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Phytoplankton community in the Anthropocene: effects of climate change and eutrophication

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Tese apresentada ao Programa de Pós-Graduação em Ecologia de Ambientes Aquáticos Continentais do Departamento de Biologia, Centro de Ciências Biológicas da Universidade Estadual de Maringá, como requisito parcial para obtenção do título de Doutor em Ecologia e Limnologia.

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Orientadora: Dr.ª Luzia Cleide Rodrigues
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“E o fim é belo e incerto, depende de como você vê...”

Fernando Anitelle

Comunidade fitoplanctônica no Antropoceno: efeitos das mudanças climáticas e eutrofização

RESUMO

Na era do Antropoceno, os efeitos das mudanças climáticas nos ecossistemas aquáticos continentais apresentam múltiplos fatores, uma vez que o aquecimento e os diferentes fatores relacionados às mudanças climáticas influenciam os processos físicos, biogeoquímicos e biológicos. As mudanças climáticas já causam impactos relevantes nos ecossistemas da Terra por meio do aumento da temperatura, mudanças nos padrões de precipitação, eventos climáticos extremos mais frequentes, mudanças nas concentrações de CO_2, entre outros. Os efeitos das mudanças climáticas somam-se aos efeitos contínuos de outros fatores globais e locais que afetam a diversidade, composição, estrutura e funcionamento das comunidades ecológicas, como eutrofização, fragmentação do habitat, mudanças no uso da terra e mudanças nos ciclos biogeoquímicos. Nesta tese, composta por dois artigos, avaliamos experimentalmente os efeitos de múltiplos fatores relacionados às mudanças climáticas na comunidade fitoplanctônica. No primeiro, conduzimos um experimento para testar como o aumento das temperaturas influencia a diversidade fitoplanctônica e as emissões de CO_2 em ambientes eutróficos. Nossos resultados mostram que, em cenários futuros de aquecimento, a composição da comunidade fitoplanctônica é alterada, afetando funções do ecossistema, como produção de biomassa, eficiência no uso de recursos e balanço de fluxo de carbono. O aquecimento agravou os efeitos negativos da eutrofização através do aumento das cianobactérias. Foi encontrado que a eutrofização pode promover mudanças climáticas, aumentando a liberação de gases de efeito estufa com evidências experimentais de um feedback positivo entre o principal sintoma de eutrofização (florações de cianobactérias) e o aquecimento, por meio de taxas de emissão de CO_2 mais altas em sistemas mais quentes dominados por cianobactérias, além de outras mudanças nas principais funções do ecossistema. No segundo artigo, conduzimos um experimento de curto prazo para testar como diferentes comunidades fitoplanctônicas naturais, promovidas por diferentes regimes de temperatura, reagiram a um evento de chuva extrema simulada e, assim, analisar a estabilidade e resiliência do ecossistema. Descobrimos que as comunidades fitoplanctônicas submetidas a diferentes temperaturas responderam de forma diferente aos distúrbios. As comunidades dominadas por florações de cianobactérias se beneficiaram e mostraram-se resilientes ao evento de chuvas extremas. Em contraste, as comunidades supostamente menos estressadas pelo aquecimento tiveram uma resposta mais lenta ao evento de chuvas extremas e não recuperaram a biomassa antes do evento de chuvas extremas. Assim, descobrimos que as comunidades aparentemente mais estressadas (ou seja, sob a temperatura mais alta) têm maior estabilidade do ecossistema (resiliência, resistência e recuperação) quando comparadas às comunidades menos estressadas. Em suma, a evidência experimental indica que as mudanças climáticas afetarão profundamente a estrutura da comunidade e algumas funções do ecossistema (por exemplo, produção de biomassa, transferência de energia e ciclo do carbono). Destaca-se a necessidade de fortalecer as políticas e medidas locais para prevenir ou mitigar os impactos ecológicos das mudanças climáticas.

Palavras-chave: Mudanças climáticas; múltiplos estressores; eutrofização; chuva; cianobactéria.

Phytoplankton community in the Anthropocene: effects of climate change and eutrophication

ABSTRACT

In the era of the Anthropocene, the effects of climate change on the freshwater ecosystems are clearly complex, since warming and different temperature related drivers influence interacting physical, biogeochemical and biological processes. Climate change is predicted to have huge impacts on the Earth's ecosystems through temperature increase, changed patterns of precipitation, more frequent extreme weather events, and combinations of these thus, climate change may become one of the major drivers affecting the diversity, composition, structure, and functioning of ecological communities over the next several decades. In this thesis, composed of two papers, we evaluate the effects of multiple factors related to climate change on the natural phytoplankton community. In the first one, we conducted an indoor experiment to test how increasing temperatures influence natural phytoplankton diversity and CO_2 emissions in eutrophic ecosystems. Our results experimentally show that, under future scenarios of climate warming, the phytoplankton community composition can respond strongly, affecting ecosystem functions such as biomass production, resource use efficiency, carbon flux balance. Warming clearly aggravated the negative effects of eutrophication through the enhancement of cyanobacteria, all other factors being equal. Since the suggestion that eutrophication may promote climate change by increasing the release of greenhouse gases from fresh waters, it has been found that eutrophication may interact with warming via a positive feedback to atmospheric CH_4 emissions. Here, we also found experimental evidence of a positive feedback between the major eutrophication symptom (cyanobacterial blooms) and warming, via higher CO_2 emission rates in cyanobacteria dominated warmer systems, besides other changes in key ecosystem functions. In the second paper, we conducted an indoor short-term experiment to test how the natural phytoplankton community subjected to different temperatures reacted to the stressors of climate change (warming, eutrophication, extremes rainfall events) can affect the ecosystem stability. We find that the phytoplankton communities responds differently to disturbances. The environments with cyanobacterial blooms have benefited and proved to be resilient to the extremes rainfall events. In contrast, environments less stressed by warming have a slower response to the event of extreme rainfall, and that they often do not recover their biomass before the extreme rainfall event. Thus, given the multiple effects of climate change, the most stressed environments have greater ecosystem stability (resilience, resistance, and recovery) when compared to the least stressed. In summary, the findings of this thesis, we have experimental evidence with phytoplankton community, that climate change will profoundly affect ecosystem functions (e.g., biomass production, energy transfer, and carbon cycle). In this sense, we hope to contribute with policies to prevent or mitigate the ecological impacts of climate change.

***Keywords*:** Climate change; multiple stressors; eutrophication; rainfall; cyanobacteria**.**

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SUMMARY

1 GENERAL INTRODUCTION

The Anthropocene term informally encompass different geological, ecological, sociological, and anthropological changes in recent Earth history (Malm and Hornborg, 2014; Steffen et al., 2011). Climate change is predicted to have huge impacts on the Earth's ecosystems through temperature increase, changed patterns of precipitation, more frequent extreme weather events, and combinations of these (Field *et al.* 2014, Lehmann *et al.* 2015). Thus, climate change may become one of the major drivers affecting the diversity, composition, structure, and functioning of ecological communities over the next several decades.

Lakes are considered good sentinels of climate change because they are sensitive to environmental changes and can integrate changes in the surrounding landscape and the atmosphere (Adrian et al., 2009). Besides climate change, freshwater ecosystems are threatened by multiple anthropogenic stressors that include, for example, habitat fragmentation and isolation, overexploitation, invasion by exotics, and eutrophication (Yvon-Durocher et al., 2011; Meerhoff et al., 2012; Birk et al., 2020; Albert et al., 2021). Biodiversity losses (e.g. due to eutrophication) is occurring as a result of global changes putting the functioning of aquatic ecosystems at risk (Cardinale et al., 2006; Dudgeon et al., 2006; Hooper et al., 2012). Together with global warming and rising atmospheric CO_2 levels, these pressures are altering life on Earth in unpredictable ways, with potentially very severe consequences for the goods and services that ecosystems provide for humanity (Steffen et al., 2015). Therefore, understanding the ecosystem responses and stability to these stressors is crucial to be able to provide better management, conservation and restoration strategies (Scheffer et al., 2001; Pecl et al., 2017; De Boeck et al., 2018).

Phytoplankton community is extremely sensitive to environmental change, responding with changes in total biomass and community composition (Litchman et al., 2015). Both changes in temperature and precipitation patterns can strongly affect phytoplankton in direct

and indirect ways. It has been predicted that warming will increase the occurrence of blooms (Paerl and Huisman, 2009, 2008; Kosten et al., 2012, Medeiros et al., 2015), or at least favor cyanobacterial dominance within phytoplankton communities (Gkelis et al., 2014; Yan et al., 2017). Climate change is also increasing the variability and extremeness of precipitation and its impacts on blooms are not well understood. Extreme rainfall events change lake abiotic conditions. The physical displacement of phytoplankton throughout the water column can alter the outcome of competition and herbivory and thus shape community composition (Reynolds et al., 2002; Paerl & Huisman, 2009).

The cyanobacterial blooms are favored because they exhibit a series of adaptations to enable survival in a range of extreme niches (Winder & Sommer, 2012) and are generally difficult for higher trophic-level consumers to assimilate. Other factors that allow the success of cyanobacteria are functional characteristics that increase their fitness in a wide range of environmental characteristics (Litchman et al., 2010). These characteristics include the presence of aerotopes that allow them to move through the water column when there is thermal stratification, the nitrogen-fixing capability, high affinity and capacity of stocking phosphorous, and the production of cysts (akinetes) (Hansson, 2000; Weyhenmeyer et al., 2007; Carey et al., 2012). Thus, it is expected that cyanobacteria blooms become more frequent and widespread in the future given the expansion of eutrophication worldwide and climate warming, as indicated by theoretical models (Mooij et al., 2005; O'Neil et al., 2012) and empirical data (De Senerpont Domis et al., 2007; Kosten et al., 2012). An expansion of cyanobacterial blooms is of great societal concern because harmful cyanobacteria can impair safe drinking, irrigation, fishing and recreational waters that are critical for the growing global human population (Heino et al., 2020).

In recent years, numerous studies have indicated that eutrophication, rising CO_2 levels, and global warming are likely to interact additively or synergistically to increase the frequency,

intensity, and duration of cyanobacterial blooms in many aquatic ecosystems across the globe (Paerl & Huisman, 2009; Wagner & Adrian, 2009; Qin et al., 2015; Yan et al., 2017). Although nutrients seem to be the most important predictor of cyanobacterial biovolume, as lakes become more eutrophic cyanobacteria become more sensitive to the interaction of nutrients and temperature (Rigosi et al., 2014).

Theoretical and experimental evidence highlight the potential reinforcing feedbacks between eutrophication and warming (Davidson et al., 2018; Moss et al., 2011; Yan et al., 2017; Li et al., 2021), among other mechanisms by altering the 'metabolic balance' of ecosystems (Allen et al., 2005) and impacts on the fluxes of greenhouse gases such as CH_4 and N_2O, besides CO_2. The metabolic balance is defined as the rate between carbon fixed through photosynthesis and its remineralization through respiration, determining whether an ecosystem acts as a net source or sink of CO_2 to the atmosphere (Del Giorgio & Duarte, 2002; Odum, 1956; Woodward, 2007). In this sense, some studies show that respiration responds more strongly to temperature change than photosynthesis (Gudasz et al., 2010; Moss et al., 2010; Yvon-Durocher et al., 2011). Current studies indicate that, in contrast to previous beliefs, freshwater ecosystems are more active in terms of carbon sequestration, processing, and burial than terrestrial and marine ecosystems (Downing, 2010; Tranvik et al., 2009; Raymond et al., 2013). Climate warming has been shown to increase greenhouse emissions and reduce carbon sequestration in these environments (López-Urrutia et al., 2006; Yvon-Durocher et al., 2011) (López-Urrutia et al., 2006; Yvon-Durocher et al., 2011). These findings are important because they imply carbon cycle responses to climate warming should be more complex than the simple temperature effect on respiration rates alone. Besides carbon cycle, climate warming can affect other ecosystem processes. Among them, resource use efficiency (RUE) is a key indicator of ecosystem functioning, since it resumes nutrient cycling and trophic transfer processes (Ptacnik et al., 2008; Filstrup et al., 2014).

The role of experiments, and of microcosms in particular, might be crucial to explore the effects and the consequences of multiple stressors, such as climate change and the effects of biodiversity loss on ecosystem functioning. Although the use of model systems might appear to be limited in scope and realism, especially compared with the infinite complexities of the real world and the spatial extent of global-scale problems, the utility of the microcosm approach lies in its ability to explore and test mechanisms (Benton et al., 2007). Together with other sources of scientific knowledge, microcosm experiments can supply robust scientific evidence to base measures and policies to prevent or mitigate the ecological impacts of environmental change.

In this sense, the experimental studies approach can offer a tool to reach "The Sustainable Development Goals", proposed by the United Nations (UN) and adopted by all UN Member States in 2015, whose action is to protect the planet and improve the lives and prospects of everyone, everywhere. The 17 Goals is part of the 2030 Agenda for Sustainable Development. The development of this thesis directly contemplates the dimension of the Biosphere but with economic and social projections, such as Goal 6: Ensure access to water for all, and Goal 13: Take urgent action to combat climate change and its impacts. The complexity of issues about climate change affects several individuals and social groups, requiring the participation of a greater number of social actors in the search of possibilities and alternatives for adaptation and mitigation for the imposed uncertainties. Thus, scientific dissemination is an indispensable tool for the connection between researchers and society, because the individual's understanding of the world directly influences their choices and decisions (Supporting Information Fig.1).

The general aim of this thesis was to test how natural freshwater phytoplankton diversity responds to components of climate change and how this response may influence some ecosystem processes. To address this general objective, two in-door microcosm experiments

were conducted. Specifically, in the first one, we tested how increasing temperatures influenced natural phytoplankton diversity and CO_2 emissions in eutrophic conditions. The potential links and feedback between eutrophication and warming were thus explored. In the second paper, we conducted an indoor short-term experiment to test how different natural phytoplankton communities, acclimatized to different temperatures, reacted to a simulated extreme rainfall event, and thus analyze ecosystem stability and resilience. We expect that under higher temperatures, rainfall extreme events in eutrophic lakes will result in increased cyanobacteria blooming.

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2 POSITIVE FEEDBACK BETWEEN WARMING AND CYANOBACTERIA BLOOMS

ABSTRACT

The synergistic effect of global warming and eutrophication favors the formation of blooms of cyanobacteria and alters the functioning of ecosystems (e.g. biogeochemical cycles and productivity). In this study, we evaluated the potential feedbacks between eutrophication and warming on the phytoplankton and how global warming will affect the metabolic balance. We conducted an indoor controlled microcosm experiment. Warming promoted species richness decreased and primary production with a 10-fold increase in the mean biomass of green algae and cyanobacteria (*Raphidiopsis raciborskii*). Resource use efficiency (RUE) increased gradually between treatments during the experimental .Maximum RUE value was obtained under the warmest treatment, dominated by few cyanobacteria genera which are more efficient in resource use and limiting the growth of other species. Although we registered high CO_2 influx values in the experiment, the ecosystem metabolic balance changed with temperature increase. The magnitude of influx decreased with warming, almost transforming the microcosms in sources of CO_2 to the atmosphere. We designed our experiment to focus on increasing mean temperature as a driver of changes in the phytoplankton community, providing non-limiting nutrients and no predation conditions, to avoid indirect or interacting effects of warming. Therefore, we can assume that warming directly drove cyanobacteria proliferation. Here, we also found experimental evidence of positive feedback between eutrophication symptoms (blooms) and warming, via higher CO_2 emission rates in cyanobacteria dominated warmer systems. Thus, we were able to capture a pure pelagic response to warming, exclusive to the phytoplankton, and how

such responses translated to ecosystem functions (e.g., biomass production, energy transfer, and carbon cycle).

Keywords: Climate change, cyanobacteria blooms, eutrophication, global warming, resource use efficiency, feedback.

2.1 Introduction

Eutrophication of surface waters has become a ubiquitous problem around the world, threatening both aquatic biodiversity and several services for the human population, such as water supply, recreation, and irrigation (Heino et al., 2020; Jeppesen et al., 2009; Moss et al., 2009). Climate change may enhance the negative effects of eutrophication through a variety of physical, chemical, and biological mechanisms (Moss et al., 2011). Warming affects biogeochemical cycles and biological processes such as respiration and decomposition rates, nutrient cycling, growth rates, individual and community body size, and environmental selection of functional traits, among other ecological processes (Jeppesen et al., 2010; Meerhoff et al., 2012; Gkelis et al., 2014). As a result, there is selective pressure on aquatic organisms, such as on groups of phytoplankton that can withstand such changes (Mouillot et al., 2013b).

Across most of the studies from laboratory and field observations, there was a general trend of enhanced cyanobacteria biomass and/or dominance with increasing water temperature. Rasconi *et* al. (2017) designed a mesocosm experiment and found a clear effect of the temperature treatments with an observed shift toward cyanobacteria dominance. However, some empirical studies have shown that not the temperature *per se*, but the interaction with the nutrient supply is important for increasing the biomass of these organisms (Thrane et al., 2017; Verbeek et al., 2018). There are still contrasting experimental evidence that showed no particular pattern of the cyanobacteria with rising temperature, at least in shallow unstratified lakes still dominated by macrophytes (Moss et al., 2003) or that the growth of cyanobacteria in a global warming scenario does not exceed the growth rates of green algae (Lürling et al., 2013). Given the wide spectrum of climate change scenarios (Moss et al., 2010), predicting global warming effects on aquatic ecosystems is still a challenge with a high level of uncertainty (Feuchtmayr et al., 2009).

Despite this, the increase in the magnitude and frequency of cyanobacteria blooms associated with higher temperatures is a global concern (Paerl & Huisman, 2008; Kosten et al., 2012; Kruk et al., 2012; Paerl & Paul, 2012; Michalak, 2016; Burford et al., 2019).

The competitive advantage that allows the success of some cyanobacteria groups are functional characteristics that increase their fitness over a wide range of environmental characteristics (Litchman et al., 2015). These characteristics include the presence of aerotopes that allow them to move through the water column when there is thermal stratification, nitrogen-fixing capacity, high affinity for and capacity of stocking phosphorous, and the production of cysts (akinetes) (Carey, Ibelings, Hoffmann, Hamilton, & Brookes, 2012; Hansson, 2000; Litchman, Pinto, Klausmeier, Thomas, & Yoshiyama, 2010; Weyhenmeyer et al., 2007). Thus, it is expected that cyanobacterial blooms become more frequent in a global warming scenario (Mooij et al., 2005; O'Neil et al., 2012; Beaulieu et al., 2013). Biodiversity losses (e.g. eutrophication) is occurring as a result of global climate change and puts at risk the functioning of the aquatic ecosystems (Cardinale et al., 2006; Dudgeon et al., 2006; Hooper et al., 2012). Among ecosystem functions, resource use efficiency (RUE) is a very important one, determining nutrient cycling, and trophic transfer processes (Ptacnik et al., 2008; Filstrup et al., 2014).

Theoretical and experimental evidence highlight the potential feedbacks between eutrophication and warming (Davidson & Janssens, 2006; Moss et al., 2011; Yan et al., 2017), among other mechanisms by altering the 'metabolic balance' of ecosystems (Allen et al., 2005). This balance is defined as the rate between carbon fixed through photosynthesis and its remineralization through respiration, determining whether an ecosystem acts as a net source or sink of CO_2 to the atmosphere (Odum, 1956; Del Giorgio & Duarte, 2002; Woodward, 2007).

Eutrophication may decrease the relative importance of the external organic matter and promote higher autotrophic fixation of CO_2 by cyanobacterial blooms, transforming the lakes into net carbon sinks. The synergistic effect of warming and eutrophication can also promote higher respiration of settling the organic matter, promoting the release of CO_2 from the lakes (Gudasz et al., 2010; Moss et al., 2010, 2011; Yvon-Durocher et al., 2010, 2011). Therefore, ecosystem respiration is affected by temperature more than photosynthesis rates and, without other interacting factors, warming may increase CO_2 emissions and reduce net carbon sequestration by eutrophic aquatic ecosystems (López-Urrutia et al., 2006; Yvon-Durocher et al., 2010). Still, it remains uncertain to what extent changes in community composition as a response to warming directly translate into changes in ecosystem function, such as CO_2 fluxes.

