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Efeito de fatores abióticos e interações bióticas nas respostas ecofisiológicas das principais espécies fitoplanctônicas de um reservatório naturalmente eutrofizado.

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2020

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Tese apresentada, como requisito parcial para obtenção do título de Doutor, ao Programa de Pós-Graduação em Biologia Vegetal, da Universidade do Estado do Rio de Janeiro

Orientador: Prof. Dr. Marcelo Manzi Marinho
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Marcella Coelho Berjante Mesquita

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Assinatura

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Marcella Coelho Berjante Mesquita

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DEDICATÓRIA

Dedico esse trabalho à minha família, porto seguro do meu viver.

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Agradeço, primeiramente a minha família, pelo apoio e amor, sem os quais não conseguiria chegar até aqui. Pela paciência em tolerar minha ausência e pela compreensão sobre minhas escolhas.

Agradeço, em especial ao meu marido, Ique, por ter me apoiado durante toda a minha jornada na pós-graduação, sobretudo nos últimos quatro anos. Agradeço imensamente pelos conselhos, pelo suporte e paciência durante à construção da tese. Obrigada por entender a minha escolha, na qual muitas vezes tivemos que sacrificar um feriado e/ou final de semana para a execução dos experimentos.

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Por fim, agradeço a todos que contribuíram de forma direta ou indiretamente para a realização deste trabalho.

Aprenda a confiar no que está acontecendo.
Se há silêncio, deixa-o aumentar, algo surgirá.
Se há tempestade, deixa-a rugir, se acalmará.
Lao Tsé

APRESENTAÇÃO

Desde a minha infância, a biologia já fazia parte da minha vida. Quando mais nova, passar as férias no interior de Minas Gerais era o acontecimento mais aguardado do ano. Não apenas por rever a família, mas pelo contato com a natureza. Era também a oportunidade de subir em árvores, de comer fruta do pé, de tomar banho de rio, entre outras sensações.

Durante a minha adolescência, a biologia que antes era uma semente dentro de mim, foi sendo cultivada e regada através dos programas de televisão sobre o mundo selvagem, do comportamento dos animais, das curiosidades sobre o mundo embaixo d'água e da possibilidade de conhecer um mundo tão extraordinário.

Em 2010, entrei para a faculdade de Ciências Biológicas na Universidade Gama Filho (UGF) e foi a melhor decisão que eu poderia ter tomado aos 17 anos. Na UGF tive a oportunidade de conhecer professores brilhantes que me apresentaram a biologia da forma mais espetacular. Além disso, conheci pessoas muito especiais que até hoje caminham ao meu lado. Ao longo de quatro anos de faculdade, tive a oportunidade de conhecer inúmeras áreas da biologia através das disciplinas ofertadas no curso, mas foi numa disciplina eletiva — Biologia Marinha que conheci o fitoplâncton e posso dizer que foi amor à primeira vista.

Em busca do meu primeiro estágio, fui até a Universidade do Estado do Rio de Janeiro (UERJ) na tentativa de encontrar uma oportunidade. Dentre alguns e-mails na porta das salas de alguns professores e de algumas ofertas de estágio coladas no mural, apenas um professor respondeu ao meu e-mail. E por ordem do destino, esse professor – Marcelo Manzi Marinho, era / é especialista em fitoplâncton. A partir desse momento (2011) até a minha conclusão na pós-graduação (2020) foi sob sua orientação.

Durante esses anos, o professor Marcelo me ensinou, me capacitou e me orientou da melhor forma possível. Ao seu lado, tive a oportunidade de apresentar diversos trabalhos, de ir à inúmeros congressos, de participar de alguns projetos e de coorientar alunos da iniciação científica. Ao longo dessa parceria, tivemos artigos publicados, uma dissertação e a conclusão de mais uma etapa — doutorado.

A tese em questão intitulada **“Efeito de fatores abióticos e interações bióticas nas respostas ecofisiológicas das principais espécies fitoplanctônicas isoladas de um reservatório naturalmente eutrofizado”** inicia-se com uma Introdução Geral, onde são apresentados i) aspectos gerais de lagos e reservatórios; ii) dinâmica da comunidade planctônica em reservatórios; iii) uma revisão bibliográfica, ainda que não exaustiva, sobre a ecologia e fisiologia de dois grupos fitoplanctônicos abundantes em reservatórios, com ênfase em duas espécies — *Raphidiopsis* (*Cylindrospermosis) raciborskii* (cianobactéria) e *Aulacoseira ambigua* (diatomácea); iv) aspectos sobre a interação entre fitoplâncton e zooplâncton, e, v) aspectos sobre diverside funcional. Segue-se o Capítulo 1 "**Functional traits as predictors of planktonic composition and interaction of a shallow tropical reservoir**", que teve como objetivo investigar a dinâmica temporal e vertical das comunidades fitoplanctônica e zooplanctônica de um reservatório tropical raso com base nos traços funcionais das espécies; Capítulo 2 "**Combined effect of light and temperature on the production of saxitoxins in *Raphidiopsis* (*Cylindrospermopsis) raciborskii* strains**", cujo objetivo foi avaliar o efeito combinado de luz e temperatura na produção de saxitoxinas em três cepas tropicais de *Raphidiopsis* (*Cylindrospermopsis) raciborskii* (cianobactéria); e o Capítulo 3 "**Combined effect of light and temperature on ecophysiology response in tropical strains of *Aulacoseira ambigua***", que teve como objetivo avaliar algumas respostas ecofisiológicas (taxa de crescimento, morfologia, capacidade fotossintética e lipídios neutros) de quatro cepas tropicais de *Aulacoseira ambigua* sob o efeito combinado de luz e temperatura. A tese ainda apresenta um tópico de Discussão Geral, onde reúne os principais resultados de cada capítulo, buscando um melhor entendimento sobre os fatores responsáveis pela estruturação da comunidade planctônica de um reservatório tropical raso naturalmente eutrofizado. Por fim, o tópico, Conclusões é apresentado, onde são sumarizados os principais achados deste estudo.

RESUMO

MESQUITA, Marcella Coelho Berjante. **Efeito de fatores abióticos e interações bióticas nas respostas ecofisiológicas das principais espécies fictoplanctônicas de um reservatório naturalmente eutrofizado.** 2020. 213f**.** Tese (Doutorado em Biologia Vegetal) – Instituto de Biologia Roberto Alcantara Gomes, Universidade do Estado do Rio de Janeiro, Rio de Janeiro, 2020.

No ambiente aquático, a comunidade planctônica é o principal responsável pela transferência de energia para os demais níveis tróficos, uma vez que o fitoplâncton (autotrófico) forma a base das cadeias tróficas enquanto o zooplâncton é o principal elo entre os produtores e consumidores. No entanto, a interação entre fitoplâncton e zooplâncton depende de características fisiológicas, morfológicas e comportamentais de ambas as comunidades. Dentre as diversas abordagens existentes para o estudo das comunidades planctônicas (taxonômica, traços e grupos funcionais e molecular), a utilização de traços e grupos funcionais têm se mostrado eficaz devido à possibilidade de um melhor entendimento sobre o papel das espécies no ecossistema aquático, assim como o vínculo entre elas. Para a comunidade fitoplanctônica, a abordagem de grupos funcionais baseados na morfologia (*morphollogically based functional groups* - MBFG) tem sido amplamente reconhecida por sua facilitação em modelar a dinâmica das comunidades fitoplanctônicas em diferentes cenários ambientais. Por outro lado, a utilização de traços funcionais para a comunidade zooplanctônica tem demonstrado ser capaz de relacionar mais claramente os requisitos ecológicos do zooplâncton e sua interação com o fitoplâncton. Num primeiro momento, esta tese avaliou a dinâmica temporal e vertical das comunidades fitoplanctônica e zooplanctônica de um reservatório tropical raso (Reservatório do Camorim) com base nos traços funcionais das espécies, visando esclarecer os principais direcionadores de sua estruturação (capítulo I). Neste capítulo, nós observamos que a biomassa e a diversidade fitoplanctônicas estiveram ligadas às variáveis ambientais, principalmente na estação quente/chuvosa, onde a estratificação térmica da coluna d'água propiciou o desenvolvimento de espécies filamentosas com aerótopos (MBFG **III)** e os altos valores de precipitação foram decisivos para a redução da biomassa total. As espécies fitoplanctônicas com tamanho corporal variando de médio a grande, sem traços especializados (MBFG **IV**), unicelulares (MBFG **V**) e com exoesqueleto de sílica (MBFG **VI**) foram os principais contribuintes para a biomassa fitoplanctônica. Os principais contribuintes para a biomassa zooplanctônica foram copépodos ciclopóide e rotíferos. A abordagem a partir dos traços funcionais demonstrou a dominância de animais de pequeno tamanho corporal, raptoriais, onívoros e de reprodução sexuada. A partir dos traços funcionais observados no fitoplâncton e zooplâncton, podemos sugerir uma fraca relação entre essas comunidades e, consequentemente, um ineficiente controle descendente. Num segundo momento, experimentos laboratoriais, combinando diferentes condições de luz e temperatura, foram realizados com o intuito de aprofundar o conhecimento sobre alguns parâmetros ecofisiológicos (crescimento, morfologia, produção de saxitoxinas, conteúdo de lipídios neutros e capacidade fotossintética) de duas espécies fitoplanctônicas dominantes no Reservatório do Camorim, *Raphidiopsis raciborskii* (cianobactéria) (capítulo II) e *Aulacoseira ambigua* (diatomácea) (capítulo III), e, ainda buscar resultados que pudessem explicar suas ocorrências no reservatório estudado, assim como nos demais ecossistemas aquáticos. No capítulo II, *Raphidiopsis raciborskii* apresentou as maiores taxas de crescimento sob altas intensidades luminosas, combinadas a temperaturas maiores que 15°C, dependendo da cepa. Essa espécie reduziu a produção e quota celular de saxitoxinas sob alta intensidade luminosa em temperatura de 30°C. Portanto, concluímos que o aumento da temperatura combinado com luz suficiente, levará ao aumento de biomassa de *R. raciborskii*, mas as florações poderão se tornar menos tóxicas nas regiões tropicais. No capítulo III, *Aulacoseira ambigua* foi sensível à alta intensidade luminosa, uma vez que não conseguiu crescer. Sua capacidade fotossintética foi reduzida e houve aumento do conteúdo de lipídios neutros. *A. ambigua* foi capaz de alterar sua morfologia sob diferentes combinações de luz e temperatura. Assim, nossos resultados demonstraram que as duas espécies fitoplanctônicas são capazes de ocorrerem e coexistirem no Reservatório do Camorim, como também em diversos ecossistemas continentais, devido aos diferentes requisitos de luz e temperatura e pela ampla plasticidade fenotípica exibida. Além disso, o sucesso de *R. raciborskii* e *A. ambigua* em diversos ecossistemas aquáticos pode estar ligado aos traços funcionais que reduzem a pressão de herbivoria (ex. toxinas, presença de sílica, respectivamente).

Palavras-chave: Diversidade funcional. Cianobactéria. Diatomácea. Variabilidade intraespecífica. Variabilidade Interespecífica.

ABSTRACT

MESQUITA, **Marcela Coelho** Ber**jante**. **Effect of abiotic factors and biotic interactions on ecophysiological responses of the main phytoplankton species in a naturally eutrophic reservoir. 2020.** 213f. Tese (Doutorado em Biologia Vegetal) – Instituto de Biologia Roberto Alcantara Gomes, Universidade do Estado do Rio de Janeiro, Rio de Janeiro, 2020.

In the aquatic environment, the planktonic community is primarily responsible for the transfer of energy to the other trophic levels, since phytoplankton (autotrophic) forms the basis of the food webs while zooplankton is the main link between producers and consumers. However, the interaction between phytoplankton and zooplankton depends on the physiological, morphological and behavioral traits of both communities. Among the several existing approaches to the study of planktonic communities (taxonomic, functional diversity and molecular), the use of functional groups and functional traits has been shown to be effective due to the possibility of a better understanding of the role of species in the aquatic ecosystem, as well as the link between them. For the phytoplankton community, the approach of morphollogically based functional groups (**MBFG**) has been widely recognized for its facilitation in modeling the dynamics of phytoplankton communities in different environmental scenarios. On the other hand, the use of functional traits for the zooplankton community has been shown to be able to relate more clearly the ecological requirements of zooplankton and its interaction with phytoplankton. At first, this thesis evaluated the temporal and vertical dynamics of the phytoplankton and zooplankton communities of a shallow tropical reservoir (Camorim Reservoir) based on the functional traits of the species, aiming to clarify the main drivers of its structuring (chapter I). In this chapter, we observed that biomass and phytoplankton diversity were linked to environmental variables, mainly in the warmer/rainy season, where the thermal stratification of the water column led to the emergence of filamentous species with aerotopes (**MBFG III**) and the high precipitation values were decisive for the reduction of total biomass. Phytoplankton species with body size ranging from medium to large, without specialized traits (**MBFG IV**), single-cell (**MBFG V**) and with silica exoskeleton (**MBFG VI**) were the main contributors to phytoplankton biomass. Cyclopoid copepod and rotifers were the main contributors to zooplankton biomass. The approach based on functional traits showed that the zooplankton community was dominated by small body size, raptorial, omnivorous and sexually reproductive. The functional traits observed in phytoplankton and zooplankton suggests a weak relationship between these communities and, consequently, an inefficient top-down control. In a second step, laboratory experiments, combining different light and temperature conditions, were carried out in order to deepen the knowledge about some ecophysiological parameters (growth, morphology, saxitoxins production, neutral lipid content and photosynthetic capacity) of two dominant phytoplanktonic species in the Camorim Reservoir, *Raphidiopsis raciborskii* (cyanobacteria) (chapter II) and *Aulacoseira ambigua* (diatom) (chapter III), and also to seek results that could explain their occurrences in the studied reservoir, as well as in the other aquatic ecosystems. In Chapter II, *Raphidiopsis raciborskii* showed the highest growth rates under high light intensities, combined at temperatures above 15°C, depending on the strain. This species reduced the production of saxitoxins and cellular quota of saxitoxins under high light intensity at 30°C. Therefore, the increase in temperature combined with sufficient light will lead to an increase in the biomass of *R. raciborskii*, but blooms may become less toxic in tropical regions. In chapter III, *Aulacoseira ambigua* was sensitive to high light intensity since it was unable to grow. Its photosynthetic capacity was reduced and there was an increase in the content of neutral lipids. *A. ambigua* was able to change its morphology under different combinations of light and temperature. Thus, our results demonstrated that the two phytoplankton species are capable of occurring and co-existing in the Camorim Reservoir, as well as in several freshwater ecosystems, due to the different light and temperature requirements and the wide phenotypic plasticity exhibited. In addition, the success of *R. raciborskii* and *A. ambigua* in several aquatic ecosystems may be linked to the functional traits that reduce herbivory pressure (e.g., toxins, presence of silica, respectively).

Key words: Functional diversity. Cyanobacteria. Diatom. Intraspecific variability. Interspecific variability.

