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DNA metabarcoding reveals the responses of prokaryotes and eukaryotes microbiota to warming: Are the patterns similar between taxonomic and trophic groups?

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ABSTRACT

The aquatic microbiota is involved in crucial ecosystem functions. Thus, investigating the effects of global warming on these organisms is highly relevant, especially given the numerous climatic changes expected by the end of the century. In this study, we used an experimental approach and high-throughput sequencing to evaluate the shortterm effect of warming predicted by different future scenarios in the composition of the planktonic freshwater bacteria and microeukaryotes, and to verify if the same effects occur for each trophic level separately (autotrophic, heterotrophic and mixotrophic). Our experiment demonstrated that the composition for eukaryotes and prokaryotes based on DNA metabarcoding is affected by the increase in temperature and these have a similar pattern of response to warming. This highlights the temperature importance in structuring the communities of different groups. Modifications in the communities were observed through the substitution of specific taxa, which occurred mainly in warmer levels. Changes in community composition were also identified when trophic levels were assessed separately. Mixotrophic eukaryotes organisms are more sensitive to warming, modifying the patterns of composition with an increase in temperature of 2 °C. Microeukaryotes and heterotrophic bacteria were more resistant, with alterations in the communities composition visualized only in higher warming levels. The composition of autotrophic organisms was not affected by the increase in water temperature in any of the biological classifications evaluated, although the richness of eukaryotic autotrophic has decreased with warming. Our results contribute to predict how different biological levels and trophic groups of the aquatic microbiota respond to global warming. This approach is relevant because warming leads to changes in community composition and affects ecosystem processes essentials to the aquatic environment.

1. Introduction

Understanding how biological communities respond to environmental variations is one of the central goals of Ecology (Smale et al., 2017). Numerous changes in environmental conditions, including those resulting from climate change, have been observed in recent years (e.g. temperature rise, change in precipitation frequency, land use and enrichment of aquatic ecosystems) and should be intensified until the end of this century (IPCC, 2014). Although many factors influence the structuring of biological communities, changes in temperature are highlighted, since this variable affects the rates of growth, reproduction, phenology and trophic dynamics of organisms (Jeppesen et al., 2010). Thus, predicting the ecological consequences of both temperature rise (e.g. Bergkemper et al., 2018) and temperature variation (e.g. Rasconi et al., 2017) on communities' composition is a highly relevant issue (Altermatt et al., 2015).

Continental aquatic ecosystems have high biodiversity but are among the most threatened in the world (Dudgeon et al., 2006). In recent years, numerous efforts have been concentrated to predict how climate change, including the increase in global temperature, should affect these environments (Woodward et al., 2010; Roland et al., 2012; Jeppesen et al., 2014). Effects have already been observed on species through changes in patterns of composition (e.g. Yvon-Durocher et al., 2015; Smale et al., 2017) and abundance (Clements et al., 2013; Bergen et al., 2016), as well as on their functional characteristics (e.g. size reduction, Sommer et al., 2015; Rasconi et al., 2017; Stefanidou et al.,

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2018). Similarly, changes in the functional characteristics of communities must alter the way they relate to other trophic levels through a top-down or bottom-up effect (Sommer and Lewandowska, 2011; Vadadi-Fülöp et al., 2012; Velthuis et al., 2017). Although many studies have investigated the effects of warming, most of them focus only on one taxonomic group and do not consider different trophic levels simultaneously, which ignores the interaction between them. In this sense, ecological studies using different trophic levels simultaneously can help to understand if different taxonomic and trophic groups respond similarly to environmental changes.

Distinct effects of warming are expected on the different trophic levels that compose the aquatic communities. According to the metabolic theory of Ecology (Brown et al., 2004), heterotrophic processes are more temperature dependent than autotrophic. Indeed, studies have shown that global warming should intensify rates of respiration and decomposition, leading to the predominance of heterotrophic organisms over the autotrophic (Hoppe et al., 2008; Lara et al., 2013; Von Scheibner et al., 2014; Bergkemper et al., 2018, but see Domaizon et al., 2012 for an opposite effect). This change in the communities' structure can increase consumer control over producers in warming scenarios (Yang et al., 2016). On the other hand, the mixotrophic microbiota shows greater plasticity in face of temperature changes, adopting, according to the situation, a producer or consumer behavior. For these organisms, a reduction in the amount of chlorophyll-a (Wilken et al., 2013) and an increase in bacterial consumption (Wilken et al., 2018) was observed, which led to the overlap of heterotrophic metabolism over autotrophic in high temperatures.

Bacteria (Fujimoto et al., 2016) and planktonic microeukaryotes (Khomich et al., 2017) have representatives at the different trophic levels (autotrophic, heterotrophic and mixotrophic), and represent evolutionarily distinct groups. These organisms play an important role in food webs (Chen et al., 2008) and ecosystems functioning through biogeochemical and nutrient cycles (Rodríguez-Varela, 2004; Chen et al., 2008). Because they have a reduced size and a short life cycle (Altermatt et al., 2015), these organisms are an excellent model for understanding the effects of global warming. For comparisons between bacterioplankton and microbial eukaryotes communities, similar responses to temperature increase have been observed (Smale et al., 2017), as well as the absence of effects at these two biological levels (Maugendre et al., 2015). However, some studies have demonstrated contrasting responses for these two groups to the warming, with abundance increased for bacteria (Domaizon et al., 2012; Lara et al., 2013; Tuyet et al., 2015; Bergen et al., 2016) and reduction of abundance (Domaizon et al., 2012; Vázquez-Domínguez et al., 2012) and biomass for protists (Lara et al., 2013), indicating that microeukaryotes organisms may be more sensitive to the effects of global warming.

Through the metabarcoding approach, many taxonomic groups with distinct biological characteristics, such as prokaryotes (e.g. Von Scheibner et al., 2014; Tuyet et al., 2015; Bergen et al., 2016) and eukaryotes (e.g. Domaizon et al., 2012; Moustaka-Gouni et al., 2016), can be evaluated simultaneously by the use of universal primers. This strategy allows for the detection of rare taxa (Medinger et al., 2010), increases the taxonomic resolution in samples screening (Deiner et al., 2017) and complements the information based on traditional morphological identification (Santoferrara et al., 2016). Thus, it can be used as a tool to detect the responses of planktonic communities to warming predicted for the future (e.g. Smale et al., 2017; Stefanidou et al., 2018).

Herein, we used an experimental approach to simulate the short-term effect of warming predicted by different future scenarios on aquatic prokaryotes (i.e. bacterioplankton) and eukaryotes (i.e. microeukaryotes) microbiota composition, and to evaluate the effects separately for each trophic level (autotrophic, heterotrophic and mixotrophic). Communities composition was obtained through high-throughput sequencing, using targeted markers for the 16S rDNA and 18S rDNA genes. This approach has been used previously in experiments involving marine environments (e.g. Domaizon et al., 2012; Bergen et al., 2016; Smale et al., 2017; Huggett et al., 2018). However, to the best of our knowledge, our experiment is one of the first to simulate the effects of temperature increase using a metabarcoding approach for the tropical freshwater microbiota of prokaryotes and eukaryotes (but see Pajares et al. (2013) and Ren et al. (2017) for bacterioplankton in temperate zones).

We hypothesized that warming has an influence on the composition of planktonic bacteria and microeukaryotes since the temperature is an important factor that determines the organization of communities in natural environments (e.g. Piwosz et al., 2018; Guo et al., 2019). Furthermore, as predicted by the metabolic theory of Ecology, we expect that heterotrophic organisms, due to their metabolism characteristics, may be favored when compared to autotrophic organisms, leading to changes in composition patterns and diversity of microbial communities. On the other hand, as bacteria and microeukaryotes have representatives on the three trophic levels and are directly associated with the food web, we expect that both biological groups, as well as the different trophic levels, respond to warming in a similar way since changes in one level may lead to modifications to the other.

2. Material and methods

2.1. Experimental design

The experiment was conducted during April 2016, at the State University of Goiás (UEG), Campus Anápolis, Goiás, Brazil (−16° 22′52.86″ S and −48° 56′45.43″ W). The microcosms were represented by rectangular glass aquariums with a capacity of 25 L of water. A water circulation pump (model JAD SP - 500) was placed in each aquarium bottom in order to avoid organism sedimentation (e.g. Maugendre et al., 2015; Bergen et al., 2016). The water temperature was manipulated using an electric heater with thermostat (model Roxin HT 1300 – 25W). Throughout the experiment, the microcosms were covered with a transparent nylon screen to prevent the entry of insects, dirt, or other residues and small animals that could interfere with the results. The mesocosms were exposed to the regime of natural light and other conditions of the environment. A full description of the experimental layout and warming system was described in Machado et al. (2019a).

Twenty microcosms were randomly distributed in four temperature treatments. The control treatment corresponded to the mean annual temperature for the region where the experiment was performed. This estimate was obtained through the Meteorology and Hydrology System of Goiás State (Simehgo, 2016). The temperature increase was established according to the forecasts proposed by the Atmospheric-Ocean Global Circulation Model (AOGCM) - Community Climate System Model (CCSM), available in the Ecoclimate database (Lima-Ribeiro et al., 2015). This model presents four future warming scenarios based on the levels of radiative forcing predicted for the year 2100, the Representative Concentration Pathways (RCP 2.6 optimistic scenario; RCP 4.5 e RCP 6.0 intermediate scenario; RCP 8.5 pessimistic scenario; Moss et al., 2008). The intermediate scenario predicts a mean increase of 2 °C in comparison to the current mean temperature, while the pessimistic scenario assumes an increase of 4 °C considering the region where the experiment was performed.

The microcosms were warmed accordingly to simulate these different temperature scenarios. The experiment was constructed with five replicates of the following treatments: Control (C – 24 °C): annual mean temperature for the region where the experiment was constructed; Intermediate (I – 26 °C): represents an average increase in temperature predicted in the optimistic and intermediate scenarios; Pessimistic (P – 28 °C): represents a pessimistic scenario of warming and Pessimistic + Pessimistic (PP – 32 °C): indicates an extreme situation in temperature increase, based on a double pessimistic scenario.