Here, we conducted an indoor experiment to test how increasing temperatures influence natural phytoplankton diversity and CO_2 emissions in eutrophic ecosystems. We expected that, as systems were isolated without immigration, water temperature increase would reduce phytoplankton richness and promote changes in composition, leading to a rapid dominance of some phytoplankton groups, mainly cyanobacteria. Furthermore, as nutrients were not limiting, we expected that warmer waters would promote higher total biomass and different functional responses, such as higher respiration rates increasing net CO_2 effluxes, and higher RUE. In summary, we expect that under higher temperatures eutrophic lakes will become dominated by cyanobacteria and act as net carbon sources to the atmosphere, creating a positive feedback between eutrophication and warming.

2.2 Methods

2.2.1 Experimental Design

We conducted an indoor controlled microcosm experiment in a laboratory located at the *campus* of the Universidad de la Republica (UdelaR), Maldonado, Uruguay (34º 54'53´S e 54 ° 56'31 " W '), between June 18 and July 19 of 2019 (southern hemisphere winter). The control treatment, (i) 17 °C, corresponded to the annual mean temperature of the aquatic ecosystems in the same region where the experiment was conducted (Pacheco et al., 2010). The second treatment, (ii) 20 °C, represented a scenario with an increase of 3 °C in relation to the annual mean temperature; and the third treatment, (iii) 23 °C, represented an increase of 6 °C in relation to the mean temperature. Room temperature was manipulated in all treatments using electronic temperature controllers (Eliwell ID Plus 961), including the control treatment.

The microcosm was cylindrical polyethylene 5-L aquaria (23.5 cm of diameter and 10 cm of height). We installed water circulation pumps (model Resun AC-9903) at the bottom of each aquarium to avoid phytoplankton sedimentation (Flury et al., 2010; Sommer et al., 2015). Besides, we moved the water manually and delicately twice a day with a stick. Both light and temperature were controlled. Phytoplankton received circa 80 lum/ft² of 12/12 ratio of light/darkness from fluorescent lamps with a light spectrum similar to that of the sun. All experimental units were placed at the same height to avoid temperature fluctuations and to homogenize the quantity of incident light. We randomly distributed thirty microcosms between the three temperature treatments (i.e. n=10 replicates each).

To prepare the natural phytoplankton inoculum for the experiment, we obtained water samples at the subsurface of the limnetic region in a series of subtropical shallow lakes situated at the Uruguayan coastline (34° S 56° W). These lakes comprise a trophic gradient from mesotrophy to hypertrophy (Kruk et al., 2009; Pacheco et al., 2010). We

collected phytoplankton using a plankton net with a mesh size of 20 µm to remove most zooplankton and added some non-filtered water to include smaller phytoplankton species (< 20 µm). We used this strategy to maximize the sampling of less abundant species and guarantee that most taxonomic groups were present in the experimental units. The mean water temperature measured in the lakes during the sampling procedure was 15 °C.

We distributed 0.5 L of concentrated inoculum for each of the 30 experimental units and added 2.5 L of dechlorinated water enriched with a nutrient solution to reach a final volume of 3 L. In the first day of the experiment, we found no significant differences among the three treatments for phytoplankton taxonomic richness ($F\text{-value}_{(2, 27)} = 1.031$; $p = 0.37$) and biomass ($F\text{-value}_{(2, 27)} = 0.086$; $p = 0.918$), showing the homogeneous phytoplankton distribution among the treatments.

To avoid losses due to heat shock due to abrupt temperature changes, the experimental units were acclimated for two weeks after the addition of the phytoplankton by increasing room temperature gradually and daily until final temperatures were reached (approximately 0.5 °C increase per day), as this allowed for changes in species abundance in response to the temperature increase but avoided heat shocks. Thus, on the 16th day of the experiment, all climatic rooms had achieved the final temperatures for each treatment. The experiment lasted for 32 days, as the short life cycle of phytoplankton allows the reproduction of several generations in a few days (Reynolds, 2006) (Fig 1, Supplementary material S1).

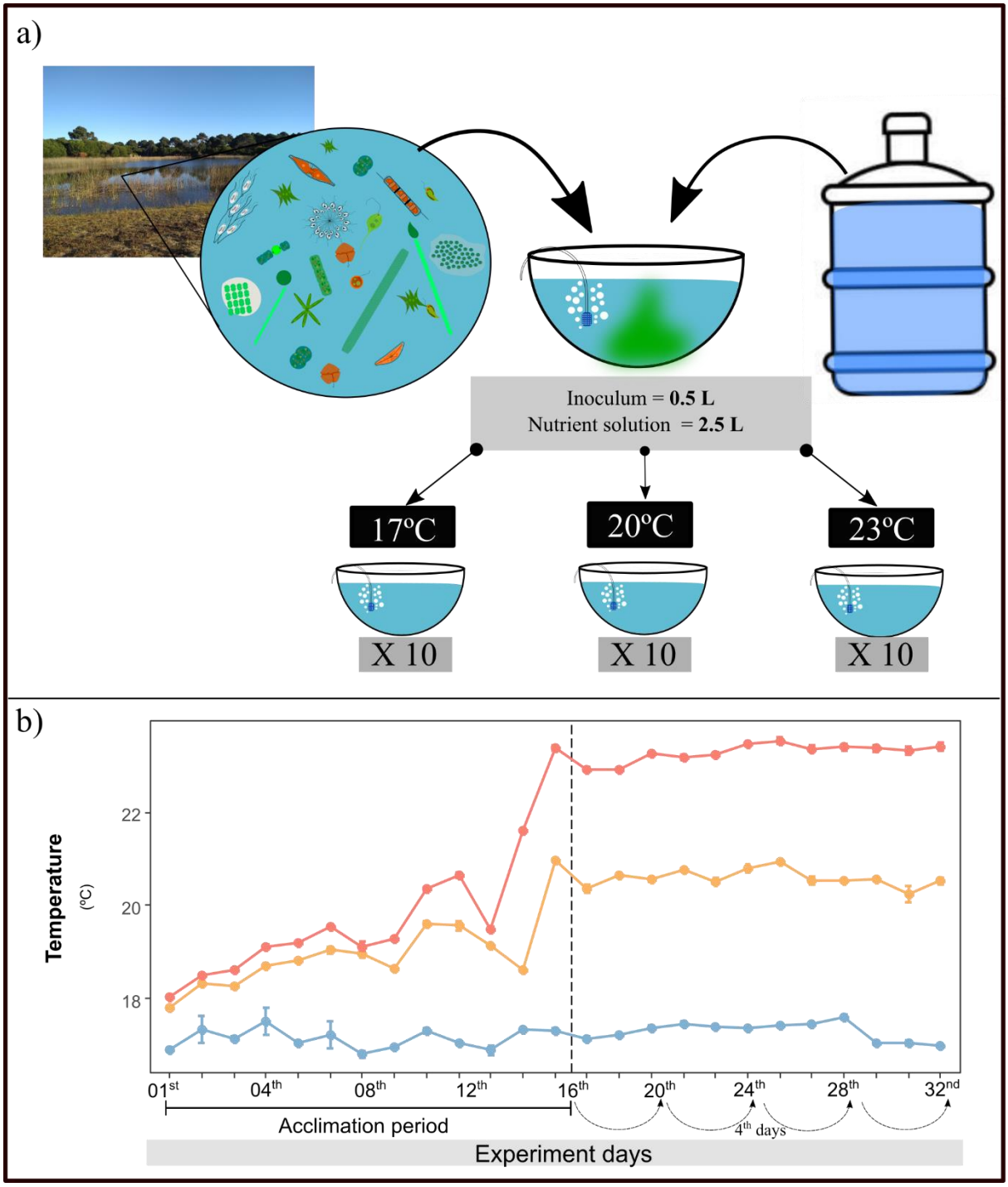

Figure 1: Experimental design. a) A concentrated phytoplankton community subtropical lakes were cultured in a nutrient solution respecting the Redfield ratio. Treatments: 17 °C, 20 °C and 23 °C, total n=30. b) Following an acclimation period, as from the 16th day of the experiment all treatments reached the wished temperature. The central point denotes the mean value and, whiskers represent standard error for each temperature in each experiment day.

Phytoplankton was maintained in a medium following the Redfield ratio. We prepared a solution composed of 200 mg L^{-1} KH_2PO_4, 100 mg L^{-1} NH_4NO_3, 177 mg L^{-1} $Ca(NO_3)_2$, 0.1 mg L^{-1} $Co(NO_3)_2$, and 250 mg L^{-1} $C_{10}H_{16}N_2O_8$. To this solution, we added 150 L of deionized water and, after intense homogenization, added 2.5 L of it to each microcosm. Weekly, the same solution was added to each microcosm to compensate for the losses due to evaporation and to reach the initial volume of 3 L (McKee et al., 2000; Ekvall & Hansson, 2012). On average, each microcosm presented 533.23 µg L^{-1} of TN and 81.2 µg L^{-1} of TP (Supplementary material Fig.S1).

2.2.2 Limnological variables

In all microcosms, we took daily measurements of water temperature (ºC), pH, and electric conductivity using a HANNA multiparametric probe, and of dissolved oxygen (mg L^{-1}) and oxygen saturation (%) using an Oxyguard Handy Polaris. We sampled water on the 4^{th}, 16^{th}, 24^{th}, and 32^{nd} day to determine total phosphorous (TP; µg L^{-1}), total nitrogen (TN; µg L^{-1}), reactive soluble phosphorous (SRP; µg L^{-1}), nitrate (NO_3; µg L^{-1}), and ammonium (NH_4^+; µg L^{-1}), as well as chlorophyll-a, according to (APHA, 2005). The limnological variables TP, TN, SRP, NO_3, NH_4^+ were log-transformed. We found no significant differences in nutrient concentration for our treatments through time, guaranteeing that these conditions were successfully controlled throughout the experiment (Supplementary material Fig.S2).

2.2.3 Phytoplankton

We sampled phytoplankton every four days (days 1, 4, 8, 12, 16, 20, 24, 28, and 32). Before taking each sample, we homogenized the water manually to avoid missing any species due to sedimentation. We sampled phytoplankton directly with flasks and

fixed them immediately with acetic Lugol solution. We estimated phytoplankton density following Utermöhl (1958) and Lund et al., (1958) and calculated density according to APHA (2005). The biomass ($mm^3.L^{-1}$) was considered as biovolume, which was estimated by multiplying the density of each taxon by its volume. We estimated the cell volume by calculating the volume of the geometric shape that was the most similar to each cell form (Sun & Liu, 2003). We also estimated the community resource use efficiency (thereafter RUE), defined as the ratio between the phytoplankton biomass production in Chl-a and TP, as a proxy for ecosystem productivity (Ptacnik et al., 2008; Olli et al., 2015; Verbeek et al., 2018).

2.2.4 Data analyses

We used 1-way ANOVA to test for differences among the treatments in nutrient concentration (TN, TP, SRP, NO_3, and NH_4) in each sampling occasion; in phytoplankton richness and biomass on the first day of the experiment; and two-way ANOVAs to test for differences in chlorophyll-a, RUE, and carbon fluxes among treatments and time. When results were significant ($p < 0.05$), we used Tukey tests, to verify the significant difference in mean among treatments and time. After the temperature was stabilized in all treatments (as from the 16^{th} day), we evaluated the effects of the temperatures and time on richness and biomass of phytoplankton groups by fitting generalized linear models (GLM), using the distribution families that better adjusted to the data: Poisson distribution for richness and Gaussian distribution for biomass. We evaluated overdispersion and corrected them when necessary.

To evaluate the effects of temperature (three levels) and sampling time (five levels) on the composition of phytoplankton (presence/absence) (after the 16^{th} day), we used a permutational multivariate analysis of variance (PERMANOVA, Anderson, 2001).

The test was based on a Jaccard dissimilarity matrix, and 999 permutations were used to test for significance. To verify patterns in species composition through time we represented our data using an analysis of principal coordinates (PCoA)(Legendre & Legendre, 1998).

The rate of net growth (NG) of the phytoplankton density was calculated per phytoplankton group as density: $\mu = (\ln N_F - \ln N_0)/t$, where it is the time of the experiment (32 days), $\ln N_F$ is the natural logarithm of the density of organisms at the end of the experiment (32nd day), and $\ln N_0$ is the natural logarithm of the density of organisms at the beginning of the experiment (1st day).

The CO_2 fluxes were measured on the 10th, 16th, 24th, and 32nd day after the beginning of the experiment in all microcosms (n = 120), using an adapted environmental CO_2 analyzer (EGM-4) with a hermetic acrylic cover. We turned the aerators off one hour before we began sampling with the EGM-4, to avoid intervention in the CO_2 emission. We sampled data in each microcosm at a frequency of 30 seconds for 5 minutes, so that we obtained 10 measurements of [CO_2] per microcosm in the light period (photosynthesis period) and 10 in the dark period (respiration period). We calculated the balance of each day of CO_2 evaluation with the equation:

CO_2 emission of the sampled time = Photosynthesis + Respiration.

We used the slope of the relationship between gas concentration and time to calculate the gas flux according to:

$$CO_2 \text{ flux} = S * \frac{V}{A} * \left({}^{P*F1*F2}/_{R*T} \right)$$

Where CO_2 flux is in $CO_2.mg^{-2}.d^{-1}$, S is the slope of the partial pressure of CO_2 in the function of time ($ppm.s^{-1}$), V is the camera volume (0.0019 m^3), A is the camera area (0.1589 m^2), P is the atmospheric pressure in KPa (102.5), F1 is the molecular weight of the gas ($44g.mol^{-1}$), F2 is the factor of conversion of seconds to days (8,64.104 $s.d^{-1}$), R is the gas constant (8.31J.mol^{-}

$^{1}.K^{-1}$) and T is the air temperature in Kelvin. We selected values of the slope only for those relationships with $R^2 \geq 0.7$. Positive values indicate CO_2 efflux (CO_2 liberation to the atmosphere: predominance of net respiration) and negative values indicate CO_2 influx (CO_2 retention in the aquatic environment: predominance of net photosynthesis).We ran all analyses in software R version 3.3.2 (R Development Core Team, 2021), using packages "vegan" (Oksanen et al., 2016) for PCoA, ANOVA, and PERMANOVA, and "MASS" (Venables & Ripley, 2002) for GLMs.

2.3 Results

To electric conductivity, there was a gradual increase through time in all treatments. The highest mean values were recorded for the treatment of 17ºC (81 µS.cm^{-1}) and minors for the treatment of 20ºC (62.1 µS.cm^{-1}). The concentrations of dissolved oxygen and pH values had little variation over the time of the experiment. However, the warming promoted environments with lower average values of oxygen concentrations (10.31 mg L^{-1}) and higher mean pH values (8.05) in relation to the lower temperature treatment (10.95 mg L^{-1} e 6.1 respectively).

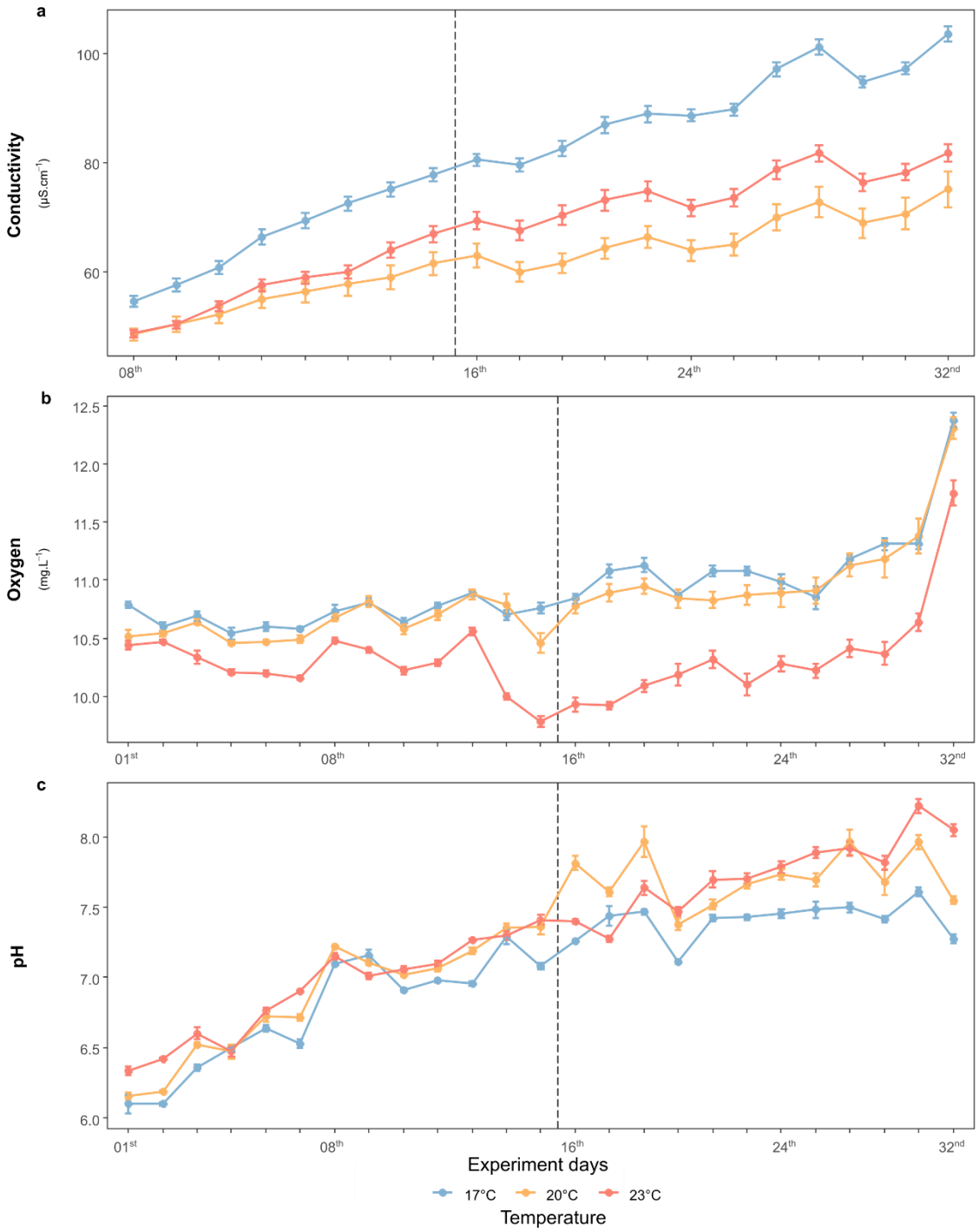

Figure 2: Variation of main limnological variables in the three treatments (17 °C, 20 °C and 23 °C) through the 32 days of the experiment. The dotted line indicates that on the 16th day of the experiment the wished temperatures were reached. The central point denotes the mean value and, whiskers represent standard error for each temperature in each experiment day.

2.3.1 Phytoplankton community

We identified 125 taxa, including green algae (62 taxa), cyanobacteria (21), diatoms (18), and phytoflagellates (16). *Scenedesmus* (Chlorophyceae) presented the highest number of taxa (7). Mean phytoplankton richness presented little oscillation through the duration of the experiment (Fig.S2). However, we registered a reduction in species richness with increasing temperature for total phytoplankton (pseudo $R^2 = 0.16$; $p = 0.001$) and also and within the main phytoplankton groups (Table 1, Supplementary material Fig. S3). The highest variability in species composition occurred in the 20 °C treatment and the lowest in the 17 °C treatment (Fig. 3). The separation detected in the PCoA was confirmed by the PERMANOVA test, which showed significant changes in phytoplankton composition with temperature ($R^2 = 0.54$; $p = 0.001$) and time ($R^2 = 0.07$; $p = 0.001$).

Cyanobacteria (as *Raphidiopsis raciborskii* (Wołoszyńska) Aguilera, Berrendero Gómez, Kastovsky, Echenique & Salerno, before *Cylindrospermopsis raciborskii*), and green algae (as *Desmodesmus magnus* (Meyen) Tsarenko and *Staurastrum* sp.) were the groups that contributed to phytoplankton biomass the most through the experiment in the three treatments compared to a more balanced community at the start of the experiment. Supporting our expectations, total phytoplankton biomass responded positively to warming (Pseudo $R^2 = 0.50$; $p < 0.001$) (Table 2, Fig.4, Supplementary material Fig.S4).

