **3-** the AEM maps were constructed, generating two matrices (one for La Niña, another for El Niño) with two columns each (C1 and C2). To calculate the AEM we used the function "aem.time" and the package "adespatial" (Dray et al. 2020) in R. In this way, we have four predictor matrix to db-RDA analysis: the first one was *limnological variables (a)*, the second was *food (b)* (consisting of the biomass of total phytoplankton, picoplankton, nanoplankton, microplankton, ciliates richness, ciliates abundance, and the first two axes from PCoA for phytoplankton and for ciliates);the third matrix was *fishpredation (c)* (composed by fish richness and abundance and, axis 1 and 2 from PCoA) and, the last predictor matrix was *Time (d)* (composed by two columns constructed by the AEM).

In a second moment, we prepared the predictor variables forFourth-corner analysis. In order to have more accuracy in the model, we first transformed all the predictor variables into distances through a PCoA and retained the first axis of each correspondent predictor variable. It was performed four PCoA, the first one contained the variables related to food availability (the biomass of microplankton, nanoplankton, picoplankton, also, ciliates abundance and richness); the second PCoA was performed with variables related to nutrients availability (total nitrogen, total phosphorus, phosphate and, nitrate); the third PCoA was related to physical variables (water temperature, wind, dissolved oxygen, turbidity, inorganic suspended matter and, transparency), and the fourth PCoA was conducted with variables related to predation (fish abundance and richness). All these variables were chosen because they were selected in the forward selection (in the db-RDA) showing that they are related to the distribution of the functional traits.

4.2.5 Data analysis

To data analysis, the first stage was performed two PERMANOVAs (Permutational analysis of variance), one for each type of lake, to analyze if the environmental heterogeneity was different among the climate seasons. For this, we transformed into distances (using the

"Euclidean" method) the 17 limnological variables cited above and used these distances as a dependent variable. The climatic seasons (El Niño, La Niña, Neutral dry and Neutral rainy, Table 1) were used as a predictor variable. We performed 999 permutations, the significance levels adopted were $p < 0.05$. Pairwise analyses were performed to analyze which seasons differed from each other, applying adjusted p-value. The distances were displayed through an NMDS (non-metric multidimensional scaling). The packages used were "vegan" (Oksanen et al. 2019), "MASS" (Venables and Ripley 2002) and, "ggplot2" (Wickham et al. 2019) in R.

In the second stage, we calculated functional beta diversity components (sites/years by sites/years dissimilarity matrices) based on the Sorensen dissimilarity coefficient using incidence data (presence/absence). We applied the approach independently proposed by Podani and Schmera (2011) and Carvalho et al. (2012), where the total beta component (βtotal) is partitioned into the replacement (βrepl) and richness difference (βrich) components. The βrepl refers to the replacement of species identities alone, and βrich relates to species loss-gain or richness differences alone. It was generated three dissimilarity matrices for each climatic season and for each type of lake (connected and isolated), totalizing 24 functional dissimilarity matrices. We also calculated the taxonomic beta diversity components following the same approach cited above, generating 24 taxonomic dissimilarity matrices. We also investigate through an ANOVA followed by posthoc analyses if the total beta-diversity (functional and taxonomic) was different among the climate seasons, using Bonferroni correction $p=0.05/6$ (Gotelli and Ellison 2004).

In the third stage, we used the three pairwise matrices (for each climatic season and type of lake) from functional beta diversity in the distance-based redundancy analysis (db-RDA, Legendre and Anderson, 1999). We applied a forward selection with two stopping rules to identify the final sets of limnological variables (Lim), food availability variables (Food), predation (Pred) and temporal process (Time) influencing the replacement component, richness difference, and total functional beta diversity in each climatic season and lake. Subsequently, to examine the relative contribution of pure and shared effects of Lim, Food, Pred and Time to explain functional beta diversity variation, we performed the variance partitioning (Peres-Neto et al. 2006). Statistical significance of the four pure fractions was tested using ANOVA and $p < 0.05$. We used the “vegan” (Oksanen et al. 2019), “FD” (Laliberté et al. 2014), “stats” (Bolar 2019) and, “BAT” (Cardoso et al. 2020) packages in R to performed these analyses.

Finally, in the last stage, we performed a Fourth-corner analysis to observe which traits are related to the predictor variables in each climatic season. Thus, three matrices were used to perform the Fourth-corner: the species distribution (L), the functional traits (Q), and the predictor variables (R - consisting in the axes of food availability, nutrients, physical variables and, fish predation from PCoA, as mentioned in the previous session). To performed the Fourth-corner we used the model type 6, more robust because permute the rows and columns of the matrix L (Dray and Legendre 2008; ter Braak et al. 2012), we performed 999 permutations, significant values were considered as $p < 0.05$ applying “Bonferroni” correction. For this analysis, we used the packages “ade4” (Bougeard and Dray 2018) and “vegan” (Oksanen et al. 2019)in R. All graphics were performed using the package “ggplot2” (Wickham et al. 2019) and, all analyses were performed in the program R Core Team (2019).

4.3 Results

4.3.1 Limnological heterogeneity

The environmental heterogeneity was different between the climatic seasons in both types of lakes (isolated F = 1.8719, p = 0.035 and, connected lakes F = 1.699, p = 0.044). During La Niña the isolated lakes presented lower heterogeneity and differed only from the neutral rainy season (p = 0.006) (Fig. 3). The La Niña and El Niño seasons differed in the connected lakes (p = 0.03), the two neutral seasons (dry and rainy) also differed (p = 0.03), but the biggest difference was between La Niña and the neutral rainy season (p = 0.004) (Fig. 3). The connected lakes presented high heterogeneity in all seasons compared to the isolated lakes.

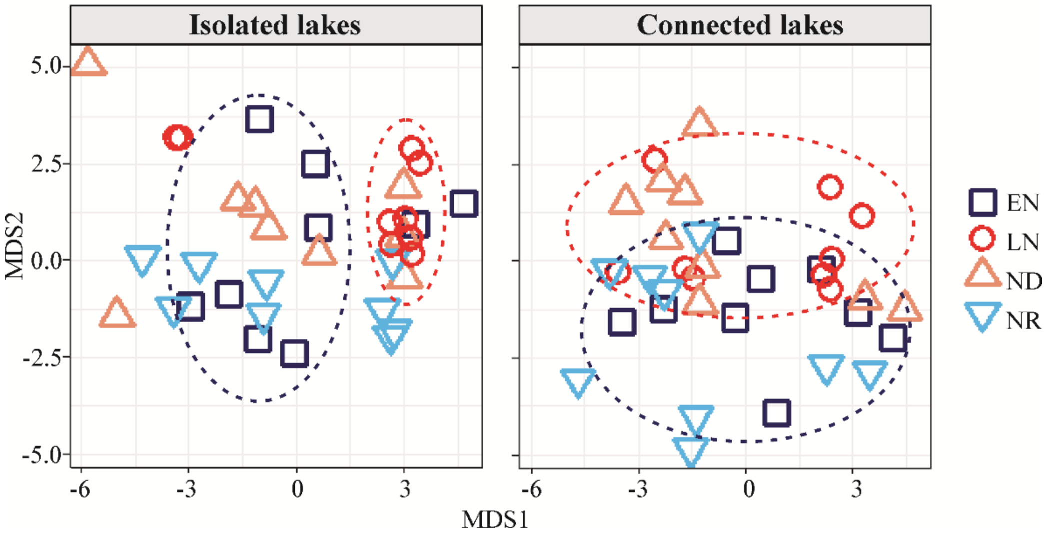

Fig. 3 Llimnological heterogeneity during El Niño (EN), La Niña (LN), neutral dry (ND) and neutral rainy (NR) seasons. Dotted circles are shown to call attention to the extremes events, blue for El Niño and red for La Niña.

4.3.2 Zooplankton taxonomic and functional β-diversity

Taxonomic and functional zooplankton β-diversity showed similar patterns when comparing the seasons and types of lakes. Both βtotal (taxonomic and functional) differed among the seasons in both types of lakes ($p<0.05$). The results revealed that taxonomic β-diversity was slightly higher during the El Niño (βSor = 0.75, $p< 0.008$) than in the other seasons in the isolated lakes. La Niña had the lowest taxonomic β-diversity (βtotal = 0.60, $p < 0.008$) in connected lakes (Fig. 4). The component βrepl had the bigger contribution to taxonomic βtotal during almost all seasons except for El Niño in connected lakes and, La Niña in isolated lakes where βrepl and βrich were similar. Also, during La Niña in connected lakes, the βtotal was most represent for βrepl as βrich had the lowest values compare to the other season and type of lake (Fig. 4). Neutral seasons had a similar pattern in the βtotal and its components in both types of lakes.

