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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
Coorientadora: Prof.ª Dr.ª Juliana Déo Dias
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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 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. Assim, esta tese é composta por dois segmentos. Para o primeiro segmento, desenvolveu-se um experimento para testar como o aumento das temperaturas influencia a diversidade fitoplanctônica e as emissões de CO_2 em ambientes eutróficos. Os resultados mostraram 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. Encontrou-se 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 segmento, conduziu-se 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. Constatou-se 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. Descobriu-se 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

283 such responses translated to ecosystem functions (e.g., biomass production, energy
284 transfer, and carbon cycle).
285
286 **Keywords:** Climate change, cyanobacteria blooms, eutrophication, global warming,
287 resource use efficiency, feedback.
288

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

388 gradient from mesotrophy to hypertrophy (Kruk et al., 2009; Pacheco et al., 2010). We
389 collected phytoplankton using a plankton net with a mesh size of 20 µm to remove most
390 zooplankton and added some non-filtered water to include smaller phytoplankton
391 species (< 20 µm). We used this strategy to maximize the sampling of less abundant
392 species and guarantee that most taxonomic groups were present in the experimental
393 units. The mean water temperature measured in the lakes during the sampling procedure
394 was 15 °C.

395 We distributed 0.5 L of concentrated inoculum for each of the 30 experimental
396 units and added 2.5 L of dechlorinated water enriched with a nutrient solution to reach a
397 final volume of 3 L. In the first day of the experiment, we found no significant
398 differences among the three treatments for phytoplankton taxonomic richness (F-value
399 $_{(2, 27)}$ = 1.031; p = 0.37) and biomass (F-value $_{(2, 27)}$ = 0.086; p = 0.918), showing the
400 homogeneous phytoplankton distribution among the treatments.

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

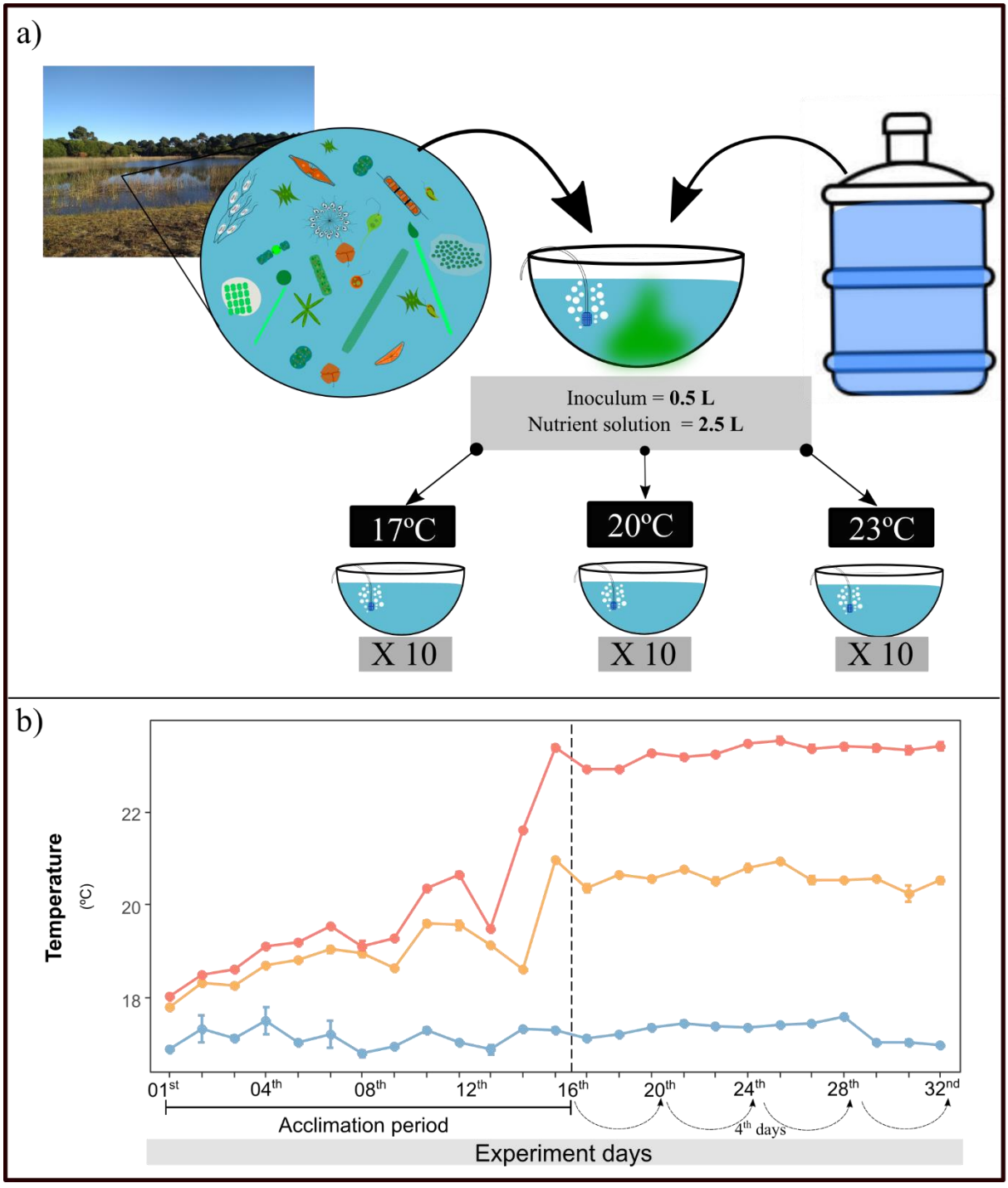


Figure 1: Experimental design. a) A concentrated phytoplankton community subtropical lakes were cultured in a nutrient solution respecting the Redfield ratio. Treatments: 17 °C, 20 °C and 23 °C, total n=30. b) Following an acclimation period, as from the 16th day of the experiment all treatments reached 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

443 fixed them immediately with acetic Lugol solution. We estimated phytoplankton density
444 following Utermöhl (1958) and Lund et al., (1958) and calculated density according to
445 APHA (2005). The biomass ($mm^3.L^{-1}$) was considered as biovolume, which was
446 estimated by multiplying the density of each taxon by its volume. We estimated the cell
447 volume by calculating the volume of the geometric shape that was the most similar to
448 each cell form (Sun & Liu, 2003). We also estimated the community resource use
449 efficiency (thereafter RUE), defined as the ratio between the phytoplankton biomass
450 production in Chl-a and TP, as a proxy for ecosystem productivity (Ptacnik et al., 2008;
451 Olli et al., 2015; Verbeek et al., 2018).
452
453 2.2.4 Data analyses
454
455 We used 1-way ANOVA to test for differences among the treatments in nutrient
456 concentration (TN, TP, SRP, NO_3, and NH_4) in each sampling occasion; in
457 phytoplankton richness and biomass on the first day of the experiment; and two-way
458 ANOVAs to test for differences in chlorophyll-a, RUE, and carbon fluxes among
459 treatments and time. When results were significant ($p < 0.05$), we used Tukey tests, to
460 verify the significant difference in mean among treatments and time. After the
461 temperature was stabilized in all treatments (as from the 16^{th} day), we evaluated the
462 effects of the temperatures and time on richness and biomass of phytoplankton groups
463 by fitting generalized linear models (GLM), using the distribution families that better
464 adjusted to the data: Poisson distribution for richness and Gaussian distribution for
465 biomass. We evaluated overdispersion and corrected them when necessary.
466 To evaluate the effects of temperature (three levels) and sampling time (five
467 levels) on the composition of phytoplankton (presence/absence) (after the 16^{th} day), we
468 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:

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

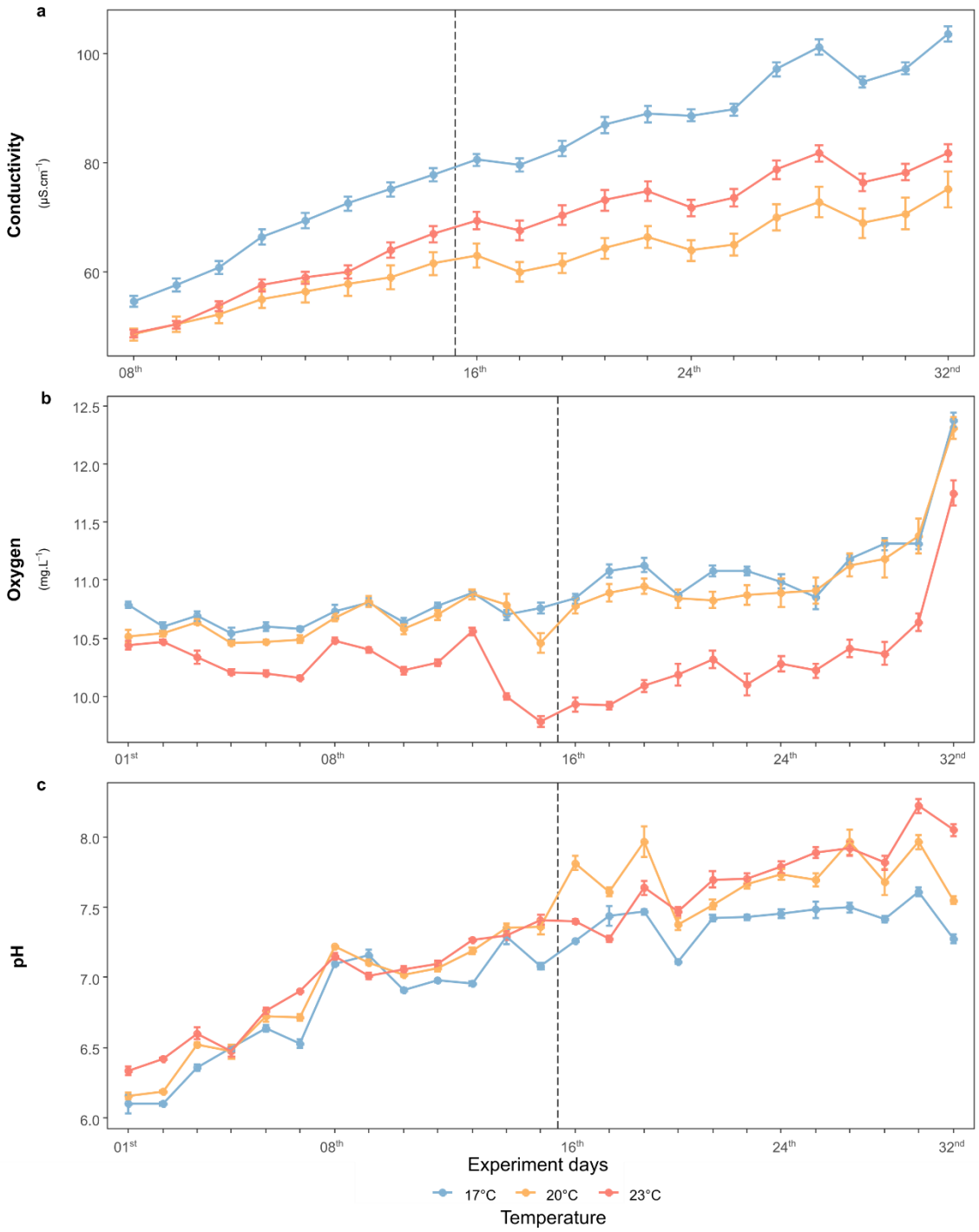
Where CO_2 flux is in $CO_2.mg^{-2}.d^{-1}$, S is the slope of the partial pressure of CO_2 in the function of time ($ppm.s^{-1}$), V is the camera volume (0.0019 m^3), A is the camera area (0.1589 m^2), P is the atmospheric pressure in KPa (102.5), F1 is the molecular weight of the gas ($44g.mol^{-1}$),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

494 ($8.31 J.mol^{-1}.K^{-1}$) and T is the air temperature in Kelvin. We selected values of the slope only
495 for those relationships with $R^2 \geq 0.7$. Positive values indicate CO_2 efflux (CO_2 liberation to
496 the atmosphere: predominance of net respiration) and negative values indicate CO_2 influx
497 (CO_2 retention in the aquatic environment: predominance of net photosynthesis).We ran all
498 analyses in software R version 3.3.2 (R Development Core Team, 2021), using packages
499 "vegan" (Oksanen et al., 2016) for PCoA, ANOVA, and PERMANOVA, and "MASS"
500 (Venables & Ripley, 2002) for GLMs.