This result was mainly driven by higher cyanobacteria growth at higher temperatures (Pseudo $R^2 = 0.41$; $p < 0.001$). Considering the three treatments evaluated, between the first and the last experiment day, we registered a 10-fold increase in the mean biomass of green algae and cyanobacteria and the highest growth rates in the 20° and 23 °C treatments (Fig. 5). Thus, warming promoted increasing of the phytoplankton biomass (F-value $_{(2,\ 85)} = 25.95$; $P < 0.001$) (Fig. 6a) reflected by higher mean values of

chlorophyll-a concentrations in the warmer treatments of the experiment over time (F-value $_{(2, 85)}$ = 12.23; P < 0.001).

The RUE changed significantly over time and between the treatments during the experiment. RUE increased gradually between treatments during the experimental period and the maximum was obtained under the warmer treatment (F-value $_{(2, 57)}$ = 13.40; P < 0.001). Furthermore, the blooms registered in this treatment, mostly of *R. raciborskii* at 23°C showed higher RUE than communities in the low temperature treatment. The differences being the largest between 17ºC and 23ºC (Tukey test p = 0.04) (Fig. 6b).

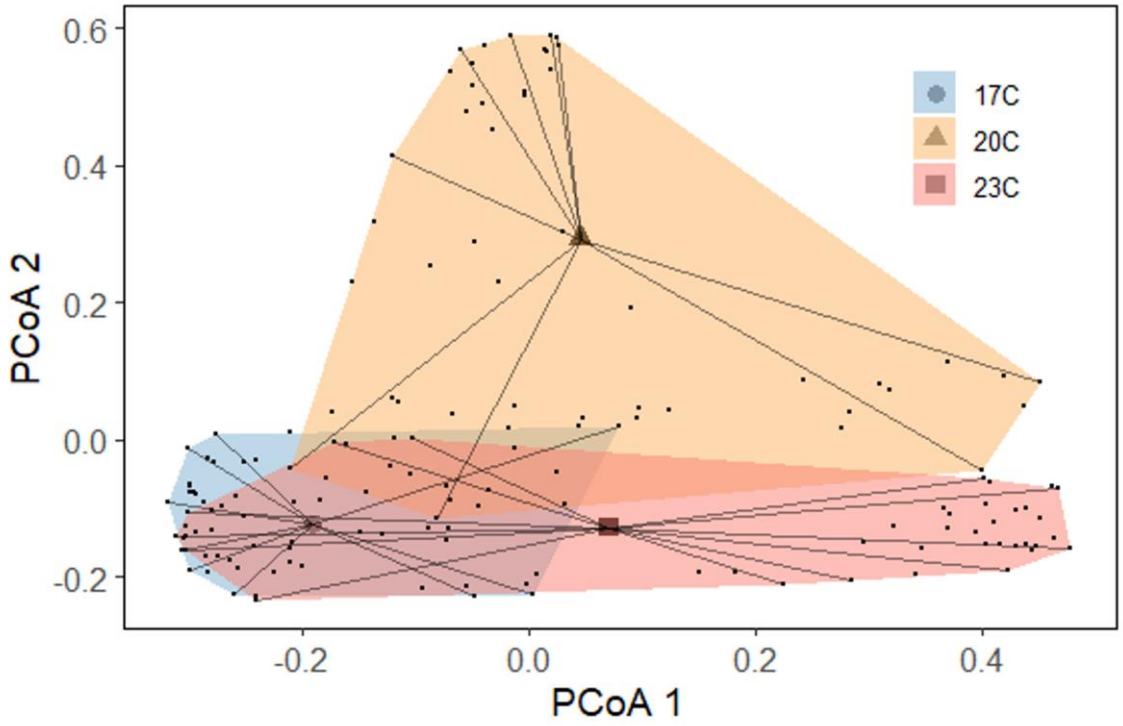

Figure 3. Principal coordinate analysis (PCoA), showing the variability in the composition of phytoplankton species from the 16th day of the experiment in the three treatments.

Table 1 Generalized linear models, indicating regression estimate, standard errors (SE), z-value, and P-values of models predicting, as from the 16th day, the taxonomic richness

of total phytoplankton, green algae, and cyanobacteria. Significant results are shown in bold.

Response variables	Predictors variables	Estimate	SE	z-value	*P*	Pseudo R^2
Total Richness	Intercept	3.907	0.164	23.72	0.000	0.16
	Temperature	**-0.033**	**0.007**	**-4.537**	**0.000**	
	Time	**-0.008**	**0.003**	**-2.440**	**0.015**	
Green algae	Intercept	**3.451**	**0.207**	**16.628**	**0.000**	0.11
	Temperature	**-0.033**	**0.009**	**-3.574**	**0.000**	
	Time	**-0.008**	**0.004**	**-2.067**	**0.039**	
Cyanobacteria	Intercept	**2.367**	**0.402**	**5.880**	**0.000**	0.08
	Temperature	**-0.063**	**0.018**	**-3.465**	**0.001**	
	Time	**0.006**	**0.008**	**0.839**	**0.402**	

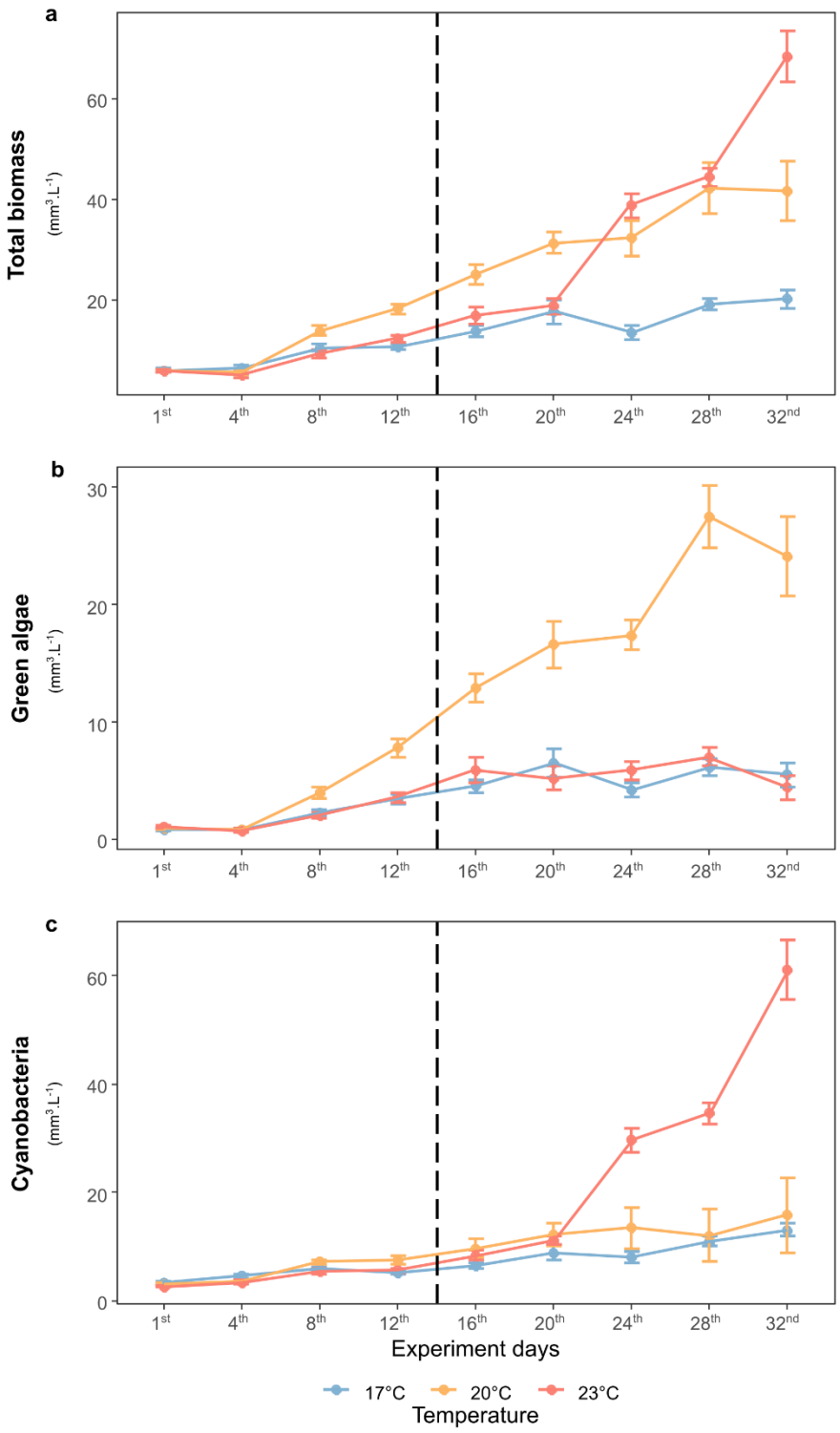

Figure 4: Variation of phytoplankton biomass (as biovolume) with temperature through the 32 days of the experiment: total (a), green algae (b), cyanobacteria (c). The dotted line indicates that on the 16th day of the experiment the final temperatures were reached. The central point denotes the mean value and, whiskers represent standard error for each temperature in each experiment day.

Table 2. Generalized linear models, indicating regression estimate, standard errors (SE), z-value, and P-values of models predicting, as from the 16th day, of the total phytoplankton biomass, green algae, and cyanobacteria. Significant results are shown in bold.

Response variable	Predictors variables	Estimate	SE	z-value	*P*	Pseudo R^2
Total Biomass	Intercept	-78.92	9.375	-8.417	0.000	0.50
	Temperature	**3.562**	**0.416**	**8.559**	**0.000**	
	Time	**1.489**	**0.177**	**8.385**	**0.000**	
Green algae	Intercept	-0.429	6.530	-0.066	0.948	0.03
	Temperature	0.197	0.290	0.679	0.499	
	Time	**0.278**	**0.124**	**2.248**	**0.026**	
Cyanobacteria	Intercept	-78.77	9.929	-7.934	0.000	0.41
	Temperature	**3.235**	**0.441**	**7.338**	**0.000**	
	Time	**1.240**	**0.188**	**6.592**	**0.000**	

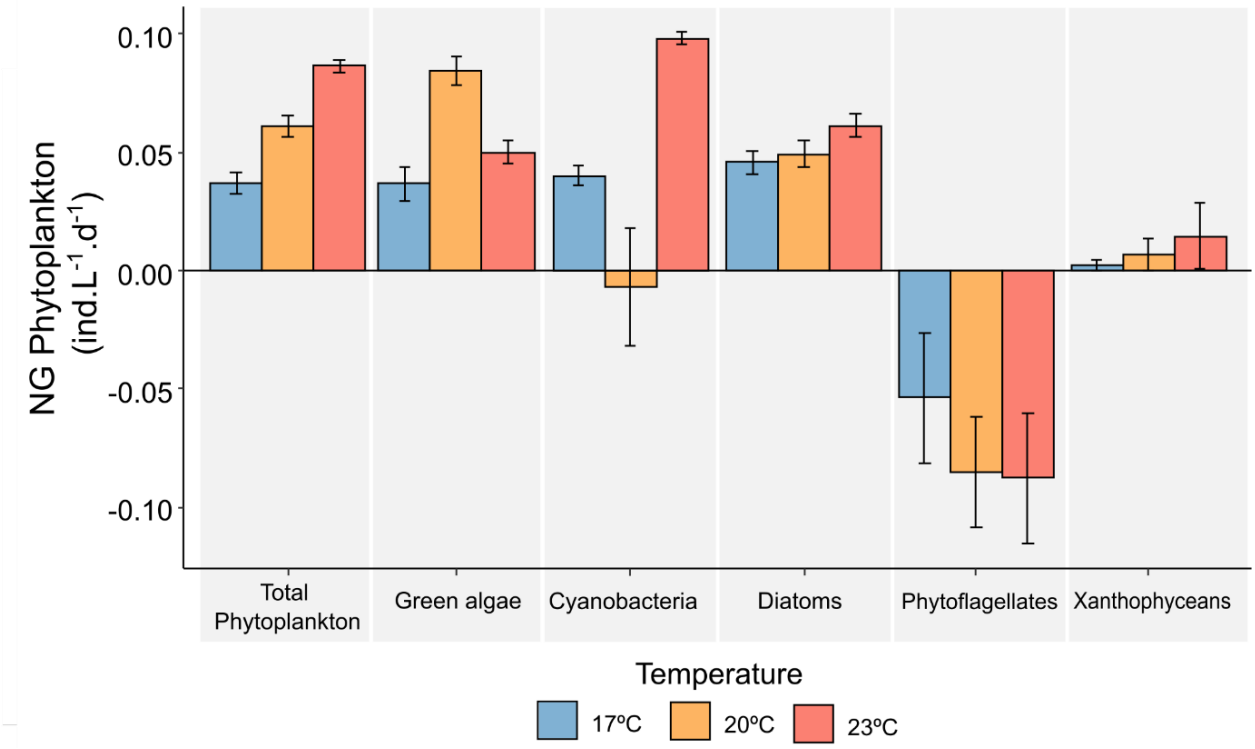

Figure 5. Net growth rate (NG) of phytoplankton density of total and main phytoplankton groups under the three temperature treatments. The bars plot denotes mean and, whiskers represent standard error.

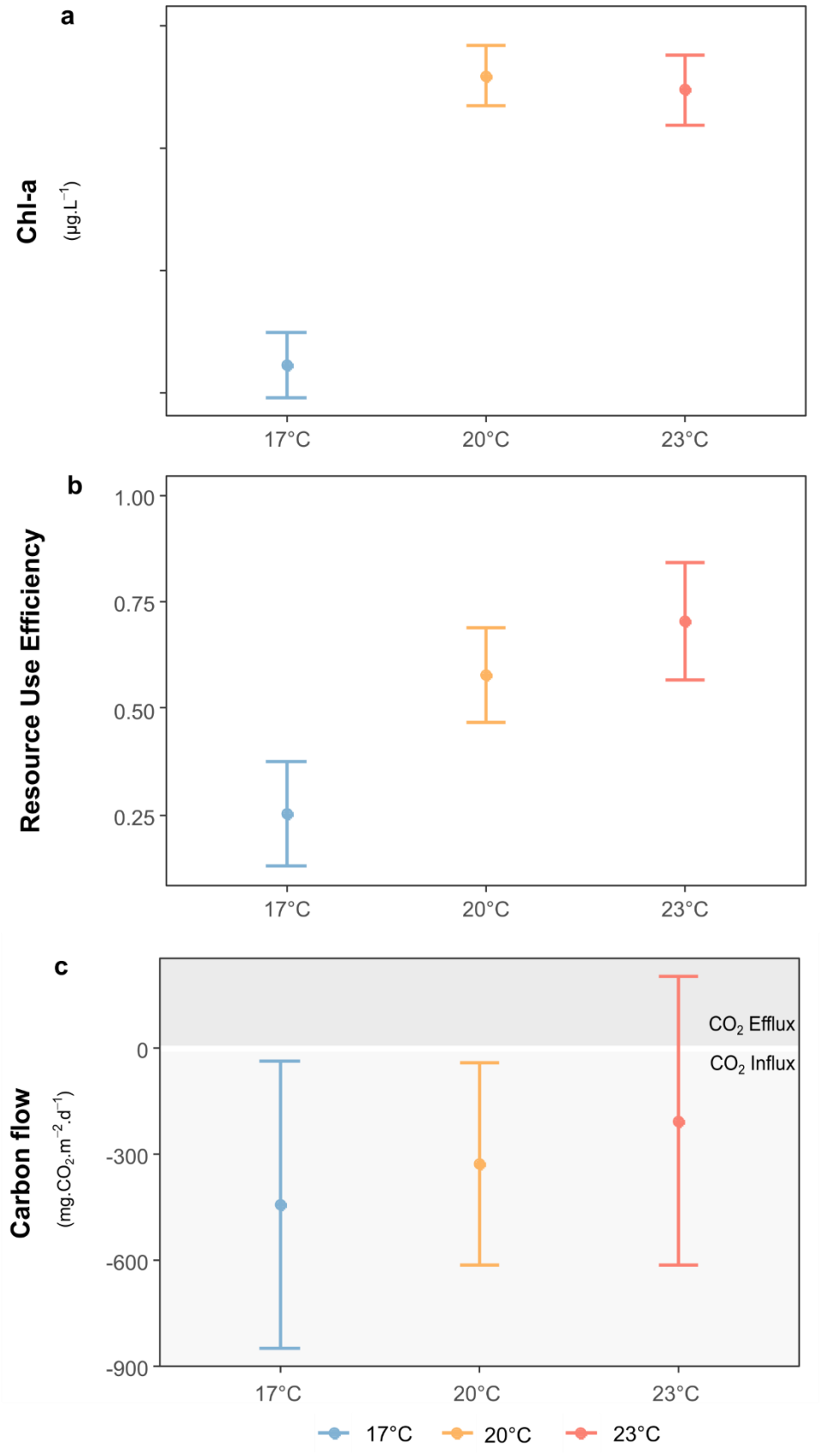

Figure 6. Different ecosystem responses to warming: variation of chlorophyll-a concentration (Chl-a - log-transformed) (a), resource use efficiency (log-transformed) (b),

and carbon flow (c). Positive values of carbon flow indicate net CO_2 emissions while negative values indicate net CO_2 sequestration by the phytoplankton communities in three treatments. The central point denotes the mean value and, whiskers represent standard error for each temperature in each experiment day.

2.3.2 CO_2 fluxes

Although we registered high CO_2 influx values in the experiment (i.e. CO_2 retention in the aquatic environment, due to net photosynthesis), the ecosystem metabolic balance changed with temperature increase. The magnitude of influx decreased with warming, almost transforming the microcosms in sources of CO_2 to the atmosphere (i.e., CO_2 liberation to the atmosphere, net respiration). These results support our hypothesis of positive feedback between blooms and warming, through an increase in CO_2 efflux to the atmosphere.

The highest daily CO_2 efflux (580.24 $CO_2.mg^{-2}.\ d^{-1}$) occurred on the 10th day of the experiment in the 23ºC treatment, and the highest CO_2 influx (-1579.58 $CO_2.mg^{-2}.\ d^{-1}$) occurred on the 25th day of the experiment in the 17 ºC treatment (Fig. 8A). We found significant differences in CO_2 concentration between the temperature treatments (F-value $_{(2, 80)}$ = 4.54; P = 0.013), the differences being the largest between 17ºC and 23ºC (Tukey test p = 0.027). The highest CO_2 mean values (i.e., the lowest influx, -184 $CO_2.mg^{-2}.\ d^{-1}$) occurred in the 23ºC treatment while the lowest (i.e., the highest influx, -445 $CO_2.mg^{-2}\ d^{-1}$) was registered in the 17ºC treatment (Fig.6c).

2.4 Discussion

Climate warming and eutrophication are two major challenges for lacustrine management worldwide (Carter & Schindler, 2012; Moe et al., 2016). Our results show experimentally that, under future scenarios of climate warming, the phytoplankton

community composition can respond strongly, affecting ecosystem functions such as biomass production, resource use efficiency, carbon flux balance.

We conducted our experiment at the community level, with a large initial species pool that represented high genetic, taxonomic, and functional variability. Warming may promote deep changes in the organization and diversity of the phytoplankton community. We found a negative effect of temperature increase on phytoplankton richness, with a functional change in the structure of the phytoplankton community and dominance patterns through time, with the substitution of eukaryotic by cyanobacteria and biotic homogenization, especially in the treatment with the highest temperature.

Besides composition changes, phytoplankton biomass increased with increasing temperature, especially green algae and cyanobacteria. In line with Verbeek et al. (2018) showing that climate change favors species with wider temperature ranges or higher temperature optima, such as green algae and cyanobacteria species in an experimental study with similar time duration to ours. Chlorophyta and Cyanobacteria are both considered fast-growing r-strategists that can endure higher temperatures and are favored in changing environments by their fast turnover, thus being able to develop blooms in a very short period.

Some studies have shown that green algae also have their optimal growth at high temperatures (De Senerpont Domis et al., 2007; Low-Décarie et al., 2011; Lürling et al., 2013). The latter experimentally found that some chlorophyceans (some of them also registered in this study, as *Desmodesmus* spp.) presented their optimal growth at high temperatures (approximately 20 °C), with no significant difference in the optimal growth temperature between cyanobacteria and green algae. Our results indicate that a community approach is needed to detect changes in dominance patterns. However, due to the competitive advantage of Cyanobacteria to rapidly sequester nutrients, they can grow

faster and outcompete other algae that are less efficient in nutrients uptake in the warming environment (Rasconi et al., 2017).