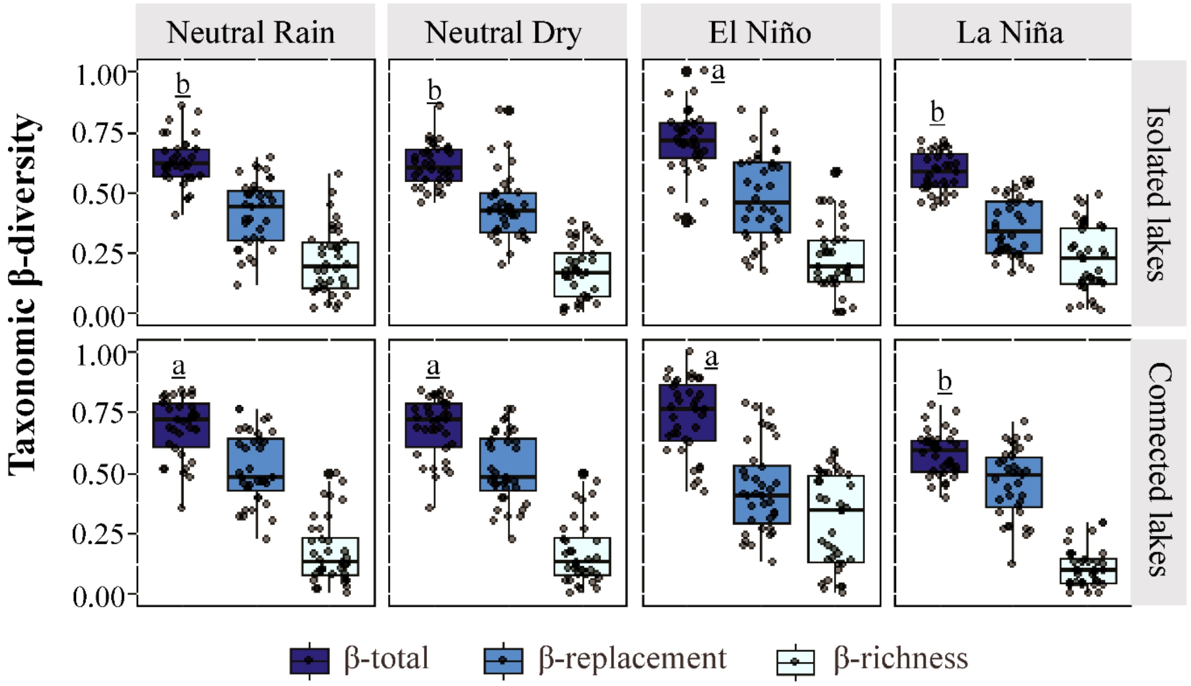

Fig. 4 Box plots of pairwise dissimilarities for the total beta taxonomic diversity (βtotal), βrepl and βrich in each climatic season and in the different lakes. The central lines denote the median value, box denotes 25th and 75th percentiles, whiskers represent respectively the smallest and largest value within 1.5 times in interquartile range below and above percentiles and dots are the observed values. Letters above the columns indicate significant differences in posthoc analyses - treatments that share a letter do not differ significantly, $p > 0.008$.

The functional β-diversity also was slightly higher during the El Niño for both types of lakes (βtotal = 0.50, $p < 0.008$) (Fig. 5). La Niña showed the lowest functional βtotal in both types of lakes, with very low βrich in connected lakes. The component βrepl was also the most representative in all seasons and type of lakes for functional diversity, although βrepl and βrich had similar values in neutral rain season (for both type of lakes), also during El Niño in connected lakes and, during La Niña in isolated lakes. The βtotal during the neutral season were similar for both type of lakes, only the proportion for each component differed (Fig. 5).

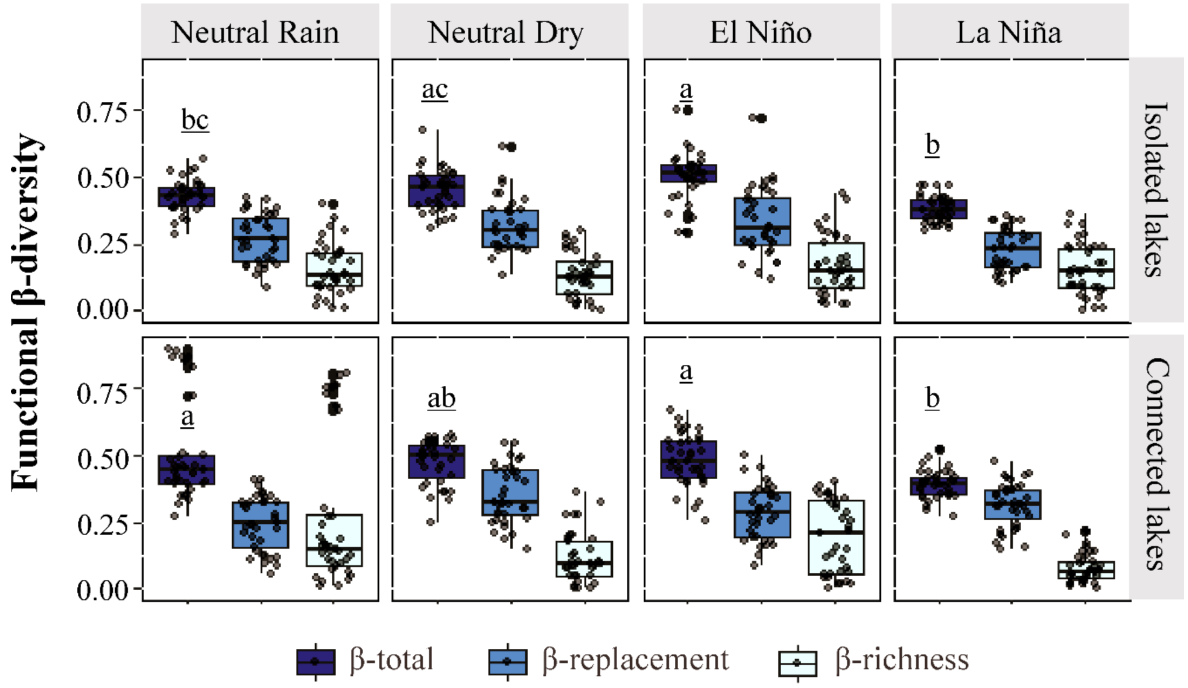

Fig. 5 Box plots of pairwise dissimilarities for the total beta functional diversity (βtotal), βrepl and βrich in each climatic season and in the different lakes. The central lines denote the median value, box denotes 25th and 75th percentiles, whiskers represent respectively the smallest and largest value within 1.5 times in interquartile range below and above percentiles and dots are the observed values. Letters above the columns indicate significant differences in posthoc analyses - treatments that share a letter do not differ significantly, p >0.008.

4.3.3 Contribution of the variables limnological, food availability, predation and temporal to zooplankton functional β-diversity variation

The variables selected by the forward selection for each predictor variable (limnological, food, fish predation and time) for each season (neutral rainy, neutral dry, El Niño and La Niña), for each lake and, for functional βtotal, βrepl, and βrich were different. In general, the limnological variables (Lim) chosen were related to nitrate, total nitrogen, phosphate, total phosphorus, water temperature, dissolved oxygen, and wind. The variables of food availability (Food) were most related to the PCoA axes, nanoplankton and picoplankton. Fish predation (Pred) variables were related to the PCoA axes and fish richness. The temporal predictor (Time) included AEM columns, which shows positive and negative temporal correlations (for more details about the selected predictors see Appendix H).

Regarding the factors shaping zooplankton functional βtotal in each season, the limnological variables explained the most part of the variation, but connected and isolated lakes had a different pattern in each season. During the neutral rainy season, all explanatory variables explained a total of 91% in isolated lakes and, 79% in connected lakes (Fig 6a, b). During the neutral dry season, all explanatory variables explained a total of 64% in isolated lakes and, 82% in connected lakes (Fig 6c, d). During El Niño all explanatory variables explained respectively, a total of 90% and 91% in isolated and connected lakes (Fig. 6e, f). And, during La Niña a total of 71% and 93% in isolated and connected lakes, respectively (Fig. 6g, h). Predation was important to explain the variation of the functional βtotal during El Niño in both types of lakes (13% and, 17%, Fig. 6e, f). Food availability was representative during the neutral dry season (20%, Fig. 6d) and, in the share proportion of Lim: Food during neutral rainy (20%, Fig. 6b). The component-time represented 20% of the β-diversity variation in the neutral rainy season (Fig. 6a) and, 18% during La Niña season (Fig. 6h)