The RCP 8.5 scenario is the most used in the literature to define a

higher warming state; however, it does not act as an upper limit for the possible emissions, since they depend directly on the uncertainties in the anthropic activities for the next century (Hayhoe et al., 2017). Although the PP treatment does not directly represent any future warming scenario predicted for Brazil, some regions in the tropical zone may show an increase above 32 °C, considering a pessimistic warming scenario (see data for tropical region in Ecoclimate database, Lima-Ribeiro et al. 2015). In addition, the increase in water temperature can also occur due to the loss of marginal vegetation (Moore et al., 2005; Caissie, 2006), causing an increase in the incidence of light and consequently in temperature. Thus, PP treatment was used to understand how an increase in temperature considering double what is expected by a pessimistic RCP scenario could affect the communities.

Each microcosm was filled with 18.2 L of water collected in an oligo-mesotrophic lake, located on the UEG Campus. These, 16 L were collected directly from the lake using a bucket without any previous filtration and 2.2 L were collected with a plankton net (20 μm mesh size), seeking to maximize the collection of species that occur in lower abundance and to ensure that most taxonomic groups were represented in the experimental units. Although this procedure can change some limnological variables in the microcosms relative to the natural environment, we need consider: i) the limnological changed (e.g. carbon, transparency) occurred similarly in all temperature treatments; ii) during the experiment (and all treatment) the trophic state of the experiment was similar with to the lake sampled (oligotrophic), thus, the experiment conditions replicated the limnological characteristic found in the lake. Furthermore, obtaining organisms directly from their natural habitat allows the use of genetically diverse populations that coexist naturally (Altermatt et al., 2015). After the microcosms filling (April 04th, 2016), we waited about 48 h for the temperature to stabilize and acclimate the organisms. Thus, the experiment started on April 6th, 2016 (day zero) and ended on April 26th, 2016, totaling 20 days. This temporal period is consistent with the generation time of organisms (Domaizon et al., 2012) and agrees with previous experimental studies involving the planktonic microbiota (e.g. Lara et al., 2013; Lindh et al., 2013; Bergen et al., 2016; Menden-Deuer et al., 2018).

To avoid depletion of nutrients, every four days, we added 5.40 mg L^{-1} of sodium nitrate ($NaNO_3^-$) and 0.34 mg L^{-1} of potassium phosphate (KH_2PO_4), which represent respectively 0.2 mg L^{-1} of nitrate (NO_3^-) and 0.002 mg L^{-1} of phosphate (PO_4^{-3}). These additions were based on the natural nitrate and phosphate concentrations of the lake from which the water was collected, considering the proportion of Redfield 16N: 1P (Reynolds, 2006). On the 10th day of the experiment, 500 mL of deionized water was also added to each aquarium, trying to recover the losses by evaporation (e.g. McKee et al., 2000; Ekvall and Hanson, 2012). During the experiment, the temperature in the treatments oscillated in average 3 °C around the previously established values (24 °C, 26 °C, 28 °C and 32 °C) following the weather oscillations (sunny or rainy days). However, the treatments with warming have always been at temperatures higher than the control (see details of the daily temperature in Machado et al., 2019a). A heater related to the control treatment presented faults in its functioning, thus, this replica was excluded from all subsequent analysis.

During the 20 days of the experiment, we daily measured water temperature, conductivity, dissolved oxygen, pH and chlorophyll-a using the Manta 2 Eureka multiparameter probe. On the last day of the experiment, we also collected 500 mL of water in each microcosm for nutrient analysis (nitrogen and phosphorus), following the protocol described in Golterman et al. (1978). Replicas of all treatments started the experiment with similar limnological conditions (pH: mean = 6.5, ± SD = 0.1; chlorophyll-a: mean = 6.5 μg L^{-1}, ± SD = 1 μg L^{-1}; conductivity: mean = 5.9 μS cm^{-1}, ± SD = 0.7 μS cm^{-1}; dissolved oxygen: mean = 7.2 mg L^{-1}, ± SD = 0.1 mg L^{-1}). Over the time, these parameters showed some variation from the initial values (see Table 1S in Supplementary Material 1). On mean, chlorophyll-a and conductivity values increased over time, being higher in the PP temperature treatment. The dissolved oxygen concentration oscillated over time and in mean was lower in the P and PP temperature treatment. The pH remained constant and with neutral values in all microcosms. We also did not observe differences in nutrient concentrations between temperature treatments (See Table 2S in Supplementary Material 1). Thus, all microcosms were maintained oligotrophic state, similar to the lake where water was collected to fill them (see more details in Machado et al., 2019a).

Due to the initial filtration to fill microcosms, zooplankton abundance could also have been increased. To ensure equal conditions between the different treatments, at the end of the experiment, samples for zooplankton analysis were obtained from each aquarium (see Additional Information topic in Supplementary Material 1). Overall, we did not find differences in zooplankton composition between the different temperatures (see Additional Information topic in Supplementary Material 1), ensuring that the differences observed in prokaryotic and eukaryotic communities were not influenced by zooplankton, since this was similar in all treatments.

2.2. DNA extraction, amplification and sequencing

At the end of the experiment, 500 mL of water was collected from each microcosm using polyethylene bottles. The samples were stored in a refrigerator (at about 2 °C) until the time of filtration, which occurred within 24 h after collection. The first filtration was carried using a vacuum pump and a Millipore cellulose filter (3 μm pore size), which enabled to capture the eukaryotic microbiota present in the sample. The water resulting from this process was again filtered, however with a Millipore cellulose filter of 0.22 μm pore size. Here, the objective was to capture the prokaryotic microbiota. Thus, the microbial community fraction investigated in this study was predominantly composed of microeukaryotes, picoprokaryotes and nanoprokaryotes (see Massana and Logares (2013) for size class classification). Each of the filters (3 μm and 0.22 μm) were placed separately in Falcon tubes and stored in liquid nitrogen at −80 °C.

The total DNA of the eukaryotes (3 μm filter) and prokaryotes (0.22 μm filter) was extracted following the protocol of the PowerWater® DNA Isolation Kit (MoBio). The extracted DNA was analyzed and quantified on 1% agarose gel. For eukaryotic DNA, a hypervariable fragment (~400 pb) 18S rDNA was amplified by the Polymerase Chain Reaction (PCR) using the primers TAReuk45FWD1 and TAReuKREV3 (Stoeck et al., 2010), following the protocol described in the kit Taq PCR Master Mix Qiagen. For prokaryotic DNA, amplification was conducted using the primers BAC341F and BAC785R (based on Klindworth et al., 2013; added with Illumina adapters) for 16S rDNA according to the Kit ReadyMix™ Taq PCR Reaction Mix. Amplifications were performed in triplicates and the resulting DNA was analyzed on 1.5% agarose gel.

During the preparation of the Illumina libraries, the triplicates were grouped into a single tube and then indexes were inserted according to the Kit Nextera XT Index 2 (Illumina), through a limited cycle PCR program. The samples were purified using the Agencourt AMPure XP Beads (Beckman Coulter). The libraries were quantified by a real-time PCR with the KAPA Library Quantification Kit, and the amplicon size was estimated using an Agilent High-Sensitivity DNA Kit on Bioanalyzer (Agilent). The libraries of each replicate were normalized to 4 nM and sequenced using MiSeq Reagent Kit v3 (600 cycles) on the Illumina MiSeq platform.

The sequences quality was evaluated using the FastQC software (Andrews, 2010). Sequences that presented size less than 100 bp or bases with a Phred score < 20 were excluded using the Trimmomatic software (Bolger et al., 2014). In this step, the adapters were also removed. The operational taxonomic units (OTUs) prediction was performed using the UPARSE pipeline (Edgar, 2013), which consists of: (i).

merging sequences; (ii). grouping the sequences of all samples; (iii). dereplication with the identification of unique sequences and chimeras filtering; (iv). clustering sequences with similarity higher 97% in the same OTU; (v). construction of OTUs table per treatment. The taxonomic prediction was performed by a BLASTn (Altschul et al., 1990) of the representative OTU sequences against the Silva 128 database using a 97% identity percentage (See Supplementary Material 2 for more details of data processing). Sequences of metazoan (e.g. fragments of animals, larvae, eggs) or non-aquatic microbiota (e.g. plants) were removed from further analysis.

The OTUs for which it was possible to assign a taxonomic group were classified into trophic groups according to their carbon source (e.g. Simon et al., 2015; Fujimoto et al., 2016; Khomich et al., 2017). Organisms that have chlorophyll-a and obtain carbon exclusively through primary productivity were considered as autotrophic (e.g. Chlorophyta, Charophyta, Cyanobacteria). The heterotrophs were represented by organisms that obtain carbon through predation, parasitism or decomposition (e.g. Ciliophora, Fungi, Amoebozoa, Proteobacteria) while organisms that acquire carbon both heterotrophically and through photosynthesis were considered as mixotrophic (e.g. putative members of Chryptophyta, Chrysophyta, Dinophyta).

Thus, nine matrices of data were used in our analysis, to evaluate the warming effects on the planktonic microorganisms. They were: (i). total prokaryotes: all OTUs obtained by sequencing the 16S rDNA; (ii). taxonomically classified prokaryotes: includes only prokaryotic OTUs for which it was possible to obtain the taxonomic classification; (iii). autotrophic prokaryotes: includes only prokaryotic primary producers; (iv). heterotrophic prokaryotes: includes only the heterotrophic prokaryotes; (v). total eukaryotes: all OTUs obtained by the sequencing of 18S rDNA after the exclusion of the Metazoa and plants; (vi). taxonomically classified eukaryotes: includes only eukaryotic OTUs for which it was possible to obtain the taxonomic classification; (vii). autotrophic eukaryotes: includes only eukaryotic primary producers; (viii). heterotrophic eukaryotes: includes only the heterotrophic eukaryotes; (ix). mixotrophic eukaryotes: includes only mixotrophic eukaryotes.

2.3. Data analysis

All analyses were performed in the R software (R Core Team, 2018) using the vegan package (Oksanen et al., 2019). We used an Analysis of Variance (ANOVA) to investigate the effect of warming on the prokaryotic and eukaryotic OTUs richness and also for the different trophic groups. Before the ANOVA, OTUs matrices were subjected to subsampling rarefaction to correct the bias that may be generated by comparing the richness of samples with different sampling depths. The rarefaction was performed through a random subsampling, in which the sample size was determined by the lowest number of sequences obtained considering the replicates of all temperature treatments (Hurlbert, 1971, see also Table 3S in Supplementary Material 1). Rarefaction was performed for each OTUs matrix separately (prokaryotic, eukaryotic and trophic groups) using the “rarefy” function. Thus, in the ANOVA, the OTUs richness normalized by rarefaction analysis represented the response variable and temperature levels represented the predictor variable. In cases where significant results were found in ANOVA, the data were submitted to a Tukey test to verify among what temperatures these differences occur.