501 **2.3 Results**

502

503 To electric conductivity, there was a gradual increase through time in all
504 treatments. The highest mean values were recorded for the treatment of 17ºC (81 $\mu S.cm^{-1}$)
505 and minors for the treatment of 20ºC (62.1 $\mu S.cm^{-1}$). The concentrations of dissolved
506 oxygen and pH values had little variation over the time of the experiment. However, the
507 warming promoted environments with lower average values of oxygen concentrations
508 (10.31 mg L^{-1}) and higher mean pH values (8.05) in relation to the lower temperature
509 treatment (10.95 mg L^{-1} e 6.1 respectively).



510

511 **Figure 2:** Variation of main limnological variables in the three treatments (17 °C, 20
512 °C and 23 °C) through the 32 days of the experiment. The dotted line indicates that on
513 the 16th day of the experiment the wished temperatures were reached. The central point
514 denotes the mean value and, whiskers represent standard error for each temperature in
515 each experiment day.

516

517 2.3.1 Phytoplankton community
518
519 We identified 125 taxa, including green algae (62 taxa), cyanobacteria (21),
520 diatoms (18), and phytoflagellates (16). *Scenedesmus* (Chlorophyceae) presented the
521 highest number of taxa (7). Mean phytoplankton richness presented little oscillation
522 through the duration of the experiment (Fig.S2). However, we registered a reduction in
523 species richness with increasing temperature for total phytoplankton (pseudo $R^2 = 0.16$;
524 $p = 0.001$) and also and within the main phytoplankton groups (Table 1, Supplementary
525 material Fig. S3). The highest variability in species composition occurred in the 20 °C
526 treatment and the lowest in the 17 °C treatment (Fig. 3). The separation detected in the
527 PCoA was confirmed by the PERMANOVA test, which showed significant changes in
528 phytoplankton composition with temperature ($R^2 = 0.54$; $p = 0.001$) and time ($R^2 =$
529 0.07; $p = 0.001$).
530 Cyanobacteria (as *Raphidiopsis raciborskii* (Wołoszyńska) Aguilera, Berrendero
531 Gómez, Kastovsky, Echenique & Salerno, before *Cylindrospermopsis raciborskii*), and
532 green algae (as *Desmodesmus magnus* (Meyen) Tsarenko and *Staurastrum* sp.) were the
533 groups that contributed to phytoplankton biomass the most through the experiment in
534 the three treatments compared to a more balanced community at the start of the
535 experiment. Supporting our expectations, total phytoplankton biomass responded
536 positively to warming (Pseudo $R^2 = 0.50$; $p < 0.001$) (Table 2, Fig.4, Supplementary
537 material Fig.S4).
538 This result was mainly driven by higher cyanobacteria growth at higher
539 temperatures (Pseudo $R^2 = 0.41$; $p < 0.001$). Considering the three treatments evaluated,
540 between the first and the last experiment day, we registered a 10-fold increase in the
541 mean biomass of green algae and cyanobacteria and the highest growth rates in the 20°
542 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).

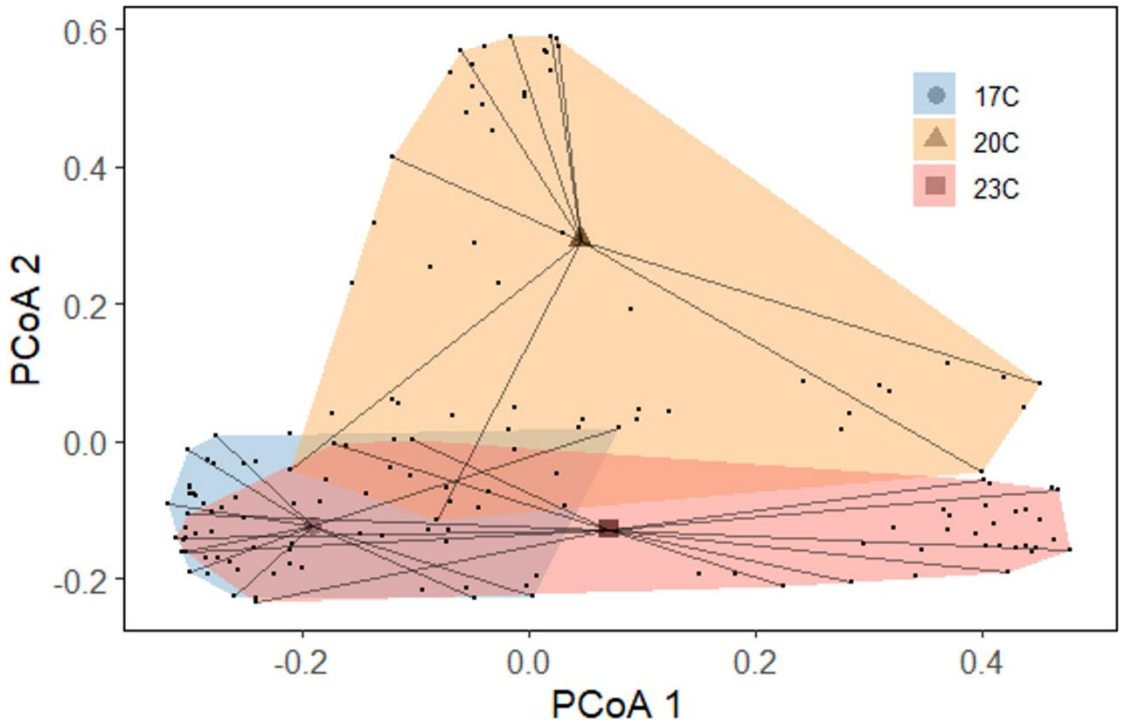
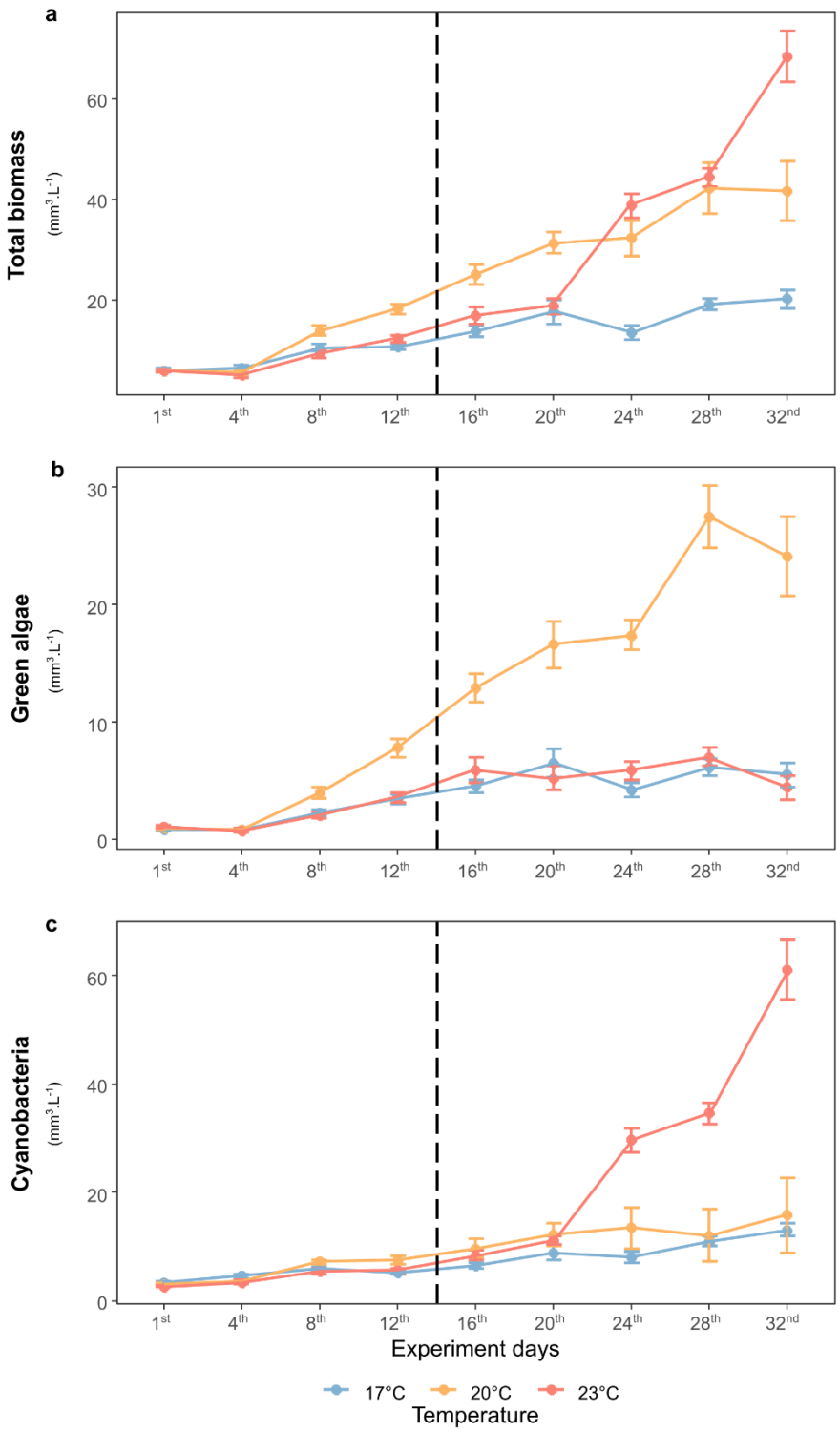


Figure 3. Principal coordinate analysis (PCoA), showing the variability in the composition of phytoplankton species from the 16th day of the experiment in the three treatments.