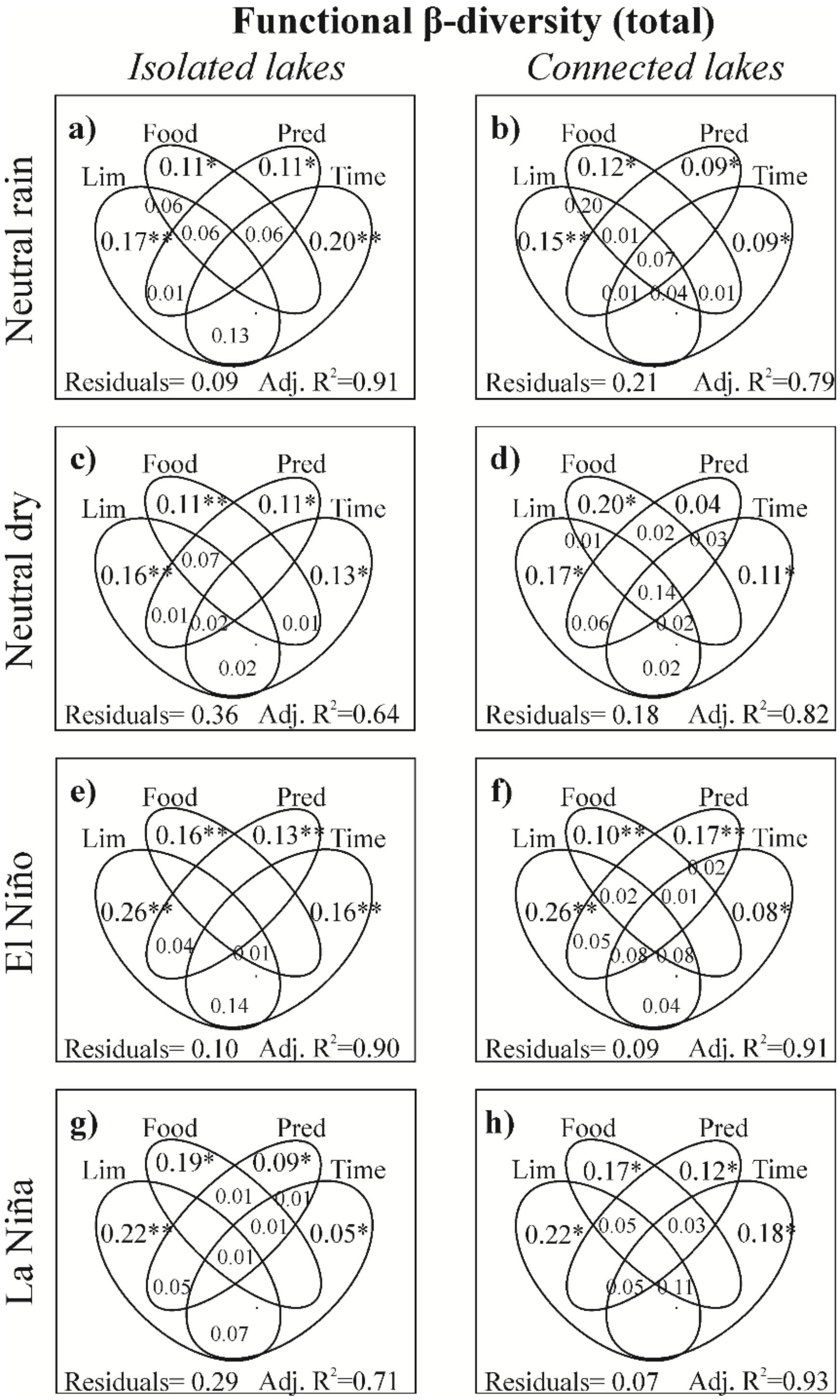

Fig. 6 Venn diagrams showing the relative contribution of limnological variables (Lim), food availability (Food), predation (Pred) and, time (Time) to zooplankton functional beta diversity in each climatic season, a, c, e, g are in isolated lakes and b, d, f, h in connected lakes. Values represent the adjusted R^2-values. Negative fraction values are not presented. Two asterisks represent significant results for $p < 0.001$ and, one asterisk represents significant results for $p < 0.05$.

Concerning the explanatory variables that explain the variation in zooplankton βrepl and βrich, limnological variables were the most representative. Fish predation was important to explain βrepl during El Niño in both types of lakes, also in neutral rainy season in isolated lakes (Table 2). Food availability alone was not representative to explain βrich variation, only in the shared portion (that was very representative in βrich db-RDA). The biggest explanations (including all variables) for βrepl variation were observed in neutral rainy (99%) and La Niña (97%) in isolated lakes; and, the biggest explanations (including all variables) for βrich were also observed during neutral rainy (96%) and La Niña (96%) in connected lakes (Table 2).

Table 2 The relative contribution of limnological variables (Lim/L), food availability (Food/F), fish predation (Pred/P) and Time (T) to zooplankton functional βrepl and βrich variation in each climatic season. Significant results are shown in bold ($p < 0.05$).

		Isolated		**Connected**	
		Brepl	βrich	βrepl	βrich
Neutral Rainy	Lim	**0.24**	**0.17**	**0.38**	0.02
	Food	**0.17**	**0.10**	**0.22**	**0.02**
	Pred	**0.22**	**0.18**	**0.12**	**0.07**
	Time	**0.21**	-	**0.01**	0.01
	L: F	-	0.05	-	0.63
	L: P	0.04	0.11	-	-
	L: T	-	-	0.01	-
	F: P	-	0.01	0.03	-
	F: T	-	0.01	0.04	-
	P: T	-	-	0.02	-
	L:F:P:T	-	0.11	-	0.12
	L:F:P	0.04	0.06	0.03	0.03
	L:F: T	0.07	0.08	0.01	0.06
	L:P:T	-	0.01	0.07	-
	F:P:T	-	0.02	0.02	-
	Residuals	0.01	0.09	0.04	0.04
Neutral Dry	Lim	**0.20**	0.06	**0.14**	-
	Food	**0.13**	**0.10**	**0.23**	0.06
	Pred	**0.08**	-	**0.06**	-
	Time	**0.03**	-	**0.07**	**0.20**
	L: F	-	0.01	0.11	-
	L: P	-	0.02	0.08	0.04
	L: T	0.12	0.01	0.07	0.08
	F: P	-	0.02	-	0.05
	F: T	-	-	-	-
	P: T	-	-	-	-
	L:F:P:T	0.03	0.27	0.14	0.07
	L:F:P	0.08	0.05	-	0.07

	L:F: T	-	-	0.03	-
	L:P:T	0.02	-	-	0.09
	F:P:T	0.04	0.18	0.01	-
	Residuals	0.27	0.28	0.06	0.34
El Niño	Lim	-	-	**0.08**	**0.16**
	Food	-	-	**0.12**	**0.01**
	Pred	**0.29**	-	**0.19**	-
	Time	**0.20**	**0.29**	**0.03**	**0.05**
	L: F	0.02	-	-	0.02
	L: P	0.01	-	0.12	-
	L: T	0.16	0.05	-	0.20
	F: P	0.01	0.01	-	-
	F: T	-	0.18	-	-
	P: T	-	-	0.12	-
	L:F:P:T	-	0.02	-	0.25
	L:F:P	-	-	0.14	0.01
	L:F: T	0.19	0.04	0.01	0.05
	L:P:T	-	-	-	0.12
	F:P:T	0.03	0.06	0.04	0.03
	Residuals	0.09	0.35	0.15	0.10
La Niña	Lim	**0.11**	**0.45**	**0.21**	**0.61**
	Food	**0.22**	-	**0.07**	0.07
	Pred	**0.18**	**0.08**	0.05	-
	Time	**0.11**	-	**0.18**	0.01
	L: F	-	0.03	0.06	0.05
	L: P	0.06	-	0.07	-
	L: T	0.14	-	-	-
	F: P	-	-	0.05	0.08
	F: T	-	0.01	0.01	0.03
	P: T	0.06	-	-	-
	L:F:P:T	-	-	-	0.08
	L:F:P	-	0.16	-	0.02
	L:F: T	0.09	-	0.17	-
	L:P:T	-	-	-	0.01
	F:P:T	-	-	-	-
	Residuals	0.03	0.27	0.13	0.04

4.3.4 Interaction between functional traits and predictor variables

Food availability and fish predation displayed the majority of the correlations with the traits. During El Niño in connected lakes, the trait body size was negatively related to food availability ($p < 0.001$), representing the only negative interaction (Fig. 7). Sixteen other positive relationships were observed between different traits and predictors, most of them (five) were during El Niño in connected lakes, the traits *habitat*, *feeding type*, *time oflife*, *reproduction*, and *predator escape* were related to food availability. During neutral rainy, the isolated lakes presented four interactions, all with predation (traits: feeding type, time of life, reproduction and predator escape). The only season with no significant interaction was neutral rainy in connected lakes (Fig. 7).

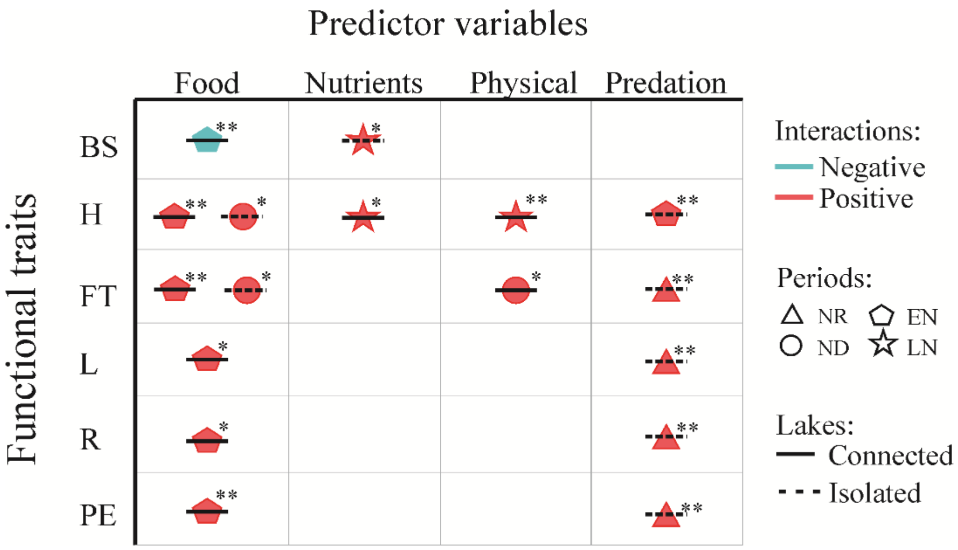

Fig 7 Fourth-corner significant results showing the interaction between the functional traits and the predictor variables in each season (geometric forms), in connected (solid line) and isolated (dotted line) lakes. The positive interactions are shown in red and negative interactions in blue. BS = body size; H = habitat, FT = feeding type; L = time of life; R = reproduction; PE = predator escape; NR = neutral rainy, ND = neutral dry, EN = El Niño and LN = La Niña. For more details of each predictor variable please see methods. One asterisk represents $p < 0.05$ and two asterisks represent $p < 0.001$.

4.4 Discussion

ENSO events are more intense due to climate change letting the aquatic environments more exposed to extremely dry and rainy periods (Marengo et al. 2012; Cai et al. 2014). The natural variation on precipitation is important to maintain the high heterogeneity and biodiversity in aquatic systems, especially in floodplains (Junk et al. 1989; Neiff 1990; Pineda et al. 2019). Here we show how neutral and extreme events in precipitation influence the limnological heterogeneity and, consequently, the distribution of functional traits and species. We also show the importance of different factors (biotic and abiotic) in structure the beta functional diversity in lakes with distinct connections to the main rivers and, which traits are related to these predictors. The limnological characteristics of the lakes fluctuateddepending on the climate event, influencing the zooplankton community and their relation with predators and food resources.