We used a Permutational Variance Analysis (PERMANOVA; Zar, 2010) to evaluate the effect of warming on the prokaryotes, eukaryotes and trophic groups composition. The assumption of variance homogeneity was tested using the “betadisper” function (Anderson, 2006). All matrices tested showed a homogeneous variation. In the PERMANOVA, the data of OTUs occurrence represents the variable response and the temperature levels the categorical predictor. In this analysis, the occurrence matrix of OTUs was converted into a Jaccard distance matrix. We used a Non-Metric Multidimensional Scaling Analysis (NMDS; Legendre and Legendre, 1998) to evaluate among what treatments the significant differences in the PERMANOVA occur. Finally, we used a Mantel test to evaluate the concordance between the composition of prokaryotic and eukaryotic OTUs. The existence of a significant relationship between the two data matrices indicates that communities respond similarly to warming. For the NMDS and Mantel test, the OTUs composition was also represented by the occurrence data converted into a Jaccard distance matrix.

3. Results

The sequencing of all samples resulted in 6,888,566 reads for the prokaryotic microbiota and 4,352,080 reads for eukaryotic microbiota. After the quality filtration, we obtained 4,506,636 and 2,708,476 reads for prokaryotes and eukaryotes, respectively (see more details in Table 4S of the Supplementary Material 1). The eukaryotes fragments presented approximately 400 bp and the prokaryotes fragments, 550 bp. The total merged sequences were 52,055 for prokaryotes, which predicted 272 OTUs, with average fragment size of 360 bp. For eukaryotes, 339,908 merged sequences were obtained and predicted 311 OTUs, with average fragment size of 408 bp. We were not able to assign a taxonomic classification for 144 prokaryotic OTUs and 170 eukaryotic OTUs, since they did not have representatives in the reference base used for BLAST in the Silva 128 database. Among the taxonomically classified OTUs, six prokaryotic OTUs and four eukaryotic OTUs represented non-planktonic organisms, while 15 eukaryotic OTUs were attributed to metazoans and therefore were not considered. Thus, 265 prokaryotic OTUs and 287 eukaryotic OTUs represented the total number of OTUs used in our analysis (See Supplementary Material 3–5), while 122 prokaryotic OTUs and 122 eukaryotic OTUs represented taxonomically classified data set, used for trophic group categorization (see Tables 5S and 6S in the Supplementary Material 1).

Among prokaryotic organisms, Proteobacteria and Bacteriodetes were the groups with the highest number of OTUs at all temperatures. As for abundance, Proteobacteria was the predominant group in all temperature treatments, followed by Cyanobacteria in the temperature C and P, Actinobacteria in the temperature I and Bacteriodetes in the temperature PP (Table 1). Eukaryotes, Fungi and Chlorophyta presented the highest number of OTUs in temperature C, I and PP while Fungi, Ciliophora and Chlorophyta in temperature P. In relation to abundance, Chlorophyta, Fungi and Chrysophyta were the most representative groups among all temperatures (Table 1). Among the prokaryotes, 104 OTUs represented heterotrophic organisms, 08 autotrophic, while 10 OTUs did not present a sufficient taxonomic resolution to be classified according to their carbon source. For eukaryotes, we found 31 autotrophic OTUs, 79 heterotrophic OTUs and 12 mixotrophic OTUs. The number of OTUs estimated by rarefaction analysis for the total microbiota, taxonomically classified microbiota and trophic groups, both of prokaryotes (Fig. 1) and eukaryotes (Fig. 2), did not differ between temperature treatments (Table 2). The only exception occurred for the richness of autotrophic eukaryotes, in which temperature C presented, on average, three OTUs more than temperature PP (Fig. 2).

Warming influenced the composition of the prokaryotic and eukaryotic microbiota, considered both the total microbiota as well as the subset of the total microbiota that was taxonomically classified (Table 3). Although no concordance was found between the prokaryotic and eukaryotic total microbiota (Mantel $r = -0.02$; $P = 0.58$), we found a concordance in patterns of response to warming between the two communities when only taxonomically classified OTUs were considered (Mantel $r = 0.37$; $P = 0.005$). We found differences between temperatures C and PP, I and PP, P and PP in both classifications, although for eukaryotes the differentiation between treatments showed a clearer pattern in the NMDS ordering (Figs. 3 and 4). Considering the taxonomically classified data set, some OTUs were directly associated

Table 1
Number of OTUs and relative abundance for prokaryotes and eukaryotes microbiota found in the different scenarios of future warming, according to the taxonomic group. These values were obtained considering all replicas of temperature treatments. C = 24 °C, I = 26 °C, P = 28 °C, PP = 32 °C, N° OTU = number of OTUs, Abund = Abundance.

	C		I		P		PP	
	N° OTU	Abund (%)	N° OTU	Abund (%)	N° OTU	Abund (%)	N° OTU	Abund (%)
Prokaryotes								
Acidobacteria	2	0.017	2	0.017	2	0.05	3	0.13
Actinobacteria	5	13.7	5	19.2	4	10.3	5	5
Armatimonadetes	3	0.40	2	0.06	1	0.06	2	0.17
Bacteroidetes	10	9.2	9	12	8	4.12	9	7.1
Cyanobacteria	7	15	5	11.3	5	12.3	6	3
Planctomycetes	2	0.10	1	0.09	1	0.08	1	0.02
Proteobacteria	64	61.4	62	57.2	55	73	52	85
Spirochaetes	1	0.06	1	0.06	1	0.05	1	0.01
Verrucomicrobia	2	0.07	2	0.017	1	0.016	1	0.074
Eukaryotes								
Amoebozoa	4	0.10	7	0.05	7	0.11	8	0.11
Apusozoa	1	0.007	0	0	0	0	0	0
Bacillariophyta	1	0.014	0	0	1	0.01	1	0.003
Bicosoecida	2	1.24	2	0.48	2	8.5	2	0.42
Cercozoa	16	9	14	4.84	13	10	13	14
Charophyta	5	10	4	3.16	6	8	7	8.7
Chlorophyta	18	13.2	17	10.8	15	29	17	35.8
Choanoflagellida	0	0	0	0	0	0	1	0.016
Chrysophyta	9	28	8	10.5	7	23.5	7	14.8
Ciliophora	16	11.5	15	2.31	16	4	15	4.96
Dinoflagellata	2	1.24	1	0.79	2	0.5	3	2.32
Eustigmatophyceae	3	0.53	2	0.21	3	0.70	3	0.22
Fungi	19	25	17	66.8	17	15.5	18	18.5
Ichthyosporea	1	0.003	1	0.005	0	0	0	0
Perkinsidae	1	0.08	0	0	0	0	0	0
Peronosporomycetes	0	0	0	0	1	0.01	0	0

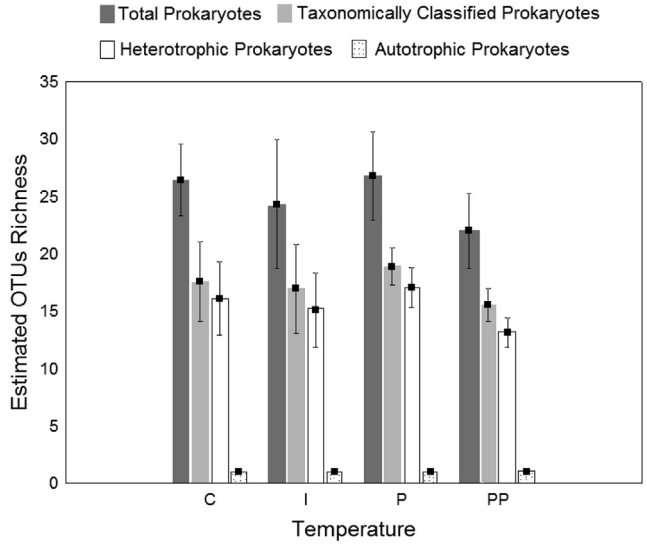

Fig. 1. OTUs richness estimated by rarefaction for prokaryotic microorganisms among different temperature treatments. The squares represent the mean value and the whiskers the ± standard deviation. C = 24 °C, I = 26 °C, P = 28 °C and PP = 32 °C.

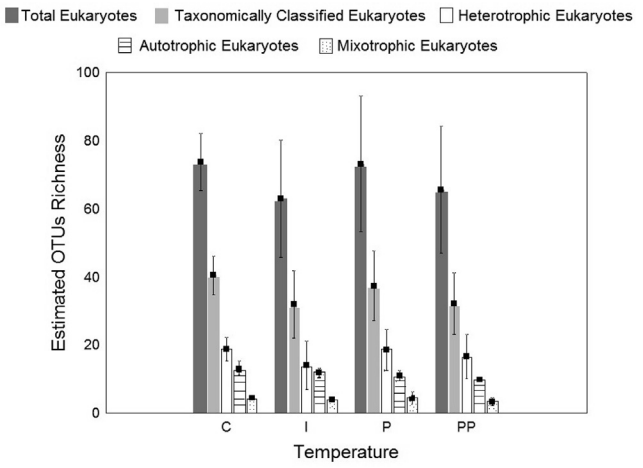

Fig. 2. OTUs richness estimated by rarefaction for eukaryotic microorganisms among different temperature treatments. The squares represent the mean value and the whiskers the ± standard deviation. C = 24 °C, I = 26 °C, P = 28 °C and PP = 32 °C.

with the PP temperature, possibly contributing to the distinction of the community composition at this temperature in relation to the others (see Tables 7S and 8S in Supplementary Material 1). Among them, we highlight the OTUs corresponding to the groups Proteobacteria and Cyanobacteria which comprehend: Rickettsiales (OTU 101, similarity 97.2%), *Pedomicrobium* sp. (OTU 145, similarity 100%), *Rhodopila* sp. (OTU 263, similarity 97.3%), *Methylobacillus* sp. (OTU 268, similarity 98.8%), Cyanophyceae (OTU 64, similarity 99.5%), *Sphingomonas* sp. (OTU 114, similarity 100%), *Acidovorax* sp. (OTU 147, similarity 100%) and *Devosia* sp. (OTU 34, similarity 100%). For eukaryotes, OTUs associated with the PP temperature refer to the Tubilinida (OTU 117, similarity 98.2%), *Spumella* sp. (OTU 90, similarity 100%), *Pseudomuriella* sp. (OTU 98, similarity 99.7%), *Orphella catalaunica* (OTU 191, similarity 98.1%), *Ochromonas sphaerocystis* (OTU 149, 100%), *Scenedesmus muspupukensis* (OTU 122, similarity 100%) and *Leptopharynx* sp. (OTU 185, similarity 99.4%).