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LISTA DE ABREVIATURAS E SIGLAS

C	Carbon
Chl-*a*	Chlorophyll *a*
CyanoHABs	Cyanobacterial harmful algal blooms
DIN	Dissolved inorganic nitrogen
DW	Dry weight
EK	Photosynthetic light saturating
MLD	Maximum linear dimension
N	Nitrogen
N_2	Atmospheric nitrogen
N-NH4+	Ammonium ion
N-NO2-	Nitrite ion
N-NO3-	Nitrate ion
DO	Dissolved oxygen
$r\mathrm{ETR}_{\mathrm{max}}$	Maximum relative electron transport rate
ROS	Reactive oxygen species
RWCS	Relative water column stability
SRP	Soluble reactive phosphorus
SRSi	Soluble reactive silica
S/V	Surface^{-}volume ratio
STX	Saxitoxin
P	Phosphorus
Zeu	Euphotic zone

LISTA DE SÍMBOLOS

°C	Degrees Celsius
CO_2	Carbon dioxide
Km^2	Square kilometer
L^{-1}	Per Liter
m	Meters
mL	Milliliter
mm	Millimeter
mm^3	Cubic millimeter
m^{-2}	Per square meter
m^{-3}	Cubic meter
nm	Nanometer
O_2	Oxygen gas
s^{-1}	Per second
PSII	Photosystem II efficiency
μ	Growth rate
μm^3	Cubic micrometer
µg	Microgram
α	Light harvesting efficiency

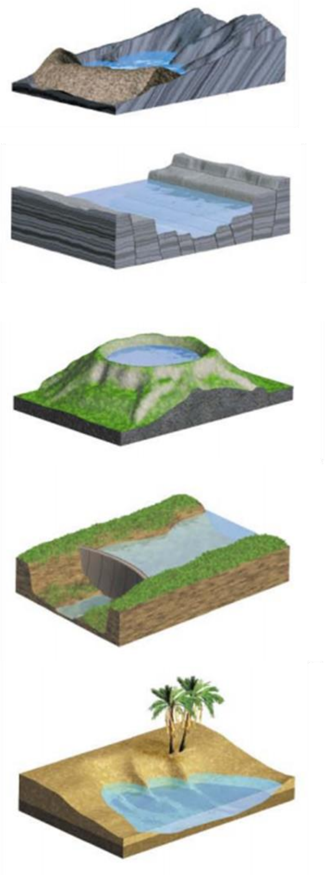
SUMÁRIO

INTRODUÇÃO

Lagos e reservatórios

Os lagos são corpos d'água interiores sem comunicação direta com o mar e, por este motivo, suas águas apresentam baixas concentrações de íons dissolvidos. No entanto, alguns lagos localizados em regiões áridas ou submetidos a longos períodos de seca podem apresentar altas concentrações de íons dissolvidos devido à intensa evaporação não ser compensada pela precipitação (ESTEVES, 1998). Além disso, os lagos apresentam curta durabilidade na escala geológica e sua formação depende tanto de elementos endógenos (originário da crosta terrestre) como exógenos (causas exteriores à crosta), o que torna cada sistema único por apresentar diferentes origens e características (Figura 1) (ESTEVES, 1998).

Figure 1– Gênese de alguns tipos de lagos



Lagos Glaciais = formado através do derretimento das geleiras.

Lagos Tectônicos = ocupam bacias naturais que resultantes dos movimentos das crostas da Terra, ao longo de dobras e falhas. Muitos estão situados abaixo do nível do mar.

Lagos de Caldeira = formado através de uma cratera ou pela destruição do cone central do vulcão. Esses lagos vulcânicos também podem se formar em vales onde os fluxos de lava retêm a água.

Lagos represados = formado naturalmente pelo represamento com restos de rocha de geleira, lava de vulcão e/ou deslizamento de terra. Os lagos também podem ser represados pela colocação de barragens, caracterizando os Reservatórios.

Oásis = lagos encontrados geralmente no deserto, onde o vento corrói o solo e expõe o lençol freático.

Fonte: Modificado de “The Visual World Atlas”, 2008.

Embora recebam diferentes denominações como, por exemplo, represas e açudes, os reservatórios apresentam a mesma origem, uma vez que são formados a partir do represamento de rios, mas possuem diferentes finalidade (ex. abastecimento de água, obtenção de energia elétrica, entre outros) (ESTEVES, 1998).

Os reservatórios podem ser vistos como um lago bastante dinâmico, onde uma parte significativa do seu volume se caracteriza e funciona como um rio, afetando diretamente seu padrão espacial (WETZEL, 1990). Além disso, os reservatórios apresentam muitas características semelhantes à de lagos naturais como, por exemplo, padrões de mistura, trocas gasosas na interface água-atmosfera, produção primária e respiração das comunidades, interações predador-presa (THORNTON; KIMMEL; PAYNE, 1990) e mecanismos relacionados à seleção de espécies fitoplanctônicas (REYNOLDS, 1999). No entanto, esses sistemas também apresentam características peculiares, se diferenciando de lagos em muitos aspectos, como origem, idade e propriedades morfológicas (STRAŠKRABA, 1999). Por serem sistemas artificiais, os reservatórios estão sujeitos a grandes variações das condições hidrológicas devido às atividades de operação das barragens, onde o manuseio acaba interferindo na vazão, nível e tempo de retenção da água, causando alterações das características limnológicas (STRAŠKRABA, 1999; STRAŠKRABA; TUNDISI, 1999).

Os reservatórios foram planejados para reter água para agricultura e/ou abastecimento humano, controlar enchentes em municípios ou para a geração de energia elétrica (TUNDISI; MATSUMURA-TUNDISI, 2014). Para a construção desses sistemas, muitos rios tiveram grande parte de seu curso segmentado em represas, o que produziu inúmeras e severas alterações no ambiente, não apenas o aquático, mas também o ambiente terrestre adjacente (ESTEVES, 1998). No Brasil, a maioria dos reservatórios está localizada no Sudeste do país, onde a demanda por energia elétrica é alta devido aos grandes centros industriais e populacionais na região. Outro importante aspecto é que muitos reservatórios são utilizados para abastecimento público, irrigação, aquicultura, navegação e recreação, aumentando a relevância de estudos nesses sistemas (TUNDISI; MATSUMURA-TUNDISI, 2014). O Reservatório do Camorim, por exemplo, foi construído em 1908 com o objetivo de abastecer uma parcela da população de Jacarepaguá, bairro localizado na Zona Oeste da cidade do Rio de Janeiro. Esse reservatório recebe água de um conjunto de rios, sendo o rio Camorim, com 6,5 km de extensão, seu principal tributário. As águas do reservatório escoam por uma escada hidráulica de declividade acentuada para uma pequena estação de tratamento da

CEDAE, empresa responsável pelo tratamento e distribuição de água e esgoto na cidade do Rio de Janeiro, que fica situada na sede do Parque Estadual da Pedra Branca (PEPB) (INEPAC, 1998). Até o ano de 2010, o Reservatório do Camorim era um dos principais recursos de fornecimento de água para a população local, abastecendo aproximadamente 20 mil habitantes (CEDAE, 2010). Atualmente, a estação de tratamento de água se encontra fechada. Além disso, o Reservatório do Camorim já foi objeto de estudo limnológico, onde o estado trófico e a comunidade fitoplanctônica foram avaliados durante o período de 2012-2013 (PEREIRA, 2018). Neste estudo, Pereira (2018) considerou o Reservatório do Camorim como eutrófico e constatou a presença de cianobactérias potencialmente produtoras de cianotoxinas como, por exemplo, *Raphidiopsis (formely Cylindrospermopsis) raciborskii* (Woloszynska) Aguilera, Berrendero Gómez, Kastovsky, Echenique & Salerno 2018.

Eutrofização e a perda na qualidade de água nos ecossistemas aquáticos

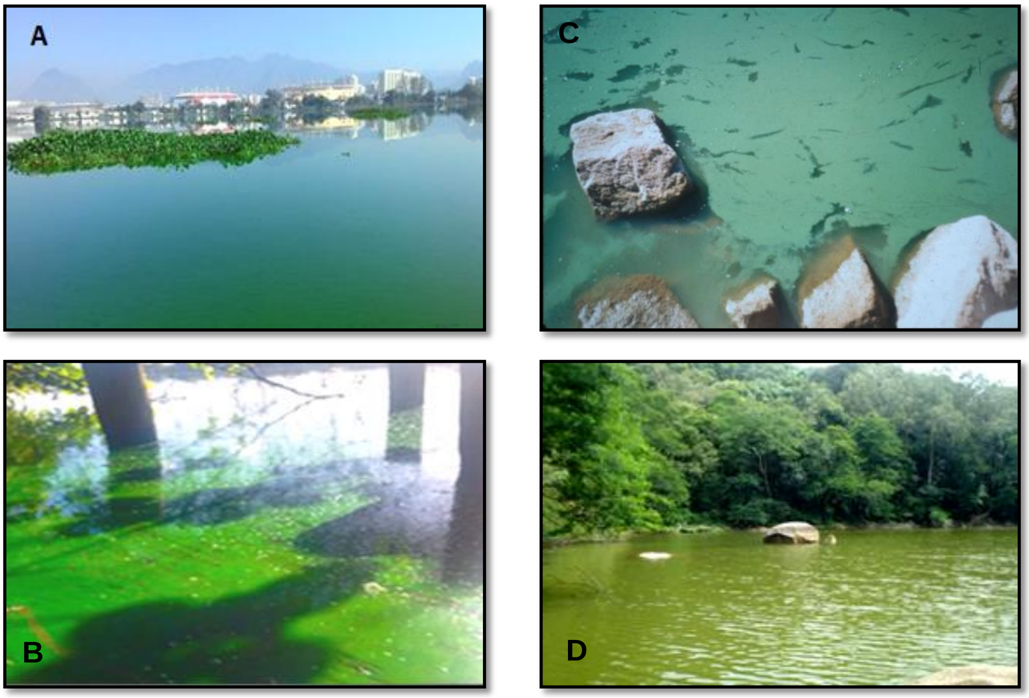
O processo de eutrofização é um problema mundial, sendo considerado um dos principais fatores responsáveis pela deterioração dos ecossistemas aquáticos. Esse processo de degradação do ambiente interfere diretamente nas propriedades físicas, químicas e biológicas da água, gerando modificações qualitativas e quantitativas nas comunidades aquáticas (HUISMAN; MATTHIJS; VISSER, 2005; TUNDISI; MATSUMURA-TUNDISI, 2014).

A eutrofização é caracterizada pelo enriquecimento da água, principalmente pelo aumento excessivo da concentração de nitrogênio e fósforo, ocasionados pelo acréscimo da carga de nutrientes de origem mineral (eutrofização natural) ou resultante da ação antrópica (eutrofização artificial) através de fontes pontuais (ex. lançamento de esgoto "*in natura*") e fontes dispersas (ex. fertilizantes provenientes dos campos agrícolas) que culminam em rápido e intenso crescimento de alguns produtores primários, tais como: cianobactérias e macrófitas aquáticas (ex. aguapé) (PAERL; PAUL, 2012; TUNDISI; MATSUMURA-TUNDISI, 2014).

Uma das principais consequências da eutrofização é o aumento das florações de cianobactérias (PEARL; HUISMAN, 2008, 2009; PAERL; OTTEN, 2013). As cianobactérias são micro-organismos fotossintetizantes que ocupam uma ampla variedade de nichos e apresentam rápido crescimento em determinadas condições ambientais (ex. incremento de nutrientes, altas temperaturas, estratificação térmica da coluna d'água), sendo capazes de acumular elevada biomassa na superfície dos ecossistemas aquáticos, caracterizando as florações (PAERL; OTTEN, 2013; REYNOLDS, 2006; TUNDISI; MATSUMURA-TUNDISI, 2014). Além disso, algumas espécies de cianobactérias apresentam estruturas que lhes conferem vantagem competitiva sobre outros organismos fitoplanctônicos, tais como: heterocito, cuja finalidade é de fixar nitrogênio atmosférico (N_2) e aerótopos (vesículas de gás). Esta última estrutura propicia a migração vertical na coluna d'água (REYNOLDS, 2006; TUNDISI; MATSUMURA-TUNDISI, 2014).

As florações de cianobactérias causam uma série de impactos negativos ao ecossistema aquático afetado, tais como: a perda da qualidade cênica (ex. águas esverdeadas e odor desagradável), impactos econômicos (ex. eleva o custo do tratamento da água, restringe a pesca e aquicultura) e ocasionam inúmeros distúrbios ecológicos (ex. queda acentuada da biodiversidade de plantas e peixes, alteração na composição das comunidades aquáticas) (PEARL; HUISMAN, 2008, 2009; TUNDISI; MATSUMURA-TUNDISI, 2014) (Figura 2).

Figure 2 – Florações de cianobactérias em diferentes ecossistemas brasileiros



Legenda: Lagoa de Jacarepaguá (A e B), Reservatório de Ingazeira (C) e Represa do Camorim (D).
Fonte: Google, 2016 (A); Marcelo Marinho – UERJ (B); Sandra Azevedo – UFRJ (C), Marcella Mesquita (D)

Em relação aos distúrbios ecológicos, as florações de cianobactérias aumentam a turbidez da coluna d'água o que reduz a penetração de luz nos ecossistemas afetados. Essa menor disponibilidade de luz suprime o estabelecimento e o crescimento de macrófitas aquáticas e microalgas bentônicas, afetando negativamente o habitat de invertebrados e peixes bentônicos (PAERL; HUISMAN, 2008, 2009; PAERL; PAUL, 2012). À medida que as florações perdem a capacidade de flutuação devido à morte, inicia-se o processo de decomposição que pode se dar no fundo ou na própria coluna d'água dos ecossistemas aquáticos. A utilização de oxigênio dissolvido (OD) para o processo de decomposição pode acarretar numa acentuada depleção desse componente na água e, em casos mais graves, o ambiente pode ficar anóxico (concentrações de OD inferiores a 3,0 mg L^{-1}). A anoxia do corpo d'água pode ocasionar a morte de diversos organismos aquáticos, especialmente os peixes (CHORUS; BARTRAM 1999; ESTEVES, 1998; PAERL; PAUL, 2012; TUNDISI; MATSUMURA-TUNDISI, 2014).

Algumas espécies de cianobactérias formadoras de floração são potencialmente produtoras de cianotoxinas (ex. microcistinas, saxitoxinas, cilindrospermopsinas e nodularina) e, quando estão em elevada biomassa caracterizam as florações de cianobactérias nocivas ("*Cyanobacteria harmful algal blooms – CyanoHABs*") (PEARL; HUISMAN, 2008, 2009; PAERL; OTTEN, 2013). As *CyanoHABs* são consideradas uma ameaça para os ecossistemas aquáticos porque, além de promoverem os mesmos distúrbios ambientais citados anteriormente, as cianotoxinas produzidas por essas espécies podem bioacumular e serem transferidas para diferentes níveis tróficos (FERRÃO-FILHO, 2009; IBELINGS et al., 2005), podendo chegar até os seres humanos através do consumo de peixe contaminado, por exemplo (MAGALHÃES; SOARES; AZEVEDO, 2001). Em casos mais graves, a intoxicação dos seres humanos pode ser de forma direta através do consumo de água contaminada, oriunda de reservatórios de abastecimento (CARMICHAEL et al., 2001; CHORUS; BARTHRAM, 1999). Isto porque algumas cianotoxinas apresentam estruturas estáveis e resistentes à hidrólise química e, portanto, sua remoção da água bruta é considerada um processo difícil.

Apesar das inúmeras consequências negativas, as florações de cianobactérias (nocivas ou não) vêm aumentando em ocorrência, frequência e magnitude em muitos ecossistemas aquáticos. A intensificação do processo de eutrofização causada principalmente pela ação antrópica através do uso indiscriminado dos recursos hídricos, somado aos efeitos causados pelas mudanças climáticas globais, como incremento da temperatura e alterações hidrológicas (ex. tempestades mais intensas e frequentes e extremas secas), propiciam condições extremamente favoráveis para o crescimento desses organismos (GOMES et al., 2020; HUISMAN; MATTHIJS; VISSER, 2005; MOSS et al., 2011; PAERL; OTTEN, 2013). Inúmeros estudos têm demonstrado que florações de cianobactérias tornam-se eventos comuns em diversos ecossistemas aquáticos, inclusive brasileiros (COSTA et al.; 2006; GOMES et al., 2009; LAGOS et al.; 1999). Tais eventos, ocasionam modificações nesses ecossistemas e causam grande preocupação para os gestores e usuários dos recursos hídricos, devido aos seus efeitos deletérios (CHORUS; BARTRAM, 1999; TUNDISI; MATSUMURA-TUNDISI, 2014).

Comunidade fitoplanctônica

Fitoplâncton pode ser definido como "coletivo de micro-organismos fotossintéticos que estão adaptados a viver total ou parcialmente suspensos na coluna d'água, cuja sua motilidade não excede aos movimentos das massas d'águas e correntezas" (REYNOLDS, 2006).