Regarding the environmental heterogeneity, isolated lakes can exhibit large seasonal changes in morphometry, wind exposure, and chemical variables during the different hydrological periods (as we observed) (Bovo-Scomparin and Train 2008), but the connected lakes are regulated daily by the rivers, which in this case present distinct characteristics among them. Paraná is a dammed river with oligotrophic characteristic (Roberto et al. 2009), Baia is a river with many backwaters and reduced water flows, whereas Ivinheima is a river that runs inside conservation units and it is considered a natural refuge for fishes reproduction (Agostinho and Zalewski 1996; Reynalte-Tataje et al. 2013). This is the possible reason that we observed more heterogeneity in connected lakes in all seasons analyzed, as they are regulated primarily by the rivers, reflecting high heterogeneity among them. The opposite of isolated lakes during La Niña, where probably similar limnological factors were acting simultaneously during the “isolation” reflecting in a lower environmental heterogeneity (Chase 2007). The high heterogeneity during El Niño and neutral seasons could be also related to intrinsic characteristics of each lake, such as allochthones inputs and, water flow that create an environmental and temporal gradient of the heterogeneity and species/traits composition (Simões et al. 2013), which also reinforces the high importance of the temporal factor during El Niño in isolated lakes on beta partitioning.

The lower heterogeneity during La Niña was linked to lower functional and taxonomic β-diversity, showing that the lakes displayed similar variation on limnological characteristics during extremes dry periods. Therefore, possibly, environmental factors acting on each lake were similar to select the traits and species, reflecting in potential homogenization (lower β-richness) with losses of traits and taxon in this season (Chase 2007). The lower β-total and β-richness could have also resulted from niche-selection, which was filtering out from the regional pool those species with adaptations to survive and persist in those conditions of environmental harshness (Chase 2007; Gianuca et al. 2017). These relations are reinforced by the high contribution of the limnological factor to explain the β-richness variation in the db-RDA during La Niña. The species that are able to persist after intense dry periods do so either being resilient and capable of rapid recolonization and/or producing resistant life stages as diapausing eggs (Wellborn et al. 1996). In the last years, the dry seasons are more common in this floodplain because of the dam regulation (Agostinho et al. 2004), which also could be leading to a lower dissimilarity among the years (Pineda et al. 2019) and, traits homogenization (Braghin et al. 2018). Moreover, it is expected that extreme dry events (La Niña) with greater frequency and duration will turn the environments, the species and traits composition more similar (Bertoncin et al. 2019). This allied to climate change effects that will turn extreme dry more frequently should be carefully analyzed for conservation actions, as we observed lower β-total and β-richness leading to a potential homogenization (biotic and functional) during extreme dry, what can reflect in losses of ecosystems functions and processes.

During the rainy season (especially under El Niño influence), the connectivity among sites is bigger, enabling greater species and traits distribution (Bozelli et al. 2015) but, in this season the heterogeneity was also greater. Thus, the species and traits were arriving in the environments (losing the replacement importance) but the environmental conditions were selecting them, i.e. more suitable environments accept more species and traits (Heino et al. 2015), increasing the richness importance, as observed during El Niño. The high β-total and β-richness during El Niño confirm the importance of this event to maintain the floodplain heterogeneity, allowing the exchange of functional traits and species among the environments and maintaining high biodiversity and the ecosystem process (Pineda et al. 2019). Especially under influence of hydrological regulation imposed by the dams upstream (Agostinho et al. 2004), thus, extreme rainy season during El Niño could reduce these negative effects allowing the connection among environments and the shifts in species composition (Stein et al. 2014). Whereas, neutral seasons here (dry and, rainy) that presented great dissimilarity, might be reflecting a legacy of previous rainy events, which were responsible for increasing the dispersion of organisms (Thomaz et al. 2007).

Concerning the factors that shaped functional beta diversity, the contribution of temporal factor, alone and in shared proportions, shows the contribution of intrinsic stochastic effects. Legendre and Gauthier (2014) assign the temporal effect in the partitioning analysis to neutral processes, such as local extinction due to demographic stochasticity and random colonization. While the other factors in our study (environmental, food availability and predation) could be representing the niche-process. The contribution of limnological variables was most related to nutrients, temperature, and, wind velocity. Wind variation is very important to planktonic communities in shallow lakes, mixing and resuspending nutrients and organic matter, influencing in this way, food availability (Serra et al. 2007) and, predation relations, especially during dry seasons. Temperature and nutrients could restrict the establishments of zooplanktonic species due to thermic tolerance and stoichiometric requirements (Brown et al. 2004). These relations were confirmed by the fourth-corner analyses, which showed that during La Niña and neutral dry seasons the traits habitat and feeding type were positively related to physical factors and, the traits body size and habitat were positively related to nutrients.

Both food resources (phytoplankton and ciliates) explained beta functional diversity and were related to the functional traits in the fourth-corner analyses. Phytoplankton, especially nanoplanktonic and picoplanktonic unicellular algae (chosen by the model) represent the zooplankton's preferred type of food (Colina et al. 2016; Bomfim et al. 2018). Protists as ciliates can also represent an important portion of food consumption for cladocerans and copepods (Auer et al. 2004). The many positive relations observed during El Niño are probably the result of the limnological heterogeneity, which provides more niche opportunities, allowing the establishment of more species and traits (Heino et al. 2015). Bovo-Scomparin and Train (2008) found low phytoplankton biovolume during rainy seasons related to ENSO, in these same environments, this could explain the negative relationship observed between body size and food availability during El Niño, as in low food availability the large-bodied zooplankton species are selected, and the inverse is also true, high food availability select the small-bodied zooplankton due to competition relations (Bomfim et al. 2018).

Fish predation was important to explain beta-diversity in almost all seasons. The highest contribution of predation was during El Niño when possibly the connectivity among the sites allowed the entry of fishes in the lakes increasing their abundances (Fernandes et al. 2009). It was also during El Niño and neutral rain that predation was positively related to the functional traits such as habitat, feeding type, time of life, reproduction and predator escape. Predation is the main force on structure the communities and shapes beta diversity (Antiqueira et al. 2018; He et al. 2018). The presence of predators alters the distribution of functional traits (Sodré and Bozelli 2019), altering the life-history parameters, such as body size, growth and, clutch-size (Santangelo et al. 2018), which reflect on changes in the community growth and establishment. The predation presence can also reduce competition allowing more species and traits to coexist (Gurevitch et al. 2000; Chase et al. 2002). Predation was also important to explain β-replacement during La Niña in isolated lakes, possibly reinforcing the relation between water reduction and ecological interactions, with local forces acting (Thomaz et al. 2007).

Our findings showed that the magnitudes of zooplankton beta diversity varied depending on the climate events and the hardness of these events. The heterogeneity and beta diversity followed a different pattern than expected with higher beta diversity and heterogeneity observed in El Niño, and lowest in La Niña. Also, limnological variables, food availability, fish predation, and temporal predictor can have distinct importance to structure beta diversity and functional traits depending on the climate season and type of lake. The functional diversity approach does a straight connection with ecosystem functioning (Hébert et al. 2017), and understand how the functional traits are distributed among aquatic environments over time enables us to link the species contribution to the ecosystem process. In this way, the potential species and traits homogenization during extreme dry season (as observed) could lead to losses in ecosystem functions and services, if these events continue to be more frequent and prolonged due the dam regulation (Agostinho et al. 2004; Braghin et al. 2018; Bertoncin et al. 2019) and/or climate change (Cai et al. 2015). Therefore, we reaffirm that the natural flood and the flood caused by the extreme rainy season (without the dam regulation) became extremely important to maintain the high heterogeneity and beta diversity (Pineda et al. 2019), allowing the coexistence of more functional traits and ecosystem functions. Maintain also the stocks of energy and the equilibrium of the ecosystem.

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APPENDIX E - Short description of the sampled lakes at the Upper Paraná River floodplain according to Souza-Filho and Stevaux (1997).

Lakes	**Environmental characterization**
	Isolated lakes
Osmar lake Paraná River sub-system	Localization: 22°46’S; 53°19’W - Mean depth: 1.13 m; area: 600 m^2; a temporary lake with dense vegetation on its banks, it gets connected to the river only during large floods.
Ventura lake Ivinheima River sub-system	Localization: 22º51' S; 53º36'W - Mean depth: 2.16 m - area: 897 m^2. This lake is 200 m far from the Ivinheima river, it gets connected to the river only during large floods.The vegetation on its banks is compound by shrubby vegetation and grasses.
Fechadalake Baia River sub-system	Localization: 22º42' S; 53º16' W - Mean depth: 2.46 m - area: 746 m^2. This lake is 100 m far from the Baia river and it gets connected to the river only during large floods.The vegetation on its banks is compound by shrubby vegetation and grasses.
	Connected lakes
Garças Paraná River sub-system	Localization: 22°43'S; 53°13'W - Mean depth: 2.03 m - area: 148 m^2; connected to Parana river through a short canal. The vegetation is arboreal in its banks.
Patos Ivinheima River sub-system	Localization: 22º49'S; 53º33’W - Mean depth: 3.50 m - area: 1,138 m^2. Presents entrances forming small bays. It is 10 m far from the Ivinheima river and it is connected to this river by a channel 8 m large. The vegetation on its banks is compound by grasses.
Guaraná Baia River sub-system	Localization: 22º43' S; 53º18' W - Mean depth: 2.06 m - area: 421 m^2. Connected to the Baia river, it is 70 m long and 18 m large, there is high macrophytes abundance in this lake. The vegetation on its banks is compound by grasses.

Souza-Filho EE, Stevaux JC (1997) Geologia e geomorfologia do complexo rio Baía, Curutuba, Ivinheima. In: Vazzoler AEAM, Agostinho ÂA, Hahn NS (eds) A Planície de Inundação do Alto Rio Paraná: aspectos físicos, biológicos e socioeconômicos. UEM, Maringa.