Raphidiopsis raciborskii (CyanoHABs), of a tropical origin but invasive over a wide distribution in temperate zones (Wiedner et al., 2007), was the main responsible for the highest biomass of cyanobacteria in the highest temperature conditions. This result corroborates other studies that showed that species of this group are favored with warming (Kosten et al., 2011; Bonilla et al., 2016; Rasconi et al., 2017; Huisman et al., 2018; Gray et al., 2019; Ho et al., 2019). This species may also use limiting resources more efficiently than other cyanobacteria species due to its high affinity for and high storage capacity of P (Istvánovics et al., 2000; Wu et al., 2012). To maintain several ecosystem functions multiple species with different traits are necessary (Hector & Bagchi, 2007; Mouillot et al., 2013a; Litchman et al., 2015). Thus, a decrease in diversity (as a consequence of the dominance of traits) has been associated with a general decrease in ecosystem functionality (Gamfeldt et al., 2008, 2013).

We found greater resource use efficiency with higher temperatures allow phytoplankton to yield a higher carbon-based biomass per unit cellular P. This suggests that RUE of phytoplankton increases with temperature, confirming earlier findings showing that primary productivity increases with temperature (Kerkhoff et al., 2005; Lovelock et al., 2007; De Senerpont Domis et al., 2014; Verbeek et al., 2018).

Ecologists have obtained inconsistent conclusions when analyzing the influence of phytoplankton diversity on RUE of phytoplankton, as this relationship depends on the composition of the community and species-specific RUE (Tian et al., 2017; Verbeek et al., 2018). Previous studies supported the existence of a positive relationship between species richness and high community RUE values (Ptacnik et al., 2008; Striebel et al., 2009; Chai et al., 2020), as a larger number of coexisting species exploit a wider range of

niches and can use limiting resources more efficiently (Tilman et al., 1997). However, our results showed an opposite relationship, with higher RUE in the communities dominated by few cyanobacteria genera which are more efficient in resource use and limiting the growth of other species (Roy & Chattopadhyay, 2007; O'Neil et al., 2012; Filstrup et al., 2014a; Sukenik et al., 2015). Lost diversity may be related to the fact that an isolated ecosystem has no chance of recovering species through regional diversity through immigration (Hillebrand et al., 2010).

Despite the higher maximum biomass of the phytoplankton community at higher temperatures, the low nutritional quality nutrient of these primary producers may negatively affect higher trophic levels (Soares et al., 2009). This may generate cascading effects on the superior trophic levels due to the reduced nutritional quality of cyanobacteria (Hassett et al., 1997). Phytoplankton is an important determinant of water quality and is key food production for heterotrophs, supporting the fish stocks in aquatic systems. Climatic effects on these autotrophs are thus of considerable interest. (Filstrup et al., 2014) for example, found lower zooplankton biomass in ecosystems dominated by a few phytoplankton genera. Specifically, the biomass stored in non-edible autotrophs (in this case, *Raphidiopsis raciborskii*, which has filaments that obstruct filtrating appendices of zooplankton species) alters the efficiency of resource transference to herbivores and the structure of trophic interactions.

Our results support previous investigations showing that warmer waters promote the dominance of cyanobacteria in phytoplankton communities in different approaches: experimental data with cultures (Staehr & Birkeland, 2006); experimental data with natural communities (De Senerpont Domis et al., 2014; Verbeek et al., 2018; Machado et al., 2019); long-term experimental data (Burgmer & Hillebrand, 2011; Yvon-Durocher et al., 2011; Rasconi et al., 2017); observational studies (Paerl & Huisman, 2008; Kosten et

al., 2012; Beaulieu et al., 2013); and paleolimnological studies (Pal et al., 2015). On the other hand, studies show that the correlation of cyanobacteria biomass and temperature in lakes is still unclear, depending on the lake trophic state or trophic interactions (e.g. resistance to grazing) (Lürling et al., 2013; Kraemer et al., 2017; Almanza et al., 2019; Gerhard et al., 2019). We designed our experiment to focus on increasing mean temperature as a driver of changes in the phytoplankton community, providing non-limiting nutrients and no predation conditions, to avoid indirect or interacting effects of warming. Therefore, we can assume that warming directly drove cyanobacteria proliferation.

Supporting our hypothesis, we found evidence of positive feedback between eutrophication and warming through changes in carbon emissions. Although all our treatments can be considered as net carbon sinks, the CO_2 sequestration in the communities with higher temperatures was severely diminished. Therefore, warming may increase CO_2 emissions from eutrophic lakes to the atmosphere (further aggravating the greenhouse effect) and reduce the role of these aquatic systems as carbon sinks (Yvon-Durocher et al., 2010, 2017). Our results are following studies realized in a variety of natural ecosystems that highlight the strong dependence between carbon efflux and temperature (Whiting & Chanton, 1993; Christensen et al., 2003; Gedney et al., 2004). A recent study suggests that there are feedbacks ("Vicious loop") among cyanobacteria blooms occurrence, lake eutrophication, and climate warming (Yan et al., 2017).

Besides, after the collapse of cyanobacteria, phytoplankton loss processes, as sedimentation and decomposition, are intensified. Many studies have suggested that organic carbon in sediments is mineralized into gas emissions of greenhouse gas, such as CO_2 (Gudasz et al., 2010; Bastviken et al., 2011; Marotta et al., 2014). Furthermore, we found that the warmest treatment caused a decrease in dissolved oxygen concentration.

This may be related to lower oxygen solubility and/or to an increase in the metabolic rates of the organisms at higher temperatures (Diaz & Breitburg, 2009). A lower dissolved oxygen concentration may, in turn, promote a stronger release of methane and nitrous oxide, further promoting warming. Besides, intensifies the respiration of planktonic organisms (Yvon-Durocher et al., 2015) and decomposition rates (Geraldes et al., 2012) both of which are processes that involve oxygen consumption.

Experiments studies are a fundamental tool to predict how scenarios of climate change will affect processes at the ecosystem levels and allow us to make predictions about how organisms may respond to changing environments (Stewart et al., 2013), despite their obvious limitations (Benton et al., 2007). In our case, the microcosms did not mimic a catchment context and, consequently, received no terrestrial organic carbon. Benthic processes and even pelagic trophic relations were purposely excluded. We used a highly diverse initial community and isolated it from potentially confounding factors. Thus, we were able to capture a pure pelagic response to warming, exclusive to the phytoplankton community, and how such responses translated to ecosystem functions (e.g., biomass production, energy transfer, and carbon cycle). Warming may aggravate the negative effects of eutrophication through the enhancement of cyanobacteria. Here, we also found experimental evidence of positive feedback between eutrophication symptoms (blooms) and warming, via higher CO_2 emission rates in cyanobacteria dominated warmer systems (Fig.7).

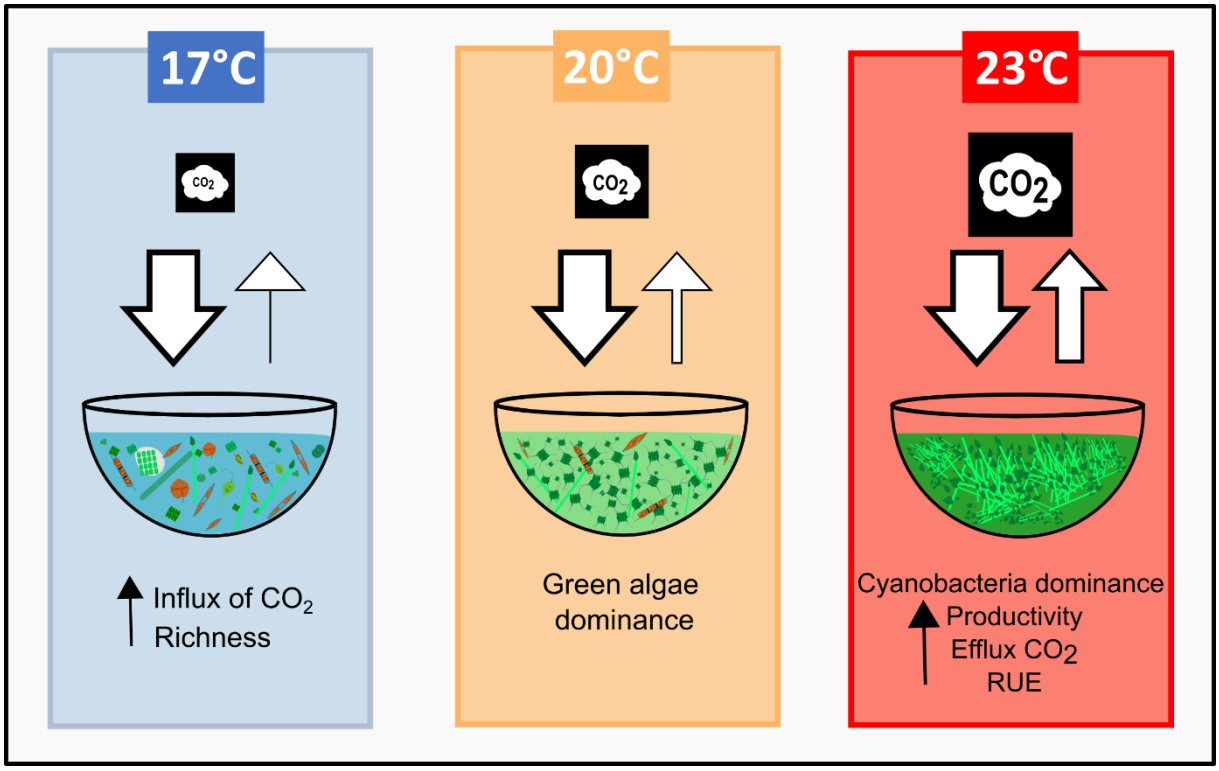

Fig.7. Under climate warming scenarios, ecosystem functions are affected. For example, with warming, environments become more productive, due to eutrophication with the dominance of cyanobacteria (less diversity), and greater resource use efficiency (RUE). This scenario gradually changes the ecosystem's metabolic balance changed, taking the lakes in the direction of being CO_2 sources for the atmosphere. We evidenced positive feedback mechanisms relationship among climate warming, lake eutrophication, and cyanobacteria blooms (vicious loop).

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3 WARMING PROMOTES RESILIENCE OF CYANOBACTERIAL BLOOMS TO SIMULATED RAINFALL EXTREME EVENTS

ABSTRACT

Cumulative stressors including climate change (warming, extremes rainfall events) and eutrophication have increased the frequency and severity of cyanobacteria blooms. In this study, we conducted an indoor short-term experiment to test how natural phytoplankton communities reacted to an extreme precipitation event under different temperature scenarios. Our main hypothesis was that communities less stressed by warming would be more resistant and would more rapidly recover their prior biomass and resource use efficient (RUE), than communities under higher temperature. Contrary to our hypothesis, in the communities exposed to lower temperature (supposedly less stressed), the effects of the disturbance were more evident. Warming promoted cyanobacteria-dominated communities showed better recovery of biomass and a thus higher ability to withstand the changes caused by the extreme rainfall event. The blooms, dominated by *Raphidiopsis raciborskii*, returned in 10 days to similar values of RUE and chlorophyll-a compared to those not subjected to the simulated rainfall event. Phytoplankton communities developed under lower temperatures did not show such resilience. Our results highlight the increasing vulnerability of freshwater ecosystems to warming as well as to rainfall extreme events. Climate change clearly aggravates the negative effects of eutrophication through the enhancement of cyanobacteria, which, as demonstrated here, has great stability and recoverability after disturbances.

Keywords: Climate change, multiple stressors, eutrophication, resilience, resistance, rainfall.

3.1 Introduction

Climate change is often studied as a single stressor (most typically the increase in temperature) impacting the natural environment, although climate change also increases the frequency and magnitude of extreme events as well as overall patterns of precipitation (Field *et al.* 2014, Lehmann *et al.* 2015). In lakes, one of the most clear and expected outcomes of climate warming is the positive interaction with eutrophication process and symptoms, leading to an increase in the distribution and intensity of cyanobacterial blooms across the globe (Garcia-Pichel et al., 2003; Paerl & Otten, 2013; Harke et al., 2016; Paerl et al., 2020; Weber et al., 2020), and the consequent major threat to freshwater quality and global water security (Codd et al., 2005; Michalak, 2016; Richardson et al., 2018; Jeppesen et al., 2021). Although nutrients seem to be the more important predictor of cyanobacterial biovolume, as lakes become more eutrophic cyanobacteria become more sensitive to the interaction of nutrients and temperature (Rigosi et al., 2014).

Associated with warming, extreme rainfall events can have multiple effects and the mechanism behind alterations in precipitation and its impacts on blooms is not well understood. The responses of phytoplankton communities to disturbances are influenced by many factors, including the type of aquatic environment (reservoirs, shallow lakes, and deep lakes) (Doubek et al., 2017; Hayes et al., 2017; Richardson et al., 2018), abiotic and biotic conditions, and extant phytoplankton community composition (Stockwell et al., 2020). Thus, changes in precipitation patterns can have multiple effects at different scales and levels, with different direct and indirect impacts on phytoplankton and particularly on cyanobacteria.

For instance, future increases in mean precipitation may promote the occurrence of cyanobacteria, due to higher nutrient input by increased runoff from the catchments (Ockenden et al., 2017), thus increasing blooms (Shaw et al., 2001; Jeppesen et al., 2011). A long-term reduction in mean precipitation can also favor cyanobacteria due to a higher stratification of the

water column and higher concentration of nutrients (Brasil et al., 2016). This may occur when extreme rainfall is followed by periods of droughts (Paerl & Huisman, 2009; Havens & Ji, 2018). Short-term changes, such as intense rainfall events can generate unfavorable conditions for cyanobacteria due to dilution and flushing; causing either a decrease in biomass or a complete collapse of the bloom due to destratification of the water column (Reynolds et al., 2012; Sadro & Melack, 2012). Indirectly, increased flow may also result in environmental changes and consequently affect biological responses, such as changes in selection pressures that affect community composition and diversity (James et al., 2008; Reichwaldt & Ghadouani, 2012). The mechanisms determining ecosystem response and recovery to climate extremes remain unclear, making vulnerability assessments uncertain (Kayler et al., 2015; De Boeck et al., 2018).

Cumulative stressors linked to climate change, as warming, anthropogenic eutrophication, extreme weather, all factors associated with promoting cyanobacterial blooms put at risk the functioning in freshwater ecosystems (e.g. resource use efficiency – RUE) (Wagner & Adrian, 2009; Kosten et al., 2012; Filstrup et al., 2014). In addition to the intensification of multiple environmental stressors, many aspects of global change are expected to alter the frequency, variance and timing of disturbances (De Laender et al., 2016; Donohue et al., 2016; Radchuk et al., 2019). After a major disturbance, the re-establishment of species is highly variable and depends on the extent of physical alteration of the environment, species growth rates, competition, predation and other factors (Ji et al., 2018).

Ecological ‘stability’ is the core concept describing potential responses to such changes, a concept of central importance for understanding present-day and predicting future ecosystem dynamics. The multifaceted concept of ecosystem stability includes: resistance, as the ability to withstand the perturbation (Pimm, 1984; Donohue et al., 2016), recovery, the ability to return

to their pre-disturbance state, and resilience, the time needed to return to their pre-disturbance state (Holling, 1973; Orians, 1975).

Understanding the ecosystem responses and stability against long-term and short-term stressors is crucial to be able to suggest better management and restoration strategies (Scheffer et al., 2001; Pecl et al., 2017; De Boeck et al., 2018). In this sense more diverse systems are expected to have greater ecosystem stability and greater resistance to a disturbance, reflecting interspecific complementarity, higher resource use efficiency (Tilman et al., 2014). Still, it is expected that eutrophic systems, due to the dominance of a single species and less diversity, may have a lower level of ecosystem stability in response to climate change (Filiz et al., 2020).

Here, we conducted an indoor short-term experiment to test how distinct natural phytoplankton communities respond to climate change (a disturbance, i.e., a simulated rainfall extreme event and a stress, i.e., different warming levels). We expected that communities less stressed by warming would be more resistant against a disturbance and would have the ability to recover their pre-disturbance biomass and functioning faster than the other phytoplankton communities.

3.2 Methods

3.2.1 Experimental Design

We conducted a short-term indoor controlled microcosm experiment in a laboratory located at the *campus* of the Universidad de la Republica (UdelaR), Maldonado, Uruguay (34º 54'53´S e 54 ° 56'31 " W '), between July 23 and August 2 of 2019 (southern hemisphere winter). We compared the response to a disturbance by three distinct phytoplankton assemblages, which resulted after four weeks of the same original community being subject to three temperature treatments. The control treatment, (i) 17 °C, corresponded to the annual mean temperature of the aquatic ecosystems in the same region where the experiment was conducted (Pacheco et al., 2010). The second treatment, (ii) 20 °C, represented a scenario with an increase of 3 °C in

relation to the annual mean temperature; and the third treatment, (iii) 23 °C, represented an increase of 6 °C in relation to the mean temperature (Fig.1). Room temperature was manipulated in all treatments using electronic temperature controllers (Eliwell ID Plus 961), including the control treatment.

The microcosm was cylindrical polyethylene 5-L aquarium (23.5 cm of diameter and 10 cm of height). We installed water circulation pumps (model Resun AC-9903) at the bottom of each aquarium to avoid phytoplankton sedimentation (Flury et al., 2010; Zhang et al., 2015). Besides, we moved the water manually and delicately twice a day with a stick. Light regime consisted of circa 80 lum/ft² of 12/12 hs ratio of light/darkness generated by fluorescent lamps with a light spectrum similar to that of the sun. All experimental units were placed at the same height to homogenize temperature and incident light. We randomly distributed twenty-four microcosms between the three temperature treatments (i.e., n=8 replicates each).

To prepare the natural phytoplankton original inoculum for the experiment, we obtained water samples at the subsurface of the limnetic region in a series of subtropical shallow lakes situated at the Uruguayan coastline (34° S 56° W). These lakes comprised a trophic gradient from mesotrophy to hypertrophy (Kruk et al., 2009). We collected phytoplankton using a plankton net with a mesh size of 20 µm to remove most zooplankton and added some non-filtered water to include smaller species (< 20 µm). We used this strategy to maximize the sampling of less abundant species and guarantee that most taxonomic groups were present in the experimental units. We distributed 0.5 L of concentrated inoculum for each of the 24 experimental units and added 2.5 L of dechlorinated water enriched with a nutrient solution to reach a final volume of 3 L.

After 4 weeks, three distinct communities emerged (summarizing 72 taxa, Supplementary Figure 1). The lowest mean values of phytoplankton biomass were recorded in the 17ºC treatment (13 $mm^3.L^{-1}$ ± 0.66 $mm^3.L^{-1}$) and the highest mean biomass values for 20ºC

treatment (23.96 $mm^3.L^{-1}$ ± 1.69 $mm^3.L^{-1}$) and 23ºC treatment (24.33 $mm^3.L^{-1}$ ± 2.27 $mm^3.L^{-1}$). Cyanobacteria (as *Raphidiopsis raciborskii* (Wołoszyńska) Aguilera, Berrendero Gómez, Kastovsky, Echenique & Salerno, before *Cylindrospermopsis*) was the main group for the treatments of 17ºC and 23ºC with mean values of 7.35 $mm^3.L^{-1}$ and 18 $mm^3.L^{-1}$, respectively whereas green algae (as *Desmodesmus magnus* (Meyen) Tsarenko and *Staurastrum* sp.) was the largest contributor under 20ºC (12 $mm^3.L^{-1}$). For more details on the methodology see Moresco et al. (*in press*)

To simulate extreme rainfall events four experimental units from each temperature were randomly selected, and, after intense homogenization, 1 L of water was removed and 1 L dechlorinated water enriched with a nutrient solution was added. The experiment lasted for 10 days, as the short life cycle of phytoplankton allows the reproduction of several generations in a few days (Reynolds, 2006).