A capacidade de sintetizar matéria orgânica através da energia luminosa – fotossíntese, torna o fitoplâncton a base de inúmeras cadeias tróficas aquáticas, onde a disponibilidade e o fluxo de recursos serão influenciados diretamente pela composição e biomassa de sua comunidade (REYNOLDS, 2006). Como característica inerente ao seu ciclo de vida, o fitoplâncton é considerado um excelente bioindicador da qualidade de água, uma vez que responde rapidamente às mudanças ambientais (CHEN et al., 2016; REYNOLDS, 2006).

A comunidade fitoplanctônica abrange alguns grupos taxonômicos que foram formados de acordo com a história evolutiva das espécies, sendo eles: Bacillarophyta, Cyanobacteria, Chlorophyta, Cryptophyta, Crysophyta, Dynophyta, Euglenophyta, Eustigmatophyta, Haptophyta, Raphidophyta, Xantophyta, doravante citadas Bacilarofíta, Cianobactéria, Clorófita, Criptofita, Crisofíta, Dinofita, Euglenofita, Eustigmatofíta, Haptofíta, Rafidofíta e Xantofíta, grafia em português (REYNOLDS, 2006). A predominância de um ou outro grupo em determinado ecossistema é em função, principalmente, das características predominantes do meio, tais como: disponibilidade de luz, temperatura, nutrientes, regime de mistura, dentre outros (ESTEVES, 1998, REYNOLDS, 2006). No entanto, cada grupo taxonômico é composto por espécies que exibem ampla diversidade morfológica (ex. tamanhos e formas) e fisiológica (ex. taxa de crescimento) e, portanto, apresentam diferentes respostas aos parâmetros físicos (ex. luz e temperatura), químicos (ex. nutrientes) e biológicos do ambiente (ex. relação predador-presa) (REYNOLDS, 2006).

Além dos parâmetros físicos e químicos, as interações biológicas são importantes fatores que atuam na dinâmica da comunidade fitoplanctônica. As espécies fitoplanctônicas interagem entre si de forma direta através da competição intraespecífica (ex. diferentes cepas de uma espécie fitoplanctônica) e interespecífica (ex. diferentes espécies) por recursos (ex. luz e nutrientes) (GOMES; AZEVEDO; LÜRLING, 2015; MESQUITA et al., 2020, TORRES; LÜRLING, MARINHO, 2016). De maneira geral, espécies ou cepas fitoplanctônicas que compartilham os mesmos recursos competem entre si, onde a competidora superior (espécie ou cepa) por aquele determinado recurso se mantém no meio, enquanto a espécie ou cepa em desvantagem é excluída (MESQUITA et al., 2020; PASSARGE et al., 2006, TORRES; LURLING; MARINHO, 2016). No entanto, a exclusão competitiva em ambientes naturais é difícil de ser observada, uma vez que o fornecimento de recursos pode variar ao longo do tempo e do espaço e, desse modo, pode promover a coexistência e aumentar a diversidade de organismos (ARMSTRONG; MCGEHEE 1980; LEVINS, 1979). Por este motivo, a exclusão competitiva é vista comumente em experimentos laboratoriais devido a capacidade de reduzir o número de variáveis ambientais que exercem efeito sobre as espécies ou cepas fitoplanctônicas (MESQUITA et al., 2020; PASSARGE et al., 2006; TORRES; LURLING; MARINHO, 2016). A competição intra / interespecífica também pode ocorrer de forma indireta através da produção de substâncias aleloquímicas (ex. toxinas) (GOMES; AZEVEDO; LÜRLING, 2015), uma vez que estas substâncias podem inibir a fotossíntese, enzimas, a síntese de ácido nucleico e podem também promover a paralisia da célula (LEFLAIVE; TEN-HAGE, 2007). O mecanismo de ação aleloquímica depende da interação entre as espécies fitoplanctônicas produtoras e as espécies-alvo, como também depende do estado fisiológico das espécies envolvidas e de condições ambientais favoráveis, como por exemplo, intensidade luminosa, temperatura, pH e nutrientes (BARREIRO; VASCONCELOS, 2014; GOMES; AZEVEDO; LÜRLING, 2015; ISSA, 1999).

As espécies fitoplanctônicas também interagem com espécies aquáticas de níveis tróficos superiores como, por exemplo, as espécies da comunidade zooplanctônica (REYNOLDS, 2006). A interação entre fitoplâncton e zooplâncton se caracteriza principalmente pelo potencial de predação através da herbivoria (efeito descendente), uma vez que a comunidade fitoplanctônica é um dos principais recursos alimentares para o zooplâncton. Desse modo, a comunidade zooplanctônica é considerada como um fator regulador da comunidade fitoplanctônica, através da clássica cascata trófica (CARVALHO, 1994; JEPPESEN et al., 1996; JURGENS; STOLPE, 1995, REYNOLDS, 2006).

Reguladores gerais da comunidade fitoplanctônica

A sobrevivência do fitoplâncton está relacionada à capacidade de adquirir energia e nutrientes suficientes para se desenvolver e se reproduzir, como também possuir estratégias que minimizem as taxas de perda (REYNOLDS, 2006). Dessa maneira, o fitoplâncton precisa ter habilidade de assimilar rapidamente os nutrientes dissolvidos na água, como também estar regularmente nas camadas superficiais do ecossistema aquático para interceptar energia luminosa (REYNOLDS, 2006). No entanto, alguns fatores ambientais (ex. regime de mistura, temperatura, escoamento hidráulico) podem alterar a disponibilidade dos recursos no meio, como também podem atuar diretamente sobre o fitoplâncton ao modificar sua distribuição, composição e abundância (ESTEVES, 1998; REYNOLDS, 2006).

A mortalidade e os processos de perdas (ex. escoamento hidráulico, sedimentação e predação) são exemplos de reguladores por atuarem diretamente sobre a densidade e composição da comunidade fitoplanctônica. Além disso, esses reguladores são imprescindíveis para o funcionamento dos ecossistemas aquáticos, uma vez que contribuem fortemente para os ciclos biogeoquímicos e, com isso, ajudam na manutenção nas teias tróficas (REYNOLDS, 2006).

A predação é um dos exemplos relacionados aos processos de perda, pois interfere diretamente sobre a densidade e composição da comunidade fitoplanctônica (REYNOLDS, 2006). Os herbívoros filtradores generalistas se destacam sobre os demais predadores por serem capazes de recolher muitas células de uma só vez, como também pela possibilidade de consumir diferentes tipos de presas e de tamanhos variados, como o fitoplâncton (ESTEVES, 1998; REYNOLDS, 2006). Na comunidade zooplanctônica, os cladóceros são considerados os principais herbívoros filtradores generalistas dos ambientes aquáticos continentais, pois são capazes de consumir amplo espectro de tamanho de partícula e, dependendo da densidade populacional podem exercer uma pressão significativa sobre a comunidade fitoplanctônica, participando efetivamente do controle descende (AMORIM et al., 2019; CARVALHO, 1994; COLINA et al., 2016; JEPPESEN et al., 1996). Por outro lado, a existência de predadores seletivos pode alterar drasticamente a composição da comunidade fitoplanctônica. Esses predadores seletivos são representados na comunidade zooplanctônica pelos copépodos devido à capacidade de selecionar sua presa (ex. tamanho da partícula e qualidade nutricional) através de um complexo aparato alimentar que inclui a presença de mecanorreceptores e quimiorreceptores (ESTEVES, 1998; HARRIS, 2000; LEITÃO; GER; PANOSSO, 2018; GER; HANSSEN; LÜRLING, 2014). Consequentemente, os copépodos podem facilitar as florações de cianobactérias, uma vez que selecionam outros componentes do fitoplâncton como diatomáceas e clorofíceas para compor sua dieta, devido ao maior teor nutricional e pela ausência de alguns traços anti-herbivoria como, por exemplo, cianotoxinas. Logo, os copépodos, através da pressão seletiva reduzem a competição interespecífica, favorecendo o desenvolvimento de cianobactérias (COLINA et al., 2016; LEITÃO; GER; PANOSSO, 2018).

O escoamento hidráulico, assim como a predação, é um processo de perda que afeta a densidade da comunidade fitoplanctônica, principalmente nos ambientes pequenos e rasos (REYNOLDS, 2006). Isto se deve ao fluxo de entrada de água que promove um aumento significativo no volume de água do corpo hídrico, excedendo a capacidade de retenção de água que esses sistemas conseguem ter. Por conseguinte, os organismos fitoplanctônicos embutidos na água são removidos no volume de saída, caracterizando as descargas, o que leva à redução da densidade fitoplanctônica (REYNOLDS, 1997, 2006). Além da redução da densidade, o escoamento hidráulico também pode promover alterações na composição da comunidade fitoplanctônica, uma vez que as descargas de água afetam mais as espécies que apresentam crescimento lento, oferecendo uma vantagem competitiva para espécies de crescimento rápido (REYNOLDS, 1997, 2006; SCHEFFER, 1998).

Outro fator regulador na comunidade fitoplanctônica é a sedimentação. Muitas espécies fitoplanctônicas são mais pesadas do que a água e tendem a afundar (PADISÁK; SORÓCZKI-PINTÉR; REZNER, 2003; REYNOLDS, 2006). Ao longo do processo evolutivo, algumas espécies desenvolveram estruturas (ex. bainha de mucilagem, presença de aerótopos, flagelos, espinhos e braços) e estratégias (ex. formação de colônia, redução do tamanho corporal) para reduzir a velocidade de sedimentação, possibilitando maior permanência na zona eufótica da coluna d'água (REYNOLDS, 2006). As demais espécies – que não apresentam tais estruturas e/ou estratégias – apresentam rápidas taxas de sedimentação e dependem exclusivamente de fatores ambientais (ex. regime de mistura, ação de ventos, correntezas e turbulências) para permanecer mais tempo suspensas na coluna d'água e/ou que sejam ressuspendidas, caso estejam no fundo dos ecossistemas aquáticos (PADISÁK; SORÓCZKI-PINTÉR; REZNER, 2003; REYNOLDS, 2006).

O regime de mistura é um dos principais fatores reguladores do fitoplâncton continental (ESTEVES, 1998; REYNOLDS, 2006) por ser capaz de alterar as propriedades físicas, químicas e biológicas da água e, por conseguinte, ocasionar mudanças na composição da comunidade fitoplanctônica (COSTA; ATTAYDE; BECKER, 2016; DENG et al., 2008; REYNOLDS, 2006). O regime de mistura é conduzido pela interação entre os fatores climatológicos (ex. radiação solar, ventos e precipitação) e as propriedades do corpo hídrico (ex. profundidade, estrutura térmica, circulação vertical e horizontal), demonstrando a inter-relação entre atmosfera e os ecossistemas aquáticos (TUNDISI; TUNDISI, 2008). Desse modo, os processos de estratificação e mistura da coluna d'água resultam dos efeitos acumulados das trocas de calor e das entradas da energia, da absorção da radiação solar com a profundidade (a qual depende das condições óticas da água na superfície), da direção e da força do vento, entre outros fatores (TUNDISI; TUNDISI, 2008). Nos ambientes rasos tropicais, por exemplo, a pouca profundidade, associada a pouca amplitude de variação da temperatura facilitam a mistura completa da coluna d'água e, com isso, esses sistemas podem sofrer misturas verticais praticamente o ano inteiro. Nestes ambientes, as alterações no corpo hídrico consequentes da mistura da coluna d'água ficam mais evidentes, uma vez que a pouca profundidade proporciona que parte ou toda a superfície do sedimento do fundo esteja frequentemente, se não continuamente, em contato com a coluna d'água (PADISÁK; REYNOLDS, 2003). Como consequência, destaca-se: o aumento da turbidez e redução da disponibilidade de luz no corpo hídrico, uma vez que os sedimentos mais finos são susceptíveis à ressuspensão pela turbulência (REYNOLDS, 2006), assim como o transporte de nutrientes de áreas mais ricas (hipolímnio) para outras mais pobres (epilímnio), proporcionando aumento na abundância da comunidade fitoplanctônica, principalmente em altas temperaturas e com maior disponibilidade de luz (SØNDERGAARD; JENSEN; JEPPESEN, 2003; ZHU, QIN, GAO, 2005). Outro fator é o recrutamento de organismos e formas de resistência depositados no fundo desses ambientes, que retornam à zona eufótica (FONSECA; BICUDO, 2008; JEWSON et al., 2010). Destaca-se também a contribuição para uma menor heterogeneidade vertical das condições ambientais, uma vez a mistura da coluna d'água proporciona uma redução de nichos (BARBOSA; BARBOSA; BICUDO, 2018; BECKER, HUSZAR; CROSSETI, 2009; LOPES; BICUDO; FERRAGUT, 2005).

Embora a temperatura não seja considerada um recurso para o fitoplâncton, ela é um importante modulador do metabolismo celular, podendo limitar ou estimular o processo fotossintético (MESQUITA et al., 2019, 2020; REYNOLDS, 2006). Além dos efeitos diretos, a temperatura também pode atuar indiretamente no crescimento fitoplanctônico como, por exemplo, ao promover estratificações térmicas da coluna d'água que, por sua vez, alteram a disponibilidade de luz e nutrientes que são recursos imprescindíveis para o fitoplâncton (REYNOLDS, 2006). Desta maneira, os recursos – luz e nutrientes – necessários para a sobrevivência do fitoplâncton podem apresentar variações no espaço (ex. distribuição vertical e horizontal) e no tempo (ex. variações diárias e sazonais) (ESTEVES, 1998; REYNDOLS, 2006), influenciando a distribuição e a composição da comunidade fitoplanctônica, uma vez que as espécies apresentam diferentes requisitos de luz e nutrientes para o crescimento (REYNOLDS, 2006).

Luz e temperatura como principais reguladores abióticos da comunidade fitoplanctônica

A luz é um fator impactante para a fisiologia dos organismos fitoplanctônicos por estar diretamente associada ao processo fotossintético. No ambiente aquático, a luz é fortemente atenuada pelas condições do meio (ex. substâncias dissolvidas e partículas em suspensão, incluindo o próprio fitoplâncton), resultando em um gradiente vertical em intensidade e distribuição espectral (KIRK, 1994). Desse modo, os organismos fitoplanctônicos estão sujeitos a flutuações diárias na disponibilidade luminosa devido às transições entre noite e dia e às mudanças menos regulares na intensidade luminosa e na qualidade do espectro (MANN, 2002).

As espécies fitoplanctônicas possuem um conjunto de traços funcionais (ex. características fenológicas, morfológicas e fisiológicas) que permitem a utilização de diferentes comprimentos de onda (400-700 *nm*) e de intensidades luminosas para a realização da fotossíntese (KIRK, 1994; REYNOLDS, 2006). Por esse motivo, as espécies fitoplanctônicas apresentam diferentes preferências em relação à luz, distribuindo-se de forma heterogênea ao longo da coluna d'água (REYNOLDS, 2006).

Por exemplo, *Aphanizomenon* sp. e *Microcystis aeruginosa* (Kützing) Kützing são espécies fitoplanctônicas que necessitam e toleram altas intensidades luminosas devido ao alto requerimento luminoso para a saturação fotossintética (I_K) (PADISÁK, 2003), enquanto espécies filamentosas como, por exemplo, *Raphidiopsis raciborskii* apresentam baixo I_K, sendo considerada boa competidora por luz (PADISÁK; REYNOLDS 1998; WU et al.; 2009).