APPENDIX F - Table with the classification of each species in the six functional traits. Filter-R= Filter rotifer, Sucker-R= Sucker rotifer, Predator-R= Predator rotifer, Filter-Clad= Filter cladoceran, Scraper-Clad= Scraper cladoceran, Raptorial-Cop= Raptorial copepod, Filter-Cop= Filter copepod.

Species	**Body length (µm)**	**Habitat**	**Feeding type**	**Time of life**	**Repro-duction**	**Predatory Escape Response**
Rotifers						
Lecane bulla (Gosse, 1886)	115	Littoral	Filter-R	Short	Asexual	Low

L. closterocerca(Schmarda, 1859)	85	Littoral	Filter-R	Short	Asexual	Low
L. cornuta (Muller, 1786)	109	Littoral	Filter-R	Short	Asexual	Low
L. curvicornis (Murray, 1913)	131	Littoral	Filter-R	Short	Asexual	Low
L. elsa Hauer, 1931	150	Littoral	Filter-R	Short	Asexual	Low
L. furcata(Murray, 1913)	655	Littoral	Filter-R	Short	Asexual	Low
*L. haliclysta*Harring & Myers, 1926	975	Littoral	Filter-R	Short	Asexual	Low
L. hastata (Murray, 1913)	86	Littoral	Filter-R	Short	Asexual	Low
L. leontina (Turner, 1892)	175	Littoral	Filter-R	Short	Asexual	Low
L. ludwigii (Eckstein, 1883)	134	Littoral	Filter-R	Short	Asexual	Low
L. luna (Müller, 1776)	127	Littoral	Filter-R	Short	Asexual	Low
L. lunaris (Ehrenberg, 1832)	102	Littoral	Filter-R	Short	Asexual	Low
L. monostyla (Daday, 1897)	69	Littoral	Filter-R	Short	Asexual	Low
L. papuana (Murray, 1913)	108	Littoral	Filter-R	Short	Asexual	Low
L. mira (Murray, 1913)	145	Littoral	Filter-R	Short	Asexual	Low
L. proiecta Hauer, 1956	113	Littoral	Filter-R	Short	Asexual	Low
L. rhytida Harring and Myers, 1926	81	Littoral	Filter-R	Short	Asexual	Low
L. quadridentata(Ehrenberg, 1830)	163	Littoral	Filter-R	Short	Asexual	Low
L. stichaea Harring, 1913	173	Littoral	Filter-R	Short	Asexual	Low
L. signifera (Jennings, 1896)	113	Littoral	Filter-R	Short	Asexual	Low
L. ungulata (Gosse, 1887)	158	Littoral	Filter-R	Short	Asexual	Low
L. stenroosi (Meissner, 1908)	118	Littoral	Filter-R	Short	Asexual	Low
Brachionus angularis Gosse, 1851	107	Pelagic	Filter-R	Short	Asexual	Low
B. bidentatus Anderson, 1889	368	Pelagic	Filter-R	Short	Asexual	Low
B. calyciflorus (Pallas, 1766)	201	Pelagic	Filter-R	Short	Asexual	Low
B. caudatus Barrois & Daday, 1894	270	Pelagic	Filter-R	Short	Asexual	Low
B. dolabratus Harring, 1914	167	Pelagic	Filter-R	Short	Asexual	Low
B. falcatus Zacharias, 1898	430	Pelagic	Filter-R	Short	Asexual	Low
B. forficula Wierzejski, 1891	145	Pelagic	Filter-R	Short	Asexual	Low
B. mirus Daday, 1905	139	Pelagic	Filter-R	Short	Asexual	Low
B. quadridentatus Hermann, 1783	144	Pelagic	Filter-R	Short	Asexual	Low
Kellicottia bostoniensis (Rousselet, 1908)	114	Pelagic	Filter-R	Short	Asexual	Low
Keratella americana Carlin, 1943	160	Pelagic	Filter-R	Short	Asexual	Low
K. cochlearis (Gosse, 1851)	107	Pelagic	Filter-R	Short	Asexual	Low
K. lenzi Hauer, 1953	112	Pelagic	Filter-R	Short	Asexual	Low
K. tropica (Apstein, 1907)	115	Pelagic	Filter-R	Short	Asexual	Low
Plationus macrachantus(Daday, 1905)	141	Littoral	Filter-R	Short	Asexual	Low
Plationus patulus (Müller,1786)	122	Littoral	Filter-R	Short	Asexual	Low
Platyias leloupi Gillard, 1967	219	Pelagic	Filter-R	Short	Asexual	Low
P. quadricornis (Ehrenberg, 1832)	142	Pelagic	Filter-R	Short	Asexual	Low
Trichocerca bicristata (Gosse, 1887)	660	Littoral	Sucker-R	Short	Asexual	Low
T. bidens (Lucks, 1912)	133	Littoral	Sucker-R	Short	Asexual	Low

T. capucina(Wierzejski & Zacharias, 1893)	325	Littoral	Sucker-R	Short	Asexual	Low
T. collaris(Rousselet, 1896)	119	Littoral	Sucker-R	Short	Asexual	Low
T. cylindrica (Imhof, 1891)	325	Littoral	Sucker-R	Short	Asexual	Low
T. flagellata Hauer, 1937	112	Littoral	Sucker-R	Short	Asexual	Low
T. dixonnuttalli (Jennings 1903)	117	Littoral	Sucker-R	Short	Asexual	Low
T. elongata (Gosse, 1886)	237	Littoral	Sucker-R	Short	Asexual	Low
T. heterodactyla (Tschugunoff, 1921)	225	Littoral	Sucker-R	Short	Asexual	Low
T. gracillis (Tessin, 1890)	141	Littoral	Sucker-R	Short	Asexual	Low
T. iernis (Gosse, 1887)	135	Littoral	Sucker-R	Short	Asexual	Low
T. inermis (Linder, 1904)	885	Littoral	Sucker-R	Short	Asexual	Low
T. macera(Gosse, 1886)	294	Littoral	Sucker-R	Short	Asexual	Low
T. insulana(Hauer, 1937)	100	Littoral	Sucker-R	Short	Asexual	Low
T. pusilla (Jennings, 1903)	175	Littoral	Sucker-R	Short	Asexual	Low
T. rosea (Stenroos, 1898)	200	Littoral	Sucker-R	Short	Asexual	Low
T. ruttneri Donner, 1953	855	Littoral	Sucker-R	Short	Asexual	Low
T. scipio(Gosse, 1886)	408	Littoral	Sucker-R	Short	Asexual	Low
T. similis (Wierzejski, 1893)	300	Littoral	Sucker-R	Short	Asexual	Low
T. tigris (Müller, 1786)	260	Littoral	Sucker-R	Short	Asexual	Low
T. stylata (Gosse, 1851)	167	Littoral	Sucker-R	Short	Asexual	Low
Beuchampiella eudactylota (Gosse, 1886)	760	Littoral	Filter-R	Short	Asexual	Low
Dipleuchlanis propatula (Gosse, 1886)	508	Littoral	Filter-R	Short	Asexual	Low
Euchlanis deflexa (Gosse, 1851)	275	Littoral	Filter-R	Short	Asexual	Low
E. dilatata Ehrenberg, 1832	188	Littoral	Filter-R	Short	Asexual	Low
E. meneta Myers, 1930	136	Littoral	Filter-R	Short	Asexual	Low
E. incisa Carlin, 1939	229	Littoral	Filter-R	Short	Asexual	Low
Lophocharis salpina (Ehrenberg, 1834)	102	Littoral	Filter-R	Short	Asexual	Low
Mytilina macrocerca (Jennings, 1894)	320	Littoral	Filter-R	Short	Asexual	Low
M. acanthophora Hauer, 1938	164	Littoral	Filter-R	Short	Asexual	Low
M. ventralis (Ehrenberg, 1830)	175	Littoral	Filter-R	Short	Asexual	Low
M. bisulcata (Lucks, 1912)	159	Littoral	Filter-R	Short	Asexual	Low
M. mucronata (Müller, 1773)	212	Littoral	Filter-R	Short	Asexual	Low
Testudinella mucronata (Gosse, 1886)	181	Littoral	Filter-R	Short	Asexual	Low
T. ohlei Koste, 1972	140	Littoral	Filter-R	Short	Asexual	Low
T. patina (Hermann, 1783)	350	Littoral	Filter-R	Short	Asexual	Low
*Pompholyx complanata*Gosse, 1851	90	Pelagic	Filter-R	Short	Asexual	Low
P. triloba Pejler, 1957	835	Pelagic	Filter-R	Short	Asexual	Low
*P. sulcata*Hudson, 1885	110	Pelagic	Filter-R	Short	Asexual	Low
Filinia longiseta (Ehrenberg, 1834)	141	Pelagic	Filter-R	Short	Asexual	Low
F. limnetica(Zacharias, 1893)	185	Pelagic	Filter-R	Short	Asexual	Low
F. opoliensis Zacharias, 1891	220	Pelagic	Filter-R	Short	Asexual	Low
*F. pjeleri*Hutchinson, 1964	179	Pelagic	Filter-R	Short	Asexual	Low
F. saltator (Gosse, 1886)	149	Pelagic	Filter-R	Short	Asexual	Low
F. terminalis (Plate, 1886)	138	Pelagic	Filter-R	Short	Asexual	Low