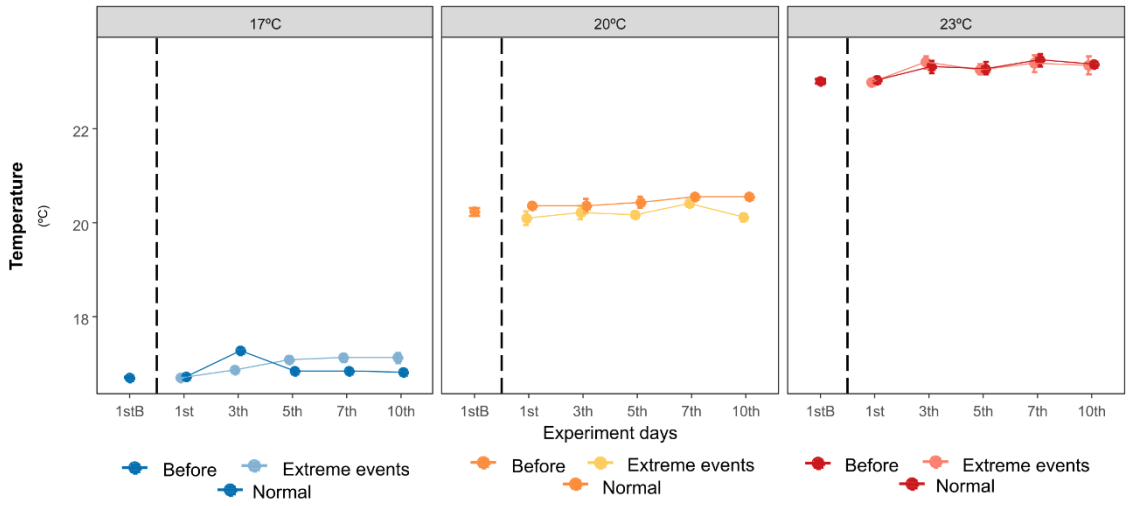

Figure 1. Mean daily temperature values in each temperature treatment. Light colors correspond to the extreme rainfall treatment and dark colors non disturbed treatments. The dotted line indicates the occurrence of the extreme rainfall event (1stB). The central point denotes mean value and whiskers represent standard error.

3.2.2 Monitoring of limnological variables

In all microcosms, every two days we measured water temperature (ºC), pH, and electric conductivity using a HANNA multiparametric probe, and dissolved oxygen (mg L^{-1}) and oxygen saturation (%) using an Oxyguard Handy Polaris. We sampled water on the first and the last day (10^{th}) to determine total phosphorous (TP; µg L^{-1}), total nitrogen (TN; µg L^{-1}), reactive soluble phosphorous (SRP; µg L^{-1}), nitrate ($N\text{-}NO_3$; µg L^{-1}), and ammonia ($N\text{-}NH_4$; µg L^{-1}), as well as chlorophyll-a, according to APHA (2005). Phytoplankton was maintained in a medium following the Redfield ratio. Weekly, a nutrient solution was added to each microcosm to compensate for the losses due to evaporation as to reach the initial volume of 3 L (McKee et al., 2000; Ekvall & Hansson, 2012). The mean and standard error of nutrients in microcosms were 2431.9± 176.2 µg L^{-1} of TN and 81.2 ± 2.3µg L^{-1} of TP.

3.2.3 Phytoplankton

We sampled phytoplankton every five days (day 1 before, and after the extreme rainfall event, day 5 and day 10). Before taking each sample, we homogenized the water manually to avoid missing any species due to sedimentation. We sampled phytoplankton directly with flasks and fixed them immediately with acetic Lugol solution. Counting of individuals (cells, colonies, and filaments) of phytoplankton followed the Utermöhl method (Utermöhl, 1958) and Lund *et al.* (1958). The biomass ($mm^3.L^{-1}$) was considered as biovolume, which was estimated by multiplying the density of each taxon by its volume. We estimated the cell volume by calculating the volume of the geometric shape that was the most similar to each cell form (Sun & Liu, 2003). We also estimated the community resource use efficiency (thereafter RUE), defined as the ratio between the phytoplankton biomass production and TP, as a proxy for ecosystem productivity (Ptacnik et al., 2008; Olli et al., 2015; Verbeek et al., 2018).

3.2.4 Data analyses

To verify the existence of significant differences between the occurrence or absence of the extreme rainfall events along time we performed two permutational multivariate analyses of variance (PERMANOVA) (Anderson, 2001b), using as response variables the biomass of different phytoplankton species. The variation in the biomass trajectory of the phytoplankton community and its capacity to return in the initial stage (i.e., resilience) was visualized using a nonmetric multidimensional scaling (NMDS). In NMDS, distances were calculated using the Bray–Curtis similarity index with the resolution distortion in two dimensions expressed by the value S (stress) (Clarke, 1993). Resistance and recovery were calculated for phytoplankton total biomass following (Hillebrand et al., 2018). Resistance and recovery were calculated with phytoplankton total biomass sampling following this ratio: ln (disturbed treatment/un disturbed treatment). We use initial biomass (after disturbance) for the calculation of the resistance and the final biomass for the recovery. We ran all analyses in software R version 3.3.2 (R Development Core Team, 2021) , using packages “vegan” (Oksanen et al., 2018).

3.3 Results

The rainfall disturbance led to different responses by the three distinctive phytoplankton communities developed under the three temperature treatments. The disturbance affected abiotic variables analyzed (Supplementary Figure 2) and had significant effects in biomass at 17°C ($P = 0.008$) and 20°C ($P = 0.01$) and not at 23°C ($P = 0.607$), according to the PERMANOVA. The non-significant effect of disturbance highlights the resilience of the phytoplankton community developed under the warmest conditions (Table 1). In the phytoplankton communities developed under the lower temperatures (i.e., supposedly less

stressed), the effects of the disturbance were more evident. Also, time had significant effects on biomass (P = 0.001), except for 20°C (Table 1).

Table 1: Effects of disturbance and time according on phytoplankton biomass under the three temperature treatments, according to PERMANOVA test. Significant P values (P<0.05) are highlighted in bold.

Temperature		17°C			20°C			23°C		
	Df	R^2	F-value	P	R^2	F-value	P	R^2	F-value	P
Time	3	0.24	2.919	**0.001**	0.09	0.958	0.472	0.27	3.528	**0.001**
Disturbance	2	0.14	2.357	**0.008**	0.16	2.763	**0.017**	0.04	0.757	0.607

Changes in the trajectory of phytoplankton biomass over time and in relationship with the disturbance indicate that the community in the warmest temperature has a greater capacity for resilience, since on the last day of the experiment, even the environments that suffered disturbance are close to those that are undisturbed. In contrast, less stressed environments had a high dispersion of points, indicating less resilience (as shown in the NMDS) (Figure 2a).

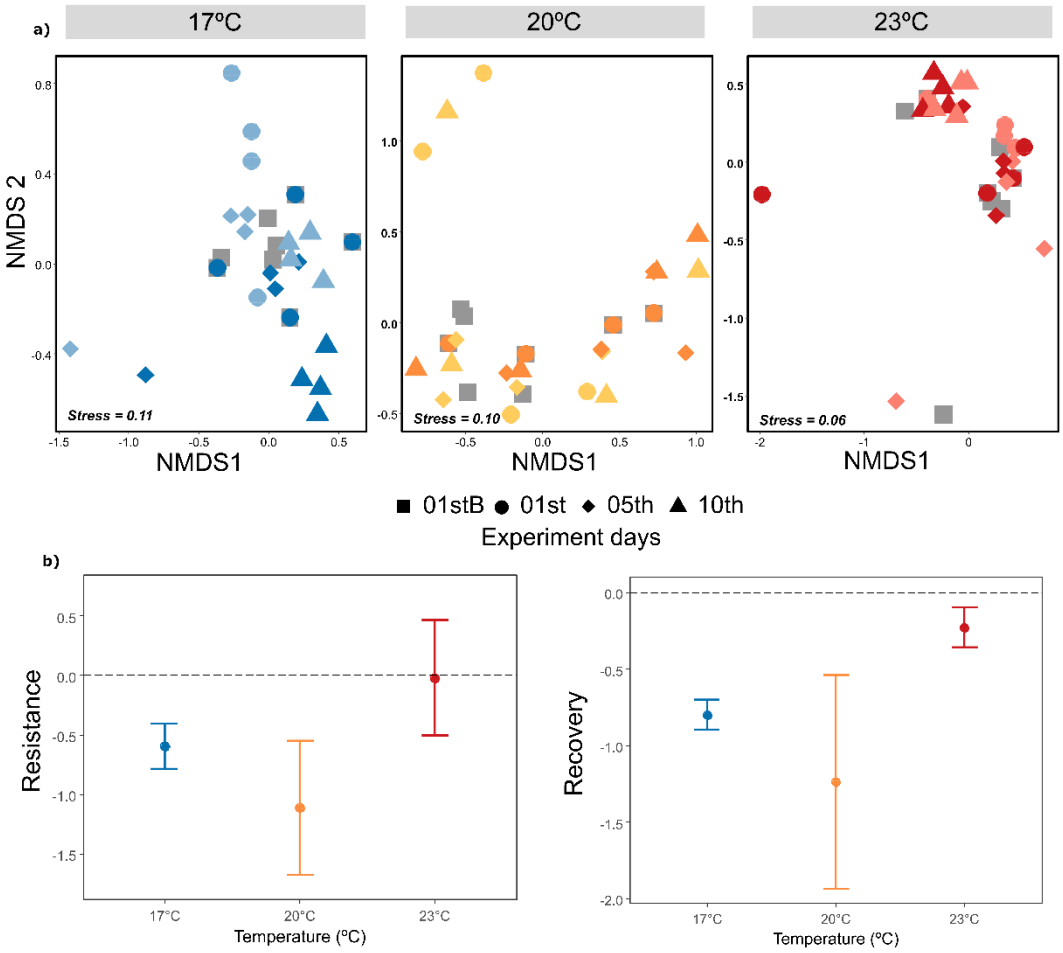

Figure 2: Trajectories (a), resistance (b), and recovery (c) of phytoplankton biomass. The gray dots in the non-metric multidimensional scaling (NMDS) (a) indicate the period before the extreme rainfall event (1stB), light colors indicate disturbed communities and dark colors the undisturbed. Dashed lines in (b) and (c) are the benchmarks. The central point denotes the mean value and whiskers represent standard error.

In contrast to our hypothesis, phytoplankton communities developed under warming conditions showed a better recovery of biomass and thus a higher ability to withstand the changes caused by the extreme rainfall event simulated in our experiment. The less stress communities (developed at low and intermediate temperatures) stayed distant from their benchmark during the entire disturbance, indicating poor resistance and poor recovery. The best recovery and higher resistance in the community developed under the warmest treatment was

led by cyanobacteria, which on the last day of the experiment had a mean biomass of 122.09 $mm^3.L^{-1}$ ± 7.32 $mm^3.L^{-1}$ in the control treatment and 99.02 $mm^3.L^{-1}$ ± 6.15 $mm^3.L^{-1}$ in the disturbed treatment (Fig. 3, Supplementary Fig. 3), even surpassing the biomass achieved in the period before the disturbance (63.86 $mm^3.L^{-1}$ Fig. 2a, Fig. 2b). Green algae did not recover their biomass at the levels before the disturbance at any temperature evaluated. The best performance for the group occurred at 20ºC, even so with a difference of approximately 10 $mm^3.L^{-1}$ between the level before the disturbance and that of the extreme rainfall event (Fig. 3)

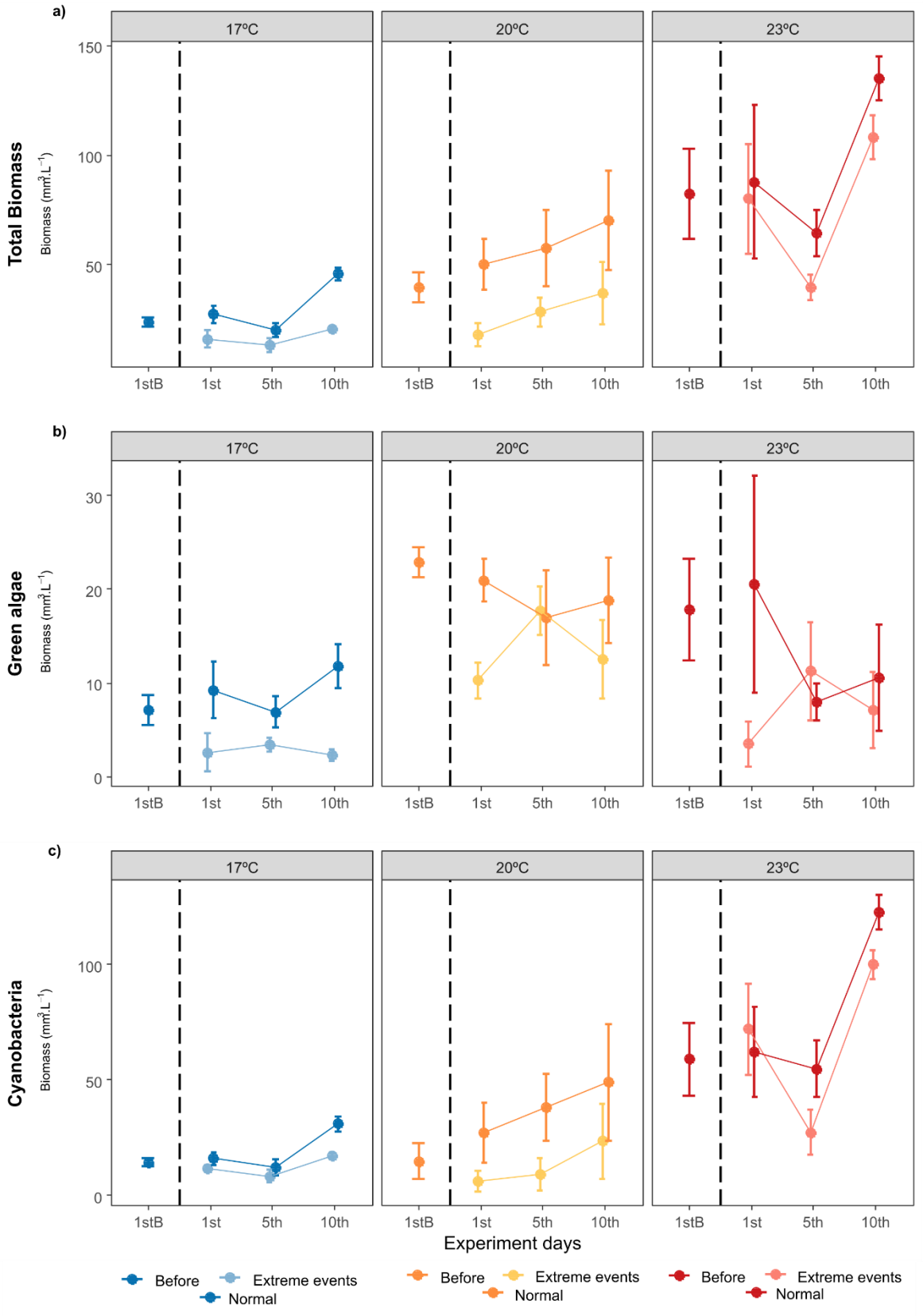
a)
b)
c)
17°C
20°C
23°C
Total Biomass
Green algae
Cyanobacteria
Biomass (mm³.L⁻¹)
1stB
1st
5th
10th
Experiment days
Before
Extreme events
Normal

Figure 3: Variation of phytoplankton biomass (biovolume) in the disturbance and temperature treatments: total (a), green algae (b), cyanobacteria (c). The dotted line indicates the occurrence of the extreme rainfall event (1stB). Light colors indicate communities subject to extreme rainfall and dark colors the undisturbed communities. The central point denotes the mean value and, whiskers represent standard error.

The RUE and final chlorophyll-a concentrations were affected by the extreme rainfall event at the three temperatures evaluated. However, the disturbed communities under the warmest treatment (with higher biomass dominated by blooms of *Raphidiopsis raciborskii*) showed values of RUE and chlorophyll-a like the undisturbed communities. In the other temperature treatments, and particularly under the lowest temperature, the disturbed communities did not achieve values similar to the disturbed ones, except for chlorophyll-a in the 20ºC (Fig. 4a, Fig. 4b). The conceptual diagram with the main results of our study is shown in Fig.5.

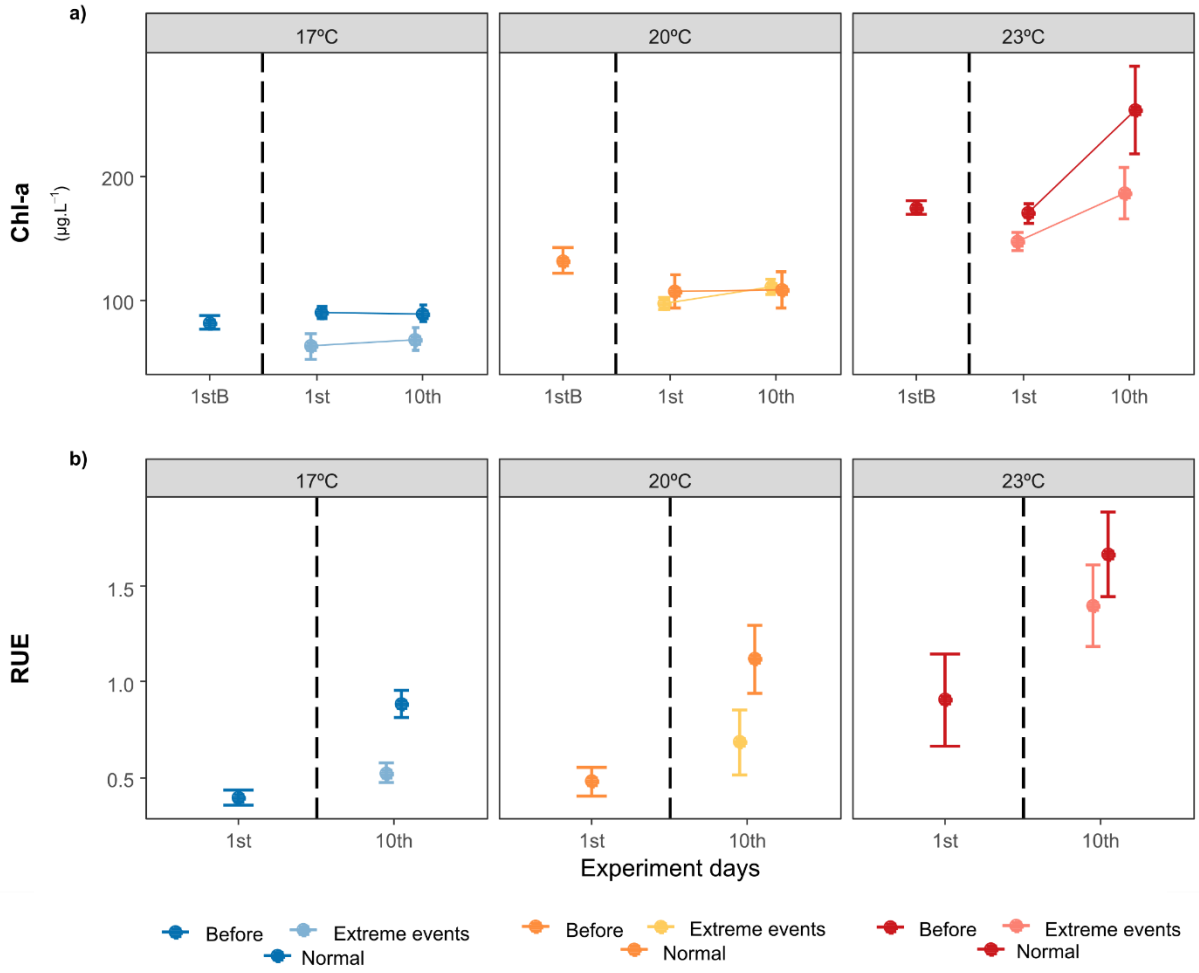

Figure 4. Different ecosystem responses to warming: variation of chlorophyll-a concentration (Chl-a) (a), resource use efficiency (RUE) (b) by the phytoplankton communities in three treatments. Light colors indicate environments with the effect of extreme rainfall events and dark colors without the effect. The central point denotes the mean value and, whiskers represent standard error.