Dentre os parâmetros relacionado à luz, a intensidade luminosa se destaca por intervir diretamente na fisiologia e na morfologia do fitoplâncton (BONILLA et al., 2016; PIERANGELINI et al., 2014, 2015). As espécies fitoplanctônicas podem crescer numa ampla faixa de intensidade luminosa devido a sua plasticidade fenotípica (BRIAND et al., 2004; BONILLA et al., 2016; MESQUITA et al., 2019). No entanto, intensidades luminosas muito altas (> 500 µmol fótons m^{-2} s^{-1}) podem ser potencialmente prejudiciais, enquanto intensidades luminosas muito baixas (10 µmol fótons m^{-2} s^{-1}) podem ser inadequadas para suportar taxas ótimas de fotossíntese (BHAYA; SCHWARZ; GROSSMAN, 2002; MESQUITA et al., 2019). Esses valores extremos de intensidades luminosas podem se configurar como condições estressantes para o fitoplâncton, desencadeando uma série de alterações fisiológicas. Por exemplo, em condições de altas intensidades luminosas, diatomáceas podem alterar a via metabólica, produzindo lipídios apolares cuja função é de reserva energética ao invés de lipídios polares que são componentes estruturais (HU et al., 2008), enquanto as cianobactérias incrementam a produção de metabólitos secundários, como por exemplo, as cianotoxinas (MESQUITA et al., 2019). Por outro lado, em condições de baixas intensidades luminosas, as espécies fitoplanctônicas podem aumentar a quantidade de pigmentos fotossintéticos acessórios (ficobilinas, carotenoides e xantofilas), permitindo a absorção de luz em comprimentos de onda diferentes à absorbância de clorofila-a (FALKOWSKI; RAVEN, 2007). Desse modo, as espécies fitoplanctônicas apresentam intensidades luminosas consideradas ideais/ótimas para o crescimento, o que permite alcançar suas taxas máximas de crescimento (BRIAND et al., 2004; BONILLA et al., 2016; MESQUITA et al., 2019).

A morfologia é um importante aspecto relacionado ao uso da luz, uma vez que a distorção da forma esférica é considerada uma adaptação que proporciona às espécies fitoplanctônicas a capacidade de utilizarem baixas intensidades de luz, tornando-as boas antena de luz (BRAZIL; HUSZAR, 2011; REYNOLDS, 1997; NASELLI-FLORES; BARONE, 2007). Corroborando com essa afirmativa, estudos têm demonstrado que espécies fitoplanctônicas filamentosas ou formadoras de cadeia apresentam maior abundância em ambientes com pouca disponibilidade de luz (MARQUARDT et al., 2018; O'FARRELL; PINTO; IZAGUIRRE, 2007). Isto porque as altas razões superfície-volume proporcionam uma maior área para a interceptação luminosa, como também permitem que as espécies fitoplanctônicas permaneçam mais tempo suspensas na zona eufótica da coluna d'água (LEWIS, 1976; REYNOLDS, 2006). Desse modo, a grande diversidade de formas e tamanhos corporais, presente na comunidade fitoplanctônica, refletem a capacidade de captação de energia luminosa e, por isso, as espécies podem ocupar diferentes nichos relacionados à luz no ambiente aquático (REYNOLDS, 2006).

Embora a morfologia seja um atributo importantíssimo para a captação de luz, determinadas condições ambientais, associadas à plasticidade fenotípica da espécie, podem levar a modificações em sua estrutura celular (ex. forma, tamanho celular e largura celular) (BITTENCOURT-OLIVEIRA et al., 2012; CHARALAMPOUS; MATTHIESSEN; SOMMER, 2014; HUANG et al., 2018). De maneira geral, as espécies fitoplanctônicas são capazes de responder fisiologicamente ao alterar sua morfologia quando as condições ambientais não estão favoráveis, como uma tentativa de se adaptar. Estudos têm demonstrado que algumas espécies fitoplanctônicas tendem a reduzir o tamanho celular quando estão submetidas à alta intensidade luminosa, enquanto tendem a aumentar o tamanho celular em condições de baixa intensidade luminosa (CHARALAMPOUS; MATTHIESSEN; SOMMER, 2014; HUANG et al., 2018). Outro exemplo, é o estudo realizado por Bittencourt-Oliveira et al. (2012) que avaliou o efeito combinado de duas condições de luz e temperatura sobre o crescimento e morfologia de tricomas do morfotipo reto e espiralado de *Raphidiopsis raciborskii* (cianobactéria). Em resposta as condições de baixa luz e alta temperatura (30 μmol fótons m–2 s–1 a 31°C) o morfotipo espiralado de *R. raciborskii* tornou-se reto ou sigmoide. Este estudo também observou maior sensibilidade do morfotipo espiralado à maior intensidade de luz, talvez devido à sua conformação espacial que permite receber luz de diferentes ângulos (BITTENCOURT-OLIVEIRA et al., 2012).

A temperatura é outro fator regulador extremamente importante na comunidade fitoplanctônica (GOMES; AZEVEDO; LÜRLING, 2015; REYNOLDS, 2006), uma vez que atua diretamente nos processos relacionados ao crescimento e biossíntese de compostos (COLES; JONES, 2000; MESQUITA et al., 2019, 2020). As espécies fitoplanctônicas exibem uma ampla plasticidade fenotípica em relação à temperatura, sendo capazes de crescer sob diferentes condições (GOMES; AZEVEDO; LÜRLING, 2015; LÜRLING et al., 2013). No entanto, para que elas alcancem suas taxas máximas de crescimento é necessário que estejam submetidas à temperatura ótima/ideal, que varia para cada espécie. Por exemplo, *Raphidiopsis raciborski* (cianobactéria), *Planktothrix agardhii* (Gomont) Anagnostidis & Komárek (cianobactéria) e *Cyclotella meneghiniana* Kützing (diatomácea*)* são capazes de crescer entre 15–30°C, mas exibem taxas máximas de crescimento em diferentes temperaturas, 30°C, 27°C e 21°C, respectivamente (GOMES; AZEVEDO; LÜRLING, 2015; MESQUITA et al., 2019).

Assim como foi descrito para a intensidade luminosa, temperaturas muito altas ou muito baixas podem ser prejudiciais para as espécies fitoplanctônicas. Isto porque, as baixas temperaturas reduzem o metabolismo celular o que leva à diminuição do processo fotossintético que, por sua vez, interfere na produção de compostos celulares necessários para a sobrevivência das espécies (FALKOWSKI; RAVEN 1997; RAVEN; GEIDER, 1988; REYNOLDS, 2006). Por outro lado, as altas temperaturas podem levar à inativação ou desnaturação de proteínas, interferindo negativamente no metabolismo celular (FALKOWSKI; RAVEN, 1997; RAVEN; GEIDER, 1988; REYNOLDS, 2006).

Algumas das alterações na morfologia das espécies fitoplanctônicas podem ser reflexo das alterações fisiológicas causadas pela temperatura (MONTAGNES; FRANKLIN, 2001; SOARES; LÜRLING, HUSZAR, 2013). Estudos têm demonstrado que as espécies fitoplanctônicas geralmente reduzem o tamanho celular quando estão submetidas a altas temperaturas devido ao forte estímulo para a replicação (MONTAGNES; FRANKLIN, 2001; SOARES; LÜRLING, HUSZAR, 2013). Além disso, a alteração no tamanho celular devido à variação induzida pela temperatura pode estar associada à estratégia de sobrevivência, uma vez que a redução no tamanho das espécies fitoplanctônicas pode conferir uma vantagem competitiva sobre espécies fitoplanctônicas maiores, que enfrentam forte competição por recursos limitados (JEWSON, 1992).

Na literatura, há inúmeros estudos que indicaram a disponibilidade de luz e variação da temperatura como os principais reguladores da comunidade fitoplanctônica (DE SENERPONT DOMIS et al., 2013; FONSECA; BICUDO, 2008; RANGEL et al., 2016; TUCCI; SANT'ANNA, 2003). Para ampliar o entendimento a respeito de como esses fatores ambientais atuam sobre a comunidade fitoplanctônica, como também aprofundar o conhecimento sobre as preferências ecológicas do fitoplâncton, muitas espécies fitoplanctônicas já foram utilizadas em experimentos laboratoriais. Estes, por sua vez, enfocaram apenas nos efeitos isolados de luz e temperatura (COLES; JONES, 2000; GOMES; AZEVEDO; LÜRLING, 2015; MESQUITA et al., 2020; SOARES; LÜRLING, HUSZAR, 2013). Todavia, alterações nas condições ambientais podem ocorrer ao longo do tempo e espaço e, portanto, a comunidade fitoplanctônica pode experimentar diferentes condições de luz e temperatura num curto período (DE SENERPONT DOMIS et al., 2013; REYNOLDS, 2006). Por esta razão, os pesquisadores começaram a estudar e aprofundar o conhecimento sobre os possíveis efeitos causados pela combinação de diferentes condições de luz e temperatura nas respostas ecofisiológicas das espécies fitoplanctônicas (BITTENCOURT-OLIVEIRA et al., 2012; BONILLA et al., 2016; SPILLING et al., 2015; XIAO; WILLIS; BURFORD, 2017).

Luz e temperatura: principais variáveis ambientais relacionadas aos futuros cenários das mudanças climáticas.

Nos últimos anos, inúmeros estudos têm avaliado os possíveis efeitos da combinação de diferentes variáveis ambientais sobre as respostas ecofisiológicas de algumas espécies fitoplanctônicas (BITTENCOURT-OLIVEIRA et al., 2012; BONILLA et al., 2016; SPILLING et al., 2015; XIAO; WILLIS; BURFORD, 2017). Dentre elas, o efeito combinado de luz e temperatura têm atraído mais a atenção dos pesquisadores porque ambas as variáveis ambientais atuam diretamente sobre a fisiologia e morfologia do fitoplâncton, como também por serem variáveis intimamente relacionadas com as mudanças climáticas (NAZARI-SHARABIAN; AHMAD; KARAKOUZIAN, 2018; PAERL, HUISMAN, 2008, 2009; PAERL; OTTEN, 2013).

O aquecimento global emerge como uma das principais consequências das mudanças climáticas por atuar sobre diferentes escalas ambientais como, por exemplo, ao englobar mudanças abióticas nos ecossistemas aquáticos (ex. incremento da temperatura e estratificação térmica mais duradoura, incremento da intensidade luminosa e maior exposição aos raios ultravioletas, alteração no ciclo hidrológico, etc.), como também alterações biológicas em suas comunidades (ex. alterações morfológicas, fisiológicas e da composição das comunidades planctônicas) (NAZARI-SHARABIAN; AHMAD; KARAKOUZIAN, 2018; PAERL, HUISMAN, 2008, 2009; PAERL; OTTEN, 2013).

As previsões climáticas apontam para um aumento da temperatura que, por sua vez, pode alterar os padrões de precipitação. Consequentemente, determinadas regiões apresentarão chuvas mais intensas o que ocasionará maior descarga de nutrientes para o ecossistema aquático, enquanto outras regiões passarão por períodos prolongados de seca, acarretando estratificações térmicas mais duradouras. Essas alterações poderão trazer sérias consequências para a comunidade fitoplanctônica, uma vez que poderão alterar as propriedades físicas, químicas e biológicas da água (ESTEVES, 1998; REYNOLDS, 2006), levando a modificações nas relações entre predador-presa e, consequentemente, alterações na dinâmica das cadeias tróficas aquáticas (BOERSMA et al., 2016; GER, HANSSON; LÜRLING, 2014; GUSHA et al., 2019**).**

O aumento da temperatura também promoverá alterações na disponibilidade e qualidade de luz nos ecossistemas aquáticos (CRAIG et al., 2014; HADER et al., 2007, 2010). Isto porque chuvas mais fortes aumentarão o carreamento de sedimento e matéria orgânica para dentro dos corpos hídricos, elevando a turbidez da água e reduzindo a disponibilidade de luz. Por outro lado, as espécies fitoplanctônicas podem ficar mais expostas à luz porque estratificações térmicas mais precoces, mais fortes e mais longas podem ocorrer com o aumento da temperatura (DE STASIO et al., 1996; PAERL; HUISMAN, 2009; PEETERS et al., 2007).

Na comunidade fitoplanctônica, as cianobactérias serão os principais organismos favorecidos com o aumento da temperatura, uma vez que são capazes de incrementar suas taxas de crescimento com o aumento da temperatura e por exibirem taxas ótimas de crescimento entre 25–30°C (GOMES; AZEVEDO; LÜRLING, 2015; LÜRLING et al., 2013; MESQUITA et al., 2019, 2020). Outros grupos fitoplanctônicos também poderão ser afetados, como por exemplo, as clorofíceas podem apresentar taxas de crescimento similares as taxas de crescimento das cianobactérias em temperaturas elevadas, enquanto o aumento da temperatura tem um impacto negativo sobre o crescimento das diatomáceas (GOMES; AZEVEDO; LÜRLING, 2015; MESQUITA et al., 2020). Além disso, os efeitos indiretos do aumento da temperatura sobre o ambiente aquático promovem condições ambientais extremamente favoráveis para o desenvolvimento das cianobactérias (ex. redução da mistura vertical e estratificação da coluna d’água mais duradoura) (DENG et al. 2018; JÖHNK et al. 2008; PAERL; PAUL 2012; RANGEL et al. 2016). Logo, mesmo que outros organismos fitoplanctônicos como, por exemplo, as clorofíceas, possam crescer na mesma velocidade que as cianobactérias, condições ambientais causadas pelo aumento da temperatura serão extremamente favoráveis para o seu desenvolvimento (LÜRLING et al., 2013).

As diatomáceas têm sido descritas como o principal grupo afetado negativamente com os futuros cenários decorrentes das mudanças climáticas. Isto porque, as diatomáceas geralmente apresentam uma redução na taxa de crescimento com o aumento da temperatura (BUTTERWICK; HEANEY; TALLING, 2005; PAERL; OTTEN, 2013), mesmo ao considerar espécies ou cepas tropicais (GOMES; LÜRLING; MARINHO, 2015; MESQUITA et al., 2020). Além disso, o aumento da temperatura irá promover alterações nas características físicas e químicas da água o que afetará o desenvolvimento das diatomáceas. O aumento da temperatura promoverá redução da viscosidade da água o que acarretará maiores perdas por sedimentação de espécies de diatomáceas (JÖHNK et al., 2008). Por outro lado, alguns estudos têm demonstrado mudanças na composição das espécies de diatomáceas em algumas condições ambientais similares às previstas pelas mudanças climáticas, ao invés das espécies de diatomáceas simplesmente desaparecerem (HSIEH et al., 2010; KEATLEY; DOUGLAS; SMOL, 2008; RUHLAND; PATERSON; SMOL, 2008; SMOL, 2005). Em alguns lagos árticos, por exemplo, a mudança na cobertura de gelo e/ou aumento da estratificação térmica devido ao aquecimento tem sido um dos principais fatores relacionados às mudanças na composição das diatomáceas, aonde foi observado um aumento pronunciado na abundância de diatomáceas planctônicas (ex. *Cyclotella* sp.) às custas de espécies bentônicas (ex. *Fragilaria* sp.) e de gêneros meroplanctônicos (ex. *Aulacoseira* sp.) (KEATLEY; DOUGLAS; SMOL, 2008; RUHLAND; PATERSON; SMOL, 2008; SMOL, 2005). Outro estudo demonstrou que o aumento da eutrofização no corpo d’água, uma das consequências das mudanças climáticas, resultou em mudanças na composição, com o aparecimento e aumento da abundância da diatomácea *Fragilaria capucina* (HSIEH et al., 2010).

As cianobactérias têm sido objeto de inúmeros estudos ecofisiológicos por serem os principais organismos beneficiadas com as mudanças climáticas (BITTENCOURT-OLIVEIRA et al., 2012; BONILLA et al., 2016; MESQUITA et al., 2019; XIAO; WILLIS; BURFORD, 2017). Para esse grupo, a interação entre luz e temperatura já demonstrou afetar o crescimento, a morfologia e a produção de cianotoxinas (BITTENCOURT-OLIVEIRA et al., 2012; BONILLA et al., 2016; MESQUITA et al., 2019; XIAO; WILLIS; BURFORD, 2017). Embora as diatomáceas não sejam beneficiadas diretamente com as mudanças climáticas, elas são importantes componentes da “atual” comunidade fitoplanctônica por co / dominar muitos ecossistemas aquáticos (BORGES, TRAIN, RODRIGUES, 2008; CHEN et al., 2016; PEREIRA, 2018). Além disso, alguns estudos demonstraram que a composição de diatomáceas pode se alterar em condições similares às previstas pelas mudanças climáticas (HSIEH et al., 2010; KEATLEY; DOUGLAS; SMOL, 2008; RUHLAND; PATERSON; SMOL, 2008; SMOL, 2005), podendo ser um indicativo benéfico para as diatomáceas num futuro cenário. No entanto, ainda são escassos os estudos que analisam os efeitos combinados de variáveis ambientais sobre alguns parâmetros ecofisiológicos em diatomáceas, principalmente em relação às preferências de luz e a temperatura (INGEBRIGTSEN et al., 2016; NISHIKAWA; YAMAGUCHI, 2006; SHEAR, NALEWAJKO; BACCHUS, 1976).