559 **Table 1** Generalized linear models, indicating regression estimate, standard errors (SE),
560 z-value, and P-values of models predicting, as from the 16th day, the taxonomic
561 richness of total phytoplankton, green algae, and cyanobacteria. Significant results are
562 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**	

563



564

565 **Figure 4**: Variation of phytoplankton biomass (as biovolume) with temperature through
566 the 32 days of the experiment: total (a), green algae (b), cyanobacteria (c). The dotted
567 line indicates that on the 16th day of the experiment the final temperatures were reached.
568 The central point denotes the mean value and, whiskers represent standard error for each
569 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**	

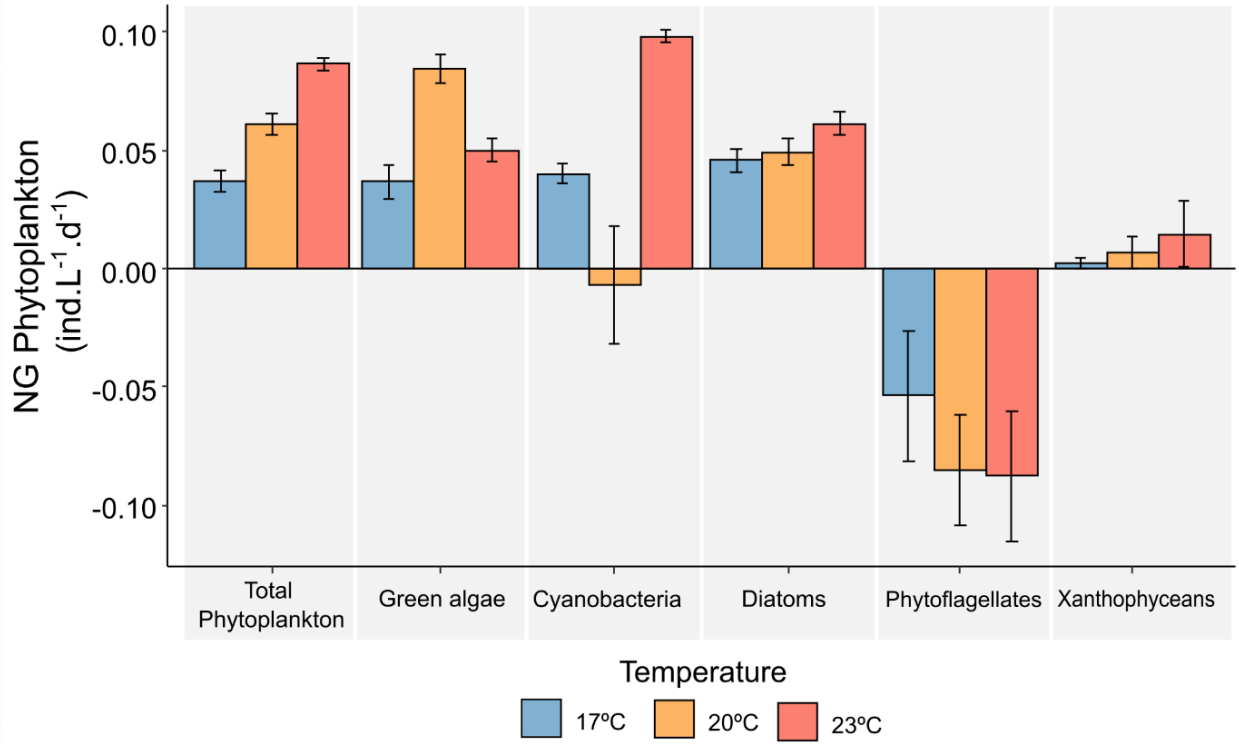
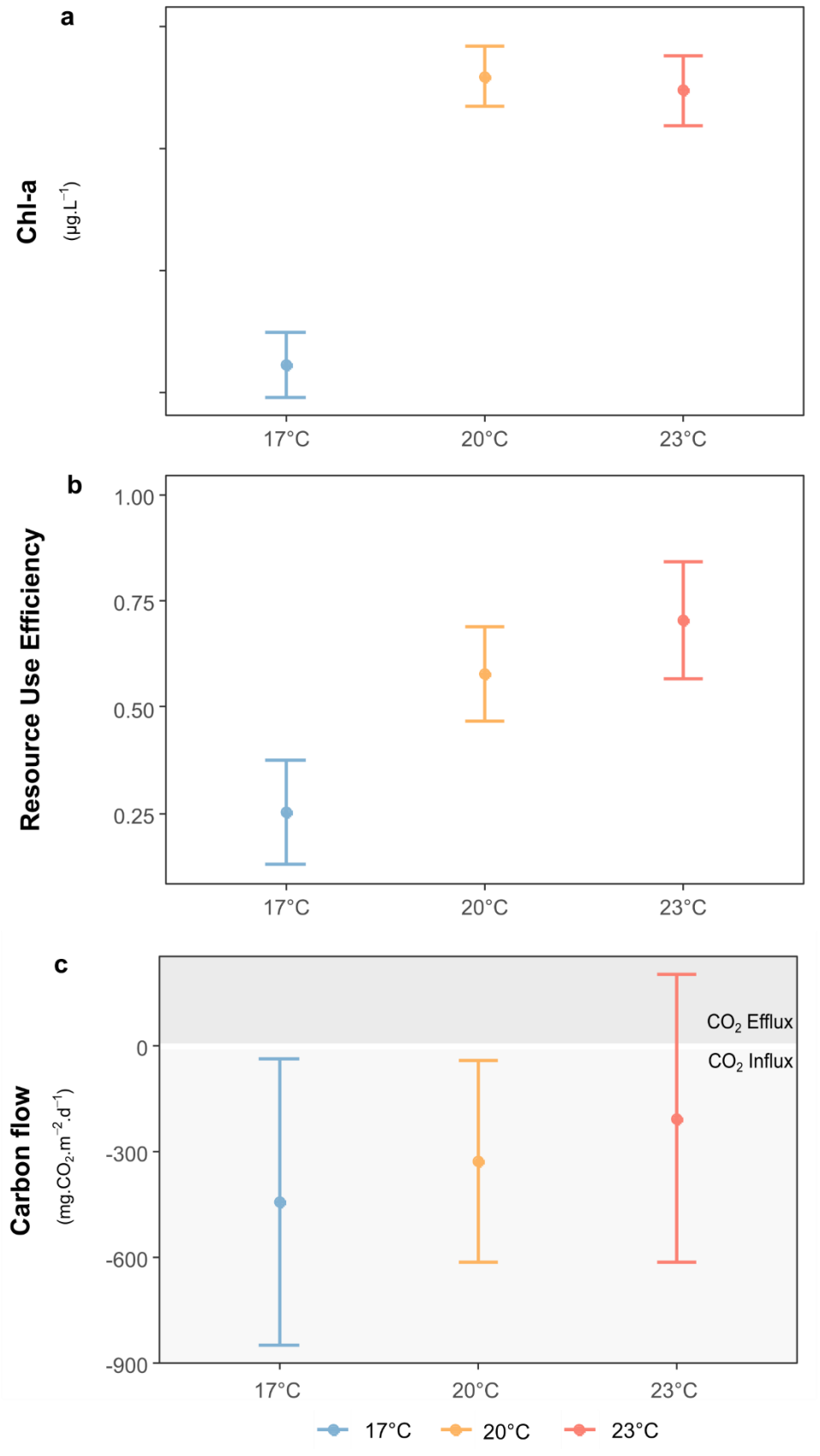


Figure 5. Net growth rate (NG) of phytoplankton density of total and main phytoplankton groups under the three temperature treatments. The bars plot denotes mean and, whiskers represent standard error.

587


a
Chl-a
(µg.L⁻¹)
17°C
20°C
23°C
b
Resource Use Efficiency
1.00
0.75
0.50
0.25
17°C
20°C
23°C
c
Carbon flow
(mg.CO2.m⁻².d⁻¹)
CO2 Efflux
CO2 Influx
0
-300
-600
-900
17°C
20°C
23°C
17°C
20°C
23°C

588

589 **Figure 6.** Different ecosystem responses to warming: variation of chlorophyll-a
590 concentration (Chl-a - log-transformed) (a), resource use efficiency (log-transformed)
591 (b), and carbon flow (c). Positive values of carbon flow indicate net CO_2 emissions
592 while negative values indicate net CO_2 sequestration by the phytoplankton communities
593 in three treatments. The central point denotes the mean value and, whiskers represent
594 standard error for each temperature in each experiment day.

595 2.3.2 CO_2 fluxes
596
597 Although we registered high CO_2 influx values in the experiment (i.e. CO_2
598 retention in the aquatic environment, due to net photosynthesis), the ecosystem
599 metabolic balance changed with temperature increase. The magnitude of influx
600 decreased with warming, almost transforming the microcosms in sources of CO_2 to the
601 atmosphere (i.e., CO_2 liberation to the atmosphere, net respiration). These results
602 support our hypothesis of positive feedback between blooms and warming, through an
603 increase in CO_2 efflux to the atmosphere.

604 The highest daily CO_2 efflux (580.24 $CO_2.mg^{-2}.\ d^{-1}$) occurred on the 10th day of
605 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
607 found significant differences in CO_2 concentration between the temperature treatments
608 (F-value $_{(2,\ 80)}$ = 4.54; P = 0.013), the differences being the largest between 17ºC and
609 23ºC (Tukey test p = 0.027). The highest CO_2 mean values (i.e., the lowest influx, -184
610 $CO_2.mg^{-2}.\ d^{-1}$) occurred in the 23ºC treatment while the lowest (i.e., the highest influx, -
611 445 $CO_2.mg^{-2}\ d^{-1}$) was registered in the 17ºC treatment (Fig.6c).

612 **2.4 Discussion**
613
614 Climate warming and eutrophication are two major challenges for lacustrine
615 management worldwide (Carter & Schindler, 2012; Moe et al., 2016). Our results show

616 experimentally that, under future scenarios of climate warming, the phytoplankton
617 community composition can respond strongly, affecting ecosystem functions such as
618 biomass production, resource use efficiency, carbon flux balance.

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

626 Besides composition changes, phytoplankton biomass increased with increasing
627 temperature, especially green algae and cyanobacteria. In line with Verbeek et al.
628 (2018) showing that climate change favors species with wider temperature ranges or
629 higher temperature optima, such as green algae and cyanobacteria species in an
630 experimental study with similar time duration to ours. Chlorophyta and Cyanobacteria
631 are both considered fast-growing r-strategists that can endure higher temperatures and
632 are favored in changing environments by their fast turnover, thus being able to develop
633 blooms in a very short period.

634 Some studies have shown that green algae also have their optimal growth at high
635 temperatures (De Senerpont Domis et al., 2007; Low-Décarie et al., 2011; Lürling et al.,
636 2013). The latter experimentally found that some chlorophyceans (some of them also
637 registered in this study, as *Desmodesmus* spp.) presented their optimal growth at high
638 temperatures (approximately 20 °C), with no significant difference in the optimal
639 growth temperature between cyanobacteria and green algae. Our results indicate that a
640 community approach is needed to detect changes in dominance patterns. However, due

641 to the competitive advantage of Cyanobacteria to rapidly sequester nutrients, they can
642 grow faster and outcompete other algae that are less efficient in nutrients uptake in the
643 warming environment (Rasconi et al., 2017).

644 *Raphidiopsis raciborskii* (CyanoHABs), of a tropical origin but invasive over a
645 wide distribution in temperate zones (Wiedner et al., 2007), was the main responsible
646 for the highest biomass of cyanobacteria in the highest temperature conditions. This
647 result corroborates other studies that showed that species of this group are favored with
648 warming (Kosten et al., 2011; Bonilla et al., 2016; Rasconi et al., 2017; Huisman et al.,
649 2018; Gray et al., 2019; Ho et al., 2019). This species may also use limiting resources
650 more efficiently than other cyanobacteria species due to its high affinity for and high
651 storage capacity of P (Isvánovics et al., 2000; Wu et al., 2012). To maintain several
652 ecosystem functions multiple species with different traits are necessary (Hector &
653 Bagchi, 2007; Mouillot et al., 2013a; Litchman et al., 2015). Thus, a decrease in
654 diversity (as a consequence of the dominance of traits) has been associated with a
655 general decrease in ecosystem functionality (Gamfeldt et al., 2008, 2013).