Some of the OTUs taxonomically classified have been observed exclusively in certain temperature treatments. Among the prokaryotes, the OTUs taxonomically classified as Armatimonadales (OTU 53, similarity 99%), Neisseriaceae (OTU 130, similarity 99.7%), *Zavarzinella* sp. (OTU 150, similarity 99.7%), *Coxiella* sp. (OTU 229, similarity

Table 2
Analysis of Variance (ANOVA) performed between the OTUs richness estimated by rarefaction and the temperature treatments. The numbers in parentheses indicate the degree of freedom for each test. Pairwise comparisons by the Tukey test were performed for situations in which ANOVA presented significant results ($P < 0.05$). N.A. = Not applicable; SS = Sum of squares; MS = Mean of squares. The bold values indicate significant results ($P < 0.05$).

	SS	MS	F	*P*	Tukey Teste
Total prokaryotes$_{(3,15)}$	70.6	23.5	1.37	0.28	N.A.
Total eukaryotes$_{(3,15)}$	411	136.9	0.46	0.70	N.A.
Taxonomically classified prokaryotes$_{(3,15)}$	28.9	9.6	1.25	0.32	N.A.
Taxonomically classified eukaryotes$_{(3,15)}$	237.7	79.2	0.95	0.43	N.A.
Autotrophic prokaryotes $_{(3,15)}$	1.84^{e-31}	6.16^{e-32}	1.31	0.30	N.A.
Heterotrophic prokaryotes$_{(3,15)}$	41.0	13.6	2.25	0.12	N.A.
Autotrophic eukaryotes$_{(3,15)}$	27.0	9.0	4.0	**0.02**	C = I, C = P, C ≠ PP, I = P, I = PP, P = PP
Heterotrophic eukaryotes$_{(3,15)}$	68.0	22.6	0.62	0.61	N.A.
Mixotrophic eukaryotes$_{(3,15)}$	4.6	1.5	0.96	0.43	N.A.

Table 3
Analysis of Permutational Variance (PERMANOVA) for OTUs composition of prokaryotes, eukaryotes and trophic groups. DF = Degrees of freedom. The bold values indicate significant results ($P < 0.05$).

	DF	R^2	F	*P*
Total prokaryotes	3,15	0.23	1.50	**0.002**
Total eukaryotes	3,15	0.24	1.64	**0.001**
Taxonomically classified prokaryotes	3,15	0.21	1.35	**0.01**
Taxonomically classified eukaryotes	3,15	0.25	1.69	**0.001**
Autotrophic prokaryotes	3,15	0.06	0.36	0.96
Heterotrophic prokaryotes	3,15	0.21	1.40	**0.01**
Autotrophic eukaryotes	3,15	0.18	1.10	0.33
Mixotrophic eukaryotes	3,15	0.32	2.35	**0.01**
Heterotrophic eukaryotes	3,15	0.26	1.81	**0.001**

97.2%) and *Chromobacterium* sp. (OTU 257, similarity 100%) were exclusive to temperature C; Fimbriimonadaceae (OTU 137, similarity 99.5%) for temperature I; no OTU occurred exclusively in temperature P, while *Acidocella* sp. (OTU 190, similarity 98.6%), Acidimicrobiaceae (OTU 241, similarity 98.2%) and *Rhodopila* sp. (OTU 263, similarity 97.3%) occurred only in the PP temperature. Among eukaryotes, the OTUs represented by *Carchesium polypinum* (OTU 114, similarity 99.1%), *Parvilucifera* sp. (OTU 157, similarity 100%), *Ancyromonas* sp. (OTU 310, similarity 99.4%) occurred only in temperature C; no OTU was exclusive to temperature I; *Aphanomyces* sp. (OTU 231, similarity 97.1%) was exclusive to temperature P and *Ophrydium versatile* (OTU 88, similarity 99.8%), *Salpingoeca* sp. (OTU 166, similarity 98.2%), *Mougeotia* sp. (OTU 266, similarity 100%), *Cladochytrium replicatum* (OTU 325, similarity 100%) and *Leptomyxa reticulata* (OTU 348, similarity 98.3%) exclusive to temperature PP.

For the prokaryotic trophic groups, warming had effect only on heterotrophic organisms (Table 3), but these differences in OTU composition were only observed between temperatures I and PP (Fig. 3). The main OTUs associated with temperature I were Sporichthyaceae (OTU 43, similarity 100%), *Rhodovarius* sp. (OTU 39, similarity 99.7%), *Bdellovibrio* sp. (OTU 20, similarity 100%), Hyphomonadaceae (OTU 238, similarity 99.7%), *Sediminibacterium* sp. (OTU 73, similarity 97.8%), while at the temperature PP, the OTUs were *Ralstonia* sp. (OTU 149, similarity 99.7%), *Pedomicrobium* sp. (OTU 145, similarity 100%), Rickettsiales (OTU 101, similarity 97.2%), *Methylobacterium* sp. (OTU 158, similarity 100%; OTU 26, similarity 100%), *Acidovorax* sp. (OTU 147, similarity 100%), *Elstera* sp. (OTU 54, similarity 99.7%), Cytophagaceae (OTU 221, similarity 99%), *Sphingomonas* sp. (OTU 114, similarity 100%), *Roseomonas* sp. (OTU 12, similarity 97.7%) and *Bradyrhizobium* sp. (OTU 112, 100%).

For eukaryotes, warming affected the heterotrophic and mixotrophic organisms (Table 3). For the heterotrophic microbiota, these differences occurred between temperatures C and P, C and PP, I and PP, P and PP, whereas for mixotrophic, the differences were evidenced between temperature C and the others temperatures (Fig. 4). Among

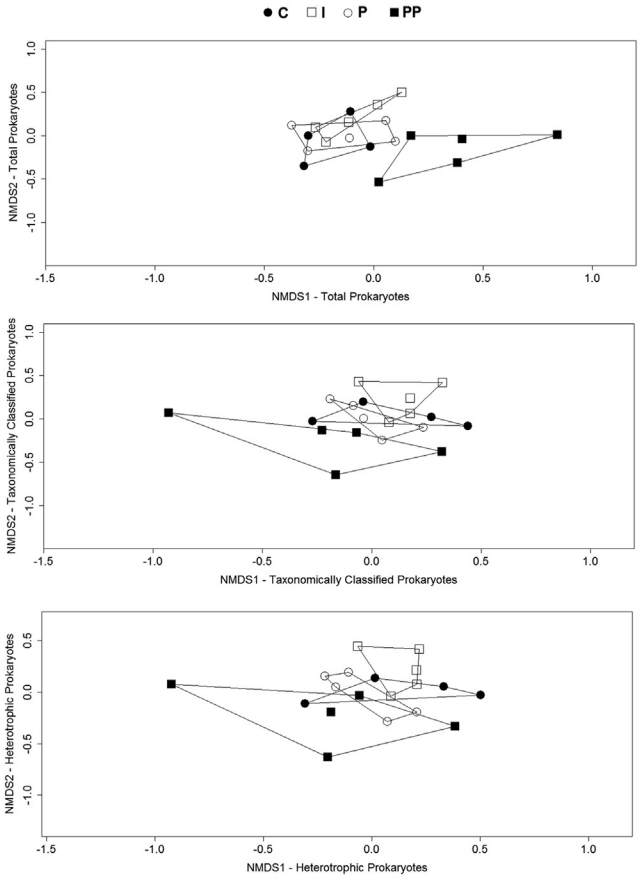

Fig. 3. Non-metric Multidimensional Scaling Analysis (NMDS) for total composition of prokaryotic OTUs and trophic groups. Here, we considered only the biological classifications that showed significant differences in the PERMANOVA. Stress for total prokaryotes = 0.166; Stress for taxonomically classified prokaryotes 0.189; Stress for heterotrophic prokaryotes = 0.188. The symbols indicate the temperature, thus C = 24 °C, I = 26 °C, P = 28 °C and PP = 32 °C.

the heterotrophic organisms, the main OTUs associated with temperature C were *Rhogostoma* sp. (OTU 106, similarity 97.4%), Cryptomycota (OTU 152, similarity 98.3%; OTU 313, similarity 100%). In temperature I we found mainly: *Paramicrosporidium* sp. (OTU 204, similarity 98.3%), *Bodomorpha* sp. (OTU 160, similarity 100%), *Rhogostoma* sp. (OTU 96, similarity 99.7%), *Hemiophrys procera* (OTU 20, similarity 97%), *Spongomonas* sp. (OTU 53, similarity 98.2%), *Loxophyllum* sp. (OTU 43, similarity 100%). For the temperature P, we highlight: *Cryptodifflugia operculata* (OTU 136, similarity 100%), Cercozoa (OTU 94, similarity 99.4%), Cryptomycota (OTU 28, similarity 100%), Bicosoecida (OTU 16, similarity 100%), *Pseudodifflugia gracilis* (OTU 36, similarity 99.2%), while for PP temperature, Tubilinida (OTU 117,