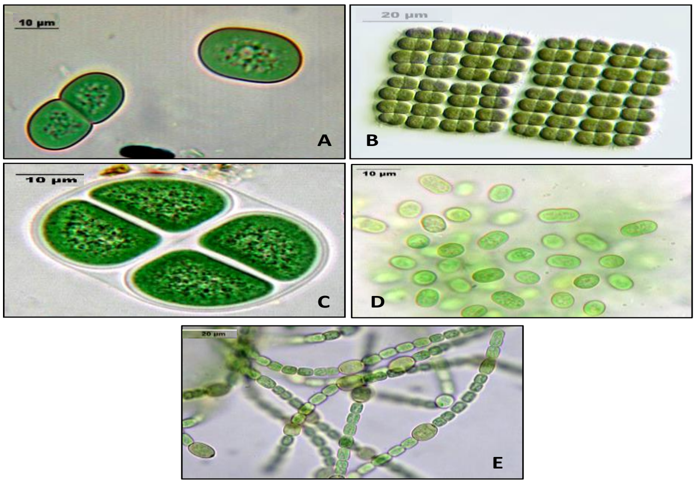
Cianobactérias

As cianobactérias são consideradas os mais antigos organismos fotoautotróficos que produzem oxigênio, tendo sua proliferação ocorrida durante a era pré-cambriana, aproximadamente há 3,5 bilhões de anos (SCHOPF, 2000). Esses organismos são produtores primários procariotas, sem membrana nuclear e nem plastídios diferenciados (BICUDO; BICUDO, 2008).

Uma das características marcantes desse grupo é a capacidade de crescimento nas mais variadas condições e recursos, sendo a grande maioria das espécies encontrada nas águas doces, onde podem viver no plâncton, no perifíton e bentos (SOUZA, 2006). Além disso, as cianobactérias apresentam morfologia bem diversificada, podendo ser unicelulares ou filamentosas, ocorrer isoladamente ou agrupados em colônias (WHITTON; POTTS, 2000) (Figura 3).

O único meio de reprodução desse grupo é assexuado, no qual a divisão binária é o processo mais comum (LEE, 2008; MUR; SKULBERG; UTKILEN, 1999). Entretanto, já foi verificada troca genética entre filamentos de cianobactérias, sendo considerada uma parassexualidade ao invés da reprodução sexuada. O fenômeno de parassexualiade ocorre seja pela passagem de um fragmento de DNA de uma célula doadora a uma célula receptora, com substituição de partes homólogas de DNA (transformação), seja por conjugação devido à transferência de DNA através de um canal estreito (REVIERS, 2006).

Figure 3 – Diversidade morfológica de cianobactérias



Legenda: (A) *Synechococcus* sp.; (B) *Merismopedia* sp.; (C) *Chroococcus* sp.; (D) *Aphanotece* sp.; (E) *Dolichorspermum* sp. (E).
Fonte: Modificado de Ralf Wagner <http://www.dr-ralf-wagner.de/>

Nas espécies filamentosas, a reprodução é feita por meio da fragmentação de tricomas ou pela formação de hormogônio (MUR; SKULBERG; UTKILEN, 1999). A formação do hormogônio geralmente se dá quando uma célula do filamento morre ou a parede entre as células se separa. Os hormogônios se diferenciam dos tricomas vegetativos por sua motilidade, pelo pequeno tamanho de suas células, a ausência de heterocitos (MEEKS; ELHAI, 2002) e, em algumas espécies, pode apresentar vacúolos de gás, responsáveis pela flutuabilidade (LEE, 2008).

As cianobactérias, assim como os outros seres autotróficos, possuem um aparato fotossintético que converte energia luminosa em energia química, tendo como produto final desse processo a síntese de compostos orgânicos (RAVEN, EVERT; EICHHORN, 2007). O aparato fotossintético das cianobactérias também apresenta ficobiliproteínas que lhes permitem captar energia luminosa em diferentes comprimentos de ondas, sendo uma vantagem competitiva em ambientes com pouca disponibilidade de luz (MOORE; CHISHOLM, 1999; MOORE; GOERICKE; CHISHOLM, 1995; PARTENSKY et al., 1999).

Em relação às preferências ecológicas, as cianobactérias são comumente encontradas no plâncton de muitos ecossistemas aquáticos (ex. rios, lagos, reservatórios), embora sejam encontradas em maior abundância nos ambientes eutrofizados (ALVAREZ COBELAS; JACOBSEN, 1992; WATSON et al., 1997). Isso porque algumas condições ambientais são extremamente favoráveis para o crescimento desse grupo (ex. elevadas temperaturas, baixa disponibilidade de luz e reduzida turbulência) e, portanto, representam vantagens competitivas em relação aos demais componentes da comunidade fitoplanctônica (PAERL et al., 2001; PAERL; PAUL, 2008, 2009).

Em elevada biomassa, as cianobactérias desencadeiam impactos negativos sobre diversos aspectos relacionados à qualidade ambiental (ver seção 1.2.1.3) (CHORUS; BARTRAM 1999; WESTRICK et. al 2010). Dentre as espécies formadoras de floração e potencialmente tóxicas, *Raphidiopsis raciborskii* tem sido registrada formando florações persistentes em reservatórios de abastecimento de água e causando preocupação generalizada quanto ao manejo e saúde pública nestes ambientes (DALU; WASSERMAN, 2018; RANGEL et al., 2012; SOARES et al., 2012).

No Brasil, a grande expansão na distribuição de *Raphidiopisis raciborskii* teve início na década de 90, coincidindo com o aumento da eutrofização dos sistemas aquáticos nas mais diversas regiões do país (SANT'ANNA; AZEVEDO, 2000). A expansão dessa espécie não apenas no Brasil, mas em diversas regiões do planeta pode ser resultado da grande plasticidade fisiológica ou da existência de diferentes ecótipos com requisitos ambientais distintos (BURFORD et al., 2018; MESQUITA et al., 2019; PICCINI et al., 2011; WILLIS et al., 2016). Por exemplo, *R. raciborskii* demonstra grande tolerância à variação de temperatura e de intensidade luminosa (MESQUITA et al., 2019; BONILLA et al., 2016) e apresenta diversas estratégias nutricionais (BURFORD et al., 2018). Além disso, a capacidade de produzir diferentes tipos de cianotoxinas (saxitoxinas e cilindrospermopsinas) (MESQUITA et al., 2019; WILLIS et al., 2016) e outros componentes alelopáticos (FIGUEREDO; GIANI; BIRD, 2007) podem contribuir significativamente para a sua expansão.

Embora as cianobactérias sejam os principais organismos relacionados a ambientes eutrofizados (ALVAREZ COBELAS; JACOBSEN, 1992; WATSON et al., 1997), outros componentes da comunidade fitoplanctônica também apresentam preferências por ambientes mais enriquecidos, como por exemplo, algumas espécies de diatomáceas (DENYS et al., 2003; HOUK, 2003; TAYLOR; HARDING; ARCHIBALD, 2007).

Diatomáceas

As diatomáceas constituem outro grupo fitoplanctônico de grande relevância em lagos e reservatórios, por serem abundantes e apresentarem muitas espécies cosmopolitas, além de demonstrar dominância ou co-dominância em comunidades fitoplanctônicas (PEREIRA, 2018). Além disso, as diatomáceas são consideradas bioindicadoras da qualidade de água por apresentarem íntima relação com a química da água (CHEN et al., 2016; WANG et al., 2017).

Dentre os gêneros de diatomáceas restritos às águas continentais, *Aulacoseira* é considerado um dos mais importantes gêneros de algas, tanto numericamente quanto em termos de sua contribuição para a produtividade primária global (HUBBLE, 2000). Além disso, *Aulacoseira* apresenta distribuição mundial, podendo ser encontrada em diversos ecossistemas aquáticos (ex. lagos, reservatórios e rios) com diferentes graus de trofia (ex. oligotrófico, mesotrófico e eutróficos) (CHEN et al., 2016; DENYS et al., 2003; WANG et al., 2017). Apesar desse gênero não produzir toxinas, como as cianobactérias (MESQUITA et al., 2019; WALLS et al., 2018; WILLIS et al., 2016), condições ambientais favoráveis podem intensificar seu crescimento, gerando consequências negativas, como o exemplo de florações de *Aulacoseira* sp. responsáveis pelo fechamento da distribuição de água na Argentina, por dois verões consecutivos (SILVA, MARTI; IMBERGER, 2014).

Algumas espécies pertencentes ao gênero *Aulacoseira*, como *Aulacoseira granulata* (Ehrenberg) Simonsen e *Aulacoseira ambigua* (Grunow) Simonsen, apresentam ampla plasticidade fisiológica em relação às condições ambientais (POISTER et al., 2012; WANG et al., 2017), podendo ser encontradas em diversos ecossistemas ao redor do mundo (CHEN et al., 2016; MARQUARDT et al., 2018; POISTER et al., 2012; SOARES et al., 2012).

A espécie *Aulacoseira ambigua* (Grunow) Simonsen tem sido frequentemente encontrada em ambientes lóticos e lênticos em várias regiões do mundo, inclusive no Brasil (BERTOLLI; TREMARIN; LUDWIG, 2010; CAVALCANTE; TREMARIN; LUDWIG, 2013; DUNCK; NOGUEIRA; MACHADO, 2012; HENRY; USHINOHAMA; FERREIRA, 2006), em águas que variam de oligotróficas a eutróficas (VAN DAM; MERTENS; SINKELDAM, 1994, STENGER-KOVACS et al. 2007). No entanto, essa espécie de diatomácea têm preferência para águas com altas concentrações de nutrientes (águas eutrofizadas) (HOUK, 2003; TAYLOR; HARDING;

ARCHIBALD, 2007), turbulentas, com baixa disponibilidade de luz e ampla variação de temperatura (BICUDO et al., 2016; FONSECA; BICUDO, 2008; HOUK, 2003; SANTOS; ROCHA; SANT'ANNA, 2012; TAYLOR; HARDING; ARCHIBALD, 2007). Embora *A. ambigua* apresente alguns requisitos ecológicos similares às cianobactérias (ex. preferência por águas eutróficas e se desenvolver numa ampla faixa de temperatura), alguns estudos já demonstraram que esses organismos fitoplanctônicos podem coexistir em alguns ecossistemas dulcícolas (NASELLI-FLORES et al., 2000; PEREIRA, 2018, TONETTA; PETRUCIO; LAUDARES-SILVA, 2013). Dentre as cianobactérias comumente observadas, destaca-se espécies potencialmente formadora de florações como, por exemplo, *Planktothrix* spp. (Naselli-flores et al., 2000) e *Raphidiopsis raciborskii* (PEREIRA, 2018, TONETTA; PETRUCIO; LAUDARES-SILVA, 2013). Essas espécies de cianobactérias são capazes de formar uma camada na superfície da água o que reduz a penetração de luz para camadas mais profundas do corpo d'água que, por sua vez, resulta em condições ambientais favoráveis para o desenvolvimento de *A. ambigua* visto que essa espécie tem preferência para baixa intensidade luminosa (MARQUARDT et al., 2018; NASELLI-FLORES et al., 2000; TAYLOR; HARDING; ARCHIBALD, 2007).

Na literatura, a maioria dos estudos com *Aulacoseira ambigua* enfoca a taxonomia ou descreve padrões temporais e espaciais de distribuição dessa espécie (POISTER et al., 2012; POTAPOVA et al. 2008; TREMARIN; LUDWIG; TORGAN, 2013, VAN VUUREN et al., 2018). Apesar de *A. ambigua* apresentar ampla plasticidade fisiológica, podendo ser encontrada em diversos ambientes aquáticos, poucos estudos avaliaram sua preferência quanto à disponibilidade de luz, temperatura e nutrientes (POISTER et al., 2012; TURKIA; LIPISTÖ, 1999) e apenas um analisou o possível efeito da interação entre luz e temperatura (SHEAR; NALEWAJKO; BACCHUS, 1976). Desse modo, ainda há uma lacuna na literatura sobre as preferências ecológicas de *A. ambigua* em relação respostas diante das alterações ambientais (ex. mudanças na disponibilidade de luz e na temperatura).

Comunidade zooplanctônica

A comunidade zooplanctônica é composta por organismos heterotróficos que apresentam ampla diversidade fisiológica, morfológica, comportamental e de história de vida, com reflexos na capacidade de alimentação, crescimento, reprodução e sobrevivência (HARRIS, 2000; HÉBERT; BEISNER; MARANGER, 2016, 2017; KIØRBOE, 2011; LITCHMAN; OHMAN; KIØRBOE, 2013). Esse conjunto de características (traços funcionais) permite que o zooplâncton desempenhe funções ecológicas extremamente importantes para a manutenção das teias tróficas no ambiente aquático, tais como: transferência energética para os demais níveis tróficos, ciclagem de nutrientes e controle da abundância de outros organismos (HARRIS, 2000; HÉBERT; BEISNER; MARANGER, 2016, 2017; LITCHMAN; OHMAN; KIØRBOE, 2013).

De maneira geral, muitos fatores ambientais (ex. luz, temperatura, nutrientes) podem atuar na estrutura e composição da comunidade zooplanctônica devido às alterações nas propriedades físicas, químicas e biológicas da água (ESTEVES, 1998; REYNOLDS, 2006), como também podem atuar diretamente sobre espécies zooplanctônicas (MAUCHLINE, 1998). Em relação aos efeitos diretos, a temperatura se destaca por atuar nos processos fisiológicos (ex. ingestão, respiração e desenvolvimento reprodutivo) (MAUCHLINE, 1998) e morfológicos (ex. tamanho corporal) (HAVENS, 2005). Estudos têm demonstrado que organismos zooplanctônicos, oriundos de ambientes mais quentes, apresentam menor tamanho corporal devido às altas temperaturas implicarem na redução do tempo de maturação, com exceção dos copépodos calanóides de água doce (ANGILLETTA; DUNHAM, 2003; HAVENS et al., 2005). Além disso, o tamanho corporal do zooplâncton é positivamente proporcional ao espectro do tamanho da presa e negativamente proporcional à taxa de ingestão específica em massa (BURNS, 1968). Desse modo, a temperatura pode promover impactos negativos na composição e na estrutura da comunidade zooplanctônica e tais efeitos podem se propagar sobre a dinâmica das teias tróficas aquáticas.

Os recursos alimentares também são importantes reguladores da comunidade zooplanctônica, uma vez que a quantidade e a qualidade das presas influenciam aspectos como: reprodução, crescimento corporal e populacional e sobrevivência (GER; LEITÃO; PANOSSO, 2016; IANORA et al., 2004). Dentre os recursos disponíveis no ambiente aquático, o fitoplâncton é diretamente e frequentemente consumido pelo mesozooplâncton (ex. rotíferos, cladóceros e copépodos), enquanto as bactérias geralmente são consumidas primeiramente pelos protozoários que, por sua vez, são consumidos pelo mesozooplâncton (BRETT et al., 2009; REYNOLDS, 2006).

No ambiente pelágico, o mesozooplâncton é o principal vínculo entre produtores (comunidade fitoplanctônica) e demais níveis tróficos, sendo responsáveis pelo fluxo de energia e manutenção das teias tróficas aquáticas (BOERSMA et al., 2016; GER, HANSSON; LÜRLING, 2014; GUSHA et al., 2019; JAGADEESAN et al., 2017). No entanto, a relação entre zooplâncton e fitoplâncton e, portanto, o potencial para o controle descendente, dependerá de características fisiológicas, morfológicas e comportamentais de ambas as comunidades (COLINA et al., 2016; GER; HANSSON; LÜRLING, 2014). Alguns organismos fitoplanctônicos apresentam traços que controlam a capacidade do zooplâncton de exercer o controle descendente, como por exemplo, as defesas morfológicas (por exemplo, colônia e filamentos), comportamentais (ex. formação de colônia) e químicas (por exemplo, toxinas) (KRUK et al., 2010; LÜRLING, 2020; RANGEL et al. 2016). Por outro lado, a pressão de herbivoria é controlada por traços funcionais do zooplâncton, como tamanho do corpo, modo de alimentação, seletividade e tolerância fisiológica às toxinas ingeridas (KIØRBOE, 2011; LITCHMAN; OHMAN; KIØRBOE, 2013).