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

661 Ecologists have obtained inconsistent conclusions when analyzing the influence
662 of phytoplankton diversity on RUE of phytoplankton, as this relationship depends on
663 the composition of the community and species-specific RUE (Tian et al., 2017; Verbeek
664 et al., 2018). Previous studies supported the existence of a positive relationship between
665 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-

691 Durocher et al., 2011; Rasconi et al., 2017); observational studies (Paerl & Huisman,
692 2008; Kosten et al., 2012; Beaulieu et al., 2013); and paleolimnological studies (Pal et
693 al., 2015). On the other hand, studies show that the correlation of cyanobacteria biomass
694 and temperature in lakes is still unclear, depending on the lake trophic state or trophic
695 interactions (e.g. resistance to grazing) (Lürling et al., 2013; Kraemer et al., 2017;
696 Almanza et al., 2019; Gerhard et al., 2019). We designed our experiment to focus on
697 increasing mean temperature as a driver of changes in the phytoplankton community,
698 providing non-limiting nutrients and no predation conditions, to avoid indirect or
699 interacting effects of warming. Therefore, we can assume that warming directly drove
700 cyanobacteria proliferation.

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

713 Besides, after the collapse of cyanobacteria, phytoplankton loss processes, as
714 sedimentation and decomposition, are intensified. Many studies have suggested that
715 organic carbon in sediments is mineralized into gas emissions of greenhouse gas, such

716 as CO_2 (Gudasz et al., 2010; Bastviken et al., 2011; Marotta et al., 2014). Furthermore,
717 we found that the warmest treatment caused a decrease in dissolved oxygen
718 concentration. This may be related to lower oxygen solubility and/or to an increase in
719 the metabolic rates of the organisms at higher temperatures (Diaz & Breitburg, 2009). A
720 lower dissolved oxygen concentration may, in turn, promote a stronger release of
721 methane and nitrous oxide, further promoting warming. Besides, intensifies the
722 respiration of planktonic organisms (Yvon-Durocher et al., 2015) and decomposition
723 rates (Geraldes et al., 2012) both of which are processes that involve oxygen
724 consumption.

725 Experiments studies are a fundamental tool to predict how scenarios of climate
726 change will affect processes at the ecosystem levels and allow us to make predictions
727 about how organisms may respond to changing environments (Stewart et al., 2013),
728 despite their obvious limitations (Benton et al., 2007). In our case, the microcosms did
729 not mimic a catchment context and, consequently, received no terrestrial organic
730 carbon. Benthic processes and even pelagic trophic relations were purposely excluded.
731 We used a highly diverse initial community and isolated it from potentially confounding
732 factors. Thus, we were able to capture a pure pelagic response to warming, exclusive to
733 the phytoplankton community, and how such responses translated to ecosystem
734 functions (e.g., biomass production, energy transfer, and carbon cycle). Warming may
735 aggravate the negative effects of eutrophication through the enhancement of
736 cyanobacteria. Here, we also found experimental evidence of positive feedback between
737 eutrophication symptoms (blooms) and warming, via higher CO_2 emission rates in
738 cyanobacteria dominated warmer systems (Fig.7).
739

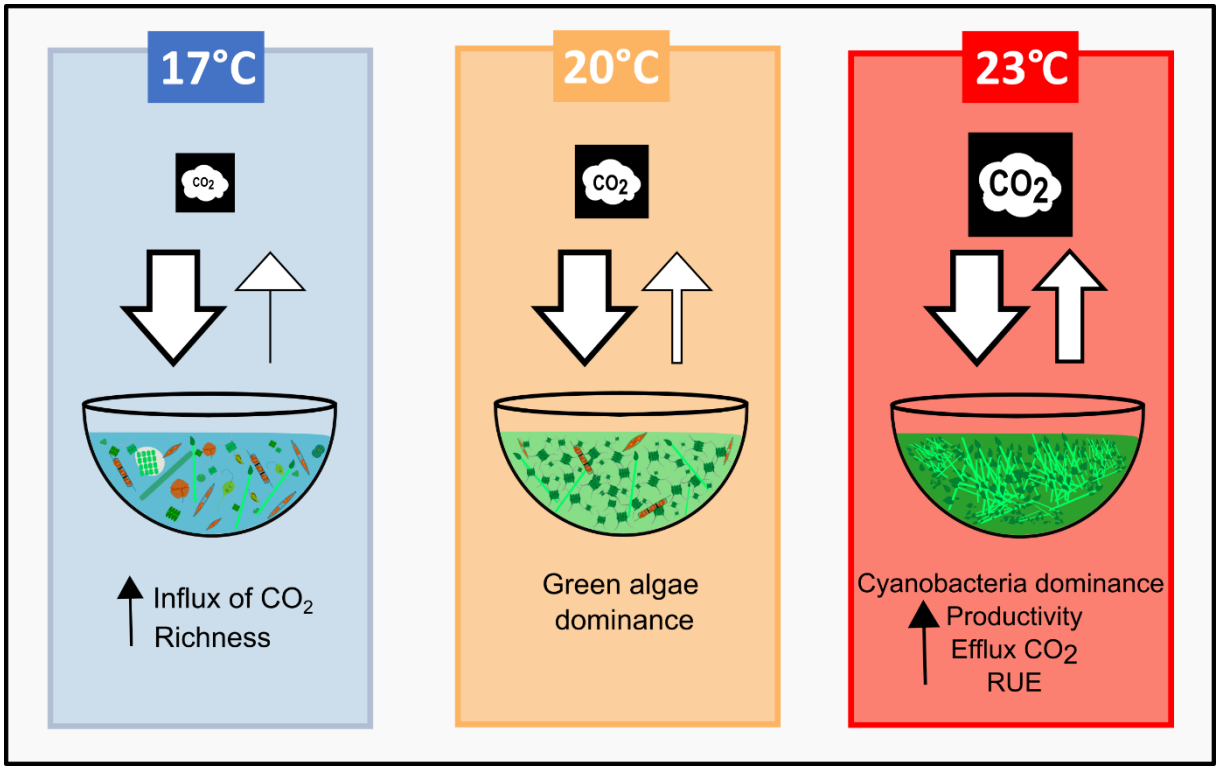


Fig.7. Under climate warming scenarios, ecosystem functions are affected. For example, with warming, environments become more productive, due to eutrophication with the dominance of cyanobacteria (less diversity), and greater resource use efficiency (RUE). This scenario gradually changes the ecosystem's 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).

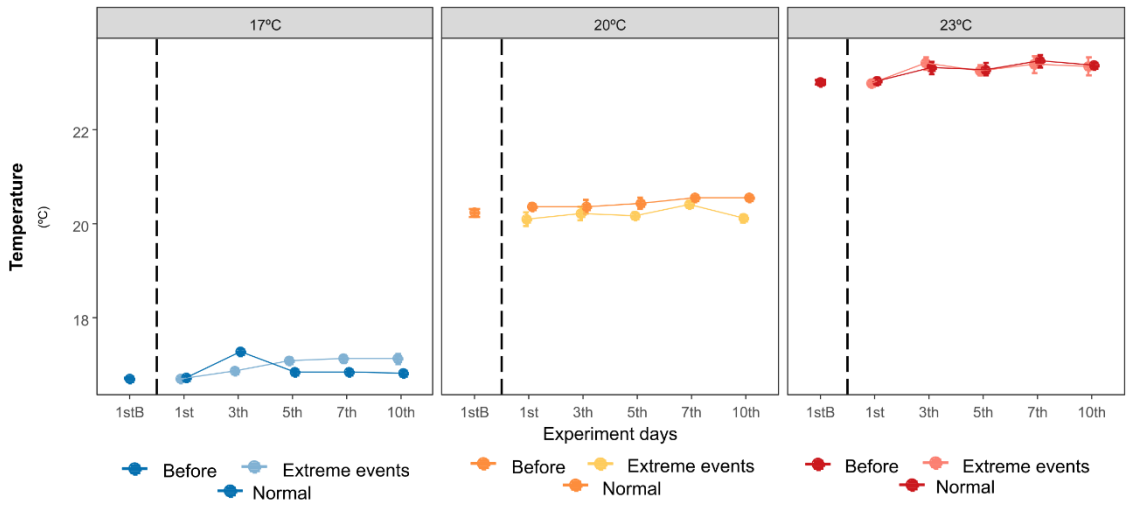


Figure 1. Mean daily temperature values in each temperature treatment. Light colors correspond to the extreme rainfall treatment and dark colors non disturbed treatments. The

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).

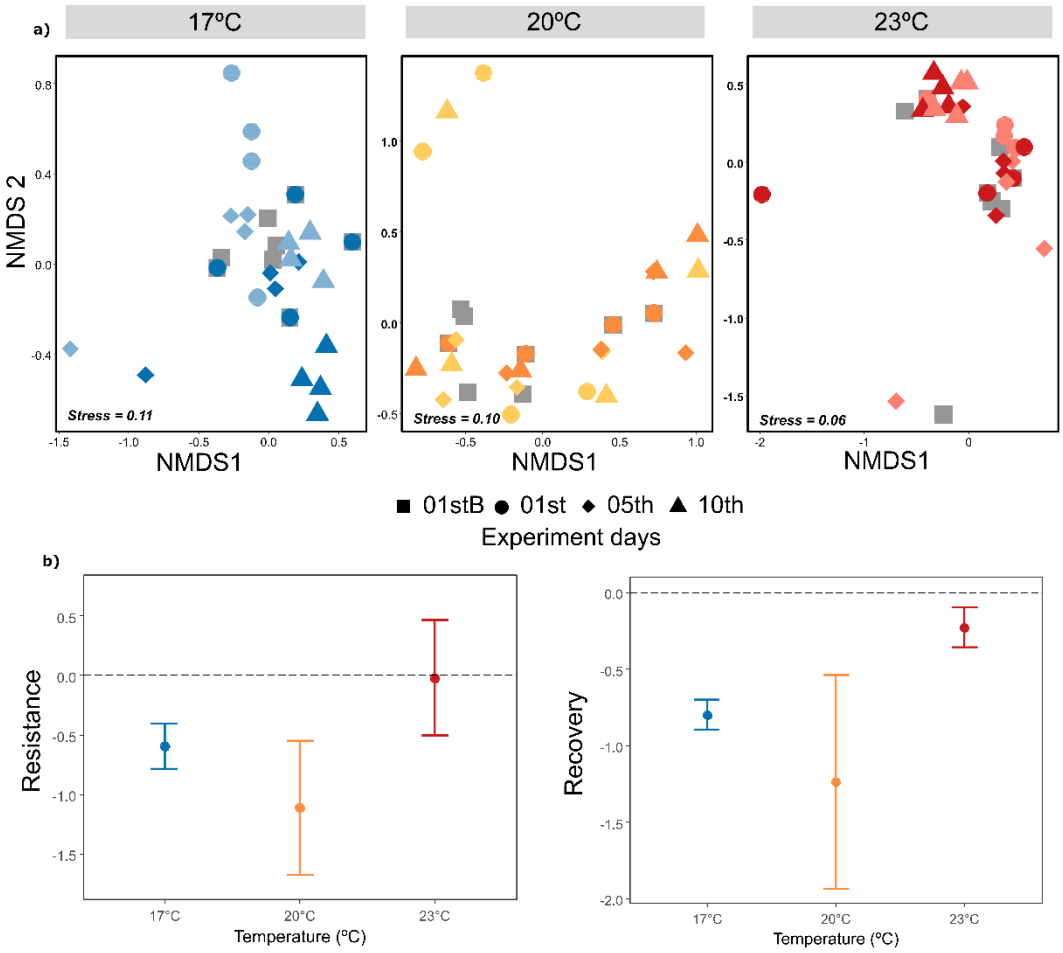
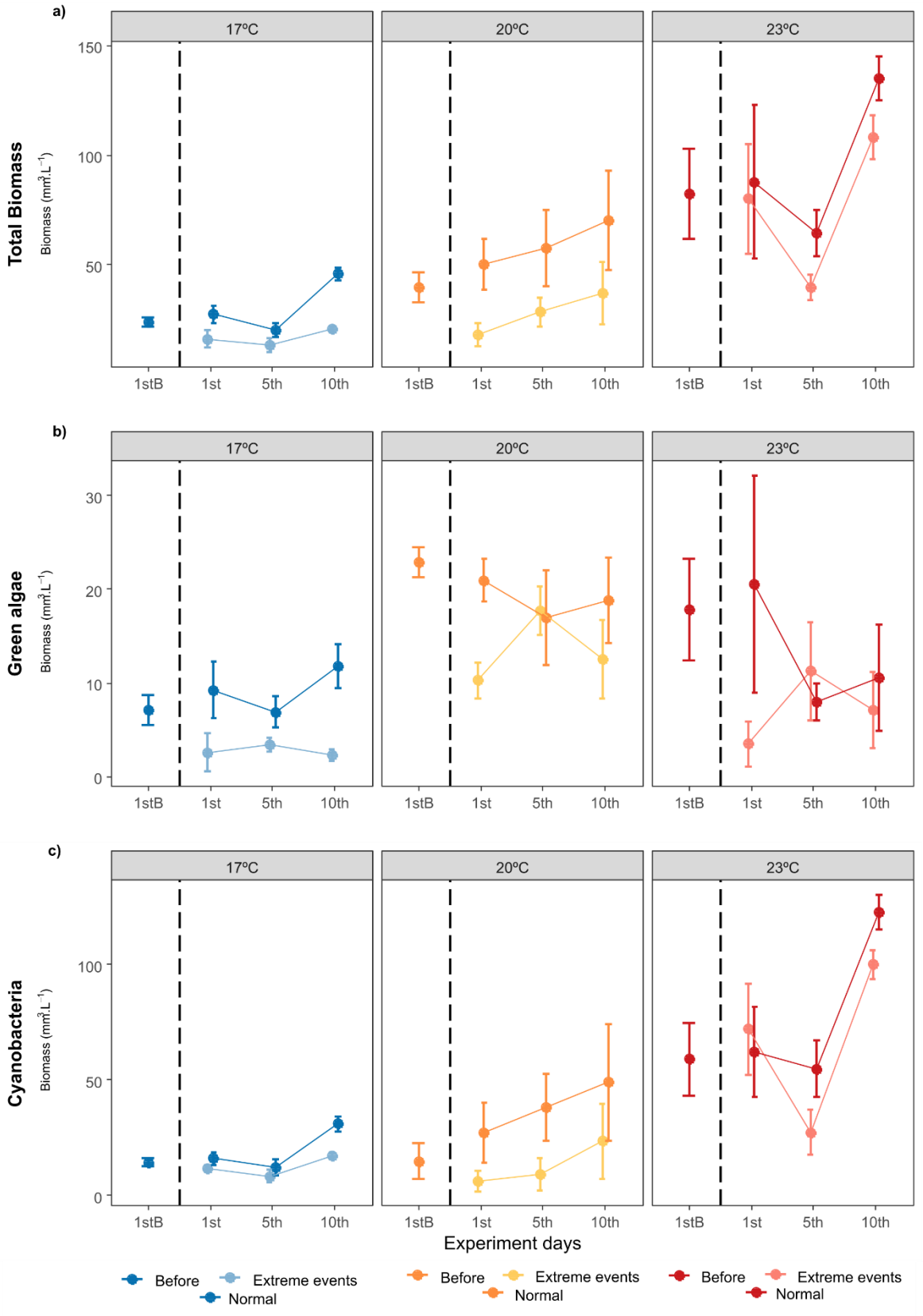