Ploesoma lenticulare Herrick, 1885	254	Pelagic	Filter-R	Short	Asexual	Low
P. truncatum (Levander, 1894)	131	Pelagic	Filter-R	Short	Asexual	Low
Polyarthra dolicoptera Idelson, 1925	965	Pelagic	Filter-R	Short	Asexual	Low
P. vulgaris (Carlin, 1943)	115	Pelagic	Filter-R	Short	Asexual	Low
*P. remata*Skorikov, 1896	925	Pelagic	Filter-R	Short	Asexual	Low
Synchaeta pectinate Ehrenberg 1832	860	Pelagic	Filter-R	Short	Asexual	Low
S. oblonga Ehrenberg, 1831	110	Pelagic	Filter-R	Short	Asexual	Low
*S. stylata*Wierzejski, 1893	238	Pelagic	Filter-R	Short	Asexual	Low
Sinantherina spinosa(Thorpe, 1893)	1,050	Littoral	Filter-R	Short	Asexual	Low
S. procera(Thorpe, 1893)	2,540	Littoral	Filter-R	Short	Asexual	Low
Floscularia ringens(Linnaeus, 1758)	1,900	Pelagic	Filter-R	Short	Asexual	Low
Ptygura sp.	350	Littoral	Filter-R	Short	Asexual	Low
Cephalodella forficula(Ehrenberg, 1830)	243	Littoral	Sucker-R	Short	Asexual	Low
*C. obvia*Donner, 1949	143	Littoral	Sucker-R	Short	Asexual	Low
C. gibba (Ehrenberg, 1830)	114	Littoral	Sucker-R	Short	Asexual	Low
*C. mucronata*Myers, 1924	209	Littoral	Sucker-R	Short	Asexual	Low
*Enteroplea lacustris*Ehrenberg, 1830	431	Littoral	Sucker-R	Short	Asexual	Low
Eothinia elongata(Ehrenberg, 1832)	410	Littoral	Sucker-R	Short	Asexual	Low
Eosphora anthadis (Harring & Myers, 1922)	312	Littoral	Sucker-R	Short	Asexual	Low
Monommata dentata Wulfert, 1940	400	Littoral	Sucker-R	Short	Asexual	Low
M. ardnti Remane, 1933	210	Littoral	Sucker-R	Short	Asexual	Low
Notommata copeus Ehrenger, 1834	544	Littoral	Sucker-R	Short	Asexual	Low
N. cerberus(Gosse, 1886)	355	Littoral	Sucker-R	Short	Asexual	Low
N. pachyura (Gosse, 1886)	482	Littoral	Sucker-R	Short	Asexual	Low
*N. prodota*Myers, 1933	328	Littoral	Sucker-R	Short	Asexual	Low
N. pseudocerberus Beauchamp, 1908	493	Littoral	Sucker-R	Short	Asexual	Low
*N. saccigera*Ehrenberg, 1830	271	Littoral	Sucker-R	Short	Asexual	Low
*Tetrasiphon hydracora*Ehrenberg, 1840	450	Littoral	Sucker-R	Short	Asexual	Low
*Taphrocampa selenura*Gosse, 1887	135	Littoral	Sucker-R	Short	Asexual	Low
*Lepadella imbricata*Harring, 1914	97	Littoral	Filter-R	Short	Asexual	Low
L. ovalis (Müller, 1786)	150	Littoral	Filter-R	Short	Asexual	Low
L. patella (Müller, 1773)	145	Littoral	Filter-R	Short	Asexual	Low
Conochilus coenobasis (Skorikov, 1914)	112	Pelagic	Filter-R	Short	Asexual	Low
*C. dossuaris*Hudson, 1885	100	Pelagic	Filter-R	Short	Asexual	Low
C. natans (Seligo, 1900)	75	Pelagic	Filter-R	Short	Asexual	Low
C. unicornis Rousselet, 1892	175	Pelagic	Filter-R	Short	Asexual	Low
Ascomorpha ecaudis Perty, 1850	170	Pelagic	Sucker-R	Short	Asexual	Low

A. ovalis (Bergendal, 1892)	176	Pelagic	Sucker-R	Short	Asexual	Low
A. saltans Bartsch, 1870	165	Pelagic	Sucker-R	Short	Asexual	Low
Gastropus hyptopus (Ehrenberg, 1938)	97	Pelagic	Sucker-R	Short	Asexual	Low
Proales sp.	120	Littoral	Filter-R	Short	Asexual	Low
Dicranophoroides caudatus (Ehrenberg, 1834)	310	Littoral	Predator-R	Short	Asexual	Low
*Dicranophorus epicharis*Harring & Myers, 1928	238	Littoral	Predator-R	Short	Asexual	Low
D. luetkeni(Bergendal, 1892)	167	Littoral	Predator-R	Short	Asexual	Low
Macrochaetus collinsii (Gosse, 1867)	250	Littoral	Filter-R	Short	Asexual	Low
M. sericus (Thorpe, 1893)	112	Littoral	Filter-R	Short	Asexual	Low
Trichotria tetractis Ehrenberg, 1830	295	Littoral	Filter-R	Short	Asexual	Low
Hexarthra intermedia (Wiszniewski, 1929)	234	Pelagic	Filter-R	Short	Asexual	Low
H. mira (Hudson, 1871)	152	Pelagic	Filter-R	Short	Asexual	Low
Collotheca sp.	380	Littoral	Filter-R	Short	Asexual	Low
*Asplanchna priodonta*Gosse 1850	323	Pelagic	Predator-R	Short	Asexual	Low
A. sieboldii (Leydig, 1854)	1,500	Pelagic	Predator-R	Short	Asexual	Low
*Harringia rousseleti*Beauchamp, 1911	383	Pelagic	Predator-R	Short	Asexual	Low
Epiphanes clavulata (Ehrenberg, 1832)	125	Littoral	Filter-R	Short	Asexual	Low
E. macrourus Barrois and Daday, 1894	187	Littoral	Filter-R	Short	Asexual	Low
Microcodides robusuts(Glascott, 1892)	252	Littoral	Filter-R	Short	Asexual	Low
Bdelloidea	625	Littoral	Filter-R	Short	Asexual	Low
*Lindia (Lindia) torulosa*Dujardin, 1841	293	Pelagic	Predator-R	Short	Asexual	Low
Scaridium longicaudatum(Müller, 1786)	400	Littoral	Predator-R	Short	Asexual	Low
Itura deridderae Segers, 1993	305	Pelagic	Predator-R	Short	Asexual	Low
*I. myersi*Wulfert, 1935	112	Pelagic	Predator-R	Short	Asexual	Low
*I. chamadis*Harring & Myers, 1928	263	Pelagic	Predator-R	Short	Asexual	Low
Cladocerans						
Moina minuta Hansen, 1899	612	Pelagic	Filter-Clad	Short	Asexual	Medium
M. reticulata (Daday, 1905)	750	Pelagic	Filter-Clad	Short	Asexual	Medium
M. rostrata McNair, 1980	760	Pelagic	Filter-Clad	Short	Asexual	Medium
M. micrura Kurz, 1874	440	Pelagic	Filter-Clad	Short	Asexual	Medium
Moinodaphnia macleayi(King, 1853)	580	Pelagic	Filter-Clad	Short	Asexual	Medium
Moina sp.	640	Pelagic	Filter-Clad	Short	Asexual	Medium
Bosmina hagmanni Stingelin, 1904	301	Pelagic	Filter-Clad	Short	Asexual	Medium
B. tubicen Brehm, 1939	294	Pelagic	Filter-Clad	Short	Asexual	Medium
B longirostris De Melo & Hebert, 1994	300	Pelagic	Filter-Clad	Short	Asexual	Medium
Bosmina sp.	300	Pelagic	Filter-Clad	Short	Asexual	Medium

Bosminopsis deitersi Richard, 1895	227	Pelagic	Filter-Clad	Short	Asexual	Medium
Ceriodaphnia cornuta Sars, 1886	289	Pelagic	Filter-Clad	Short	Asexual	Medium
C. reticulata (Jurine, 1820)	1,000	Pelagic	Filter-Clad	Short	Asexual	Medium
C. silvestrii Daday, 1902	450	Pelagic	Filter-Clad	Short	Asexual	Medium
C. richardi Sars, 1901	647	Pelagic	Filter-Clad	Short	Asexual	Medium
Daphnia gessneri (Herbst, 1967)	812	Pelagic	Filter-Clad	Short	Asexual	Medium
*D. ambigua*Scourfield, 1947	1,051	Pelagic	Filter-Clad	Short	Asexual	Medium
Simocephalus serrulatus(Koch, 1841)	2,005	Pelagic	Filter-Clad	Short	Asexual	Medium
S. latirostris Stingelin, 1906	1,600	Pelagic	Filter-Clad	Short	Asexual	Medium
S. vetulus(Müller, 1776)	1,850	Pelagic	Filter-Clad	Short	Asexual	Medium
*S. iheringi*Richard, 1897	1,200	Pelagic	Filter-Clad	Short	Asexual	Medium
Simocephalus sp.	1,641	Pelagic	Filter-Clad	Short	Asexual	Medium
Diaphanosoma birgei Korineck, 1981	506	Pelagic	Filter-Clad	Short	Asexual	Medium
D. brevireme Sars, 1901	612	Pelagic	Filter-Clad	Short	Asexual	Medium
D. spinulosum Herbst, 1967	550	Pelagic	Filter-Clad	Short	Asexual	Medium
D. fluviatile Hansen, 1899	538	Pelagic	Filter-Clad	Short	Asexual	Medium
D. polyspina Korovchink, 1982	630	Pelagic	Filter-Clad	Short	Asexual	Medium
Sarsilatona serricauda(Sars, 1901)	1,920	Pelagic	Filter-Clad	Short	Asexual	Medium
Alonella clathratula Sars, 1896	450	Littoral	Scraper-Clad	Short	Asexual	Low
A. dadayi Birge, 1910	213	Littoral	Scraper-Clad	Short	Asexual	Low
Alonella sp.	282	Littoral	Scraper-Clad	Short	Asexual	Low
Acroperus tupinamba Sinev & Elmoor-Loureiro, 2010	350	Pelagic	Scraper-Clad	Short	Asexual	Low
Ovalona glabra (Sars, 1901)	325	Littoral	Scraper-Clad	Short	Asexual	Low
Alona ossiani Sinev, 1998	800	Littoral	Scraper-Clad	Short	Asexual	Low
A. guttata Sars, 1862	250	Littoral	Scraper-Clad	Short	Asexual	Low
A. intermedia Sars, 1862	425	Littoral	Scraper-Clad	Short	Asexual	Low
*A. yara*Sinev & Elmoor-Loureiro, 2010	580	Littoral	Scraper-Clad	Short	Asexual	Low
Alona sp.	450	Littoral	Scraper-Clad	Short	Asexual	Low
Flavalona iheringula (Kotov & Sinev, 2004)	300	Littoral	Scraper-Clad	Short	Asexual	Low
Karualona muelleri (Richard, 1897)	462	Littoral	Scraper-Clad	Short	Asexual	Low
Coronatella monocantha (Sars, 1901)	264	Littoral	Scraper-Clad	Short	Asexual	Low
Magnospina dentifera (Sars, 1901)	480	Littoral	Scraper-Clad	Short	Asexual	Low
Nicsmirnovius paggii Sousa & Elmoor-Loureiro, 2017	325	Littoral	Scraper-Clad	Short	Asexual	Low
Nicsmirnovius incredibilis (Smirnov, 1984)	440	Littoral	Scraper-Clad	Short	Asexual	Low
Anthalona verrucosa Sars, 1901	300	Littoral	Scraper-Clad	Short	Asexual	Low
Coronatella poppei (Richard, 1897)	393	Littoral	Scraper-Clad	Short	Asexual	Low