Inúmeros estudos têm demonstrado o potencial de controle descendente do zooplâncton sobre o fitoplâncton (AMORIM et al, 2019; CARVALHO, 1994; COLINA et al., 2016; JEPPESEN et al., 1996; JURGENS; STOLPE, 1995), especialmente em ambientes temperados (CARVALHO, 1994; JEPPESEN et al., 1996; JURGENS; STOLPE, 1995). Nestes ambientes, a menor densidade de peixes zooplanctívoros permite que os cladóceros se desenvolvam, atingindo maior tamanho corporal e maiores densidades populacionais (CARVALHO, 1994; JEPPESEN et al., 1996; JURGENS; STOLPE, 1995). Os cladóceros exibem um espectro mais amplo de tamanhos de presas, quando comparados aos rotíferos e normalmente exibem um comportamento alimentar de suspensão mais passivo e, portanto, com menor seletividade de presas, sendo considerados animais generalistas (HANSEN, 1994; LAMPERT; SOMMER, 2007; REYNOLDS, 2006). Desse modo, os traços funcionais do cladócero (ex. maior tamanho corporal, menor seletividade à presa, dentre outros) permitem maior pressão de herbivoria sobre o fitoplâncton, refletindo numa forte relação fitoplâncton-zooplâncton e, consequentemente, num eficiente controle descendente.

Em ambientes tropicais, o menor tamanho corporal da comunidade zooplanctônica associado à dominância de rotíferos e copépodos promovem uma redução na pressão de herbivoria (GOMES et al., 2009; HAVENS et al., 2005; JOSUÉ et al., 2019; PEREIRA, 2018). Embora os rotíferos apresentem o comportamento alimentar generalista, o espectro de tamanho de presas é reduzido, quando comparados aos cladóceros, e, por este motivo, são capazes de consumir pequenos organismos fitoplanctônicos. Por outro lado, os copépodos consomem espécies fitoplanctônicas maiores, o que poderia resultar numa maior pressão de herbivoria sobre a comunidade fitoplanctônica. No entanto, o aparato de alimentação complexo através da presença de quimiorreceptores e mecanorreceptores faz com que os copépodos sejam capazes de selecionar a presa. Deste modo, os copépodos apresentam comportamento alimentar seletivo, diferindo dos rotíferos e cladóceros (BARNETT; FINLAY; BEISNER, 2007; HANSEN, 1994; LAMPERT; SOMMER, 2007; REYNOLDS, 2006). A fraca relação entre zooplâncton-fitoplâncton também pode estar associada à alta densidade de cianobactérias (JOSUÉ et al., 2019; GOMES et al., 2009), uma vez que são consideradas alimento de baixo valor nutricional e apresentam traços funcionais (ex. morfológicos e fisiológicos) que reduzem a pressão de herbivoria do zooplâncton (GER; LEITÃO, PANOSSO, 2016; LAMPERT, 1987). Além disso, estudos já demonstraram uma relação positiva entre cianobactérias e organismos zooplanctônicos seletivos, onde a comunidade zooplanctônica composta por organismos filtradores generalistas de grande porte (ex. *Daphnia*) modifica-se para seletores de partículas de pequeno porte (HANSSON et al. 2007; LEONARD; PEARL, 2005) devido a alta densidade de cianobactérias (HANSSON et al. 2007; LEONARD; PEARL, 2005).

Diversidade funcional

Ao longo dos últimos 20 anos, o uso do termo “diversidade funcional” ganhou notoriedade nos trabalhos científicos por considerar a função da espécie numa comunidade e/ou ecossistema ao invés de sua história evolutiva (DÍAS; CABIDO, 2001; PETCHEY; GASTON, 2006; TILMAN, 2001). Desse modo, estudos que enfocam a diversidade funcional, modificam o foco da espécie em si para seus respectivos traços funcionais, que serão os principais componentes selecionados através dos filtros ambientais (ex. fatores abióticos, fatores bióticos e barreiras à dispersão) (VIOLLE et al., 2007).

O termo diversidade funcional apresenta algumas definições como "a multiplicidade funcional dentro de uma comunidade" (TESFAYE et al., 2003) e "o número, tipo e distribuição de funções desempenhadas por organismos dentro de um ecossistema" (DÍAZ; CABIDO, 2001). Embora a generalidade dessas definições seja aceitável, alguns estudos que consideraram as consequências potenciais da biodiversidade para os processos do ecossistema (CHAPIN et al., 2000; GRIME, 2001;

HOOPER et al., 2005; LOREAU et al., 2001) levaram à uma definição mais específica – “o valor e a extensão dessas espécies e características do organismo que influenciam o funcionamento do ecossistema" (TILMAN, 2001). De maneira geral, a diversidade funcional baseia-se no pressuposto de que a capacidade de desenvolvimento de uma espécie em um determinado habitat está relacionada com sua estratégia de uso dos recursos, que pode se sintetizada a partir de seus traços funcionais (LAURETO et al., 2005). Logo, traço funcional pode ser definido como qualquer característica morfológica, fisiológica ou fenológica mensurável em nível de indivíduo, que afeta diretamente a eficácia biológica via seus efeitos sobre crescimento, reprodução e sobrevivência, influenciando, assim, na aptidão dos mesmos a uma determinada condição ambiental (VIOLLE et al., 2007). Ao inserir num contexto comunitário e ecossistêmico, os traços funcionais fornecem informações sobre o papel da espécie para o funcionamento do ecossistema, incluindo as interações das espécies dentro das cadeias alimentares e com seu ambiente (DÍAZ; CABIDO, 2001). Desse modo, os traços funcionais emergem como uma ferramenta eficaz para relacionar mais prontamente a estrutura da comunidade à função ecológica do ecossistema (HÉBERT; BEISNER; MARANGER, 2016, 2017).

É importante ressaltar que alguns mecanismos da diversidade funcional vão além das relações entre diversidade e função ecossistêmica, baseando-se em padrões de distribuição de espécies em nicho. Segundo Hutchinson (1957), nicho ecológico pode ser definido como um conjunto de condições bióticas e abióticas que determinam os limites dentro dos quais as espécies podem persistir e manter populações viáveis, ou seja, é uma região de um hiperespaço n-dimensional envolvendo as tolerâncias e requerimentos de um organismo, onde *n* é o número de eixos ecológicos que o constitui, tais como: as condições ambientais e os recursos. De acordo com a hipótese de diversificação de nicho, comunidades que apresentam maior diversidade funcional poderiam tirar maior vantagem das oportunidades de nicho por serem capaz de capturar uma proporção maior de recurso (CONNEL, 1978). Desse modo, pode-se esperar que a diversidade funcional, através dos traços funcionais, desempenhe um papel importante na determinação do uso de recurso (CARDINALLI, 2001; MCGILL et al., 2006; YE et al., 2019). Isto porque espécies com diferentes traços funcionais complementam-se aumentando a eficiência dos processos (complementaridade de nicho), enquanto espécies com traços funcionais semelhantes substituem umas às outras aumentando a resiliência do ecossistema (BLÜTHGEN; KLEIN, 2011). Logo, espera-se que uma comunidade com maior diversidade funcional tenha um maior particionamento de nicho de recurso e, portanto, maior eficiência no uso de recurso (GARCÍA-COMAS et al., 2016; GROSS et al., 2017; YE et al., 2019).

Utilização da diversidade funcional nas comunidades fitoplanctônicas e zooplanctônicas

Na comunidade planctônica, estudos que abordam a diversidade funcional têm se concentrado na investigação de traços funcionais por meio de índices funcionais (ex. riqueza funcional, divergência funcional, dentre outros) (ABONYI; HORVÁTH; PTACNIK, 2018; JOSUÉ et al., 2019; YE et al., 2019) ou agrupando espécies em grupos funcionais de acordo com a composição de características das espécies (REYNOLDS et al., 2002; KRUK et al., 2010).

Para a comunidade fitoplanctônica, os grupos funcionais têm sido amplamente utilizados devido à sua precisão em modelar a dinâmica dessa comunidade em diferentes cenários ambientais (KRUK et al., 2011; KRUK; SEGURA, 2012; RANGEL et al., 2016; SALMASO; NASELLI-FLORES; PADISÁK, 2015). Dentre as classificações de grupo funcional disponíveis na literatura (KRUK et al. 2010; MIELEITNER et al., 2008; REYNOLDS et al., 2002; SALMASO; PADISÁK, 2007), os grupos funcionais baseados na morfologia (“*morphologically based functional group – MBFG*”) emergiram da forte correlação entre as principais características fisiológicas e morfológicas das espécies fitoplanctônicas (KRUK et al., 2010) (Quadro 1 e 2). Esse sistema de classificação apresenta algumas vantagens em relação aos demais, tais como: a formação dos grupos independe do conhecimento de traços fisiológicos das espécies, condicionamento ambiental e afiliação taxonômica, como também independe das condições ambientais para a inclusão de novas espécies (BRASIL; HUSZAR, 2011; KRUK et al., 2010, REYNOLDS et al., 2002).

A abordagem de grupos funcionais baseados na morfologia utiliza nove traços funcionais individuais, sendo eles: volume; área superficial, razão superfície-volume, a máxima dimensão linear e a presença de mucilagem, de aerótopos, de flagelo de heterocito e de exoesqueleto silicioso (KRUK et al., 2010) (Quadro 1 e 2). A máxima dimensão linear se correlaciona com o tamanho das espécies de fitoplâncton e suas atividades fisiológicas, como crescimento e velocidade de afundamento (KRUK et al., 2010). A razão superfície-volume fornece informações sobre a capacidade de uma espécie fitoplanctônica de interceptar e assimilar recursos (luz e nutrientes), bem como sobre suas taxas de afundamento (KIRK, 1996; PADISÁK et al. 2003; REYNOLDS, 1988). A produção de mucilagem afeta positivamente a aquisição de nutrientes e a flutuabilidade das espécies fitoplanctônicas (REYNOLDS, 2006) e negativamente a pressão de herbivoria do zooplâncton (GER; LEITÃO; PANOSSO, 2016; GER, PANOSSO, LÜRLING, 2011). Os aerótopos fornecem a capacidade de flutuação, controlando a posição das espécies fitoplanctônicas na coluna d'água (PADISÁK; SORÓCZKI-PINTÉR; REZNER, 2003). Os flagelos estão relacionados à locomoção e, para algumas espécies de fitoplâncton, são considerados uma estratégia de aquisição de recursos (por exemplo, mixotrofia e fagotrofia) (GRAHAM; WILCOX, 2000). Por fim, as estruturas exoesqueléticas de silício estão relacionadas à taxa de afundamento (KRUK et al., 2010), bem como à resistência a pressão de herbivoria do zooplâncton (HAMM et al., 2003). A partir dos nove traços funcionais utilizados na proposta de grupos funcionais baseados na morfologia são formados sete grupos descritos no quadro 1 e as bases para a classificação de novas espécies estão resumidas em forma de chave dicotômica no quadro 2 (KRUK et al., 2010).

A grande complexidade dos organismos da comunidade zooplanctônica reflete a ampla diversidade de traços funcionais, categorizados em traços fisiológicos, morfológicos, comportamentais e de história de vida. Estes, por sua vez, refletem a capacidade do zooplâncton em se alimentar, crescer, se reproduzir e sobreviver (Quadro 3) (HÉBERT; BEISNER; MARANGER, 2016, 2017; KIØRBOE, 2011; LITCHMAN; OHMAN; KIØRBOE, 2013). A grande vantagem de utilizar traços funcionais ao invés de grupos funcionais é que permite a redução da complexidade, mantendo uma representação adequada da diversidade e, além disso, permite o surgimento de espécies e grupos com novas combinações de características que podem surgir sob condições ambientais mutáveis (LITCHMAN; OHMAN KIØRBOE, 2013). Assim, a abordagem por traços funcionais permite relacionar mais claramente os requisitos ecológicos das espécies zooplanctônicas (KIØRBOE, 2011; LITCHMAN; OHMAN KIØRBOE, 2013; LOKKO; VIRRO; KOTTA, 2017; MA et al., 2019; TAVSANOGLU; AKBULUT, 2019), assim como sua interação com a comunidade fitoplanctônica (JOSUÉ et al., 2019).

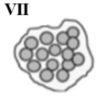
Quadro 1 – Chave para classificar as espécies fitoplanctônicas em grupos funcionais baseados na morfologia.

1 Presença de flagelo	2 Presença de estrutura silicosa	**Grupo II**			
	2’ Ausência de estrutura silicosa	3 MDL < 2 µm	**Grupo I**		
		3’ MDL > 2 µm	**Grupo V**		
1’ Ausência de flagelo	2 Presença de estrutura silicosa	**Grupo VI**			
	2’ Ausência de estrutura silicosa	3 Presença de mucilagem	4 Presença de aerótopos	5 S/V > 0,6 µm^{-1}	**Grupo III**
				5’ S/V < 0,6 µm^{-1}	**Grupo VII**
			4’ Ausência de aerótopos	5 V < 10 µm^{3}	**Grupo I**
				5’ V > 10 µm^{3}	**Grupo VII**
		3’ Ausência de mucilagem	4 V < 30 µm^{3}	5 MDL < 20 µm	**Grupo I**
				5’ MDL > 20 µm	**Grupo IV**
			4’ V > 30 µm^{3}	5 Presença de aerótopos	**Grupo III**
				5’ Ausência de aerótopos	**Grupo IV**

Legenda: V = volume (µm^{3}); S/V = razão superfície-volume (µm^{-1}); MDL = máxima dimensão linear (µm).

Fonte: Modificado de Kruk et al., (2010).

Quadro 2 – Descrição morfológica e taxa representativos dos sete grupos funcionais baseados na morfologia do fitoplâncton obtido a partir de mais de 700 espécies e 200 lagos.