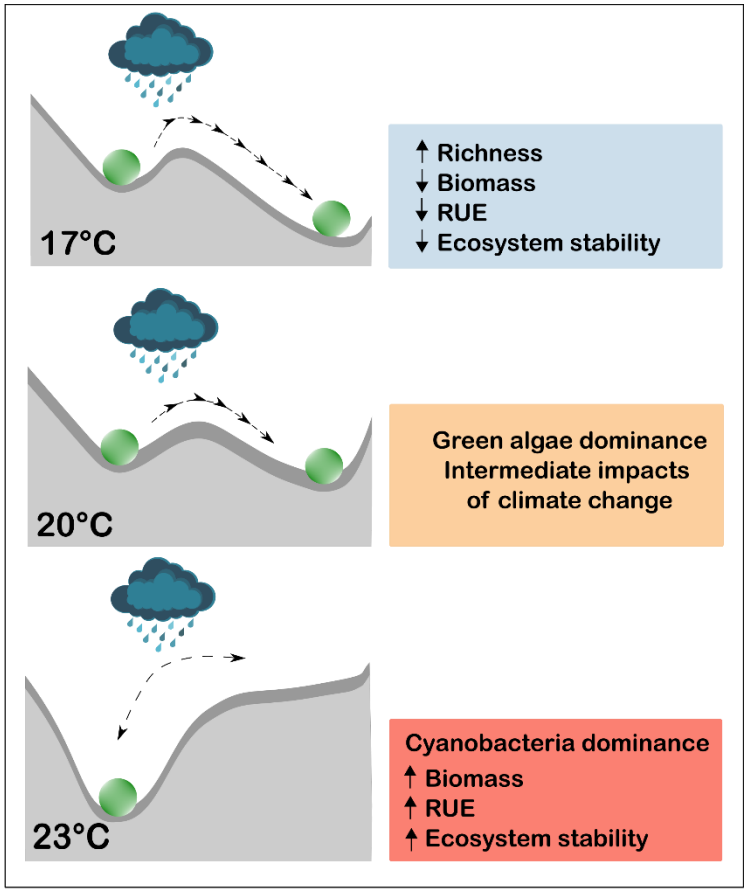

Figure 5. Under different experimental climate change scenarios, community structure and ecosystem functions were affected. Different warming scenarios lead to different phytoplankton communities (represent by ball), which had different resistance and resilience against a short-term disturbance, as indicated by the biomass and resource use efficiency (RUE) recovery.

3.4 Discussion

We avaliated the effects of strong disturbance simulating an extreme climatic event, on three phytoplankton communities pre-adapted to different levels of warming and under non-limiting nutrient conditions. The study revealed that ecosystem stability and resilience (recoverability of biomass and ecosystem function) depended on the dominant group. Cyanobacteria-dominated communities, developed under the warmest treatment, were the most resilient. Our results indicate that climate change, both via long-term stressors such as an

increase in mean temperature and short-term perturbations such as intense rainfall events, may aggravate the negative effects of eutrophication through the enhancement of cyanobacteria.

Freshwater ecosystems are becoming more vulnerable to multiple disturbances due to the combined and often synergistic effects of climate change and several human activities (Evtimova & Donohue, 2014; Hunter-Cevera et al., 2016; Paerl et al., 2016; Yang et al., 2017). Eutrophic environments seem particularly vulnerable to climate warming (Rigosi et al., 2014). In this sense, we found that phytoplankton communities developed under different temperatures respond differently to disturbances. Cyanobacteria blooms benefited and proved to be more resilient to a pulse disturbance than communities expectedly less stressed by warming, which in contrast, showed a slower response to the simulated extreme rainfall event.

This indicates that the synergistic effect of multiple stressors is important in shaping the response of cyanobacteria and that a multiple factors approach could help better predict community responses to future environmental change. Several studies have shown that higher water temperature and rainfall regimes may play a key role in the proliferation of cyanobacteria, especially under eutrophic conditions (Bormans et al., 2005; Paerl & Huisman, 2009; O'Neil et al., 2012; Wood et al., 2017).

Our earlier results (Moresco et al., *in press*) had confirmed previous empirical findings that higher water temperatures promote cyanobacterial blooms (Paerl & Huisman, 2008; Kosten et al., 2012; Richardson et al., 2019), in our case through direct effects on phytoplankton community structure and performance. Many species of cyanobacteria may benefit in warmer conditions due to several traits (Carey et al., 2012; Mantzouki et al., 2016).

Although the phytoplankton community is sensitive to losses due to extreme rainfall event (Reynolds et al., 2002; Carvalho et al., 2011; Stockwell et al., 2020), cyanobacteria can grow more efficiently at higher temperatures and persist longer under extreme wet/dry cycles (Paerl et al., 2016). Besides changes in the environmental conditions in aquatic systems caused

by extreme rainfall events depends of others characteristics (hydrology of the catchment, waterbody type), eutrophic systems lead to higher biomass production (Reichwaldt & Ghadouani, 2012). This eutrophication process generally favors cyanobacterial blooms due to physiological characteristics that include the presence of aerotopes that allow them to move through the water column when there is thermal stratification, nitrogen-fixing capability, high affinity and phosphorus storage abilities, and the production of cysts (akinetes) (Weyhenmeyer et al., 2007; Salmaso et al., 2015). In addition, this process can be greatly intensified if after a large pulse of nutrients (via extreme rainfall event) followed by a dry period could benefit cyanobacteria (‘perfect storm’, Paerl *et al.* 2016). Thus, the survival chance of sensitive groups is much lower than that of adaptive groups (Filiz et al., 2020; Stockwell et al., 2020).

Empirical and theoretical evidence suggests that more diverse assemblages show more stable productivity over time (Tilman, 1996; Isbell et al., 2015)). Although the environments with lower temperatures present greater species richness, they were not very resistant and had a low capacity to recover after the extreme rainfall event. A number of studies have shown a positive relationship between phytoplankton species richness and RUE which has been attributed to a more efficient use of resources in more species rich communities (Ptacnik et al., 2008; Striebel et al., 2009; Chai et al., 2020). However, we found that the RUE of phytoplankton increased with temperature, confirming earlier findings showing that primary productivity increases with temperature (Kerkhoff et al., 2005; Lovelock et al., 2007; De Senerpont Domis et al., 2014; Verbeek et al., 2018). Besides, after the dilution effect, the productivity of the ecosystem was only recovered in the cyanobacteria-dominated community developed under the warmest treatment. This result demonstrates that the most diverse communities do not necessarily better buffer the impacts of climate extremes on ecosystem functioning than less diverse communities. A reason for the faster recovery in the warmest conditions might be that the dominant *R. raciborskii* is able to use phosphorous more efficiently than other

phytoplankton taxa, which concurs with studies finding strong effects of cyanobacteria on RUE in eutrophic lakes (Roy & Chattopadhyay, 2007; O'Neil et al., 2012; Filstrup et al., 2014; Sukenik et al., 2015). The temperatures used in our study were, however, in the range where no major differences in cyanobacterial or eukaryote algal growth rates were expected (Lürling et al., 2013).

Experimental studies are a fundamental tool for understanding the complexity of how global climate change may impact freshwater ecosystems (Stewart et al., 2013), despite their obvious limitations (Benton et al., 2007). They allow developing a clearer mechanistic understanding of the interactions between multiple stressors, allowing quantification and comparison of individual stressor effects and their interactions (Crain et al., 2008; Piggott et al., 2015). In relation to limitations, in our case, the microcosms are isolated and, changes in the environmental conditions in aquatic systems caused by rainfall events will mainly depend on the hydrology characteristics of the catchment and waterbody, and the land use in area. Because benthic processes and even pelagic trophic interactions were purposely excluded, we were able to capture a pure pelagic response to the combined effects of climate change, exclusive to the phytoplankton community, and how such responses translated to ecosystem functions (e.g., biomass production, and ecosystem stability).

We examined the response of phytoplankton communities subjected to stressors of climate changes, such as eutrophication, warming, and extreme rainfall events. The study revealed the increasing vulnerability of freshwater ecosystems phytoplankton community to warming as well as rainfall extreme events. Ecosystem stability in the warming microcosms was affected differently by the rainfall disturbance. Climate change stressors may aggravate the negative effects of eutrophication through the enhancement of cyanobacteria and, with increased frequency of extreme events, freshwater ecosystems may not have sufficient time to

recover, and this could possibly lead to regime shifts. Besides, eutrophic and warm environments, dominated by cyanobacteria, showed greater stability and recoverability.

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4 FINAL CONSIDERATIONS

In this thesis, composed of two papers, we evaluated the effects of multiple factors related to climate change effects on natural freshwater phytoplankton community. Together with land use change, climate change is expectedly one of the major drivers threatening both aquatic biodiversity and several ecosystem services for the human population, such as water supply, recreation, culture, and irrigation. Thus, in the first paper, we tested how increasing temperatures influence natural phytoplankton diversity and CO_2 emissions in eutrophic ecosystems. Besides, we evaluated the potential feedbacks between eutrophication and climate warming of a natural phytoplankton community and how increasing temperatures could affect the metabolic balance. For instance, we found that under no nutrient limitation, warming promoted an increase in productivity, with a dominance of cyanobacteria (with less overall diversity), and greater resource use efficiency. More interestingly, the ecosystem's metabolic balance changed, taking the microlakes in the direction of being CO_2 sources to the atmosphere. This finding gives experimental evidence of a positive feedback between eutrophication symptoms (cyanobacteria blooms) and warming, via higher CO_2 emission rates in cyanobacteria dominated warmer systems, adding to current research highlighting such self-reinforcing feedbacks.

In the second paper, we verified how cumulative stressors climate change (warming, eutrophication, and extremes rainfall events) can affect community and ecosystem stability. To do that, we used three phytoplankton communities already established and adapted to three different temperature scenarios. Our results showed experimentally that, environments stressed by warming combined with extreme rainfall events, seem to have a greater capacity to recover to the stage before the disturbance, such as restoring their biomass production and resource use efficiency. Thus, eutrophication symptoms are exacerbated by rising temperatures and are resilient to rainfall extreme events, reinforcing the dominance of cyanobacteria blooms.

In conclusion, we experimentally demonstrated the effects of climate change components and the vulnerability of the freshwater phytoplankton community as well as its enormous potential to test relevant ecological hypotheses in relatively simple experiments. We used a highly diverse initial community and isolated it from potentially confounding factors. Thus, we were able to capture a pure pelagic response to climate change stressors, exclusive to the phytoplankton community, and how such responses translated to ecosystem functions (e.g., biomass production, energy transfer, and carbon cycle).

APPENDIX A - Scientific Dissemination

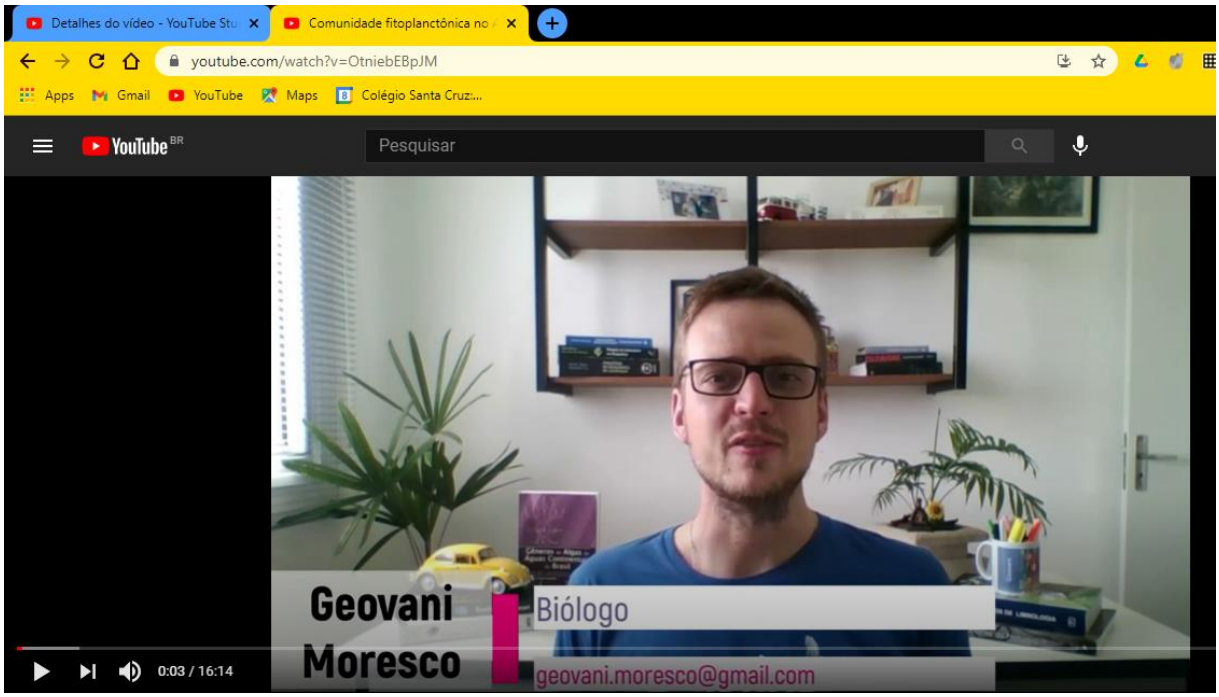


Fig S1. Scientific dissemination of the main results of the thesis for the participation of society on the theme of climate change. Video available at: https://www.youtube.com/watch?v=OtniebEBpJM

APPENDIX B - Experimental design

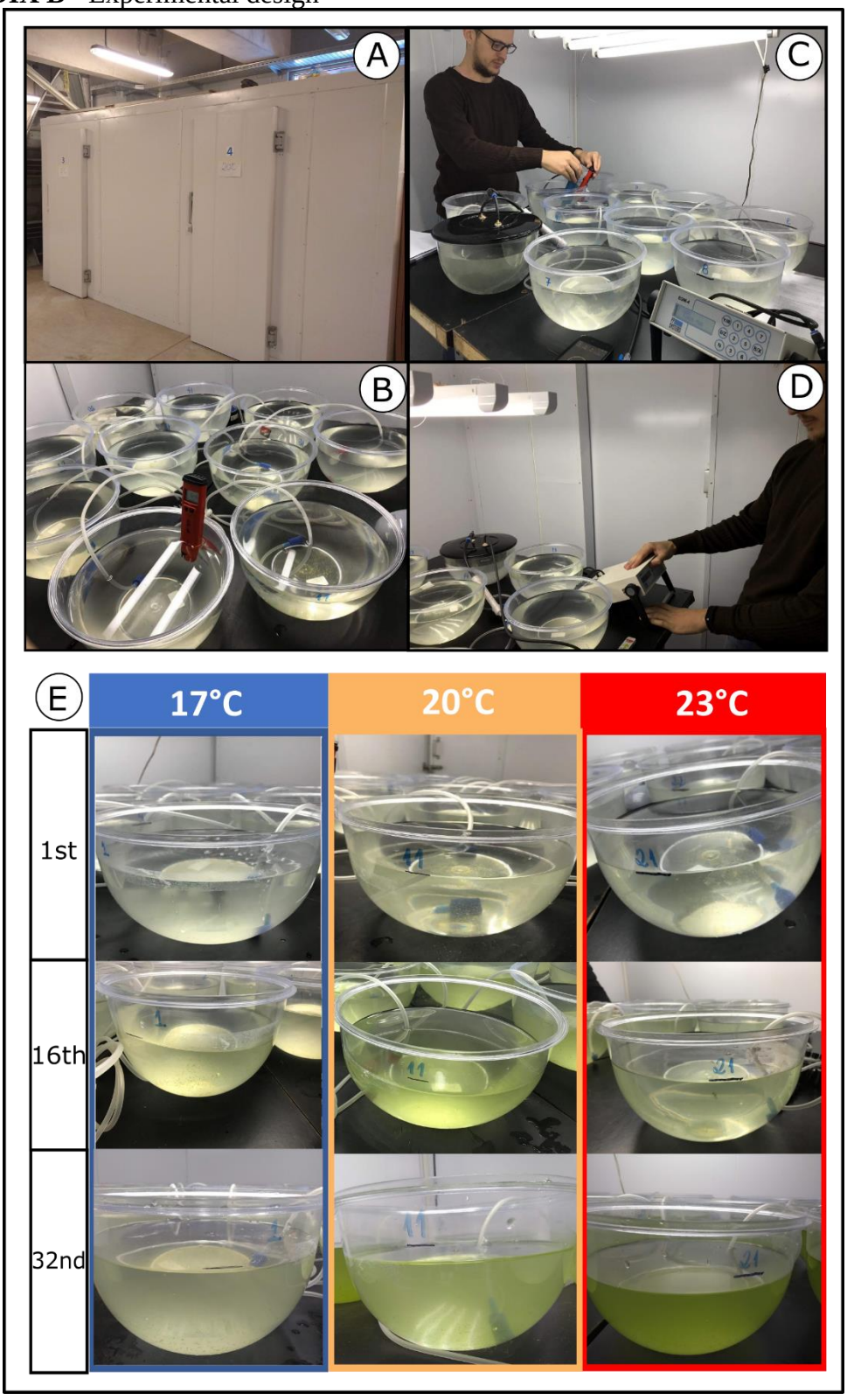


Figure S1. Experimental Design. A) Rooms temperature; B) overview of the experiment; C) Monitoring of limnological variables; D) CO_2 fluxes measured; E) Development of the phytoplankton community at the three temperatures evaluated (17ºC, 20ºC, and 23ºC). Photos were taken on the 1st day, on the 16th day (when temperatures reached the desirable), and on the 32nd day.

APPENDIX C – Nutrient concentrations

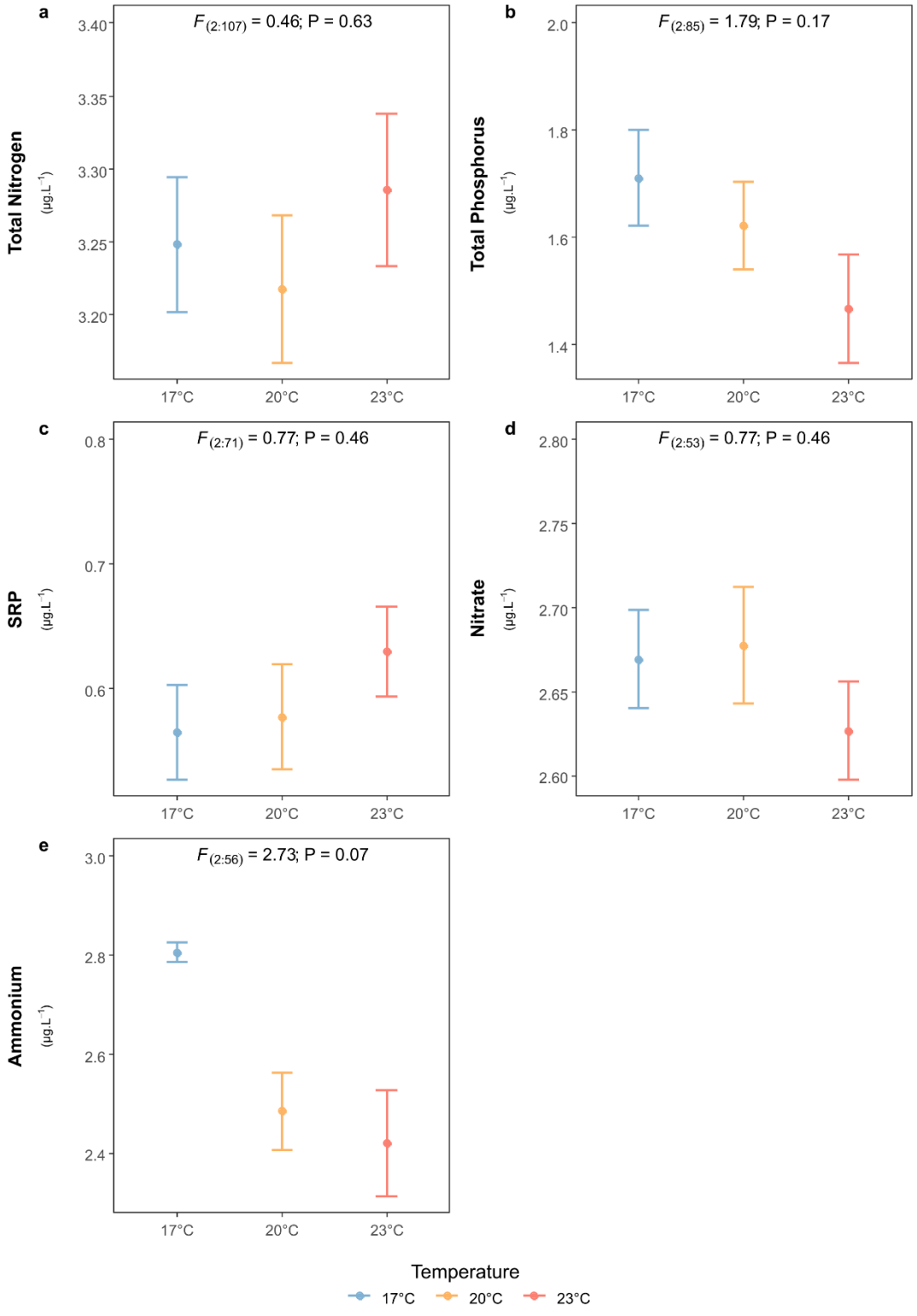


Figure S2: Nutrient concentrations in the three temperature treatments obtained. The non-significant result in the analysis of variance ($P > 0.05$) indicated that nutrient concentrations were similar between treatments. Note that the scale varies among limnological variables. The central point denotes the mean value and, whiskers represent standard error for each temperature.