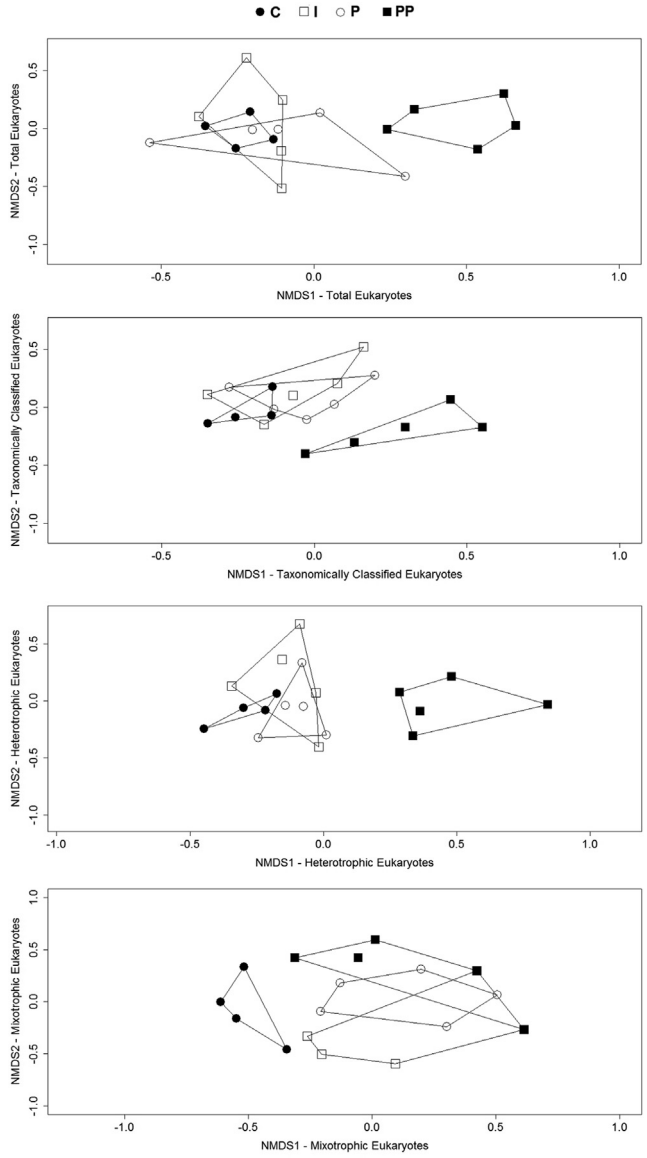

Fig. 4. Non-Metric Multidimensional Scaling analysis (NMDS) for total composition of eukaryotic OTUs and trophic groups. Here, we considered only the biological classifications that showed significant differences in the PERMANOVA. Stress for total eukaryotes = 0.171; Stress for taxonomically classified eukaryotes = 0.189; stress for heterotrophic eukaryotes = 0.196; stress for mixotrophic eukaryotes = 0.145. The symbols indicate the temperature, thus C = 24 °C, I = 26 °C, P = 28 °C and PP = 32 °C.

similarity 98.2%), *Paramicrosporidium* sp. (OTU 332, similarity 100%), Cryptomycota (OTU 201, similarity 97.8%) and Cercozoa (OTU 327, similarity 97.7%). For mixotrophic organisms, the main OTUs associated with temperature C, allowing their differentiation from the other treatments were *Lagynion scherffelii* (OTU 283, similarity 99.4%) and Chromulinales (OTU 85, similarity 97.6%), both belonging to the class Chrysophyceae.

4. Discussion

It is relevant to predict how microorganisms respond to variations in temperature, especially due to the numerous environmental disturbances currently observed and expected by the end of the century (Menden-Deuer et al., 2018). In this study, we evaluated the warming effects on different biological groups and trophic levels for planktonic microbiota using DNA metabarcoding in the experimental approach. We did not observe differences in OTUs richness between different temperatures for most of the classifications adopted. However, we found that the increase in temperature influences both prokaryotic and eukaryotic microorganisms, altering the patterns of community composition in a pessimistic warming scenario. In addition, bacteria and microeukaryotes have a consistent pattern of response. The effects of warming seem to be more severe on the mixotrophic eukaryotes, since differences in composition have already been observed with the increase of 2 °C. For heterotrophic organisms, the differences were observed only between the extreme temperature PP and the others temperatures. However, we did not observe effects of temperature increase on both autotrophic eukaryotes and prokaryotes composition.

Overall, the total number of OTUs found for both eukaryotic and prokaryotic organisms can be considered low compared to other studies conducted in natural environments (Machado et al., 2019; Tandon et al., 2018), artificial (Lee et al., 2016) and experimental (e.g. Smale et al., 2017; Stefanidou et al., 2018). This may be due collection in a single lake to fill the microcosms. This lake was resulted of damming part of the Barreiro stream channel, a small stream that runs through the State University of Goiás campus (Curado and Angeline, 2006). This lake has a small size, low depth and therefore can naturally harbor a low diversity of OTUs. Another hypothesis that can be used to explain this low diversity is experimental manipulation. When exposed the experimental conditions, some OTUs, especially those that occur naturally at low frequency, may be lost or not detected. However, this is an effect that occurs for both warm and control treatments, since the samples for filling were obtained in the same place and exposed the same environmental conditions.

Temperature is a limiting factor for most aquatic organisms, determining important ecological processes in communities, such as primary productivity (e.g. Häder et al., 2014; Yvon-Durocher et al., 2015), decomposition (e.g. Geraldes et al., 2012), respiration (e.g. Hoppe et al., 2008; Panigrahi et al., 2013) and carbon cycle (e.g. Wohlers et al., 2009; Yvon-Durocher et al., 2010). In our experiment, we observed no differences in OTU richness between temperatures for most of the biological groups and trophic levels evaluated, except for the richness of autotrophic eukaryotes. This indicates that warming probably did not lead to a change in the number of OTUs that could reflect in the richness, but rather their substitution promoting the permanence of those who represent groups more adapted to high temperatures. Another factor that can be considered is the presence of dormant, dead or lysed cells. Since the amplicon sequencing will cover all of the previous mentioned DNA types, changes in diversity may not be captured. However, our initial premise is confirmed when we evaluated community composition, in which the responses were specific to each taxonomic group, with some OTUs occurring in only a few temperatures.

In our study, the major taxonomic groups were not found exclusively at a single temperature level. The OTUs corresponding to Proteobacteria occurred in all treatments for prokaryotes, while eukaryotes were represented mainly by OTUs attributed to Chlorophyta and Fungi. This indicates that changes in the composition may have occurred due to the presence of specific taxa (e.g. Lindh et al., 2013; Bergen et al., 2016). In fact, some prokaryotic and eukaryotic OTUs occurred exclusively in the PP treatment or were more associated with this temperature, which may have contributed to differentiate this treatment from the others. Many of these OTUs represent taxa that can tolerate high temperatures, such as *Sphingomonas* sp. (Feng et al., 2014) and *Methylobacillus* sp. (Kaparullina et al., 2017) or that has growth positively associated with warming such as *Spumella* sp. (Weisse, 1997). In addition, among the OTUs found in the PP treatment, there are some taxa capable of causing diseases in humans, such as prokaryotes of the order Ricktissiales (Schrallhammer et al., 2013) and the genus *Sphingomonas* (Beaino et al., 2018). Thus, besides to generating ecological consequences with the substitution of species, warming predicted for the next decades can also bring threats to human health and well-being

through the proliferation of pathogenic organisms favored by warming (Ichiro Kurame, 2010).

Although some studies have already demonstrated the effect of warming on primary producers (e.g. Domaizon et al., 2012; Yvon-Durocher et al., 2015; Pulina et al., 2016; Rasconi et al., 2017), we did not observe differences for the autotrophic organism composition in any of the biological classifications. In all treatments, autotrophic eukaryotes were represented mainly by Chlorophyceae while autotrophic prokaryotes by Cyanobacteria. Previous studies indicate that the optimal temperature for the growth of these two groups is very similar, especially in tropical regions (Thomas et al., 2016). Cyanobacteria have optimal growth between 25 °C and 35 °C while Chlorophyceae grows between 27.5 °C and 35 °C (Lürling et al., 2013). Thus, the two groups seem to exhibit a thermal tolerance that allows them to survive at both high and mild temperatures, contributing to the absence of effects of the different temperatures used in our experiment.

On the other hand, the heterotrophic organisms had their composition influenced by temperature increase for both prokaryotes and eukaryotes. In fact, the heterotrophic processes must be more affected by the increase in temperature than the autotrophic. (e.g. Hoppe et al., 2008; Panigrahi et al., 2013; Von Scheibner et al., 2014; Huete-Stauffer et al., 2018). Due to the linkage of heterotrophic organisms to other communities within the food chain, changes in their composition can have serious consequences for food webs (Domaizon et al., 2012), leading, for example, to an increase in consumption within the ecosystems (Yang et al., 2016). These organisms are responsible for organic matter remineralization and CO_2 recycling through respiration (Hoppe et al., 2008). Thus, changes in their composition can have consequences for ecosystem functions such as decomposition and nutrient cycling.

In our study, we found mixotrophic representatives only for eukaryotes and this was the most sensitive trophic level, with differences in composition evidenced between treatments C and I. This trophic level was represented mainly by OTUs classified as Chrysophyta (about 75% of the mixotrophic OTUs), whose optimum temperature for growth is around 10 to 25 °C, depending on the latitude (Thomas et al., 2016). All the temperatures used in our experiment were above the optimal temperature for the growth of this group, thus justifying an alteration in the mixotrophic composition with an increment of only 2 °C in comparison to the control temperature.

Mixotrophic organisms combine the photoautotrophic and heterotrophic habit for the production or consumption of organic matter (Flynn et al., 2013). The occurrence of primary or secondary productivity depends on both temperature and light availability in the environment (e.g. Princiotta et al., 2016). Thus, in most aquatic ecosystems with sufficient light availability, there is likely to be a continuum between the two habits (Flynn et al., 2013). Experimental evidence indicates that warming promotes the predominance of a heterotrophic behavior in mixotrophic (Wilken et al., 2013; Wilken et al., 2018). Although our experiment was not designed to quantify these changes of habits, we can suggest that due to the plasticity to obtain organic matter, small changes in temperature might be able to influence the mixotrophic microbiota composition. However, future studies evaluating these changes at the community level and regarding the influence of other environmental factors are needed to better understand the effects of warming on this trophic level.

An important result was the similar response obtained between the prokaryotic and eukaryotic microbiota composition classified taxonomically to warming. The two biological levels showed species substitution between the PP temperature and the others temperatures, although the eukaryotic organisms showed a clearer differentiation pattern in the NMDS. Evidence indicates that eukaryotes have a lower thermal tolerance in comparison to prokaryotes (Clarke, 2014). This fact may be due to the biological characteristics of each group. Bacteria emerged and evolved in a period in which the earth was subjected to high temperatures (Oschmann et al., 2002) and nowadays, there are representatives of bacteria that can survive in extreme temperatures (Clarke, 2014). On the other hand, microeukaryotes are composed of numerous taxonomic groups (Pawlowski, 2014) that have different thermal tolerances. This indicates a similar response between the two levels, yet the substitution pattern probably did not occur at the same intensity between the biological groups.