Figure 2: Trajectories (a), resistance (b), and recovery (c) of phytoplankton biomass. The gray dots in the non-metric multidimensional scaling (NMDS) (a) indicate the period before the extreme rainfall event (1stB), light colors indicate disturbed communities and dark colors the undisturbed. Dashed 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

1287 was led by cyanobacteria, which on the last day of the experiment had a mean biomass of
1288 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
1289 the disturbed treatment (Fig. 3, Supplementary Fig. 3), even surpassing the biomass achieved
1290 in the period before the disturbance (63.86 $mm^3.L^{-1}$ Fig. 2a, Fig. 2b). Green algae did not
1291 recover their biomass at the levels before the disturbance at any temperature evaluated. The
1292 best performance for the group occurred at 20ºC, even so with a difference of approximately
1293 10 $mm^3.L^{-1}$ between the level before the disturbance and that of the extreme rainfall event
1294 (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

1295

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.

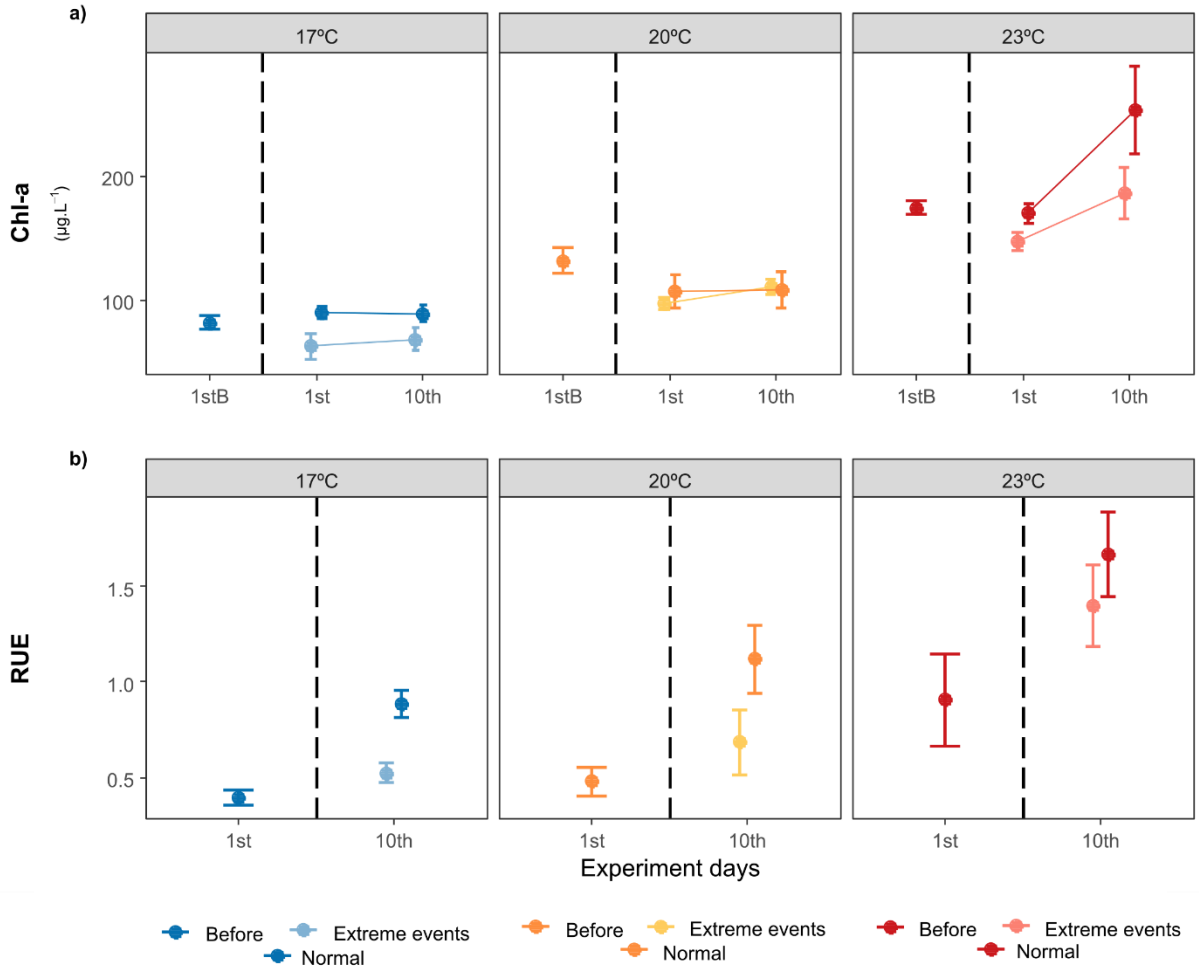
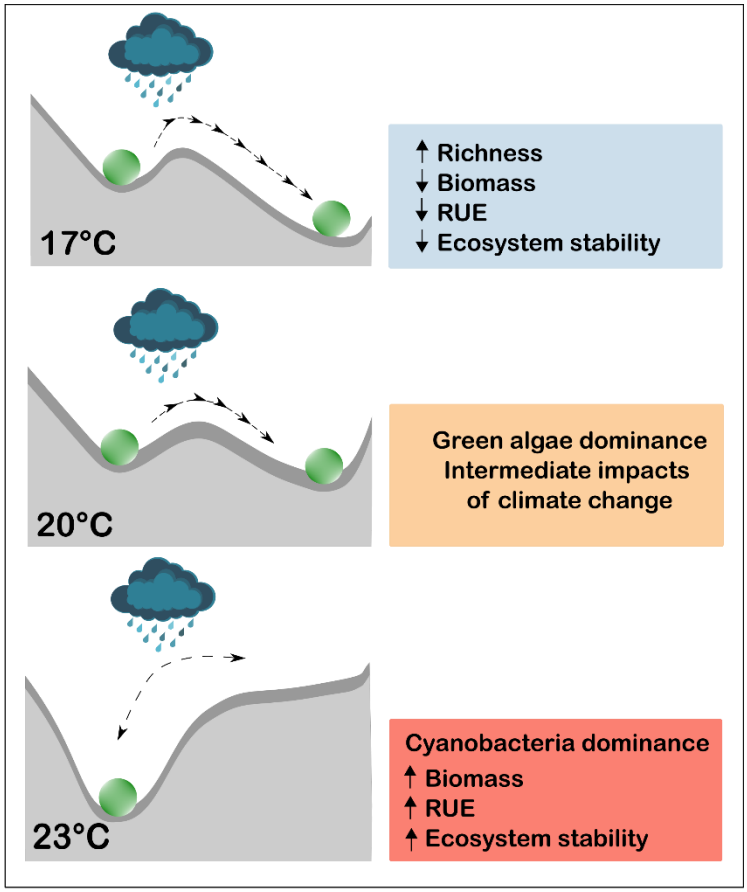


Figure 4. Different ecosystem responses to warming: variation of chlorophyll-a concentration (Chl-a) (a), resource use efficiency (RUE) (b) by the phytoplankton communities in three treatments. Light colors indicate environments with the effect of extreme rainfall events and dark colors without the effect.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



1317 .

1318 **Figure 5.** Under different experimental climate change scenarios, community structure and
1319 ecosystem functions were affected. Different warming scenarios lead to different
1320 phytoplankton communities (represent by ball), which had different resistance and resilience
1321 against a short-term disturbance, as indicated by the biomass and resource use efficiency
1322 (RUE) recovery.