APPENDIX D Variation of phytoplankton taxonomic richness

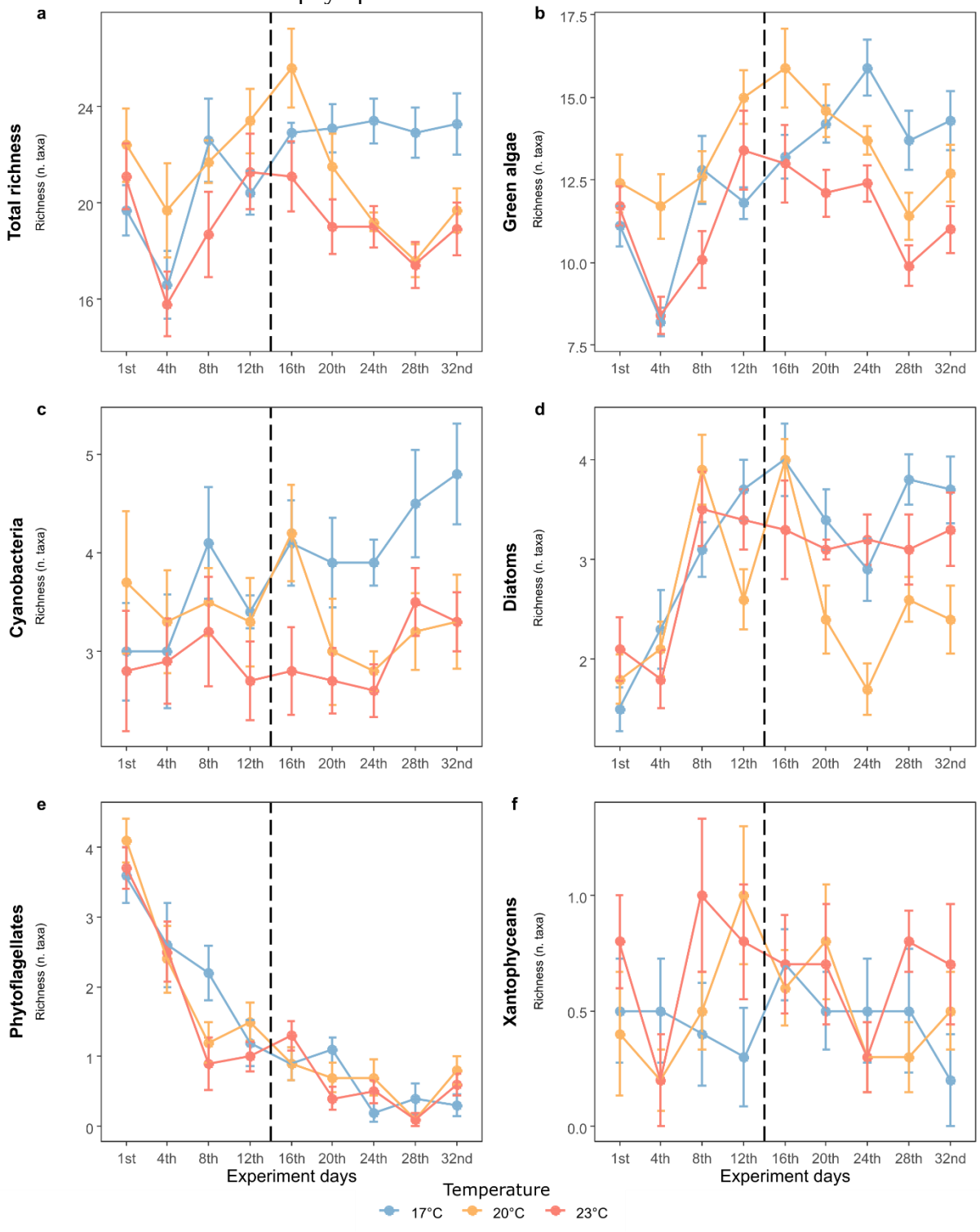


Figure S3: Variation of phytoplankton taxonomic richness with temperature through the 32 days of the experiment: total (a), green algae (b), cyanobacteria (c), diatoms (d), phytoflagellates (e) and xanthophyceans (f). The dotted line indicates that on the 16th day of the experiment the wished temperatures were reached. The central point denotes the mean value and, whiskers represent standard error for each temperature in each experiment day.

APPENDIX E List of phytoplankton taxa recorded in the experiment through the 32 days of the experiment.

Table S1: Phytoplankton taxa identified during the 32-days of the experiment.

Popular name/ Taxonomic Class /Taxon
Green algae
Chlorophyceae
Ankistrodesmus bernardii Komárek
Ankistrodesmus gracilis (Reinsch) Korshikov
Coelastrum microporum Nägeli
Coelastrum pseudomicroporum Korshikov
Coelastrum pulchrum Schmidle
Coelastrum reticulatum (P.A.Dangeard) Senn
Coelastrum sphaericum Nägeli
Desmodesmus communis (E.Hegewald) E.Hegewald
Desmodesmus magnus (Meyen) Tsarenko
Eutetramorus fottii (Hind.) Kom. Sensu Kom.
Kirchneriella contorta Schm. Bohl.
Kirchneriella irregularis (G. M. Schm.) Kors.
Kirchneriella lunaris (Kirchn.) Möb.
Monoraphidium arcuatum (Kors.) Hind.
Monoraphidium contortum (Thur.) Kom. – Legn.
Monoraphidium griffithii (Berk.) Kom.-Legn.
Pediastrum cf. *boryanum* (Turp.) Menegh.
Pediastrum duplex Mey. var. *duplex*
Scenedesmus acutus Mey.
Scenedesmus alternans Reins.
Scenedesmus ecornis var. *ecornis* (Ehrenb. ex Ralfs) Chodat
Scenedesmus obliquus (Turpin) Kützing
Scenedesmus obtusus Mey.
Scenedesmus ovalternus Chod.
Scenedesmus acuminatus (Lagerheim) Chodat
Schroederia setigera (Schröd.) Lemm.
Selenastrum bibraianum Reins.
Selenastrum gracile Reinsch
Stauridium tetras (Ehrenberg) E. Hegewald
Tetraedron caudatum (Cor.) Hansg.
Tetraedron minimum (A. Br.) Hansg.
Tetrastrum heteracanthum (Nordst.) Chod.
Tetrastrum komarekii Hindák
Treubaria triappendiculata Bern.
Chlorococcales unicelular não identificada
Quadrigula sp.
Tetrastrum sp.
Klebsormidiophyceae
Elakatothrix sp.
Trebouxiophyceae

Actinastrum aciculare Playf.
Actinastrum gracillimum G. M. Sm.
Botryococcus braunii Kütz.
Dictyosphaerium pulchellum Wood
Lemmermannia tetrapedia (Kirchner) Lemmermann
Micractinium pusillum Fres.
Closteriopsis sp.
Nephrocytium sp.
Oocystis sp.
Zygnematophyceae
Closterium lineatum Ehr. ex Ralfs
Closterium setaceum Ehr. ex Ralfs
Cosmarium regnesi Reins.
Euastrum gayanum De Toni
Staurastrum boergesenii W.B.Turner
Staurastrum rotula Nordst.
Staurodesmus cuspidatus (Bréb.) Teil.
Staurodesmus dejectus (Bréb.) Teil.
Staurodesmus glaber (Ehr.) Teil.
Staurodesmus mucronatus (Nägeli) Thomasson
Staurodesmus triangularis (Lagerh.) Teil.
Cosmarium sp.
Cosmarium sp1
Staurastrum sp.
Staurastrum sp1
Cyanobacteria
Cyanobacteria
Aphanizomenon gracile Lemm.
Aphanocapsa delicatissima W. Et G. S. West
Aphanocapsa elachista W. e G. S. West
Aphanocapsa holsatica (Lemm.) Cronb. e Kom.
Aphanocapsa incerta (Lemm.) Cronb. e Kom.
Chroococcus minimus (Keis.) Lemm.
Cylindrospermopsis raciborskii (W.) Seen. e Sub. Rajú
Dolichospermum circinalis (Rabenh. ex Bornet & Flahault) Wacklin et al.
*Dolichospermum planctonicum (*Brunnthaler) Wacklin et al.
Dolichospermum spiroides (Kleb.) Wacklin et al.
Lemmermanniella pallida (Lemmermann) Geitler
*Merismopedia glauca (*Ehr.) Kütz.
Merismopedia tenuissima Lemm.
Microcystis aeruginosa Kütz.
Pseudanabaena mucicola (Hüb.-Pest. e Naum.) Bourr.
Pseudanabaena limnetica
Romeria gracilis (Koczw.) Koczw. ex. Geit.
Snowella atomus Kom. e Hindák
Aphanocapsa sp.
Chroococcales not identificaded 1
Phormidium sp.

Diatoms
Bacillariophyceae
Achnanthidium minutissimum (Kütz.) Czarn.
Nitzschia palea (Kütz.) W. Sm.
Ulnaria ulna (Nitzch.) Comp.
Amphora sp.
Eunotia sp.
Eunotia sp1
Fragilaria sp.
Fragilaria sp1
Navicula sp.
Pinnularia sp.
Surirella sp.
Surirella sp1
Synedra sp.
Ulnaria sp.
Coscinodiscophyceae
Aulacoseira ambigua (Grun.) Sim. var. *ambigua*
Aulacoseira granulata (Ehr.) Sim. var. *angustissima* (O. Mül.) Sim.
Aulacoseira granulata (Ehrenberg) Simonsen (células curtas)
Cyclotella sp.
Phytoflagellates
Chlamydophyceae
Eudorina elegans Ehr.
Spermatozopsis exsultans Korshikov
Chlamydomonas sp.
Phytoflagellates not identificaded 1
Phytoflagellates not identificaded 2
Chrysophyceae
Dinobryon divergens Imh.
Dinobryon sertularia Ehr.
Cryptophyceae
Chroomonas acuta Uterm.
Cryptomonas marssonii Skuja
Dinophyceae
Ceratium furcoides (Levander) Langhans
Peridinium sp.
Peridinium sp1
Euglenophyceae
Phacus horridus Pochm. *(Lepocinclis spinosa* N.S. BENNTT & Triemerrhn*)*
Phacus longicauda var. *longicauda* (Ehr.) Duj.
Trachelomonas hispida (Perty) Stein emend Defl. var. *coronata* Lemm.
Trachelomonas volvocinopsis Swir.
Xantoficeans
Xanthophyceae
Centritractus belenophorus Lemm.
Goniochloris contorta (Bourr.) Ettl
Goniochloris mutica (A. Braun) Fott

Goniochloris spinosa Pasch.
Isthmochloron gracile (Reins.) Skuja
Pseudostaurastrum limneticum (Bor.) Cout. e Rous.
Tetraplektron torsum (Skuja) Dedus. Sceg.
Tetraplektron tribulus (Pasch.) A R. Loeb.

APPENDIX F Variation of phytoplankton biomass

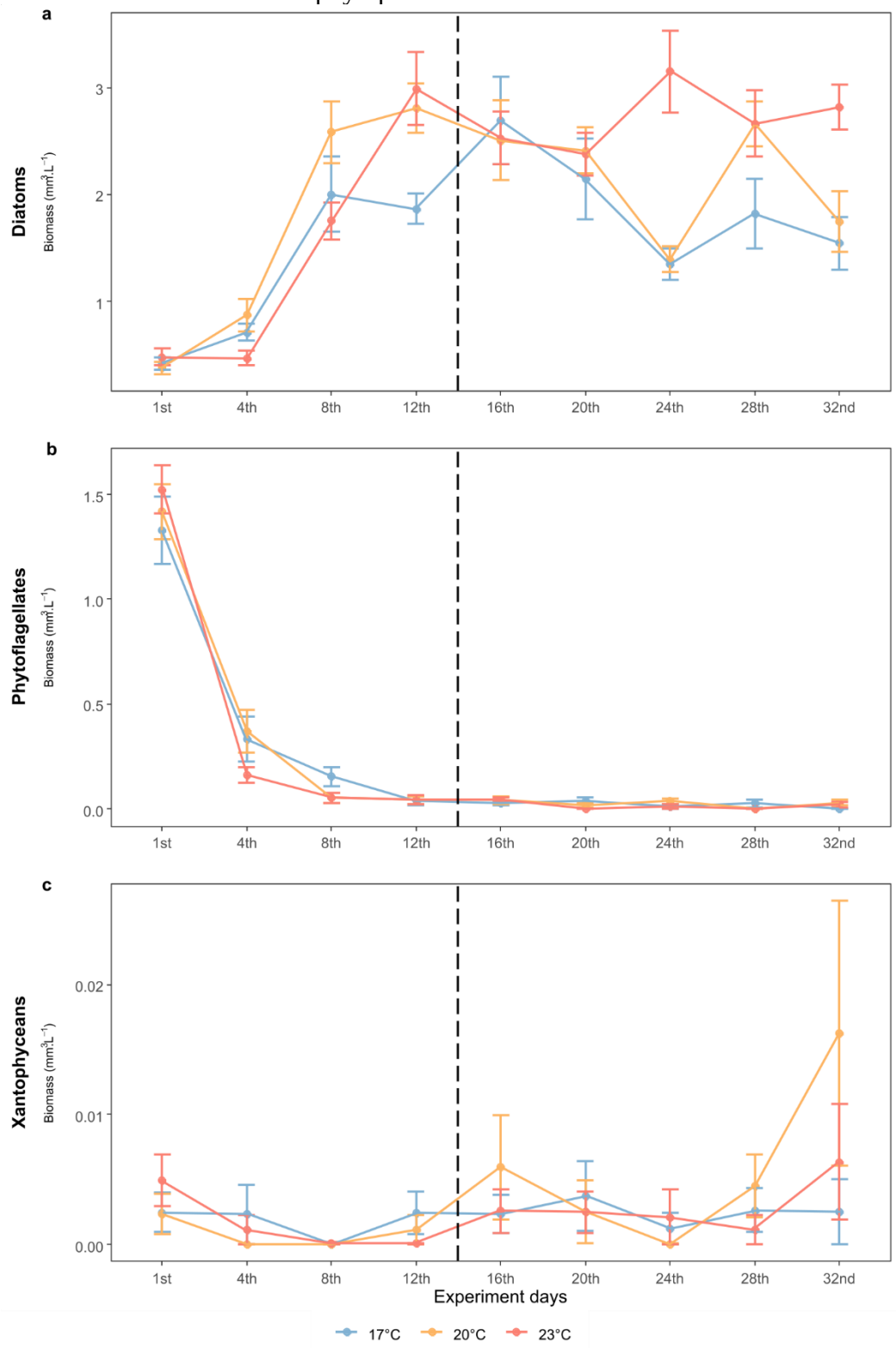
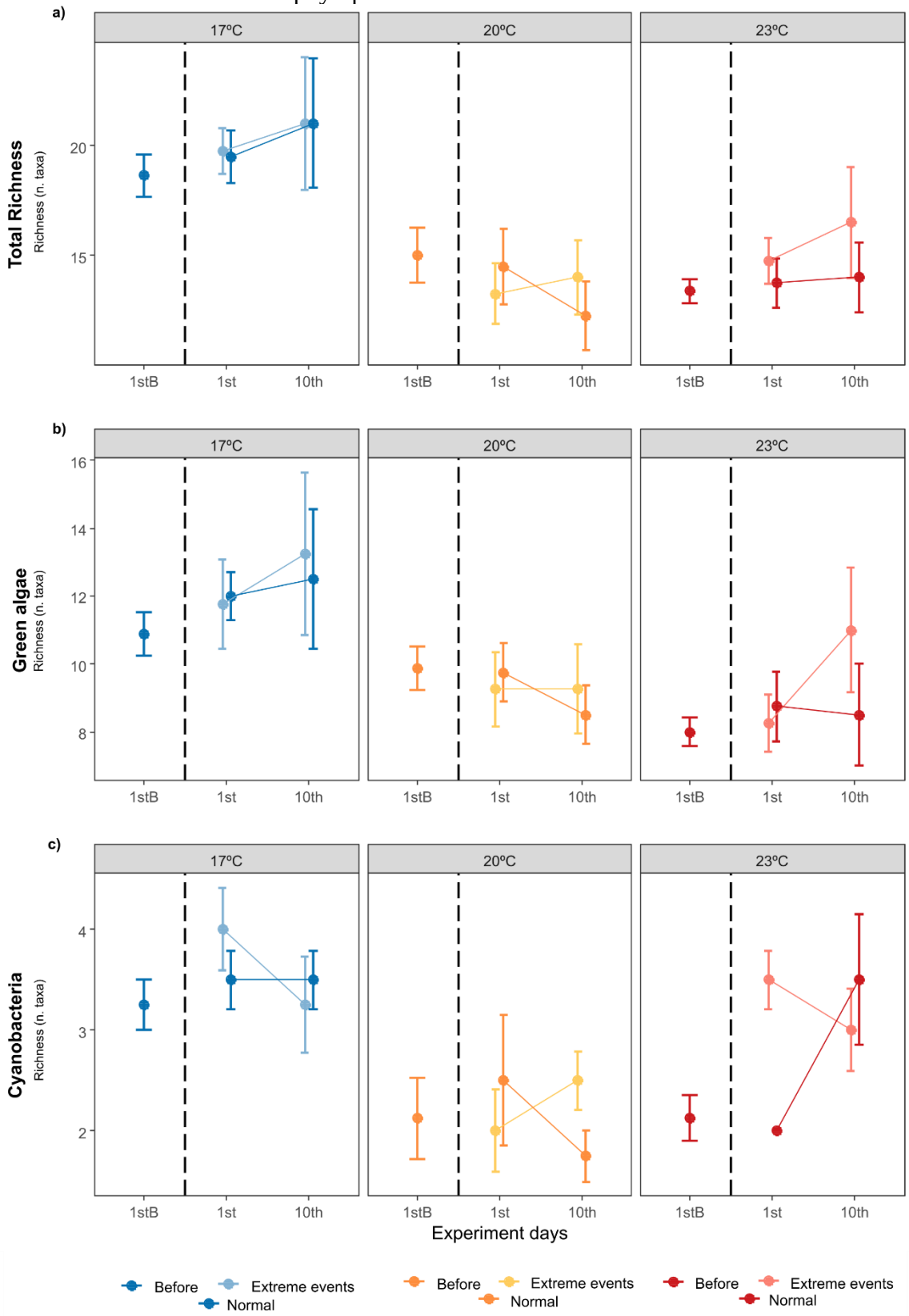
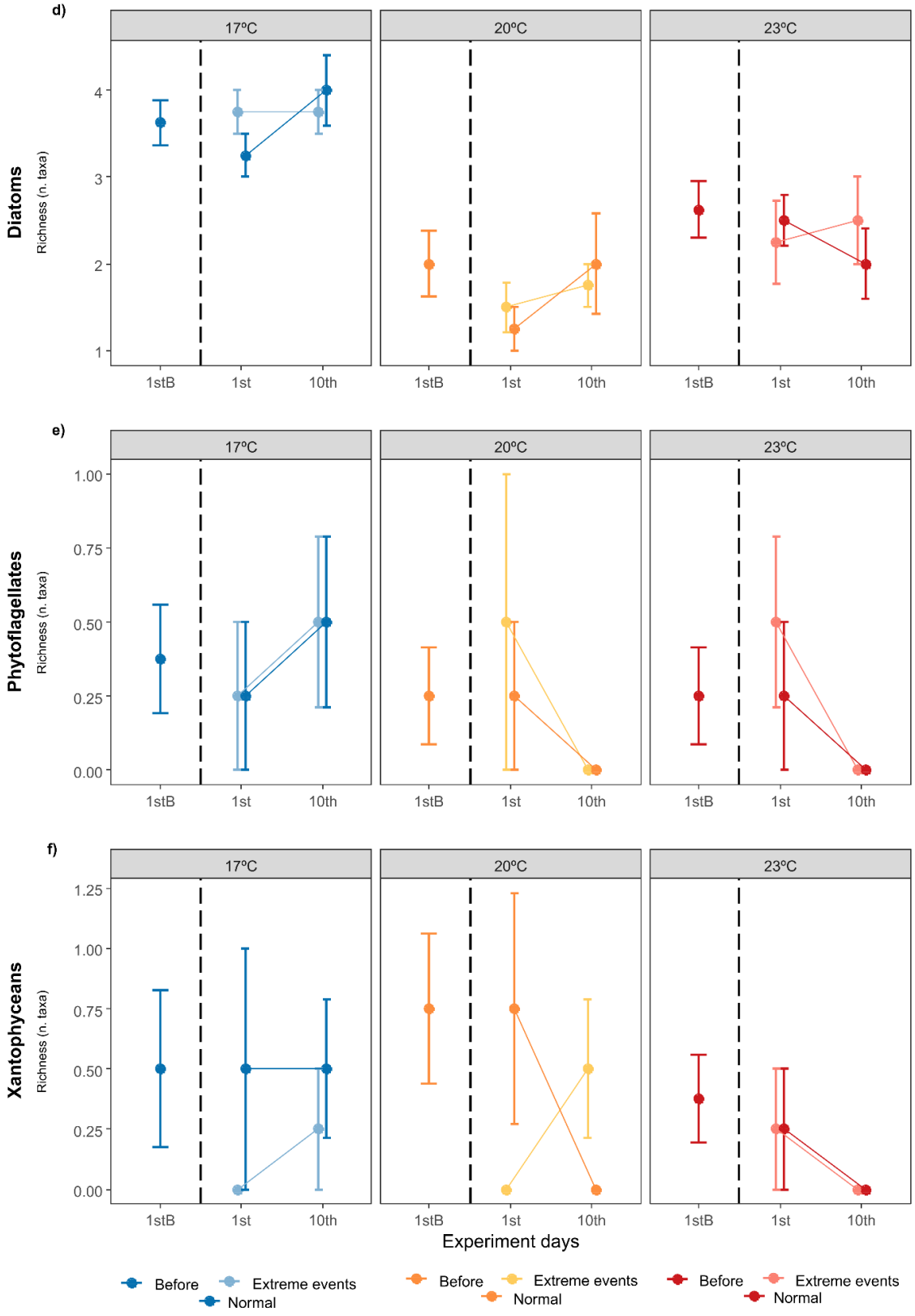