Bergamina lineolata (Sars, 1901)	310	Littoral	Scraper-Clad	Short	Asexual	Low
Camptocercus australis Sars, 1896	680	Littoral	Scraper-Clad	Short	Asexual	Low
Chydorus eurynotus Sars, 1901	242	Littoral	Scraper-Clad	Short	Asexual	Low
C. parvireticulatus Frey, 1897	300	Littoral	Scraper-Clad	Short	Asexual	Low
C. nitidulus(Sars, 1901)	260	Littoral	Scraper-Clad	Short	Asexual	Low
C. pubescens Sars, 1901	287	Littoral	Scraper-Clad	Short	Asexual	Low
C. sphaericus(Müller, 1776)	500	Littoral	Scraper-Clad	Short	Asexual	Low
Chydorus sp.	317	Littoral	Scraper-Clad	Short	Asexual	Low
Ephemeroporus barroisi (Richard, 1894)	270	Littoral	Scraper-Clad	Short	Asexual	Low
E. tridentatus (Bergamin, 1931)	310	Littoral	Scraper-Clad	Short	Asexual	Low
E. hybridus (Daday, 1905)	260	Littoral	Scraper-Clad	Short	Asexual	Low
Dunhevedia odontoplax Sars, 1901	460	Littoral	Scraper-Clad	Short	Asexual	Low
Euryalona brasiliensis Brehm & Thomsen, 1936	362	Littoral	Scraper-Clad	Short	Asexual	Low
E. orientalis (Daday, 1898)	450	Littoral	Scraper-Clad	Short	Asexual	Low
Graptoleberis occidentalis Sars, 1901	391	Littoral	Scraper-Clad	Short	Asexual	Low
Kurzia polyspina Hudec, 2000	600	Littoral	Scraper-Clad	Short	Asexual	Low
K. longirostris Daday, 1898	420	Littoral	Scraper-Clad	Short	Asexual	Low
*Leydigia striata*Berabén, 1939	631	Littoral	Scraper-Clad	Short	Asexual	Low
*L. curvirostris*Sars, 1901	850	Littoral	Scraper-Clad	Short	Asexual	Low
*L. propinqua*Sars, 1903	730	Littoral	Scraper-Clad	Short	Asexual	Low
Notoalona sculpta (Sars, 1901)	430	Littoral	Scraper-Clad	Short	Asexual	Low
*Oxyurella ciliata*Bergamin, 1939	440	Littoral	Scraper-Clad	Short	Asexual	Low
Ilyocryptus spinifer Herrich, 1882	266	Littoral	Filter-Clad	Short	Asexual	Low
Guernella raphaellis Richard, 1892	415	Littoral	Scraper-Clad	Short	Asexual	Low
Grimaldina brazzai Richard, 1892	775	Littoral	Scraper-Clad	Short	Asexual	Low
Macrothryx elegans (Sars, 1901)	300	Littoral	Scraper-Clad	Short	Asexual	Low
M. superaculeata (Smirnov, 1982)	350	Littoral	Scraper-Clad	Short	Asexual	Low
M. laticornis (Jurine, 1820)	375	Littoral	Scraper-Clad	Short	Asexual	Low
M. squamosa Sars, 1901	400	Littoral	Scraper-Clad	Short	Asexual	Low
*Streblocerus cf. pygmaeus*Sars, 1901	225	Littoral	Scraper-Clad	Short	Asexual	Low
Copepods						
Ectocyclops rubescens(Brady 1904)	944	Littoral	Raptorial-Cop	Long	Sexual	Big
*Eucyclops ensifer*Kiefer, 1936	962	Littoral	Raptorial-Cop	Long	Sexual	Big
E. elegans(Herrick, 1884)	945	Littoral	Raptorial-Cop	Long	Sexual	Big
Eucyclops sp.	1,005	Littoral	Raptorial-Cop	Long	Sexual	Big
Macrocyclops albidus (Jurine, 1820)	1,285	Littoral	Raptorial-Cop	Long	Sexual	Big
Mesocyclops	1,302	Littoral	Raptorial-Cop	Long	Sexual	Big

aspericornis(Daday, 1906)						
*M. ogunnus*Onabamiro1957	1,185	Littoral	Raptorial-Cop	Long	Sexual	Big
M. longisetus curvatus Dussart, 1987	1,597	Littoral	Raptorial-Cop	Long	Sexual	Big
M. meridianus (Kiefer, 1926)	767	Pelagic	Raptorial-Cop	Long	Sexual	Big
*M. ellipticus*Kiefer, 1936	965	Pelagic	Raptorial-Cop	Long	Sexual	Big
Microcyclops anceps (Richard, 1887)	750	Littoral	Raptorial-Cop	Long	Sexual	Big
M. alius (Kiefer, 1935)	575	Littoral	Raptorial-Cop	Long	Sexual	Big
M. ceibaensis(Marsh, 1919)	650	Littoral	Raptorial-Cop	Long	Sexual	Big
*M. finitimus*Dussart, 1984	578	Littoral	Raptorial-Cop	Long	Sexual	Big
Paracyclops chiltoni(Thomson, 1883)	739	Littoral	Raptorial-Cop	Long	Sexual	Big
Paracyclops sp.	819	Littoral	Raptorial-Cop	Long	Sexual	Big
Metacyclops laticornis(Lowndes, 1934)	772	Pelagic	Raptorial-Cop	Long	Sexual	Big
M. mendocinus(Wierzejski, 1892)	1,145	Pelagic	Raptorial-Cop	Long	Sexual	Big
Thermocyclops decipiens (Kiefer, 1929)	691	Pelagic	Raptorial-Cop	Long	Sexual	Big
T. minutus (Lowndes, 1934)	523	Pelagic	Raptorial-Cop	Long	Sexual	Big
T. inversus(Kiefer, 1936)	527	Pelagic	Raptorial-Cop	Long	Sexual	Big
Argyrodiaptomus azevedoi(Wright, 1935)	1,704	Pelagic	Filter-Cop	Long	Sexual	Max
Notodiaptomus cearensis (Wright, 1936)	1,100	Pelagic	Filter-Cop	Long	Sexual	Max
N. henseni (Dahl, 1894)	1,209	Pelagic	Filter-Cop	Long	Sexual	Max
N. iheringi (Wright, 1935)	922	Pelagic	Filter-Cop	Long	Sexual	Max
N. jatobensis(Wright, 1936)	1,073	Pelagic	Filter-Cop	Long	Sexual	Max
N. coniferoides(Wright, 1927)	1,548	Pelagic	Filter-Cop	Long	Sexual	Max
N. deitersi (Poppe, 1891)	1,240	Pelagic	Filter-Cop	Long	Sexual	Max
N. incompositus(Brian, 1925*)*	1,029	Pelagic	Filter-Cop	Long	Sexual	Max
N. isabelae (Wright, 1936)	1,058	Pelagic	Filter-Cop	Long	Sexual	Max
N. spiniger(Brian, 1926)	952	Pelagic	Filter-Cop	Long	Sexual	Max

APPENDIX G - Contribution of the most representative functional traits in each period and in each lake. Con= connected, Iso= isolated, Filt-R = filter-rotifer, Suc-Rot = sucker-rotifer, Filt-Clad = filter-cladoceran, Scra-Clad = scraper-cladoceran. * Time of life and reproduction are shown together as they had the same percentage in these periods.