1323

1324 **3.4 Discussion**

1325 We avaliated the effects of strong disturbance simulating an extreme climatic event,
1326 on three phytoplankton communities pre-adapted to different levels of warming and under
1327 non-limiting nutrient conditions. The study revealed that ecosystem stability and resilience
1328 (recoverability of biomass and ecosystem function) depended on the dominant group.
1329 Cyanobacteria-dominated communities, developed under the warmest treatment, were the
1330 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, 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

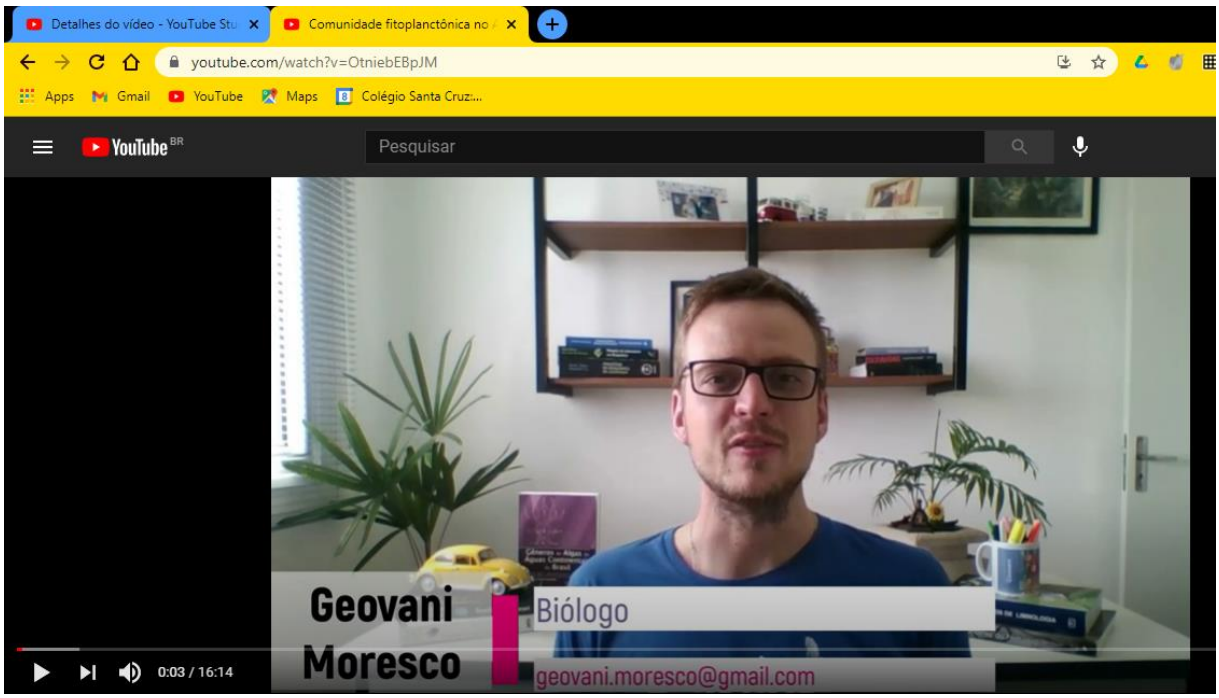


Fig S1. Scientific dissemination of the main results of the thesis for the participation of society on the theme of climate change. Video available at: https://www.youtube.com/watch?v=OtniebEBpJM

APPENDIX B - Experimental design

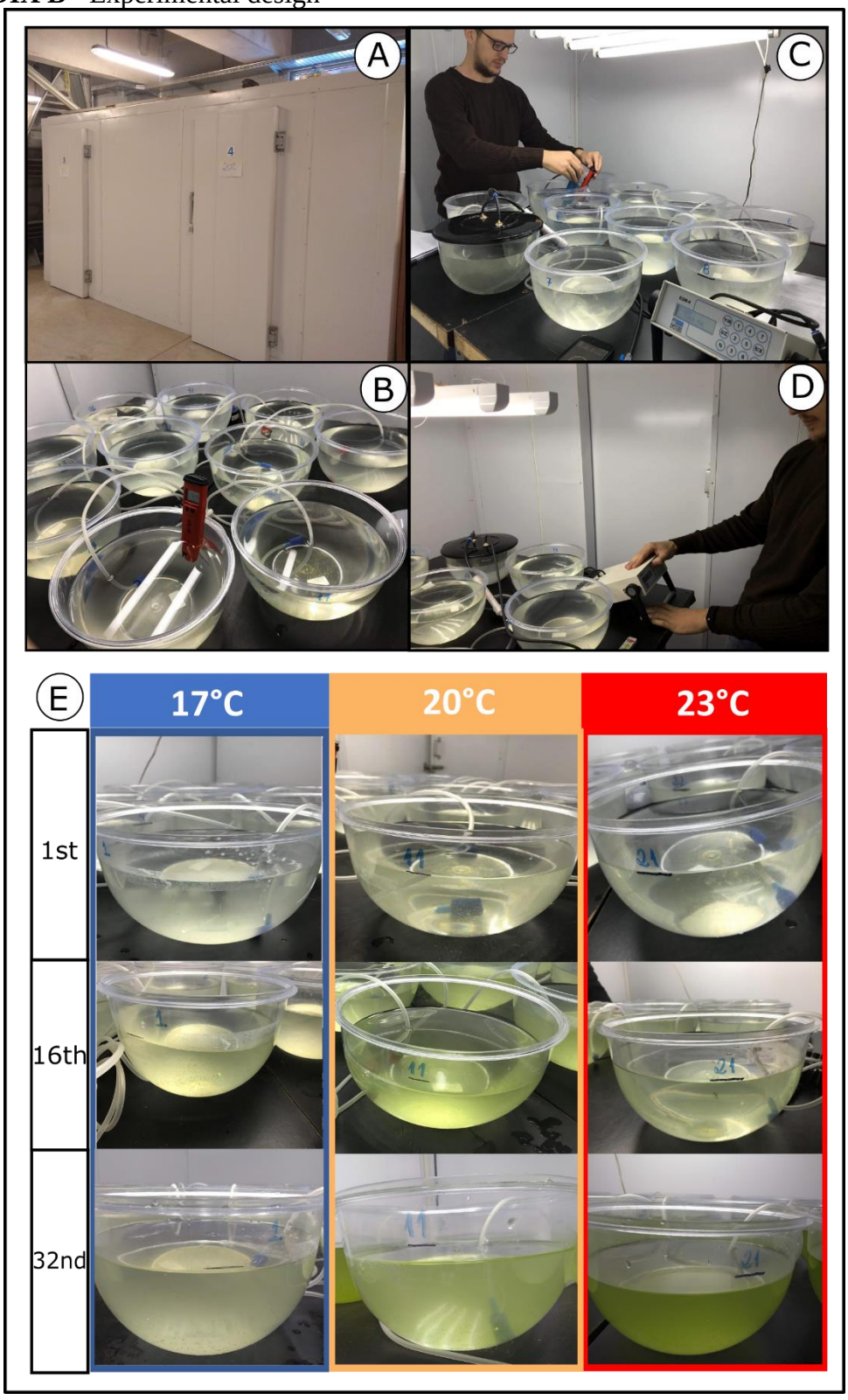


Figure S1. Experimental Design. A) Rooms temperature; B) overview of the experiment; C) Monitoring of limnological variables; D) CO_2 fluxes measured; E) Development of the phytoplankton community at the three temperatures evaluated (17ºC, 20ºC, and 23ºC). Photos were taken on the 1st day, on the 16th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

APPENDIX C – Nutrient concentrations

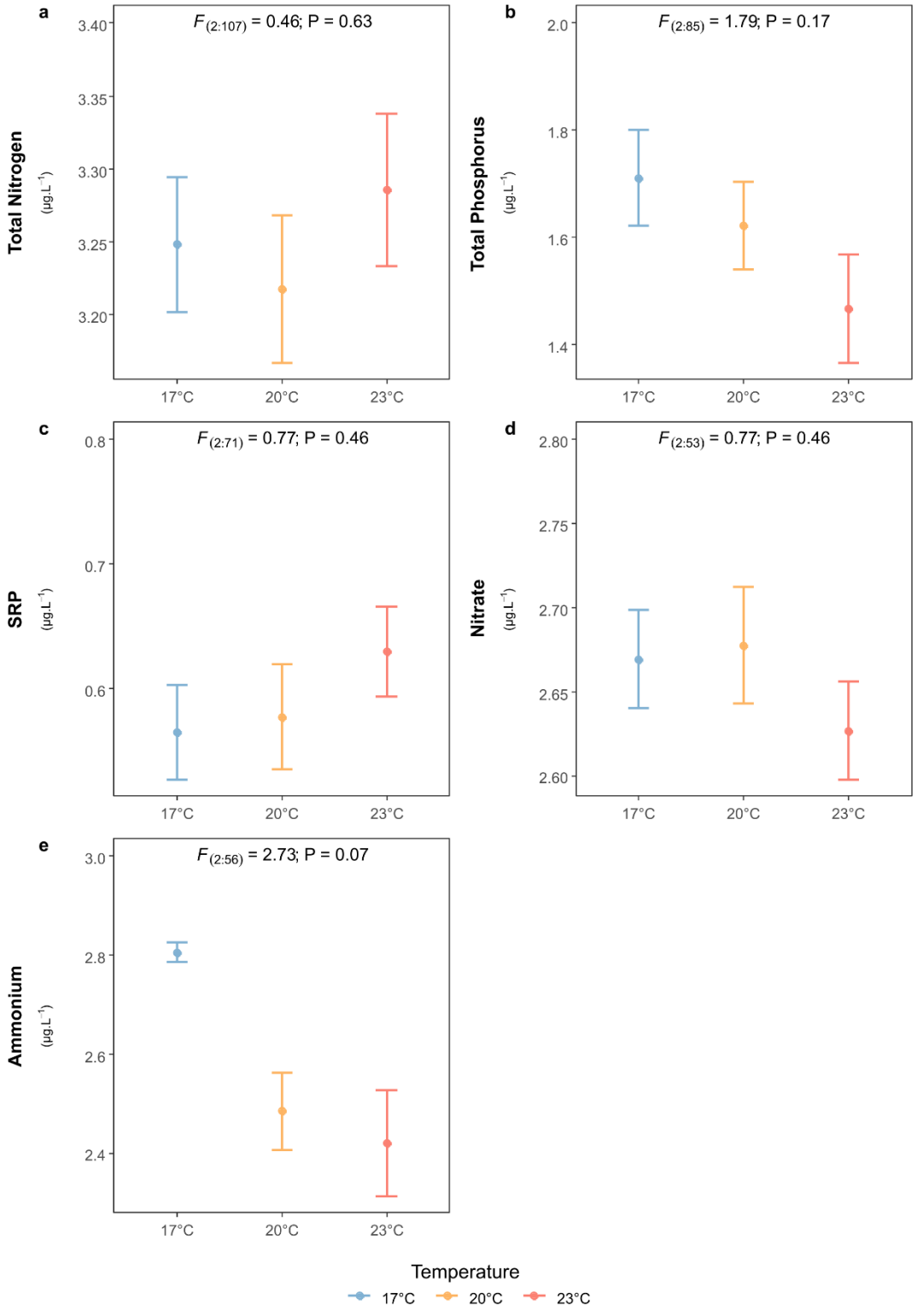


Figure S2: Nutrient concentrations in the three temperature treatments obtained. The non-significant result in the analysis of variance ($P > 0.05$) indicated that nutrient concentrations were similar between treatments. Note that the scale varies among limnological variables. The central point denotes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