Understanding the responses of different taxonomic and trophic groups to temperature increases is a crucial factor for predicting the community's dynamics in a global warming scenario (Smale et al., 2017). The climate changes simulated here represent a change in temperature that must occur over decades and thus, the time scale evaluated may not be sufficient to determine the adaptation of microorganisms to high temperatures (e.g. Bergen et al., 2016). However, extreme events in temperature can be used to predict the effects of heatwaves (e.g. Audet et al., 2017), since the warming forecast for the future should not only increase average temperatures, but also intensify the occurrence of summers more hots, and increase number of days with maximum temperature (IPCC, 2014). In fact, heatwaves and other extreme events have already been verified for Brazil (Orlowsky and Seneviratne, 2012; Bitencourt et al., 2016; Geirinhas et al., 2018). Furthermore, the experiments provide a link between the theory and natural ecosystems in which the conditions (e.g. temperature effect) are simulated in a simplified way, but with a high level of control (Altermatt et al., 2015). Therefore, our experiment simulates the shortterm effect of warming, contributes to predicting how the aquatic microbiota should be affected by global warming and also indicates a similar response pattern among the different trophic levels.

5. Conclusions

In general, our results indicate that the temperature increase should affect different levels of biological organization, altering the heterotrophic and mixotrophic microbiota composition. Prokaryotes and eukaryotes have a similar response to warming, but the change patterns do not occur at the same intensity. This probably occurred because, even though the biological levels respond to warming, the pattern of species substitution between different trophic levels and biological classifications occurred differently. This study highlights the temperature importance in structuring the communities of different groups and reveals similar patterns of response between them. Thus, our results contribute to predict the warming effects at different levels of biological organization and trophic groups, especially for continental aquatic ecosystems in tropical regions that, have vast biodiversity but are still poorly studied.

6. Data archiving

The sequences used in this study were deposited in the GenBank's Sequence Reads Archive database (https://submit.ncbi.nlm.nih.gov/) under the access number SUB5769264 for Eukaryotes and SUB5772232 for prokaryotes (Bioproject Accession PRJNA548125; BioSample Accession: SAMN12003851 - SAMN12003869 for microeukaryotes and SAMN12004256 – SAMN12004274 for bacteria).

Credit authorship contribution statement

Karine Borges Machado: Conceptualization, Writing - original draft, Methodology, Formal analysis, Investigation, Writing - review & editing. **Adriana Maria Antunes:** Methodology, Formal analysis, Writing - review & editing. **Cíntia Pelegrineti Targueta:** Methodology, Formal analysis, Writing - review & editing. **Jordana Gontijo Fernandes:** Methodology, Formal analysis, Writing - review & editing. **Thannya Nascimento Soares:** Conceptualization, Methodology, Writing - review & editing, Resources. **João Carlos Nabout:** Conceptualization, Writing - original draft, Investigation, Writing - review & editing, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ecolind.2020.106452.

References

Altermatt, F., Fronhofer, E.A., Garnier, A., Giometto, A., Hammes, F., Klecka, J., Legrand, D., Mächler, E., Massie, T.M., Pennekamp, F., Peblani, M., Pontarp, M., Schtickzelle, N., Thuillier, V., Petchey, O.W., 2015. Big answers form small worlds: a useŕs guide for protist microcosms as a model system in ecology and evolution. Methods Ecol. Evol. 6, 218–231. https://doi.org/10.1111/2041-210X.12312.

Altschul, S.F., Gish, W., Miller, W., Myers, E.W., Lipman, D.J., 1990. Basic local alignment seash tool. J. Mol. Biol. 215, 403–410.

Anderson, M.J., 2006. Distance-based tests for homogeneity of multivariate dispersions. Biometrics 62, 245–253. https://doi.org/10.1111/j.1541-0420.2005.00440.x.

Andrews, S., 2010. FastQC: a quality control tool for high throughput sequence data. http://www.bioinformatics.babraham.ac.uk/projects/fastqc (Accessed 07 December 2017).

Audet, J., Neif, E.M., Cao, Y., Hoffmann, C.C., Lauridsen, T.L., Larsen, S.E., Søndergaard, M., Jeppesen, E., Davidson, T.A., 2017. Heat-wave effects on greenhouse gas emissions from shallow lake mesocosms. Freshwater Biol. 62, 1130–1142. https://doi.org/10.1111/fwb.12930.

Beaino, M.E., Fares, J., Malek, A., Hachem, R., 2018. Sphingomonas paucimobilis-related bone and soft-tissue infections: a systematic review. Int. J. Infect. Dis. 77, 68–73. https://doi.org/10.1016/j.ijid.2018.09.021.

Bergen, B., Endres, S., Engel, A., Zark, M., Dittmar, T., Sommer, U., Jürgens, K., 2016. Acidification and warming affect prominent bacteria in two seasonal phytoplankton bloom mesocosms. Environ. Microbiol. 18, 4579–4595. https://doi.org/10.1111/1462-2920.13549.

Bergkemper, V., Stadler, P., Weisse, T., 2018. Moderate weather extremes alter phytoplankton diversity - a microcosm study. Freshwater Biol. 63, 1211–1224. https://doi.org/10.1111/fwb.13127.

Bitencourt, D.P., Fuentes, M.V., Maia, P.A., Amorin, F.T., 2016. Frequência, duração, abrangência espacial e intensidade das ondas de calor no Brasil. Rev. Bras. Meteorol. 31, 506–517. https://doi.org/10.1590/0102-778631231420150077.

Bolger, A.M., Lohse, M., Usadel, B., 2014. Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics 30, 2114–2120. https://doi.org/10.1093/bioinformatics/btu170.

Brown, J.H., Gillooly, J.F., Allen, A.P., Savage, V.M., West, G.B., 2004. Toward a metabolic theory of ecology. Ecology 87, 1771–1789. https://doi.org/10.1890/03-9000.

Caissie, D., 2006. The thermal regime of rivers: a review. Freshwater Biol. 51, 1389–1406. https://doi.org/10.1111/j.1365-2427.2006.01597.x.

Chen, M., Chen, F., Yu, Y., Ji, J., Kong, F., 2008. Genetic diversity of eukaryotic microorganism in Lake Taihu, a large shallow subtropical lake in China. Microb. Ecol. 56, 572–583. https://doi.org/10.1007/s00248-008-9377-8.

Clarke, A., 2014. Thermal limits to life on Earth. Int. J. Astrobiol. 12, 141–154. https://doi.org/10.1017/S1473550413000438.

Clements, C.F., Warren, P.H., Collen, B., Blackburn, T., Worsfold, N., Petchey, O., 2013. Interactions between assembly order and temperature can alter both short and long-term community composition. Ecol. Evol. 3, 5201–5208. https://doi.org/10.1002/ece3.901.

Curado, P.M., Angeline, R., 2006. Avaliação de atividade de educação ambiental em trilha interpretativa, dois a três anos após sua realização. Acta Sci. Biol. Sci. 28, 395–401.

Deiner, K., Bik, H.M., Mächler, E., Seymour, M., Lacoursière-Roussel, A., Altermatt, F., Creer, S., Bista, L., Lodge, D.M., Vere, N., Pfrender, M.E., Bernatchez, L., 2017. Environmental DNA metabarcoding: transforming how we survey animal and plant communities. Mol. Ecol. 26, 5872–5895. https://doi.org/10.1111/mec.14350.

Domaizon, I., Lepère, C., Debroas, D., Bouvy, M., Ghiglione, J.F., Jacquet, S., Bettarel, Y., Bouvier, C., Torréton, J.P., Vidussi, F., Mostajir, B., Kirkham, A., LeFloc'h, E., Fouilland, E., Montanié, H., Bouvier, T., 2012. Shrotterm responses of unicellular planktonic eukaryotes to increases in temperature and UVB radiation. BMC Microbiol. 12, 202. https://doi.org/10.1186/1471-2180-12-202.

Dudgeon, D., Arthington, A.H., Gessner, M.O., Kawabata, Z.I., Knowler, D.J., Lévêque, C., Naiman, R.J., Prieur-Richard, A.H., Soto, D., Stiassny, M.L.J., Sullivan, C.A., 2006. Freshwater biodiversity: importance, threats, status and conservation challenges. Biol. Rev. 81, 163–182. https://doi.org/10.1017/S1464793105006950.

Edgar, R.C., 2013. UParse: highly accurate out sequences from microbial amplicon reads. Nat. Methods 10, 996–998.

Ekvall, M.K., Hanson, L.A., 2012. Differences in recruitment and life-history strategy alter zooplankton spring dynamics under climate-change conditions. PLoS One 7, e44614. https://doi.org/10.1371/journal.pone.0044614.

Feng, G.D., Yang, S.Z., Wang, Y.H., Zhang, X.X., Zhao, G.Z., Deng, M.R., Zhu, H.H., 2014. Description of a gram-negative bacterium, Sphingomonas guangdongensis sp. nov. Int. J. Syst. Evol. Microbiol. 64, 1697–1702. https://doi.org/10.1099/ijs.0.056853-0.

Flynn, K.J., Stoecker, D., Mitra, A., Raven, J.A., Glibert, P.M., Hansen, P.J., Granéli, E., Brukholder, J.M., 2013. Minuse of the phytoplankton-zooplankton dichotomy: the need to assign organisms as mixotrophs within plankton functional types. J. Plankton Res. 35, 3–11. https://doi.org/10.1093/plankt/fbs062.

Fujimoto, M., Cavaletto, J., Liebig, J.R., McCarthy, A., Vanderploeg, H.A., Denef, V.J., 2016. Spatiotemporal distribution of bacterioplankton functional groups along a freshwater estuary to pelagic gradient in Lake Michigan. J. Great Lakes Res. 42, 1036–1048. https://doi.org/10.1016/j.jglr.2016.07.029.

Geirinhas, J.L., Trigo, R.M., Libonati, R., Coelho, C.A.S., Palmeira, A.C., 2018. Climate and synoptic characterization of heat waves in Brazil. Int. J. Climatol. 38, 1760–1776. https://doi.org/10.1002/joc.5294.

Geraldes, P., Pascoal, C., Cássio, F., 2012. Effects of increased temperature and aquatic fungal diversity on litter decomposition. Fungal Ecol. 5, 734–740. https://doi.org/10.1016/j.funeco.2012.05.007.