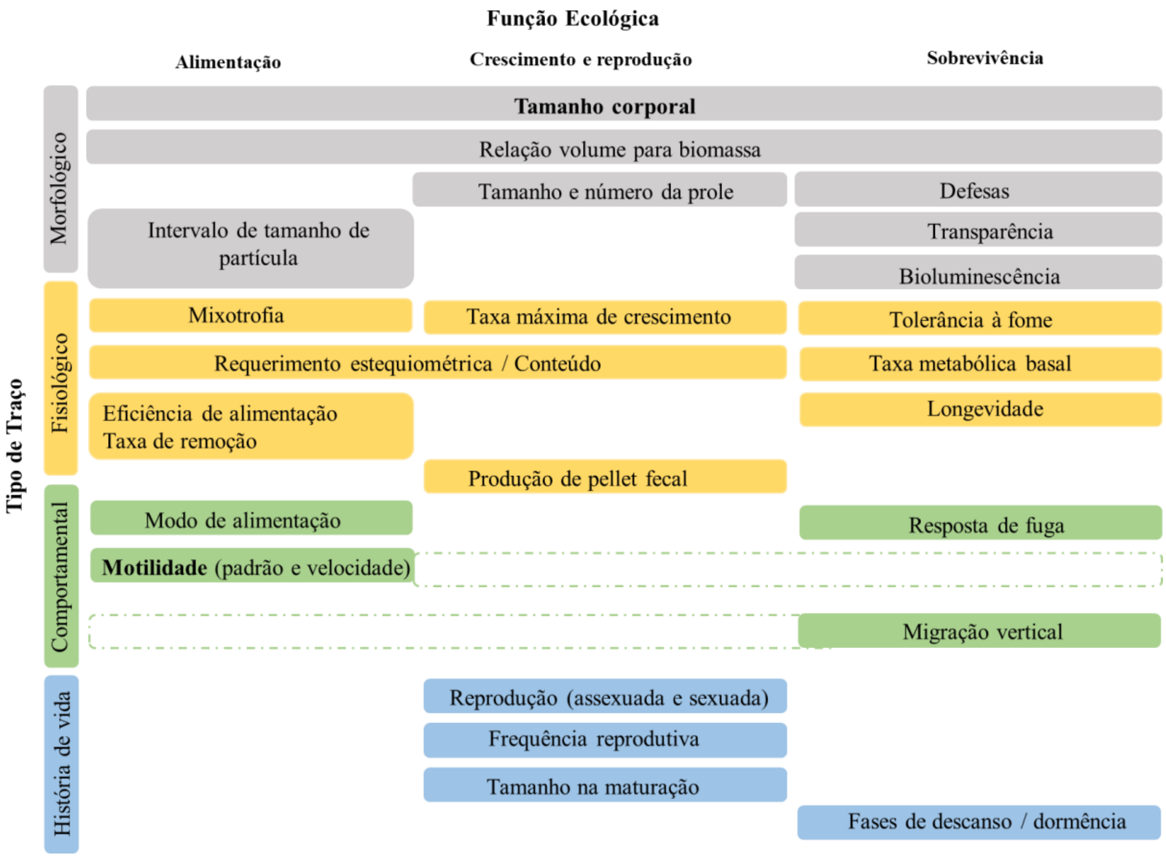
MBFG	Descrição	Táxons representativos	V (µm³)	S (µm²)	S/V (µm²)	MDL (µm)	Aer	Fla	Muc	Het	Si	Toxicidade	Suscetibilidade à herbivoria
I 	Organismos pequenos com alta razão superfície-volume	*Chlorella minutíssima* *Monoraphidium minutum*	12,9	134	5,1	7,8	0,02	0,03	0,06	0	Não	Não	Alta
II 	Pequenos organismos flagelados com estruturas exoesqueléticas siliciosas	*Chromulina gyrans* *Dinobryon cylindricum*	626	308	1,6	17,1	0	1	0	0	Sim	Não	Baixa
III 	Filamentos grandes com aerótopos	*Dolicospermum sp.* *Cylindrospermopsis raciborskii*	1541	1553	1,7	95,7	1	0	0,27	0,64	Não	Sim	Baixa
IV 	Organismos de tamanho médio sem características especializadas	*Scenedesmus acutus* *Chlorella* sp	1543	791	1,6	37	0	0	0	0	Não	Não	Alta
V 	Flagelados unicelulares de médio a grande porte	*Chlamydomonas reinhardii* *Rhodomonas* sp	2444	764	1,2	17,5	0	1	0	0	Não	Não	Média
VI 	Organismos não flagelados com exoesqueletos siliciosos	*Thalassiosira weissflogi* *Cyclotella* sp	3143	1344	1,1	44,1	0	0	0	0	Sim	Não	Média
VII 	Grandes colônias mucilaginosas	*Microcystis aeruginosa* *Aphanocapsa delicatissima*	43,15	3062	1	30,1	0,04	0	1	0	Não	Sim	Baixa

Legenda: V= volume, S = área superficial, S/V = razão superfície-volume, MDL = máxima dimensão linear, Era = aerótopo, Fla = flagelo, Muc = mucilagem, Het = heterocito, Si = sílica

Fonte: Modificado de Kruk et al. (2010) e Colina et al. (2016).

Os traços funcionais mais utilizados para a comunidade zooplanctônica são: o tamanho corporal, modo de alimentação (raptorial, filtradores micrófagos, filtradores tipos *Bosminidae* ou de suspensão estacionários) e posição trófica (carnívoro, detritívoro, herbívoro e onívoro) (KIØRBOE, 2011; LITCHMAN; OHMAN KIØRBOE, 2013; OBERTEGGER et al., 2011). Isto porque, estes traços funcionais estão diretamente relacionados a pressão de herbivoria do zooplâncton, assim como o seu efeito em múltiplas funções ecológicas, como ciclagem de nutrientes, respiração e manutenção das cadeias tróficas aquáticas (HÉBERT; BEISNER; MARANGER, 2016, 2017; LITCHMAN; OHMAN; KIØRBOE, 2013). Além disso, os traços funcionais têm sido usados como parâmetros para calcular índices de diversidade funcional do zooplâncton (JOSUÉ et al., 2019).

Quadro 3 – Classificação das características do zooplâncton de acordo com sua função ecológica e tipo de traço funcional



Legenda: Traços-chave que transcendem várias funções e influenciam muitos outros traços estão indicados em negrito. As linhas pontilhadas indicam características que podem ter uma importância secundária para outras funções.
Fonte: Modificado de Litchman; Ohman; Kiørboe (2013).

Desse modo, a utilização da diversidade funcional seja por meio de grupos funcionais (KRUK et al., 2010; REYNDOLS et al., 2002) ou traços funcionais (ABONYI; HORVÁTH; PTACNIK, 2018; JOSUÉ et al., 2019; YE et al., 2019) tem permitido um maior entendimento sobre a relação entre a biodiversidade e o funcionamento do ecossistema, uma vez que ressalta as funções das espécies e suas interações (JOSUÉ et al., 2019), como também tem sido utilizada como ferramenta para uma maior compreensão sobre a dinâmica das comunidades planctônicas em diferentes cenários ambientais (KRUK et al., 2011; KRUK; SEGURA, 2012; RANGEL et al., 2016; SALMASO; NASELLI-FLORES; PADISÁK, 2015)

Objetivos e Hipóteses

Os objetivos gerais desta tese foram i) investigar a dinâmica temporal e vertical das comunidades fitoplanctônica e zooplanctônica de um reservatório tropical raso com base nos traços funcionais das espécies (capítulo I), ii) avaliar o efeito combinado de luz e temperatura na produção de saxitoxinas em três cepas tropicais de *Raphidiopsis raciborskii* (cianobactéria) (capítulo II) e iii) avaliar o efeito combinado de luz e temperatura sobre alguns parâmetros ecofisiológicos (taxa de crescimento, morfologia, capacidade fotossintética e conteúdo de lipídios neutros) de quatro cepas tropicais de *Aulacoseira ambigua* (diatomácea) (capítulo III).

Capítulo I – Traços funcionais como preditores da composição e interação planctônica num reservatório tropical raso (“Functional traits as predictors of planktonic composition and interaction of a shallow tropical reservoir”)

A utilização da diversidade funcional através de traços funcionais ou grupos funcionais tem se mostrado uma abordagem eficaz no monitoramento da comunidade planctônica em diversos ecossistemas aquáticos (JOSUÉ et al., 2019; RANGEL et al., 2016). Tal abordagem consegue identificar alterações no padrão da distribuição da comunidade planctônica em relação às condições ambientais, como também permite um melhor entendimento da funcionalidade de cada organismo no ecossistema (KIØRBOE, 2011; KRUK et al., 2010; LITCHMAN; OHMAN KIØRBOE, 2013; OBERTEGGER et al., 2011).

Em ambientes rasos, mudanças no padrão de distribuição da comunidade planctônica podem ser observadas rapidamente em função das alterações ambientais (ex. aumento da temperatura, aumento de nutrientes, diminuição de ventos e correnteza). Inúmeros estudos têm demonstrado que os ambientes rasos apresentam variação temporal e vertical das variáveis ambientais (abióticas e bióticas) (BARBOSA; BARBOSA; BICUDO, 2018; LOPES; BICUDO; FERRAGUT, 2005), sendo o regime de mistura da coluna d'água um dos principais direcionadores da comunidade fitoplanctônica nestes ambientes (BARBOSA; BARBOSA; BICUDO, 2018; COSTA; ATTAYDE; BECKER, 2016; HUSZAR et al., 2000). Desse modo, ambientes mais heterogêneos como, por exemplo, ambientes estratificados tornam os recursos (ex. luz e nutrientes) mais disponíveis aumentando o número de nichos e, consequentemente, suportam maior diversidade de espécies do que ambientes menos heterogêneos (MACARTHUR; MACARTHUR, 1961). Logo, nós esperamos que (H_{1a}) que a estratificação da coluna d'água causada pela variação temporal e vertical das condições ambientais leve ao aumento da diversidade funcional do fitoplâncton. Consequentemente, a alta diversidade funcional do fitoplâncton (H2) aumentará o nicho-espaço da comunidade zooplanctônica, o que permitirá a coexistência de espécies com diferentes traços funcionais. O objetivo geral deste capítulo foi investigar a dinâmica temporal e vertical das comunidades fitoplanctônica e zooplanctônica de um reservatório tropical raso com base em características funcionais das espécies.

Capítulo II – Efeito combinado de luz e temperatura na produção de saxitoxinas em cepas de *Raphidiopsis raciborskii* ("Combined effect of light and temperature on the production of saxitoxins in *Raphidiopsis raciborskii* strains")

Luz e temperatura desempenham papel fundamental na comunidade fitoplanctônica, uma vez que atuam diretamente na fotossíntese e regula o metabolismo (ex. taxa de crescimento) desses organismos (KIRK, 1994; MESQUITA et al., 2019; PIERANGELINI et al., 2014; REYNOLDS, 2006). Em condições de pouca (limitação) ou excesso (fotoinibição) de luminosidade, espécies fitoplanctônicas podem apresentar redução e até limitar o crescimento, como também alterar a produção de metabólitos secundários (ex. cianotoxinas) (CARNEIRO et al., 2009; REYNOLDS, 2006). Estas respostas também podem ocorrer frente às variações na temperatura (RANGEL et al., 2016; SOARES; LÜRLING, HUSZAR, 2013; WALLS et al., 2018). No entanto, espécies de cianobactérias formadoras de florações, potencialmente produtoras de cianotoxinas (ex. saxitoxinas) apresentam ampla tolerância à intensidade luminosa e temperatura, podendo incrementar suas taxas de crescimento e produção de cianotoxina em condições de alta intensidade luminosa e alta temperatura (BITTENCOURT-OLIVEIRA et al., 2012; BONILLA et al., 2016; WALLS et al., 2018). Dentre essas espécies, *Raphidiopsis raciborskii* apresenta ampla tolerância a variações na intensidade luminosa e temperatura, além de produzir cianotoxinas, o que garantiria vantagem competitiva, servindo de explicação para sua ampla distribuição global (ANTUNES, LEÃO, VASCONCELOS, 2015; BONILLA et al., 2011, 2016; RANGEL et al., 2016). Nossa hipótese é que sob condições de alta luminosidade e temperaturas elevadas, cepas tropicais de *R. raciborskii* apresentarão maior crescimento e maior produção de saxitoxinas devido ao aumento do metabolismo celular.

Capítulo III – Efeito combinado de luz e temperatura nas respostas ecofisiológicas de cepas tropicais de *Aulacoseira ambigua* ("Combined effect of light and temperature on ecophysiology response in tropical strains of *Aulacoseira ambigua*")

As diatomáceas apresentam ampla diversidade morfológica (ex. tamanho e forma) e fisiológica (ex. temperatura e nutrientes) o que possibilita ampla distribuição global. Algumas condições ambientais como elevadas temperaturas e estratificação da coluna d'água podem impactar negativamente a abundância das diatomáceas no ambiente devido à redução na taxa de crescimento (GOMES; LÜRLING, MARINHO, 2015; MESQUITA et al., 2020) e às altas taxas de sedimentação causadas pelo esqueleto de sílica denso, respectivamente (KRUK et al., 2010), como também as diatomáceas podem responder fisiologicamente às condições ambientais ao alterar sua morfologia (CHARALAMPOUS; MATTHIESSEN; SOMMER, 2018; JUNG et al., 2013). Uma das estratégias para evitar as perdas por sedimentação é através da produção de lipídio apolar, conhecido também como lipídio neutro. O aumento da produção de lipídios neutros ocorre quando as diatomáceas estão submetidas a condições estressantes, tais como: alta intensidade luminosa ou alta temperatura (HU et al., 2008).

Dentre as espécies de diatomáceas, *Aulacoseira ambigua* é um importante componente da comunidade fitoplanctônica nos ambientes aquáticos continentais (BERTOLLI; TREMARIN; LUDWIG, 2010; CAVALCANTE; TREMARIN;

LUDWIG, 2013; DUNCK; NOGUEIRA; MACHADO, 2012; HENRY; USHINOHAMA; FERREIRA, 2006). Geralmente, a alta abundância dessa espécie está associado a ambientes eutróficos, com pouca disponibilidade de luz e temperaturas relativamente baixas (BICUDO et al., 2016; HOUK, 2003; FONSECA; BICUDO, 2008; SANTOS et al., 2012; TAYLOR; HARDING; ARCHIBALD, 2007). Apesar da grande plasticidade fisiológica dessa espécie (POISTER et al., 2012; WANG et al., 2017), há pouquíssimos estudos ecofisiológicos em relação às preferências de luz e temperatura, assim como o efeito combinado dessas duas variáveis ambientais sobre suas respostas ecofisiológicas, como crescimento, morfologia e a produção de compostos orgânicos (ex. lipídios) (TURKIA; LEPISTÖ, 1999; SHEAR; NALEWAJKO; BACCHUS, 1976). Logo**,** nós esperamos que (H1a) a combinação de alta intensidade luminosa e altas temperaturas reduzirá a taxa de crescimento, a capacidade fotossintética, o tamanho do tricoma e razão superfície volume das cepas de *A. ambigua*, enquanto proporcionará o incremento do volume celular e do conteúdo de lipídios neutros.

1. FUNCTIONAL TRAITS AS PREDICTORS OF PLANKTONIC COMPOSITION AND INTERACTION IN A SHALLOW TROPICAL RESERVOIR

Abstract

In this study, we investigate the temporal and vertical dynamics of the phytoplankton and zooplankton communities of a shallow tropical reservoir based on species functional traits. We expected that the (H_1) stratification of water column caused by temporal and vertical variation in environmental conditions would lead to an increase functional diversity of phytoplankton. Consequently, the high functional diversity of phytoplankton (H_2) would increase the space-niche for zooplankton community, which will allow species with different functional traits to co-exist. Phytoplanktonic and zooplanktonic communities and limnological variables were sampled from 2017 to 2018 in a shallow tropical reservoir. The phytoplankton was classified into morphologically based functional groups (MBFG) and the zooplankton based on the community-weighted mean trait value (CWM). Phytoplankton biomass and diversity were linked to environmental variables, where the thermal stratification of the water column observed at the warmer/rainy season led to the appearance of **MBFG III** and the rainy season were decisive for the reduction of total biomass. Also, the availability of light to the bottom of the reservoir combined with low/limiting values of dissolved nutrients were determinant for the high biomass of **MBFG V**. Regarding the zooplankton community, it was dominated by copepod cyclopoid. The omnivorous functional trait observed in cyclopoid copepods and rotifers was responsible for maintaining the biomass of the zooplankton community. The functional traits exhibited in the zooplankton (small body size, omnivorous, raptorial and sexual reproduction) and phytoplankton (medium to large size, siliceous skeleton and the presence of flagella) were determinant for the weak relationship between those communities and, consequently, inefficient trophic transfer. Hence, environmental conditions are the main driver to the phytoplankton community in shallow tropical eutrophic reservoir.

Keywords: Phytoplankton, Zooplankton, Freshwater, Functional Diversity.

1.1 Introduction

The planktonic community is composed of a wide and expressive diversity of autotrophic (e.g., phytoplankton) and heterotrophic (e.g., zooplankton) species that have morphological, physiological and behavioral differences that reflect the ability to assimilate resources, and consequently to growth, reproduction, and survive (KIØRBOE, 2011; LITCHMAN; OHMAN KIØRBOE, 2013; REYNOLDS, 2006). This community is responsible for the functioning of the many aquatic ecosystems, as phytoplankton form the base of the food web (primary producer) while zooplankton is responsible for the flow of energy and matter to other trophic levels as it is the main link between producers and consumers (KIØRBOE, 2011; LITCHMAN; OHMAN KIØRBOE, 2013; REYNOLDS, 2006).