APPENDIX D - Variation of phytoplankton taxonomic richness

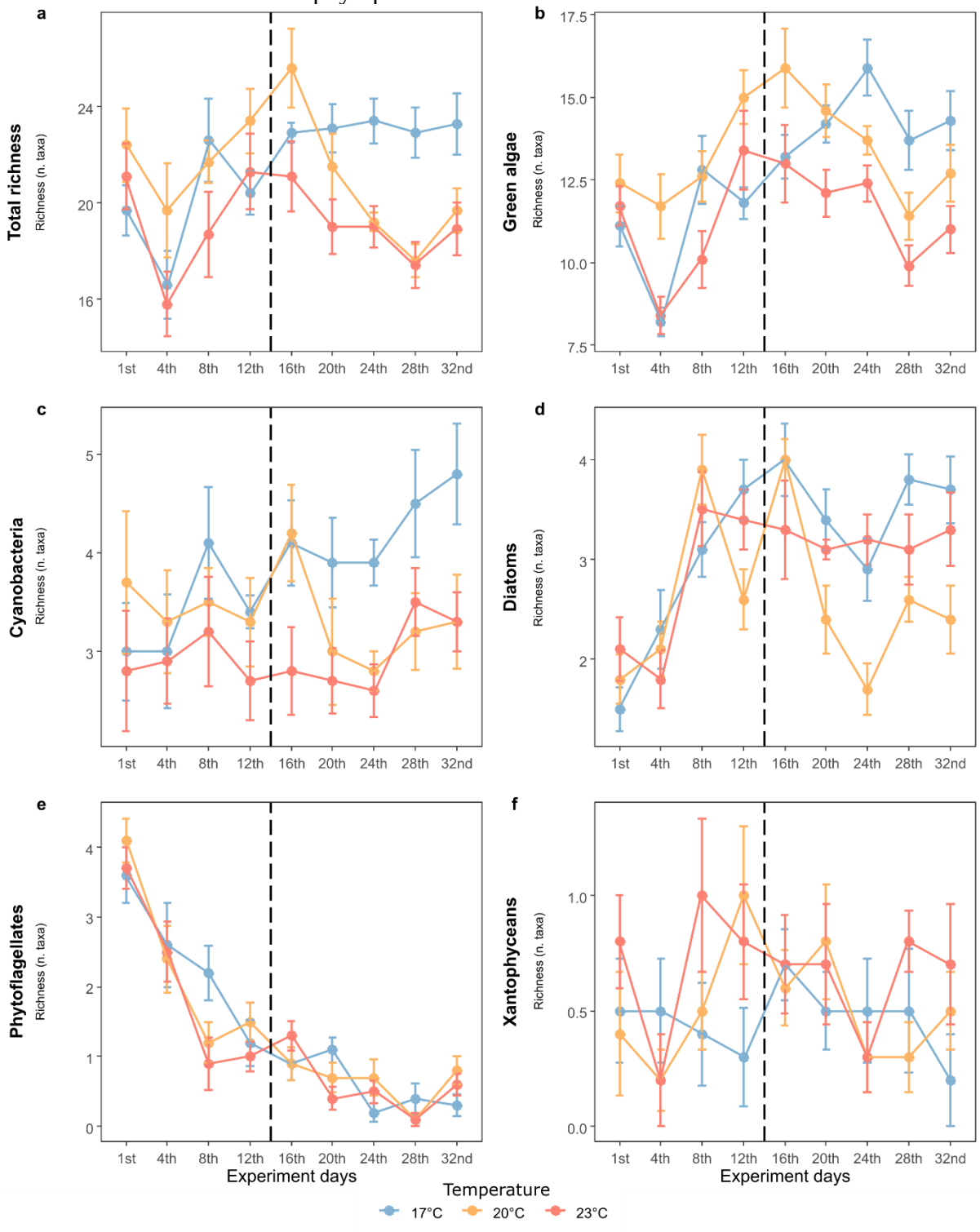


Figure S3: Variation of phytoplankton taxonomic richness with temperature through the 32 days of the experiment: total (a), green algae (b), cyanobacteria (c), diatoms (d), phytoflagellates (e) and xanthophyceans (f). The dotted line indicates that on the 16th day of the experiment the wished temperatures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

APPENDIX E - Variation of phytoplankton biomass

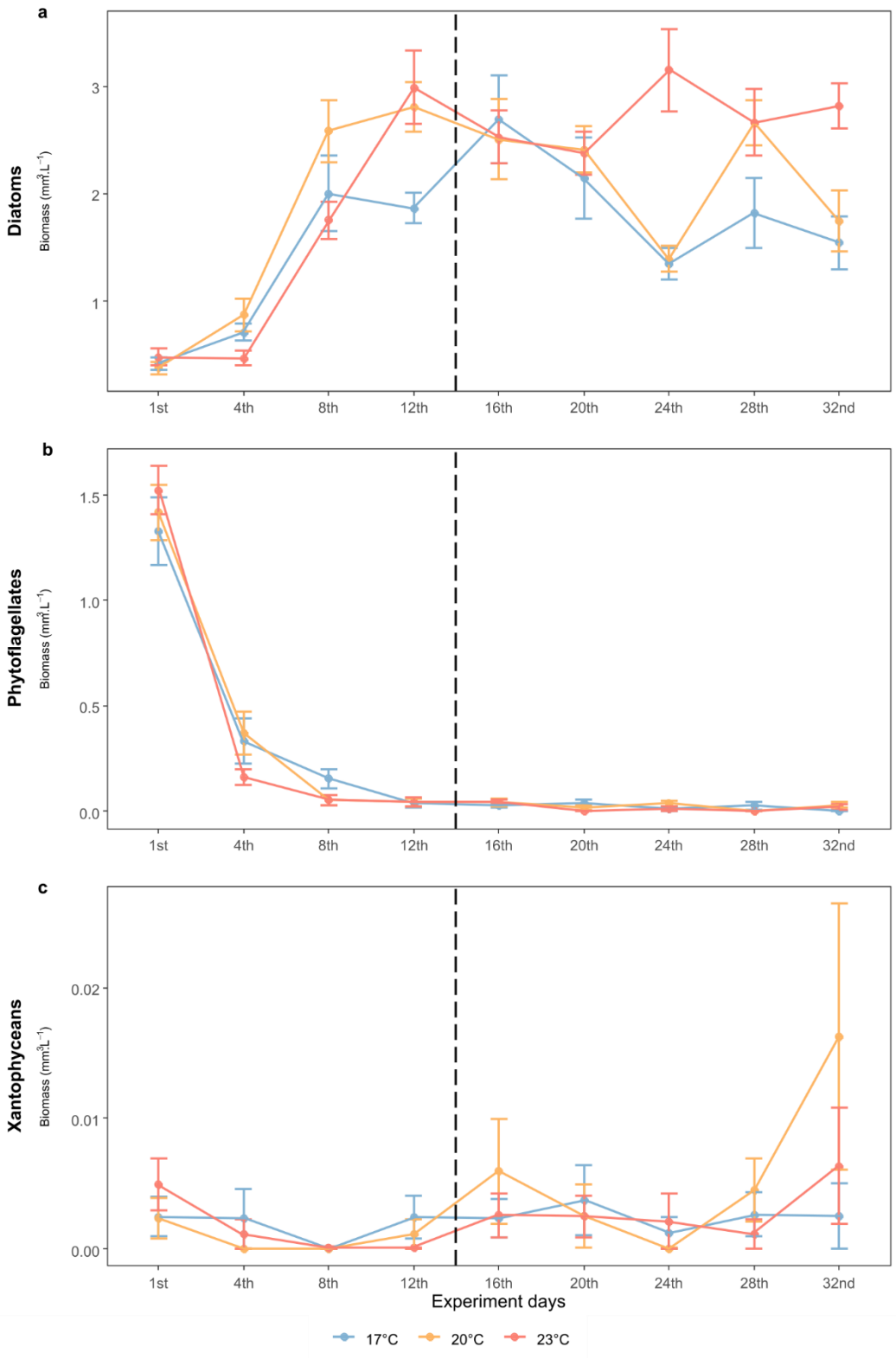
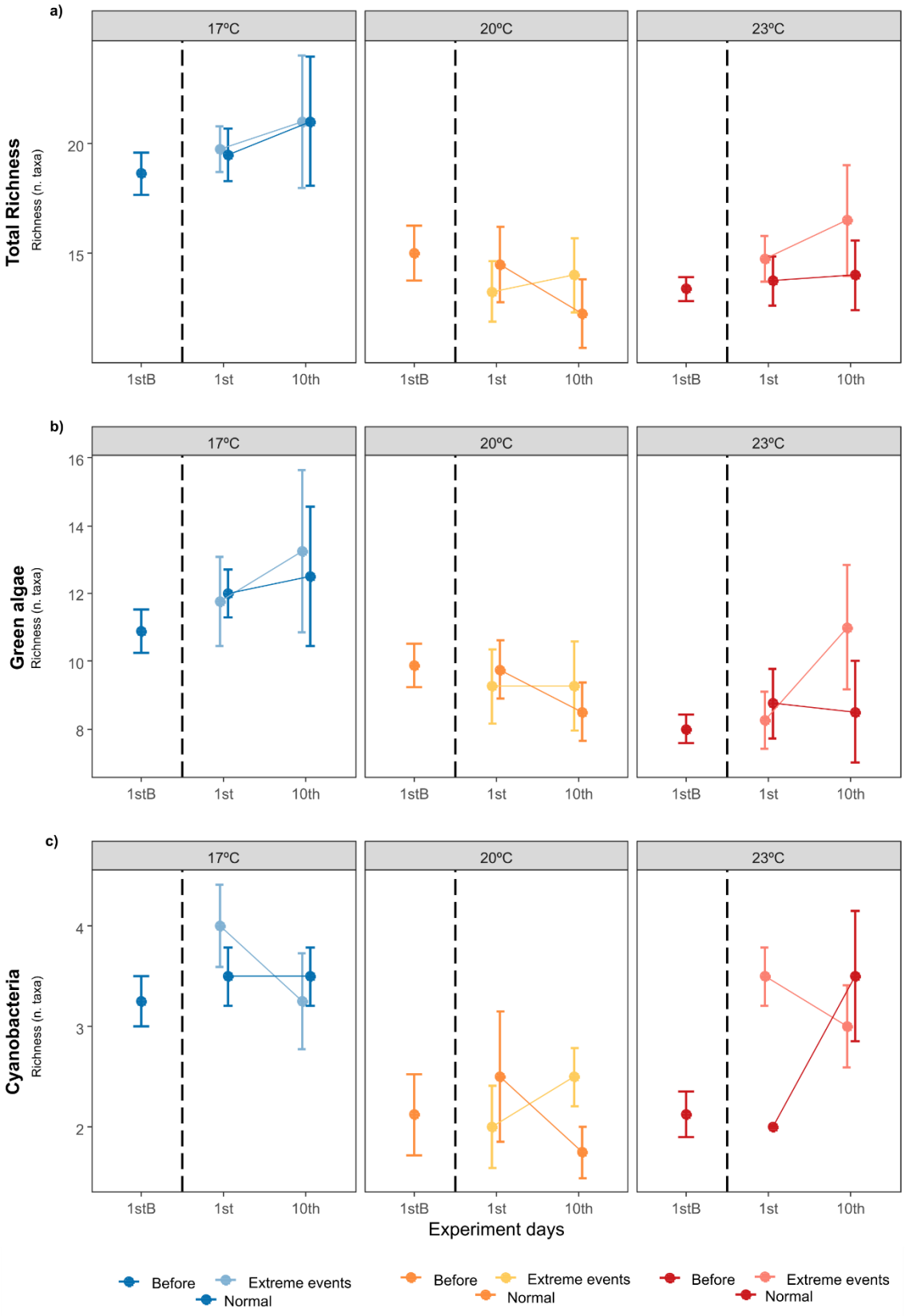
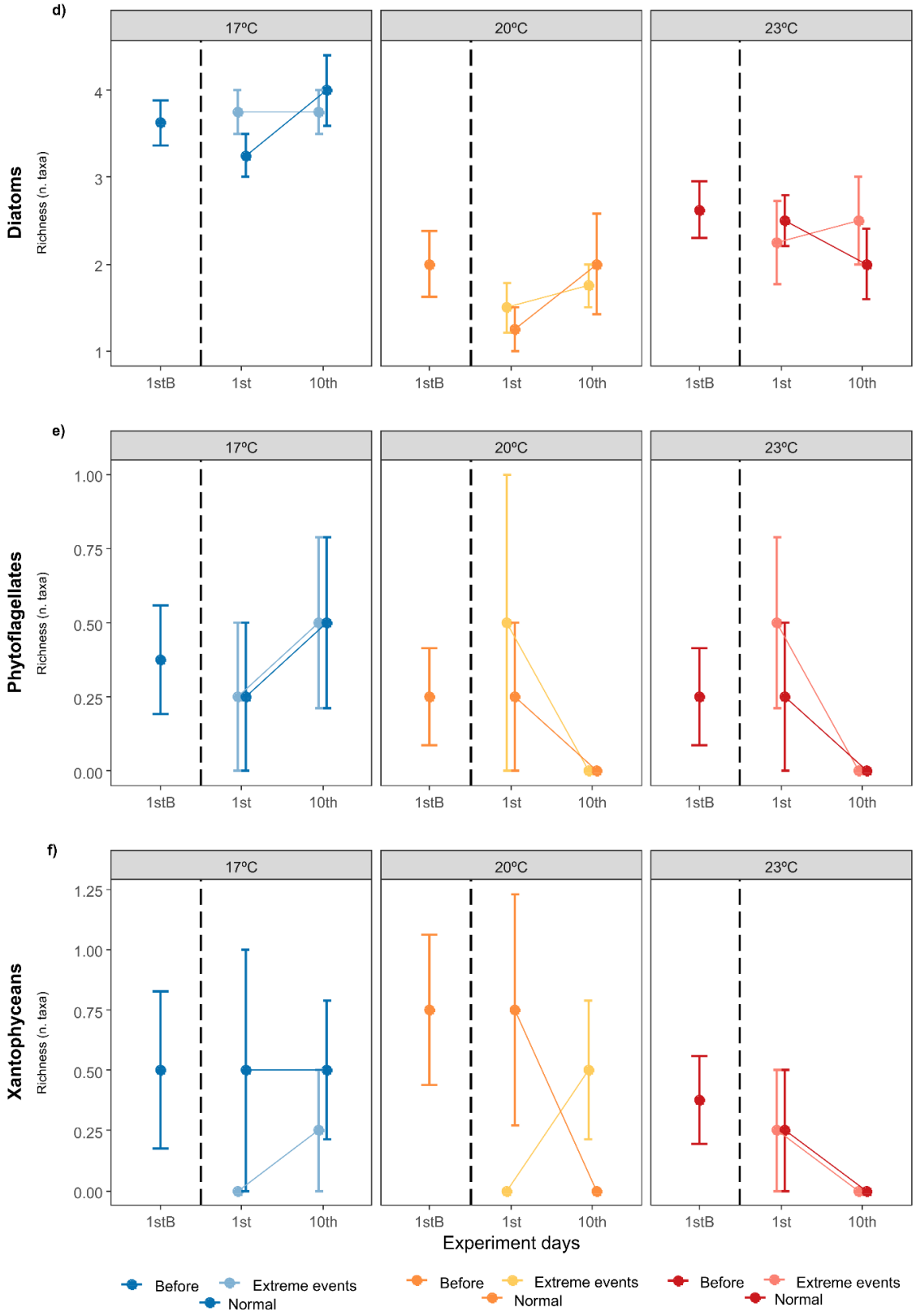