Golterman, H.L., Clymo, R.S., Ohnstad, M.A.M., 1978. Methods for Physical and Chemical Analysis of Freshwaters. Blackwell Scientific Publication, Oxford.

Guo, C., Zhu, G., Qin, B., Zhang, Y., Zhu, M., Xu, H., Chen, Y., Paerl, H.W., 2019. Climate exerts a greater modulating effect on the phytoplankton community after 2007 in eutrophic lake Taihu, China: evidence from 25 years of recordings. Ecol. Ind. 105, 82–91. https://doi.org/10.1016/j.ecolind.2019.05.034.

Häder, D.P., Villafañe, V.E., Helbling, W., 2014. Productivity of aquatic primary producers under global climate change. Photochem. Photobiol. Sci. 13, 1370–1392. https://doi.org/10.1039/C3PP50418B.

Hayhoe, K., Edmonds, J., Kopp, R.E., LeGrande, A.N., Sanderson, B.M., Wehner, M.F., Wuebbles, D. J., 2017. Climate models, scenarios, and projections. In: Wuebbles, D. J., Fahey, D.W., Hibbard, K. A., Dokken, D. J., Stewart, B.C., Maycock, T.K. (Eds.), Climate Science Special Report: Fourth National Climate Assessment, Volume I, Global Change Research Program, Washington, DC, USA, pp. 133-160.

Hoppe, H.G., Breithaupt, P., Walther, K., Koppe, R., Bleck, S., Sommer, U., Jürgens, K., 2008. Climate warming in winter affects the coupling between phytoplankton and bacteria during the spring bloom: a mesocosm study. Aquat. Microb. Ecol. 51, 105–115. https://doi.org/10.3354/ame01198.

Huete-Stauffer, T.M., Arandia-Gorostidi, N., González-Benítez, N., Díaz-Pérez, L., Calvo-Díaz, A., Morán, X.A.G., 2018. Large plankton enhance heterotrophy under experimental warming a temperate coastal ecosystem. Ecosystems 21, 1139–1154.

Huggett, M.J., McMahon, K., Bernasconi, R., 2018. Future warming and acidification result in multiple ecological impacts to a temperate coralline alga. Environ. Microbiol. 28, 2769–2782. https://doi.org/10.1111/1462-2920.14113.

Hurlbert, S.H., 1971. The nonconcept of species diversity: a critique and alternative parameters. Ecology 52, 577–586.

Ichiro Kurame, M.D., 2010. The effect of global warming on infectious diseases. Public Health Res. Perspect. 1, 4–9.

IPCC, 2014. Climate change 2014: Synthesis report. Contribution of Working Groups I, II and III to the Fifth Assessment Report of IPCC. Switzerland, Geneva.

Jeppesen, E., Moss, B., Bennion, H., Carvalho, L., DeMeester, L., Feuchtmayr, H., Friberg, N., Gessner, M.O., Hefting, M., Lauridsen, T.L., Liboriussen, L., Malmquist, H.J., May, L., Meerhoff, M., Olafsson, J.S., Soons, M.B., Verhoeven, T.A., 2010. Interaction of climate change and eutrophication. In: Kernan, M., Battarbee, R. W., Moss, B. Climate change impacts on freshwater ecosystems. Blackwell Publishing, Hoboken, pp. 120-151.

Jeppesen, E., Meerhoff, M., Davidson, T.A., Trolle, D., Søndergaard, M., Lauridsen, T.L., Beklioglu, M., Brucet, S., Volta, P., Gonzalez-Bergonzoni, I., Nielsen, A., 2014. Climate change impacts on lakes: an integrate ecological perspective based on a multi-faceted approach, with special focus on shallow lakes. J. Limnol. 73, 84–107.

Kaparullina, E.N., Trotsenko, Y.A., Doronina, N.V., 2017. Methylobacillus methanolivorans sp. nov., a novel non-pigmented obligately methylotrophic bacterium. Int. J. Syst. Evol. Microbiol. 67, 425–431. https://doi.org/10.1099/ijsem.0.001646.

Khomich, M., Kauserud, H., Logares, R., Rasconi, S., Andersen, T., 2017. Planktonic protistan communities in lakes along a large-scale environmental gradient. Microbiol. Ecol. 93, fiw231. https://doi.org/10.1093/femsec/fiw231.

Klindworth, A., Pruesse, E., Schweer, T., Peplies, J., Quast, C., Horn, M., Glockner, F.O., 2013. Evaluation of general 16S ribosomal RNA gene PCR primers for classical and next-generation sequencing-based diversity studies. Nucl. Acids Res. 41, e1. https://doi.org/10.1093/nar/gks808.

Lara, E., Arrieta, J.M., Garcia-Zarandona, I., Boras, J.A., Duarte, C.M., Agustí, S., Wassmann, P.F., Vaqué, D., 2013. Experimental evaluation of the warming effect on viral, bacterial and protistan communities in two contrasting Arctic systems. Aquat. Microb. Ecol. 70, 17–32. https://doi.org/10.3354/ame01636.

Lee, C.S., Kim, M., Lee, C., Yu, Z., Lee, J., 2016. The microbiota of recreational freshwaters and the implications for environmental and public health. Front. Microbiol. 7, 1826. https://doi.org/10.3389/fmicb.2016.01826.

Legendre, P., Legendre, L., 1998. Numerical Ecology. Elsevier Science, Amsterdam.

Lima-Ribeiro, M.S., Varela, S., González-Hernández, J., Oliveira, G., Diniz-Filho, J.A.F., Terribile, L.C., 2015. EcoClimate: a database of climate data from multiple models for past, present, and future for Macro ecologists and Biogeographers. Biodivers. Inf. 10, 1–21. https://doi.org/10.17161/bi.v10i0.4955.

Lindh, M.V., Riemann, L., Baltar, F., Romero-Oliva, C., Salomon, P.S., Granéli, E., Pinhassi, J., 2013. Consequences of increased temperature and acidification on bacterioplankton community composition during a mesocosm spring Bloom in the Baltic Sea. Environ. Microbiol. Rep. 5, 252–262. https://doi.org/10.1111/1758-2229.12009.

Lürling, M., Eshetu, F., Faassen, E.J., Kosten, S., Huszar, V.L.M., 2013. Comparison of cyanobacterial and green algal growth rates at different temperatures. Freshwater Biol. 58, 552–559. https://doi.org/10.1111/j.1365-2427.2012.02866.x.

Machado, K.B., Targueta, C.P., Antunes, A.M., Soares, T.N., Telles, M.P.C., Logares, R., Vieira, L.C.G., Huszar, V.L.M., Nabout, J.C., 2019. Diversity patterns of planktonic microeukaryote communities in tropical floodplain lakes based on 18S rDNA gene sequence. J. Plankton Res. 41, 241–256.

Machado, K.B., Vieira, L.C.G., Nabout, J.C., 2019a. Predicting the dynamics of taxonomic and functional phytoplankton compositions in different global warming scenarios. Hydrobiologia 830, 115–134. https://doi.org/10.1007/s10750-018-3858-7.

Massana, R., Logares, R., 2013. Eukaryotic versus prokaryotic marine picoplankton ecology. Environ. Microbiol. 15 (5), 1254–1261. https://doi.org/10.1111/1462-2920.12043.

Maugendre, L., Gattuso, J.P., Louis, J., Kluijver, A., Marro, S., Soetaert, K., Gazeau, F., 2015. Effect of ocean warming and acidification on a plankton community in the NW Mediterranean Sea. J. Mar. Sci. 72, 1744–1755. https://doi.org/10.1093/icesjms/fsu161.

McKee, D., Atkinson, D., Collings, S., Eaton, J., Wolstenholme, L., Moos, B., 2000. Heated aquatic microcosms for climate change experiments. Freshwater Forum 14, 51–58.

Medinger, R., Nolte, V., Pandey, R.V., Jost, S., Ottenwälder, B., Schlötterer, C., Boenigk, J., 2010. Diversity in a hidden word: potential and limitation of next-generation sequencing for surveys of molecular diversity of eukaryotic microorganisms. Mol. Ecol. 19, 32–40. https://doi.org/10.1111/j.1365-294X.2009.04478.x.

Menden-Deuer, S., Lawrence, C., Franzé, G., 2018. Herbivorous protist growth and grazing rates at in situ and artificially elevated temperatures during an Arctic phytoplankton spring bloom. Peer J. 6, e5264. https://doi.org/10.7717/peerj.5264.

Moore, R.D., Spittlehouse, D.L., Story, A., 2005. Riparian microclimate and stream temperature response to forest harvesting: a review. J. Am. Water Resour. Assoc. 41, 813–834. https://doi.org/10.1111/j.1752-1688.2005.tb03772.x.

Moss, R., Babiker, M., Brinkman, S., Calvo, E., Cater, T., Edmonds, J., Elgizouli, I., Emori, S., Erda, L., Hibbard, K., Jones, R., Kainuma, M., Kelleher, J., Lamarque, J. F., Manning, M., Matthews, B., Meehl, J., Meyer, L., Mitchell, J., Nakicenovic, N., O'Neill, B., Pichs, R., Riahi, K., Rose, S., Runci, P., Stouffer, R., Vuuren, D. V., Weyant, J., Wilbanks, T., Ypersele, J. P. V., Zurek, M., 2008. Towards new scenarios for analysis of emissions, climate change, impacts and response strategies. Technical Summary. Intergovernmental Panel on Climate Change, Geneva.

Moustaka-Gouni, M., Kormas, K.A., Scotti, M., Vardaka, E., Sommer, U., 2016. Warming and acidification effects on planktonic heterotrophic pico-and nanoflagellates in a mesocosm experiment. Protist 167, 389–410. https://doi.org/10.1016/j.protis.2016.06.004.

Oksanen, J., Blanchet, F. G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., Minchin, P. R., O'Hara, R. B., Simpson, G. L., Solymos, P., Stevens, M. H. H., Szoecs, E., Wagner, H., 2019. Vegan: Community Ecology Package. R Package Version 2.5-5. http:// CRAN.R-project.org/package = vegan.

Orlowsky, B., Seneviratne, S.I., 2012. Global changes in extreme events: regional and seasonal dimension. Clim. Change 110, 669–696. https://doi.org/10.1007/s10584-011-0122-9.

Oschmann, W., Grasshof, M., Gudo, M., 2002. The early evolution on the planet earth and the origin of life. Senckenb. lethaea. 82, 285-294.