Figure S4: Variation of phytoplankton biomass with temperature through the 32 days of the experiment: diatoms (a), phytoflagellates (b) and xanthophyceans (c). The dotted line indicates that on the 16th day of the experiment the wished temperatures were reached. The central point denotes the mean value and, whiskers represent standard error for each temperature in each experiment day.

APPENDIX G Variation of phytoplankton taxonomic richness





Supplementary Figure 1: Variation of phytoplankton taxonomic richness with temperature through the 10 days of the experiment: total (a), green algae (b), cyanobacteria (c), diatoms (d), phytoflagellates (e), xanthophyceans (f). The dotted line indicates the occurrence of the extreme rainfall event (1stB). Light colors indicate communities subject to extreme rainfall and dark colors the undisturbed communities. The central point denotes the mean value and, whiskers represent standard error.

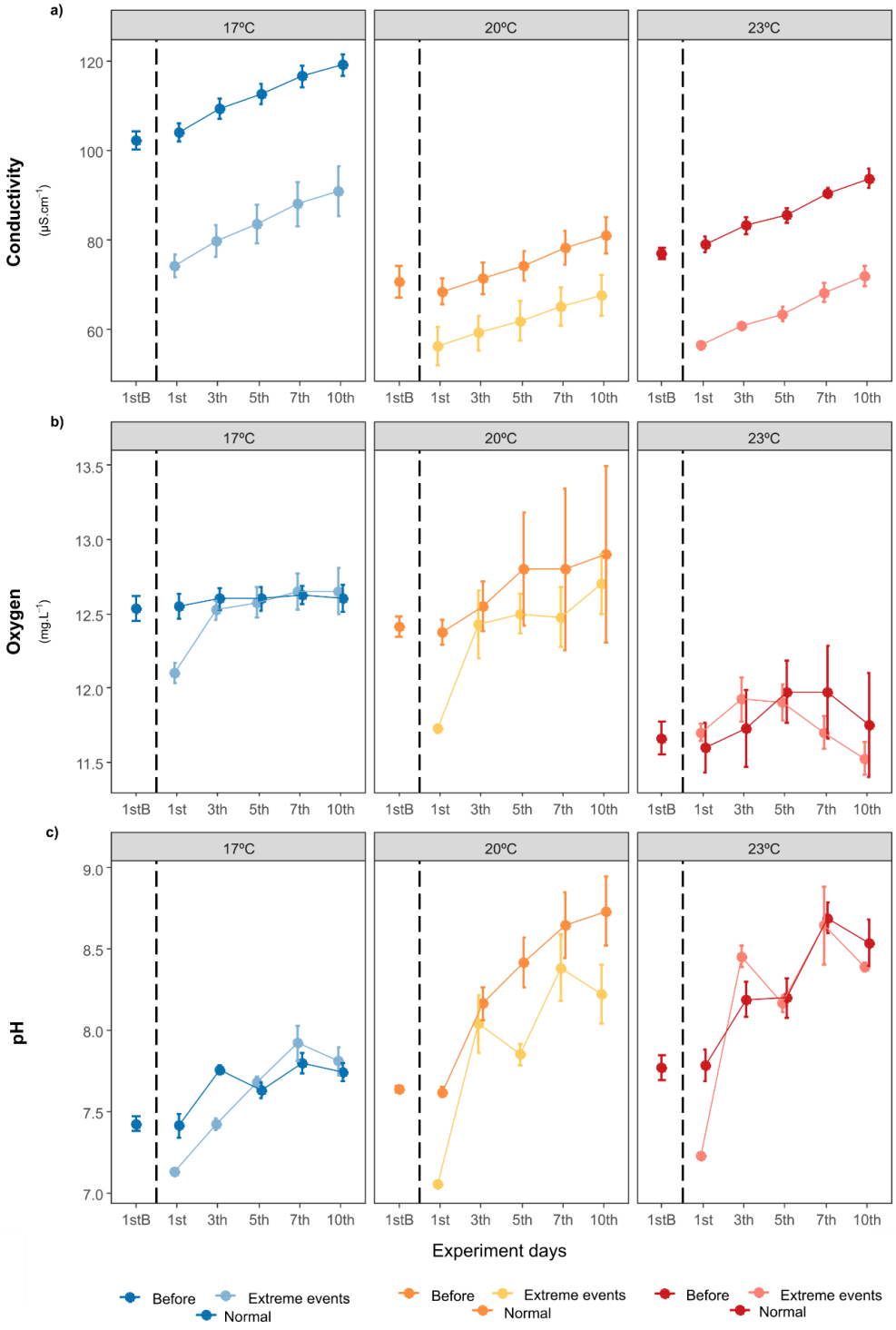
APPENDIX H List of phytoplankton taxa recorded in the experiment through the 10 days of the experiment.

Table S2: Phytoplankton taxa identified during the 10 days of the experiment.

Popular name/ Taxonomic Class /Taxon
Green algae
Chlorophyceae
Ankistrodesmus fusiformes Cor.
Ankistrodesmus gracilis (Reinsch) Korshikov
Coelastrum pulchrum Schm.
Coelastrum sphaericum Nägeli
Desmodesmus armatus (Chod.) Hegew.
Desmodesmus communis (Hegew.) Hegew
Desmodesmus magnus (Meyen) Tsarenko
Eutetramorus fottii (Hind.) Kom. Sensu Kom.
Kirchneriella contorta (Schmidle) Bohlin
Kirchneriella irregularis (G. M. Schm.) Kors.
Kirchneriella lunaris (Kirchner) Möbius
Monoraphidium arcuatum (Kors.) Hind.
Monoraphidium contortum (Thur.) Kom. - Legn.
Monoraphidium griffithii (Berk.) Kom.-Legn.
Pediastrum boryanum (Turp.) Menegh.
Pediastrum duplex Mey.
Quadrigula sp.
Scenedesmus acuminatus (Lagerh.) Chod.
Scenedesmus acunae Com.
Scenedesmus acutus Meyen
Scenedesmus alternans Reins.
Schroederia setigera (Schröd.) Lemmerm.
Selenastrum bibraianum Reinsch
Tetraëdron minutum (A.Braun) Hansgirg
Chlorophyceae unicelular not identificated
Trebouxiophyceae
Botryococcus braunii Kütz.
Closteriopsis scolia A.Comas
Crucigenia tetrapedia (Kirch.) W. e G.S. West
Dictyosphaerium pulchellum Wood
Nephrocytium sp.
Oocystis sp.
Zygnematophyceae
Closterium lineatum Ehr. ex Ralfs
Closterium incurvum Ehr. ex Ralfs
Cosmarium sp.
Mougeotia sp.
Staurastrum sp.
Staurastrum sp1

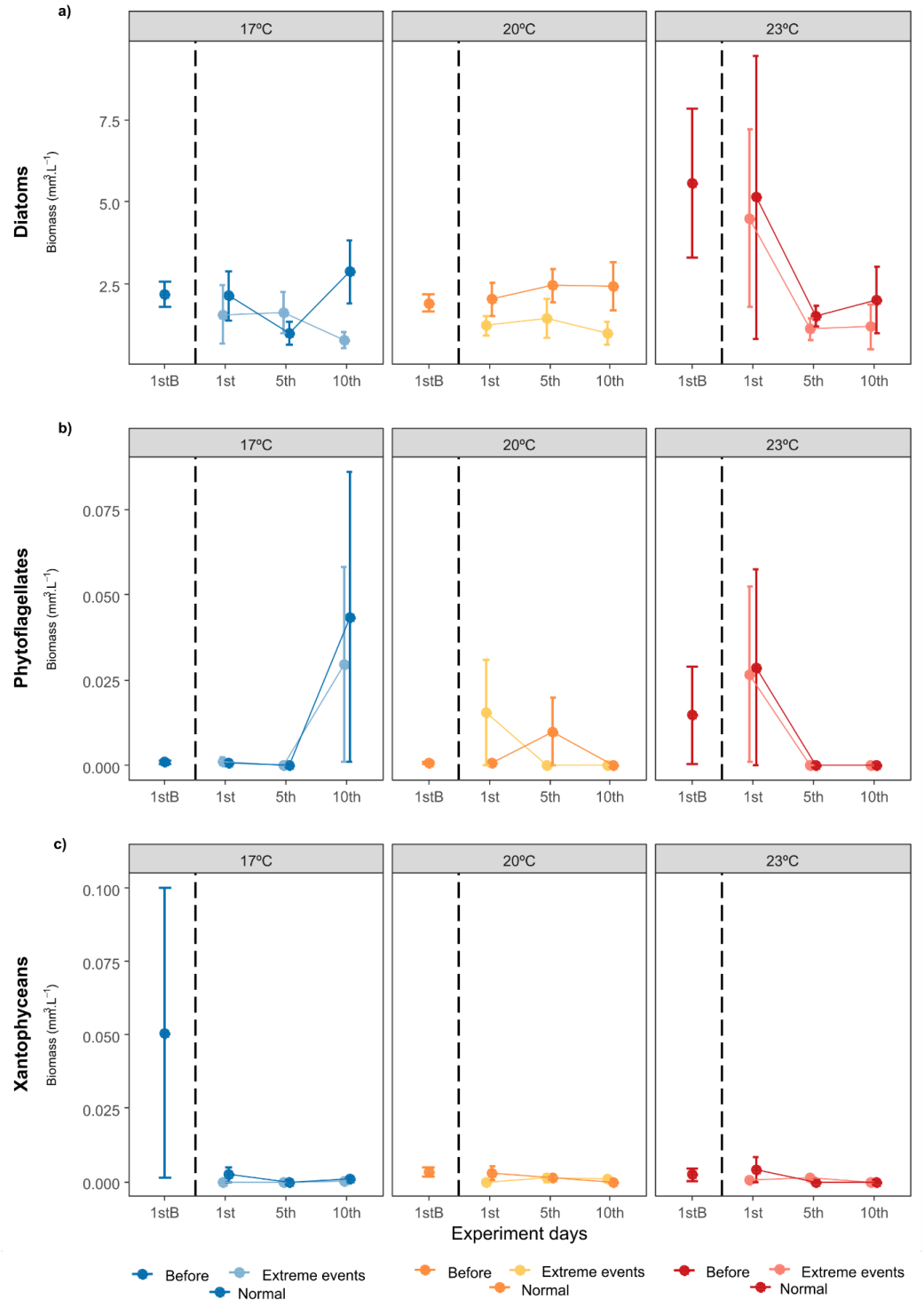
Staurodesmus dejectus (Bréb.) Teil.
Staurodesmus glaber (Ralfs) Teiling
Staurodesmus mamilatus (Lagerh.) Teil.
Staurodesmus triangularis (Lagerh.) Teil.
Cyanobacteria
Cyanobacteria
Aphanizomenon gracile Lemm.
Aphanocapsa delicatissima W. et G. S. West
Aphanocapsa elachista W. e G. S. West
Aphanocapsa holsatica (Lemm.) Cronb. & Kom.
Aphanocapsa sp.
Chroococcus minutus (Kütz.) Näg.
Cylindrospermopsis raciborskii (W.) Seen. & Sub. Rajú
Dolichospermum circinale (Rabenhorst ex Bornet & Flahault) P.Wacklin, L.Hoffmann & J.Komárek
Dolichospermum planctonicum (Brunnthaler) Wacklin, L.Hoffmann & Komárek
Microcystis aeruginosa Kütz.
Oscillatoria sp
Phormidium sp
Planktolyngbia limninetica (Lemm.) Kom.-Legn. e Cronb.
Pseudanabaena limnetica (Lemm.) Kom.
Snowella atomus Kom. & Hind
Diatoms
Bacillariophyceae
Achnanthidium minutissimum (Kütz.) Czarn.
Eunotia sp.
Fragilaria sp.
Navicula sp.
Nitzschia palea (Kütz.) W. Sm.
Coscinodiscophyceae
Aulacoseira ambigua (Grun.) Sim. var. *ambigua*
Aulacoseira granulata (Ehr.) Sim. var. *angustissima* (O. Müller) Sim.
Aulacoseira granulata (Ehr.) Sim. var. *granulata*
Phytoflagellates
Cryptophyceae
Cryptomonas marssonii Skuja
Euglenophyceae
Trachelomonas volvocinopsis Swir.
Dinophyceae
Peridinium sp.
Xantoficeans
Xanthophyceae
Goniochloris mutica (A.Braun) Fott
Goniochloris spinosa Pascher
Isthmochloron gracile (Reins.) Skuja
Tetraplektron torsum (W.B.Turner)
Pseudostaurastrum limneticum (Bor.) Cout. e Rous.

APPENDIX I Variation of abiotic variables



Supplementary Figure 2: Variation of abiotic variables in the three temperature treatments (17 °C, 20 °C, and 23 °C) through the 10 days of the experiment. The dotted line indicates the occurrence of the extreme rainfall event (1stB). Light colors indicate communities subject to extreme rainfall and dark colors the undisturbed communities. The central point denotes the mean value and, whiskers represent standard error.

APPENDIX J. Variation of phytoplankton biomass



Supplementary Figure 3. Variation of phytoplankton biomass with temperature through the 10 days of the experiment: diatoms (a), phytoflagellates (b), xanthophyceans (c). The dotted line indicates the environments before the period of extreme rainfall event (1stB) of the period after the disturbance. Light colors indicate environments with the effect of extreme rainfall and dark colors without the effect. The central point denotes the mean value and, whiskers represent standard error.

ANNEX A - Research papers accepted or published during the doctoral development period that contributed to the execution of this thesis

Dunck, B., M. G. Junqueira, M. V. da Silva, and others. 2018. Periphytic and planktonic algae records Dunck, B., M. G. Junqueira, M. V. da Silva, A. Pineda, A. C. M. de Paula, B. F. Zanco, **G. A. Moresco**, P. Iatskiu, J. C. Bortolini, Y. R. De Souza, S. Train, L. C. Rodrigues, S. Jati, & L. Rodrigues, 2018. Periphytic and planktonic algae records from the upper Paraná river fl oodplain , Brazil : an update. Hoehnea 45: 560–590.

Jati, S., J. C. Bortolini, **G. A. Moresco**, A. C. M. de Paula, L. C. Rodrigues, P. Iatskiu, A. Pineda, B. F. Zanco, M. V. da Silva, & Y. R. Souza, 2017. Phytoplankton community in the last undammed stretch of the Paraná River: considerations on the distance from the dam. Acta Limnologica Brasiliensia 29: e112.

Lansac-Tôha, F. M., J. Heino, B. A. Quirino, **G. A. Moresco,** O. E. P. Zapata, B. R. Meira, L. C. Rodrigues, S. Jati, F. A. Lansac-Tôha, & L. F. M. Velho, 2019. Differently dispersing organism groups show contrasting beta diversity patterns in a dammed subtropical river basin. Science of The Total Environment Elsevier B.V 691: 1271–1281,

Moresco, G. A., J. C. Bortolini, J. D. Dias, A. Pineda, S. Jati, & L. C. Rodrigues, 2017. Drivers of phytoplankton richness and diversity components in Neotropical floodplain lakes, from small to large spatial scales. Hydrobiologia 799: 203–215.

Moresco, G. A., J. C. Bortolini, L. C. Rodrigues, S. Jati, & L. F. Machado Velho, 2020. A functional deconstructive approach to mixotrophic phytoplankton responds better to local, regional and biogeographic predictors than species. Austral Ecology 45: 249–263.

Pineda, A., P. Iatskiu, S. Jati, A. C. M. Paula, B. F. Zanco, C. C. Bonecker, **G. A. Moresco**, L. A. Ortega, Y. R. Souza, & L. C. Rodrigues, 2020. Damming reduced the functional richness and caused the shift to a new functional state of the phytoplankton in a subtropical region. Hydrobiologia

Pineda, A., **G. A. Moresco**, A. Caroline, M. De Paula, L. M. Nogueira, P. Iatskiu, Y. R. De Souza, L. M. Reis, & L. C. Rodrigues, 2017. Rivers affect the biovolume and functional traits of phytoplankton in floodplain lakes. Acta Limnologica Brasiliensia 29: e113.

Ruaro, R., E. O. Conceição, J. C. Silva, E. G. Cafofo, M. A. Angulo-Valencia, T. Mantovano, A. Pineda, A. C. M. de Paula, B. F. Zanco, E. M. Capparros, **G. A. Moresco**, I. J. de Oliveira, J. L. Antiqueira, J. Ernandes-Silva, J. V. F. da Silva, J. R. P. Adelino, J. A. dos Santos, M. J. M. Ganassin, M. S. Iquematsu, G. O. Landgraf, P. Lemes, F. A. S. Cassemiro, V. F. Batista-Silva, J. A. F. Diniz-Filho, T. F. Rangel, A. A. Agostinho, & D. Bailly, 2019. Climate change will decrease the range of a keystone fish species in La Plata River Basin, South America.

Moresco, G.A.. J.D. Dias, L.C. Lamanna, C. Baladán, L. C. Rodrigues, M. Meerhoff. Positive feedback between warming and cyanobacteria blooms. Nature Climate Change (*in press*).