Figure S4: Variation of phytoplankton biomass with temperature through the 32 days of the experiment: diatoms (a), phytoflagellates (b) and xanthophyceans (c). The dotted line indicates that on the 16th day of the experiment the wished temperatures were reached. The central point denotes the mean value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

APPENDIX F - Variation of phytoplankton taxonomic richness

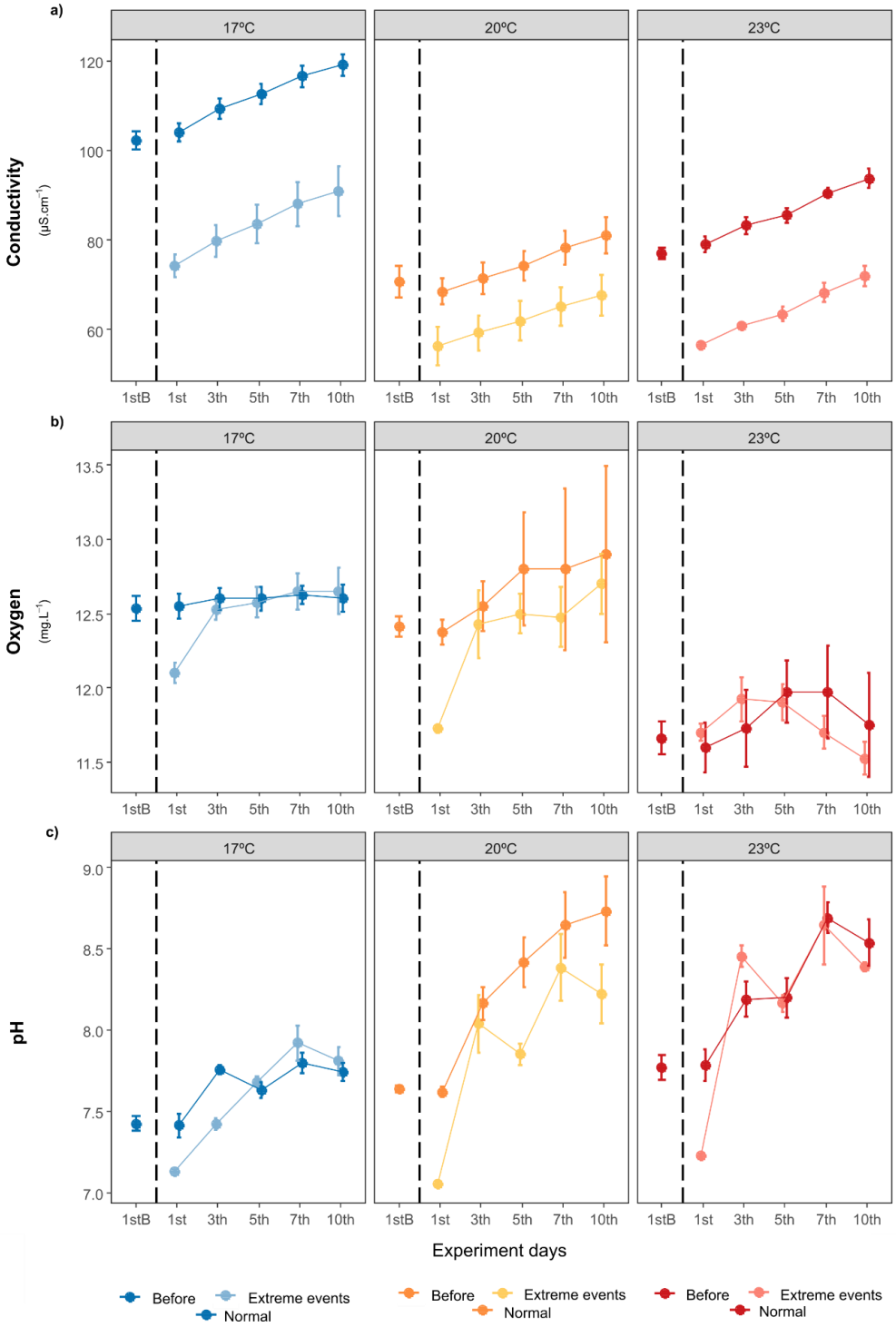




Supplementary Figure 1: Variation of phytoplankton taxonomic richness with temperature through the 10 days of the experiment: total (a), green algae (b), cyanobacteria (c), diatoms (d), phytoflagellates (e), xanthophyceans (f). The dotted line indicates the occurrence of the extreme rainfall event (1stB). 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.

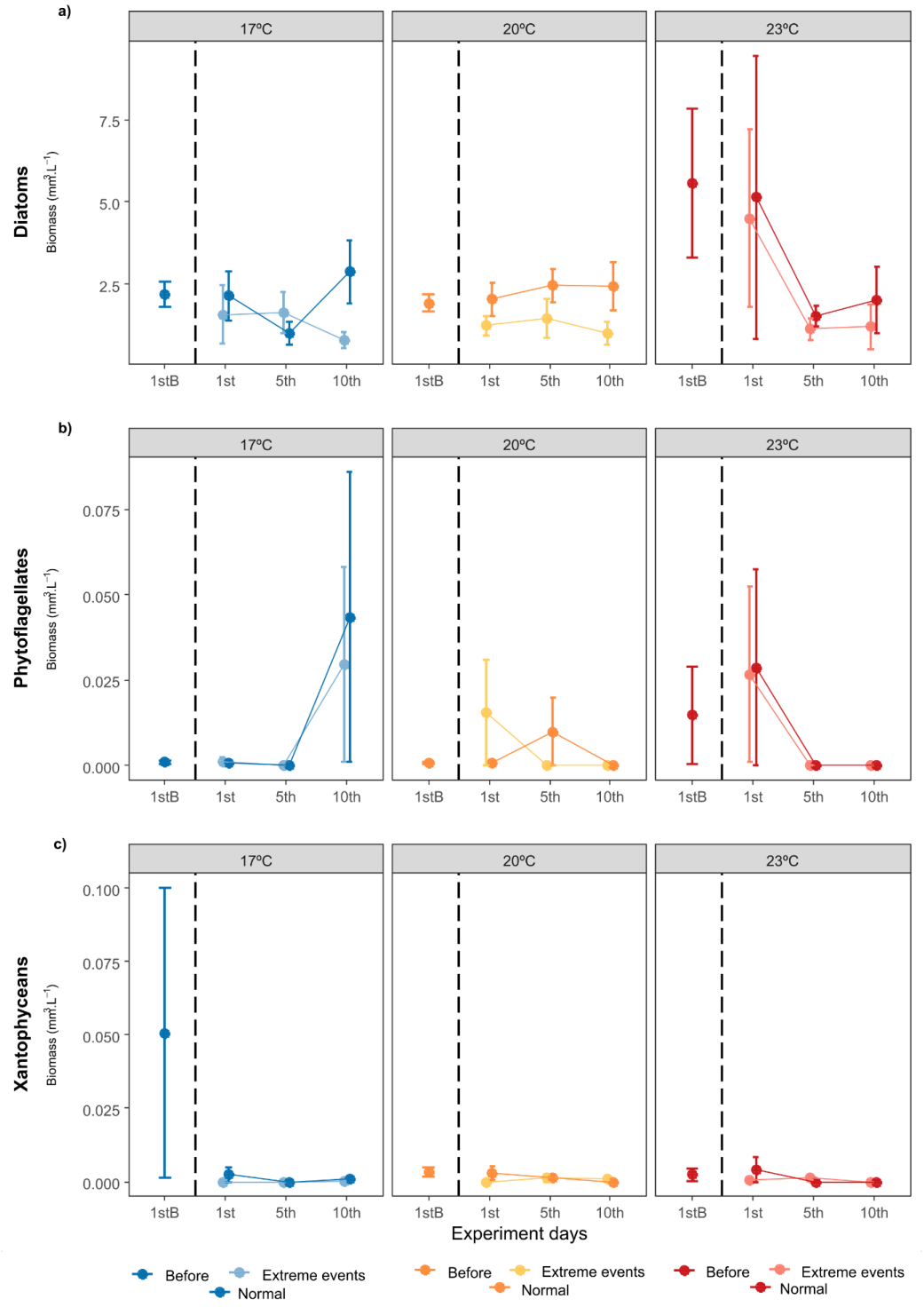
APPENDIX G - Variation of abiotic variables



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 H - 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*).