Pajares, S., Eguiarte, L.E., Bonilla-Rosso, G., Souza, V., 2013. Drastic changes in aquatic bacterial populations from the Cuatro Cienegas basin (Mexico) in response to long-term environmental stress. Antonie van Leeuwenhoek 104, 1159–1175. https://doi.org/10.1007/s10482-013-0038-7.

Panigrahi, S., Nydal, A., Anton, P., Wikner, J., 2013. Strong seasonal effect of moderate experimental warming on plankton respiration in a temperate estuarine plankton community. Estuar. Coast. Shelf Sci. 135, 269–279. https://doi.org/10.1016/j.ecss.2013.10.029.

Pawlowski, J., 2014. Protist evolution and phylogeny. eLS John Wiley & Sons. DOI:10.1002/9780470015902.a0001935.pub2.

Piwosz, K., Calkiewicz, J., Golebiewski, M., Greer, S., 2018. Diversity and community composition of pico and nanoplanktonic protists in the Vistula River estuary (Gulf of Gdánsk, Baltic Sea). Estuar. Coast. Shelf Sci. 207, 242–249. https://doi.org/10.1016/j.ecss.2018.04.013.

Princiotta, S.D., Smith, B.T., Sanders, R.W., 2016. Temperature-dependent phagotrophy and phototrophy in a mixotrophic chrysophyte. J. Phycol. 52, 432–440. https://doi.org/10.1111/jpy.12405.

Pulina, S., Brutemark, A., Suikkanen, S., Padedda, B.M., Grubisic, L.M., Satta, C.T., Caddeo, T., Farina, P., Sechi, N., Lugliè, A., 2016. Effects of warming on a Mediterranean phytoplankton community. Web Ecol. 16, 89–92. https://doi.org/10.5194/we-16-89-2016.

R Core Team, 2018. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing. Vienna, Austria. http://www.R-project.org/.

Rasconi, S., Winter, K., Kainz, M.J., 2017. Temperature increase and fluctuation induce phytoplankton biodiversity loss – evidence from a multi-seasonal mesocosm experiment. Ecol. Evol. 7, 2936–2946. https://doi.org/10.1002/ece3.2889.

Ren, L., He, D., Chen, Z., Jeppesen, E., Lauridsen, T.L., Søndergaard, M., Liu, Z., Wu, Q.L., 2017. Warming and nutrient enrichment in combination increase stochasticity and beta diversity of bacterioplankton assemblages across freshwater mesocosms. ISME J. 11, 613–625. https://doi.org/10.1038/ismej.2016.159.

Reynolds, C.S., 2006. Ecology of Phytoplankton. Cambridge University Press, Cambridge.

Rodríguez-Varela, F., 2004. Environmental genomics, the big picture? Microbiol. Lett. 231, 153–158. https://doi.org/10.1016/S0378-1097(04)00006-0.

Roland, F., Huszar, V.L.M., Farjalla, V.F., Enrich-Prast, A., Amado, A.M., Ometto, J.P.H.B., 2012. Climate change in Brazil: perspective on the biogeochemistry of inland waters. Braz. J. Biol. 72, 709–722. https://doi.org/10.1590/S1519-69842012000400009.

Santoferrara, L.F., Grattepanche, J.D., Katz, L.A., McManus, G.B., 2016. Patterns and processes in microbial biogeography: do molecules and morphologies give the same answers? ISME J. 10, 1779–1790. https://doi.org/10.1038/ismej.2015.224.

Schrallhammer, M., Ferrantini, F., Vannini, C., Galati, S., Schweikert, M., Görtz, H.D., Verni, F., Petroni, G., 2013. Candidatus Megaira polyxenophila gen. nov., sp. nov.: considerations on evolutionary history, host range and shift of early divergent Rickttsiae. PLoS One 8, e72581. https://doi.org/10.1371/journal.pone.0072581.

Simehgo, 2016. Sistema de Meteorologia e Hidrologia do Estado de Goiás. http://www.simehgo.sectec.go.gov.br/ (accessed February 2016).

Simon, M., López-García, P., Deschamps, P., Moreira, D., Restoux, G., Bertolino, P., Jardillier, L., 2015. Marked seasonality and high spatial variability of protist communities in shallow freshwater systems. ISME J. 9, 1941–1953. https://doi.org/10.1038/ismej.2015.

Smale, D.A., Taylor, J.D., Coombs, S.T., Moore, G., Cunlifee, M., 2017. Community responses to seawater warming are conserved across diverse biological groupings and taxonomic resolutions. Proc. R. Soc. B 284, 20170534. https://doi.org/10.1098/rspb.2017.0534.

Sommer, U., Lewandowska, A., 2011. Climate change and the phytoplankton spring bloom: warming and overwintering zooplankton have similar effects on phytoplankton. Glob. Change Biol. 17, 154–162. https://doi.org/10.1111/j.1365-2486.2010.02182.x.

Sommer, U., Paul, C., Moustaka-Gouni, M., 2015. Warming and ocean acidification effects on phytoplankton – from species shifts to size shifts within species in a mesocosm experiment. PLoS One 10, e0125239. https://doi.org/10.1371/journal.pone.0125239.

Stefanidou, N., Genetsaris, S., Lopez-Bautista, J., Sommer, U., Moustaka-Gouni, M., 2018. Unicellular eukaryotic community response to temperature and salinity variation in mesocosm experiments. Front. Microbiol. 9, 2444. https://doi.org/10.3389/fmicb.2018.02444.

Stoeck, T., Bass, D., Nebel, M., Christen, R., Jones, M.D.M., Breiner, H., Richards, T.A., 2010. Multiple marker parallel tag environmental DNA sequencing reveals a highly complex eukaryotic community in marine anoxic water. Mol. Ecol. 19, 21–31. https://doi.org/10.1111/j.1365-294X.2009.04480.x.

Tandon, K., Yang, S., Wan, M., Yang, C., Baatar, B., Chiu, C., Tsai, J., Liu, W., Tang, S., 2018. Bacterial community in water and air of two sub-alpine lakes in Taiwan. Microbes Environ. 33 (2), 120–126. https://doi.org/10.1264/jsme2.ME17148.

Thomas, M.K., Kremer, C.T., Litchman, E., 2016. Environment and evolutionary history determine the global biogeography of phytoplankton temperature traits. Glob. Ecol. Biogeogr. 25, 75–86. https://doi.org/10.1111/geb.12387.

Tuyet, D.T.A., Tanaka, T., Sohin, R., Hao, M., Nagaosa, K., Kato, K., 2015. Effects of warming on microbial communities in the coastal waters of temperature and subtropical zones in the Northern Hemisphere, with a focus on Gammaproteobacteria. J. Oceanogr. 71, 91–103. https://doi.org/10.1007/s10872-014-0264-2.

Vadadi-Fülöp, C., Spikay, C., Mészáros, G., Hufnagel, L., 2012. Climate change and freshwater zooplankton: what does it boil down to? Aquat. Ecol. 46, 501–519.

Vázquez-Domínguez, E., Vaqué, D., Gasol, J.M., 2012. Temperature effects on the heterotrophic bacteria nanoflagellates, and microbial top predators of the NW Mediterranean. Aquat. Microb. Ecol. 67, 107–121. https://doi.org/10.3354/ame01583.

Velthuis, M., Senerpont-Domis, L.N., Frenken, T., Stephan, S., Kazanjian, G., Aben, R., Hilth, S., Kosten, S., Van Donk, E., Van de Waal, D.B., 2017. Warming advances top-down control and reduces producer biomass in a freshwater plankton community. Ecosphere 8, 01651. https://doi.org/10.1002/ecs2.1651.

Von Scheibner, M., Dörge, P., Biermann, A., Sommer, U., Hoppe, H.G., Jürgens, K., 2014. Impact of warming on phyto-bacterioplankton coupling and bacterial community composition in experimental mesocosms. Environ. Microbiol. 16, 718–733. https://doi.org/10.1111/1462-2920.

Weisse, T., 1997. Growth and reproduction of heterotrophic nanoflagellates in a meso-eutrophic lake. J. Plankton Res. 19, 703–722. https://doi.org/10.1093/plankt/19.6.703.

Wilken, S., Huisman, J., Naus-Wiezer, S., Van Donk, E., 2013. Mixotrophic organisms become more heterotrophic with rising temperature. Ecol. Lett. 16, 225–233. https://doi.org/10.1111/ele.12033.

Wilken, S., Soares, M., Urrutia-Cordero, P., Ratcovich, J., Ekavall, M.K., Van Donk, E., Hasson, L.A., 2018. Primary producers or consumers? Increasing phytoplankton

bacterivory along gradient of lake warming and browning. Limnol. Oceanogr. 63, S142–S155. https://doi.org/10.1002/lno.10728.
Wohlers, J., Engel, A., Zöllner, E., Breithaupt, P., Jürgens, K., Hoppe, H.G., Sommer, U., Riebessell, U., 2009. Changes in biogenic carbon flow in response to sea surface warming. Proc. Nat. Acad. Sci. 106, 7067–7072. https://doi.org/10.1073/pnas.0812743106.
Woodward, G., Perkins, D.M., Brown, L.E., 2010. Climate change and freshwater ecosystems: impacts across multiple levels of organization. Phil. Trans. R. Soc. B 365, 2093–2106. https://doi.org/10.1098/rstb.2010.0055.
Yang, Z., Zhang, L., Zhu, X., Wang, J., Montagnes, D.J.S., 2016. An evidence-based framework for predicting the impact of differing autotroph-heterotroph thermal sensitivities on consumer-prey dynamics. ISME J. 10, 1767–1778.
Yvon-Durocher, G., Allen, A.P., Montoya, J.M., Trimmer, M., Woodward, G., 2010. The temperature dependence of the carbon cycle in aquatic ecosystems. Adv. Ecol. Res. 43, 268–309. https://doi.org/10.1016/B978-0-12-385005-8.00007-1.
Yvon-Durocher, G., Allen, A.P., Cellamare, M., Dossena, M., Gaston, K.J., Leitão, M., Montoya, J.M., Reuman, D.C., Woodward, G., Trimmer, M., 2015. Five years of experimental warming increases the biodiversity and productivity of phytoplankton. PLoS Biol. 13, e1002324. https://doi.org/10.1371/journal.pbio.1002324.
Zar, J.H., 2010. Biostatistical Analysis. Pearson Print Hall, New Jersey.