	El Niño		**Neutral dry**		**Neutral rain**		**La Niña**	
Lakes	Con	Iso	Con	Iso	Con	Iso	Con	Isso
Species number	137	136	116	137	99	113	88	85
TRAITS CONTRIBUTION								
***Body-size* (mm)(mean ±SD)**	380.± 363	424± 377	369 ± 318	339 ± 305	348 ± 336	389 ± 353	351 ± 310	365 ± 337

Habitat	58.4 % littoral	51.4% littoral	57.7% littoral	62% littoral	54.5% littoral	58.4% littoral	53.4% pelagic	65% pelagic
Feeding type	44.5% Filt-R, 15% Suc-Rot	42% Filt-R, 17% Filt-Clad	47.4% Filt-R, 14.6% Scra-Clad	43% Filt-R, 18% Scra-Clad	47.4% Filt-R, 18.1% Filt-Clad	47% Filt-R, 21% Scra-Clad	51.13% Filt-R, 12.5% Suc-Rot	51.7% Filt-R, 16.4% Filt-Clad
Time of life and Reproduction *	92.7 % short and asexual	91 % short and asexual	86 % short and asexual	93 % short and asexual	91 % short and asexual	91% short and asexual	88 % short and asexual	89 % short and asexual
Predator escape	79 % low	75% low	75% low	84% low	76% low	79% low	77 % low	75% low

APPENDIX H - Selected variables by forward selection methods which compose limnological, food, fish and time variables on functional beta diversity of zooplankton in the climatic events. Iso.= isolated lakes, Con.= connected lakes, TN = total nitrogen, NO3 = nitrate, PO4 = phosphate, TP = total phosphorous, Turb. = turbidity, temp= water temperature, wind= wind velocity, ISM = inorganic suspended matter, transp. = water transparency, DO = dissolved oxygen, Phyto = phytoplankton biovolume, nanop. = nanoplankton biovolume, picop. = picoplankton biovolume, abund.=individuals abundance, richn=species richness, axis = refers to PCoA axes; C1 and C2 are the first and second columns created by the AEM, respectively; $^{(a)}$ = β-total, $^{(b)}$ = β-replacement, $^{(c)}$ = β-richness.

Period	**Limnological variables**		**Food availability**		**Fish predation**		**Time**	
	Iso.	Con.	Iso.	Con.	Iso.	Con.	Iso.	Con.
Neutral rain	TN $^{(a, b)}$ PO4 $^{(a,c)}$	Wind $^{(a, c)}$ TP $^{(a, c)}$ Temp$^{(b, c)}$ DO$^{(b)}$ PO4$^{(b)}$ TN$^{(c)}$	Ciliate axis 2 $^{(a)}$, axis 1 $^{(b)}$ Nanop. $^{(c)}$	Nanop. $^{(a, c)}$ Ciliate axis 1 and 2 $^{(b)}$	Fish axis 1$^{(a, c)}$ Richn.$^{(b)}$	Fish axis 1 $^{(a, c)}$ richn.$^{(b)}$	C1 $^{(a,b)}$ C2 $^{(a,b,c)}$	C1 $^{(a,b,c)}$
Neutral Dry	NO3 $^{(a, b, c)}$ Turb. $^{(b)}$	Temp $^{(a, b)}$ Wind $^{(a, b)}$ PO4 $^{(b)}$ TP $^{(c)}$	Phyto axis 1 $^{(a, c)}$ Ciliate axis 2 $^{(b)}$	Phyto axis 1$^{(a)}$ axis 2 $^{(a, b)}$ Nanop. $^{(b)}$ Picop. $^{(c)}$	Fish axis 1 $^{(a, c)}$ Density $^{(b)}$	Fish axis 2 $^{(a, b, c)}$	C1 $^{(a, c)}$ C2 $^{(a, b)}$	C1 $^{(a, b)}$ C2 $^{(c)}$
El Niño	PO4 $^{(a, c)}$ Wind $^{(a, b)}$	Wind $^{(a, c)}$ Temp $^{(a)}$	Phyto axis 2 $^{(c)}$ Nanop	Ciliate axis 2 $^{(a)}$ Nanop	Fish axis 2 $^{(a, b, c)}$	Fish axis 2 $^{(a, c)}$ richn.	C2 $^{(a, b)}$ C1 $^{(c)}$	C1 $^{(a, b, c)}$ C2 $^{(b)}$

	Temp $^{(a)}$	NO3 $^{(b)}$	. $^{(a, b)}$	. $^{(b)}$ Picop. $^{(c)}$ Ciliate axis 1$^{(c)}$		and density $^{(b)}$		
La Niña	Temp $^{(a, b, c)}$ DO $^{(a)}$ ISM $^{(c)}$ TN $^{(c)}$	Wind $^{(a, b, c)}$ Transp. $^{(a, b)}$ TN $^{(b)}$ DO $^{(c)}$	Ciliate axis 2 $^{(a)}$ Phyto axis 2 $^{(b)}$ Nanop. $^{(c)}$	Ciliate abund$^{(a, b)}$ Picop. $^{(a,c)}$ Nanop. $^{(b)}$	Fish richn.$^{(a)}$ axis 2 $^{(b, c)}$	Fish richn.$^{(a, b, c)}$	C1 $^{(a, b)}$ C2 $^{(c)}$	C1 $^{(a, b)}$ C2 $^{(c)}$

5 FINAL CONSIDERATIONS

Several physical and biological factors can act in isolation or together on zooplanktonic organisms. In our experiments we observed that the increase in temperature actually leads to more complex relationships inside food webs with possible negative consequences for aquatic ecosystems. However, temperature was not the predominant factor in determining the performance of cladoceran species of temperate and subtropical climate, and predation was the most important factor.

High temperatures also attenuated the dominance in competition among cladoceran species. However, larger-bodied species (Daphnia magna and Daphnia pulex) are better competitors in temperate environments, and are only suppressed in the presence of fish predation. Thus, temperature, competition and predation act together altering the composition of species and changing the body size patterns of cladocerans, which can lead to changes in ecosystem services, such as primary, secondary productivity, and the energy stock available inside aquatic food-webs.

Fish larvae is an effective predator of small and medium-bodied subtropical cladocerans. *Ceriodaphnia silvestrii*, *Daphnia laevis* and *Simocephalus serrulatus* responded to fish chemical signal increasing their population growth and biomass production in an effort to leave as many offspring as possible to survive predation. In this study, temperature and predation reduced the performance of subtropical cladocerans, which altered the relationship between the micro-crustaceans and algae, especially at high temperatures. Because of the central position that zooplankton occupies in aquatic food-webs, the alteration in their growth, reproduction rates, and feeding rates can affect many ecosystem functions, including the availability of fish stocks, the control on algae bloom (especially cyanobacteria), and the water quality for human use.

The extreme climatic events of El Niño and La Niña can alter the limnological heterogeneity of shallow lakes, which acts as an environmental filter, altering the distribution of species and functional traits of zooplankton. Extreme dry periods (La Niña) have reduced the zooplankton functional and taxonomic beta diversity, with potential for loss of species and traits (homogenization), while during rainy periods we observed the highest beta diversity values, evidencing extremely importance to maintaining limnological heterogeneity, species biodiversity and thus ecosystem services, especially in aquatic environments that suffer from dams. Several factors were responsible for changing the distribution of species and functional traits, but the limnological variables explained the most community variation, although the availability of food resources (phytoplankton and ciliates), and predation by fish also explained the distribution of functional traits.

As we can see, changes in functional zooplankton responses, such as distribution of functional traits, biomass production, and feeding rates driven by changes in natural temperature and/or precipitation patterns lead to changes in the availability of energy and matter inside aquatic food-webs with negative consequences for functioning and ecosystem equilibrium. We emphasize the importance of conservation measures to maintain the natural biodiversity of aquatic environments, as a way to maintain the services offered by these ecosystems. As well, more investments in research to help understand the effects of global warming on aquatic ecosystems.

ANNEX A – Scientific articles published during the doctoral development period that contribute to the execution of this thesis

Fabiana Palazzo, **Francieli de Fátima** Bomfim, Juliana Dias, Nadson Ressyé Simões, Fábio A. Lansac-Tôha, Claudia C. Bonecker. Functional feeding traits of rotifers structured bytime, chlorophyll and suspended inorganic matter. Submetido narevista*International Review of Hydrobiology,* v. 106, p. e010, 2020.

Claudia Costa Bonecker, Leidiane Perreira Diniz, Louizi de Souza Magalhaes Braghin,Tatiane Mantovano, João Vitor Fonseca da Silva, **Francieli Fátima Bomfim** et al. SynergicEffects of Natural and Anthropogenic Impacts on Zooplankton Diversity in a SubtropicalFloodplain: A Long-Term Study.*Oecologia Australis*, v. 24, p. 524-537, 2020.

Francieli de Fátima Bomfim, Maria da Graça Gama Melão, Renan Castelhano Gebara and Fábio Amodêo Lansac-Tôha. Linking scenarios of global warming to matter cycling:consequences of high temperatures on *Ceriodaphnia silvestrii* metabolic rates and life history parameters. *Annals of the Brazilian Academy of Sciences*, 2020.

Diogo Castanho Amaral, **Francieli de Fátima Bomfim** and Fábio Amodêo Lansac-Tôha. Environmental heterogeneity drives the distribution of copepods (Crustacea: Copepoda) inthe Amazon, Araguaia, Pantanal, and Upper Paraná floodplains. *Annals of the Brazilian Academy of Sciences*, 2020.

Bomfim, Francieli F.; Braghin, Louizi S. M.; Bonecker, Claudia C.; Lansac-Tôha, Fábio A. High food availability linked to the dominance of small zooplankton in a subtropicalfloodplain. *International Review of Hydrobiology*, v. 103, p. 26-34, 2018.

Bomfim, F. F.; Mantovano, T.; Amaral, D. C.; Palhiarini, W. S.; Bonecker, C. C.; LansacToha, F. A. Adjacent environments contribute to the increase of zooplankton species in aneotropical river. *Acta Limnologica Brasiliensia*, v. 29, p. 1/e103, 2017.

Bomfim, F. F.; Mantovano, T.; Schwind, L. T. F.; Palazzo, F.; Bonecker, C. C.; LansacToha, F. A. Geographical spread of the invasive species Kellicottia longispina (Kellicott,1879) and K. bostoniensis (Rousselet, 1908): A scientometric approach. *Acta Scientiarum*. Biological Sciences, v. 38, p. 29, 2016.