The dynamic of plankton community rely on environmental conditions (e.g., availability of light and nutrients, mixing regime and hydrological events) and biotic interactions (e.g., herbivory; availability and quality of prey), since both aspects affect the structure of communities (e.g., phytoplankton and zooplankton), including relevant processes in species turnover and habitat selection (DOMIS et al., 2013; REYNOLDS, 2006; TONETTA; PETRUCCIO; LAUDRES-SILVA, 2013; WANG et al., 2015; ZOHARY; PADISÁK; NASELLI-FLORES, 2010). For the phytoplankton community, the availability of light and nutrients affects the growth conditions of this community, selecting species able to adapt to environmental change (COSTA; ATTAYDE; BECKER, 2016; DOMIS et al., 2013; WANG et al., 2015). Also, physiological and phenotypical characteristics of the phytoplankton species affect their palatability (e.g., size and shape) and food quality (e.g., sterols and lipids) for zooplankton communities, affecting the efficiency of energy trophic transfer to other trophic level (COLINA et al., 2016; REYNOLDS, 2006, BRETT et al. 2009). On the other hand, the zooplankton community is mainly affected by temperature (environmental condition), predation and the food resources available in the environment (e.g., availability and quality of prey), which act synergistically in the structuring and composition. Regarding the environmental conditions, temperature stands out for acting on the physiological processes, such as: ingestion, breathing and reproductive development of zooplankton species (KIØRBOE, 2011; MAUCHLINE, 1998; REYNOLDS, 2006). For example, higher temperatures imply a shorter maturation time, resulting in adult organisms of smaller body size (ANGILLETTA & DUNHAM, 2003; GILOOLEY et al., 2002). Also, body size reflects other ecological attributes such as predation, since this functional trait is positively proportional to the prey size spectrum and negatively proportional to the specific mass rate (BURNS, 1968). Meanwhile, biotic interactions such as predation and food resources (e.g., quantity and quality) can regulate the density and composition of zooplankton community because directly influence some aspects for zooplankton fitness (e.g., reproduction, individual and population growth and survival) (GER; LEITÃO; PANOSSO, 2016; IANORA et al., 2004; REYNOLDS, 2006), and consequently trigger changes in the trophic cascade (REYNOLDS, 2006). Among the available food resources, phytoplankton are often directly consumed by the mesozooplankton (e.g., rotifers, cladocerans and copepods), while bacteria are usually consumed primarily by the protozoa which, in turn, are consumed by the mesozooplankton (BRETT et al., 2009; REYNOLDS, 2006).

The relationship between zooplankton and phytoplankton and, therefore, the potential for top-down control and, consequently trophic energy transfer will depend on the functional traits present in both communities (COLINA et al., 2016; GER; HANSSON; LÜRLING, 2014; LÜRLING, 2020). Some phytoplankton species have traits that influence the palatability and food quality which affect the capacity of zooplankton to exercise top-down control, such as, morphological (e.g., colony, filament, and spines), physiological (e.g., rapid growth, production of toxins, and thick cell wall), and behavioral defenses (e.g., migration, mobility) (KRUK et al., 2010; LÜRLING, 2020; REYNOLDS, 2006). On the other hand, grazing pressure is controlled by some traits of zooplankton, such as body size, feeding type (e.g., raptorial and filter feeders), trophic group (e.g., omnivorous, herbivorous, carnivorous) and physiological tolerance (e.g., resistance to cyanotoxins) (BARNETT; FINLAY; BEISNER, 2007; KIØRBOE, 2011; LITCHMAN; OHMAN; KIØRBOE, 2013). For instance, laboratory studies have shown that zooplankton grazing pressure (e.g., clearance rate) reduce with diets composed mostly or exclusively of cyanobacteria species, since some cyanobacteria species present functional anti-herbivory traits that directly affects its palatability (e.g., long filament, larger colonies, toxin production etc.) (GER; LEITÃO; PANOSSO, 2016; GER, PANOSSO; LÜRLING, 2011; RANGEL et al., 2016, 2020). Also, the dominance of cyanobacteria in natural communities may change the composition of functional traits of the zooplankton community by promoting the development some more selective species (e.g., rotifers and copepods) (HANSSON et al., 2007; JOSUÉ et al., 2019; KRZTON; KOSIBA, 2020) effectively feeding on an alternative food source (e.g., bacteria and organic detritus) (ZÖLLNER et al. 2003; ARNDT, 1993). In this context, the trophic energy transfer can be directly affected by the composition of the functional traits of the planktonic community since the weak relationship between zooplankton and phytoplankton can trigger changes in the trophic cascade.

One of the possibilities of summarizing the wide diversity of phytoplankton-zooplankton interaction without losing much information is the use of approaches based on functional traits (KIØRBOE, 2011; KRUK et al., 2010; LITCHMAN; OHMAN; KIØRBOE, 2013). Functional trait is defined as any morphological, physiological, or phenological characteristic that is expressed in the individual level, which is affects the performance of organisms to the environment and/or their effects on ecosystem properties (VIOLLE et al. 2007). Thus, the trait-based approach appears as a tool that allows us to better understand and predict the structure and functioning of ecosystems (LITCHMAN; OHMAN KIØRBOE, 2013; CADOTTE; CARSCADDEN; MIROTCHNICK, 2011). Overall, studies have focused on the investigation of functional traits via functional indices (e.g., functional richness, functional evenness etc.) (ABONYI; HORVÁTH; PTACNIK, 2018; YE et al., 2019) or by clustering species into functional groups according to their trait composition (REYNOLDS et al., 2002; KRUK et al., 2010). For phytoplankton community, the functional group approach has been widely used, because its groups species with or without phylogenetic affinities that share similar functional traits and that respond similarly to environmental conditions (KRUK et al., 2010; REYNOLDS et al., 2002). Among them, the morphology-based functional groups (**MBFG**) emerged from the strong correlation between the main physiological and morphological traits of phytoplankton species (see KRUK et al., 2010). Moreover, MBFGs are widely acknowledged by their accuracy to model the dynamics of phytoplankton communities under different scenarios (KRUK et al., 2011; KRUK; SEGURA, 2012; RANGEL et al., 2016; SALMASO; NASELLI-FLORES; PADISÁK, 2015). On the other hand, the use of functional traits appears to be a useful tool to study zooplankton community, mainly because it relates more clearly the ecological requirements of zooplankton species (KIØRBOE, 2011; LITCHMAN; OHMAN KIØRBOE, 2013; LOKKO; VIRRO; KOTTA, 2017; MA et al., 2019; TAVSANOGLU; AKBULUT, 2019) and their interaction with phytoplankton community (JOSUÉ et al., 2019).

The structure and dynamic of planktonic community (phytoplankton and zooplankton) are also influenced by physical (e.g., light availability and mixing regime), chemical (e.g., nutrient availability, concentration of dissolved oxygen etc.), biological (e.g., predation) and hydrological (e.g., water residence time) factors, which show temporal and spatial changes (LOPES; BICUDO; FERRAGUT, 2005; BECKER et al. 2010, 2009; RANGEL et al. 2012). In shallow ecosystem, the mixing regime is one main environmental driver that act on the dynamic of the planktonic community, especially for phytoplankton, as it promotes physical (e.g., temperature and light), chemical (e.g., nutrient availability) and biological (e.g., composition and abundance) changes in the water body (COSTA; ATAYDE; BECKER, 2016; DENG et al., 2018; FONSECA; BICUDO, 2008). The mixing regime is driven by the interaction between climatological factors (e.g., solar radiation, winds and precipitation) and water body properties (e.g., depth, thermal structure, vertical and horizontal circulation), demonstrating the interrelationship between atmosphere and aquatic ecosystems (TUNDISI; TUNDISI, 2008). Thus, the stratification and mixing process (de-stratification) of the water column result from the accumulated effects of heat exchanges and energy inputs, from the absorption of solar radiation with depth (which depends on the optical conditions of the water on the surface), wind direction and strength, among other factors (TUNDISI; TUNDISI, 2008).

In shallow tropical environments, for example, the shallow depth coupled with the slight range of temperature throughout the year facilitates the complete mixing of the water column and, therefore, these systems can undergo vertical mixtures practically all year round. Furthermore, changes in the water body (e.g., physical, chemical and biological) are more evident in this kind of system, since the shallow depth provides that part or all of the bottom sediment surface is frequently, if not continuously, in contact with the water column (PADISÁK; REYNOLDS, 2003). Consequently, it is possible to observe i) an increase in turbidity and a reduction in the availability of light in the water body, since the finer sediments are susceptible to resuspension by turbulence (REYNOLDS, 2006), as well as the transport of nutrients from richer areas (hypolimnion) to others that are poorer (epilimnion), providing an increase in the abundance of the phytoplankton community, mainly at high temperatures and with greater availability of light (SØNDERGAARD; JENSEN; JEPPESEN, 2003; ZHU, QIN, GAO, 2005); ii) the recruitment of organisms and forms of resistance deposited at the bottom of these environments, which return to the euphotic zone (FONSECA;

BICUDO, 2008; JEWSON et al., 2010); and iii) a reduction in functional diversity, since the vertical mixture leads to less heterogeneity of environmental conditions (BARBOSA; BARBOSA; BICUDO, 2018; BECKER, HUSZAR; CROSSETI, 2009; LOPES; BICUDO; FERRAGUT, 2005). Shallow tropical systems also have the ability to make rapid changes with the environment (TALLING et al., 2001), allowing to observe superficial stratification of the water column during the day, followed by night mixing (atelomyxia) (BARBOSA; BARBOSA; BARBOSA, 2011; DOMIS et al., 2013). However, longer periods of thermal stratification of the water column can occur, especially in the summer where high temperatures are recorded (FONSECA; BICUDO, 2008; LOPES; BICUDO; FERRAGUT, 2005). As a result of thermal stratification, greater heterogeneity of environmental conditions can be observed, which allows the coexistence of species with different functional traits and, consequently, increasing the functionality of the ecosystem (BARBOSA, BARBOSA, BICUDO, 2018; REYNOLDS, 2006).

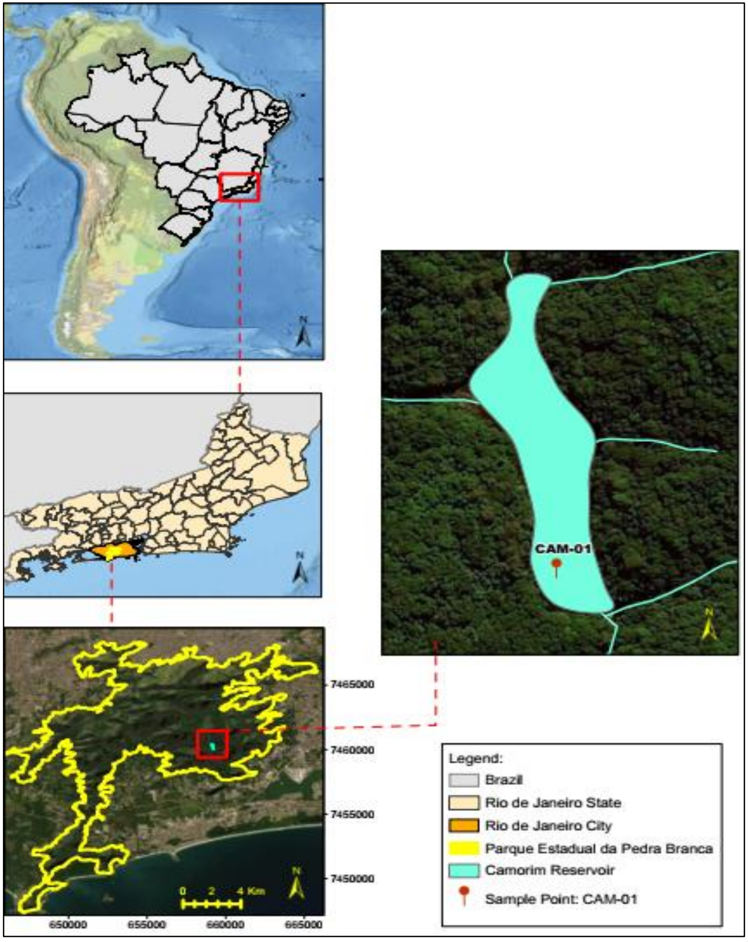
Considering the functioning of the aquatic ecosystem depends on the composition and interaction of the functional traits arising from phytoplankton and zooplankton and the structure and dynamics of the planktonic community is strongly modulated by physical, chemical and hydrological factors; we assessed the temporal and vertical dynamics of the phytoplankton and zooplankton communities of a shallow tropical reservoir based on species functional. We hypothesis that (H_1) stratification of water column caused by temporal variation in environmental conditions would lead to an increase functional diversity of phytoplankton community. Consequently, the high functional diversity of phytoplankton (H_2) would increase the space-niche for zooplankton communities, which will allow species with different functional traits to co-exist.

1.2 Materials and methods

1.2.1 Study site

The Camorim Reservoir is located on the southeastern slope of the Pedra Branca massif, 436m above sea level, in the western region of the city of Rio de Janeiro, Brazil (22°57’31’’S and 43°26’47’’W). This reservoir is located within an environmental protection area (Parque Estadual da Pedra Branca (PEPB) - State Law nº 2.377 / 1974) and, for this reason it is surrounded by forest. Also, Camorim Reservoir is considered a shallow (maximum depth of 3 m) and relatively small system (surface area = 26,000 m^2 and volume = 210,000 m^3), with a storage capacity of 2 million and 400 thousand m^3 of water (INEPAC, 1998; MENEZES, 1996; MENEZES; BICUDO, 2008). The Camorim Reservoir receives the waters of a set of rivers, in which the Camorim River is the main tributary with 6.5 km length. The waters of this reservoir flow through a hydraulic ladder steep slope for a small water treatment station located at the PEPB (INEPAC, 1998), at an average flow rate of 60 liters per second. After treatment, the water supplies approximately 20 thousand inhabitants in the western region of the city of Rio de Janeiro (CEDAE, 2010). According to the Köppen classification, the region's climate is Aw, that is, humid tropical climate without a dry, megathermic season, with maximum rainfall from December to March (summer season) and minimum rainfall from June to August (winter season). In general, rainfall ranges from 1,500 to 2,500 mm, with the rainiest periods in summer and the driest in winter (INEPC, 1998).

Figure 4 – Camorim Reservoir location map



Source: The author (2020)

1.2.2 Field Sampling

Samples of phytoplankton and zooplankton communities and limnological variables were taken at one sample point in the mild-cold/dry season (May/2017, August/2017, April/2018) and warmer/rainy season (December/2017, January/2018 and March/2018). Vertical profiles of water temperature, pH, conductivity, and dissolved oxygen were obtained with a multiparameter sonde (YSI model 600R), every 0.5 m. The depth of sampling station was measured with a portable handheld depth sounder (Hondex PS-7). Water transparency was estimated by the Secchi disk extinction depth. The precipitation data was obtained from Alerta Rio Data Center (Sistema Alerta Rio, 2020) and covered daily observations registered in the nearest weather monitoring station. Qualitative and quantitative samples from the phytoplankton community were assembled by Van Dorn like bottle at the surface (0.1 m) and the bottom (0.2 – 0.3 m above sediment). The quantitative phytoplankton samples were fixed with 2% lugol. Zooplankton samples were collected using a conical net (20 cm mouth diameter and 50 µm mesh size) and vertical hauls from 1 m depth to the surface and fixed with a 4% formaldehyde solution. Nutrients samples were collected concurrently with phytoplankton community, using the same method. Water aliquots for dissolved nutrients determinations were filtered in glass-microfilter discs (grade: MGC and diameter: 47 mm) and kept frozen until analysis.

1.2.3 Sample analysis

Soluble reactive phosphorus (SRP), nitrite (NO_2^-), nitrate (NO_3^-), ammonium (NH_4^+) and soluble reactive silicate (SRSi) were measured using flow injection analysis according to manufacturer instructions (FIAlab 2500, FIALab Instruments Inc., Seattle, Washington). Dissolved inorganic nitrogen (DIN) was considered as the sum of nitrite, nitrate and ammonium. Algal nitrogen (N) and phosphorus (P) requirements were accessed by DIN and SRP concentrations, comparing them to the semi-saturation constants for phytoplankton growth (<10 µg P/L, considered as limiting by P, SAS, 1989; and <100 µg N/L by N, REYNOLDS et al., 1997).

Phytoplankton population densities (ind. mL^{-1}) was estimated from samples fixed in 2% lugol through sedimentation (UTERMÖHL, 1958) and counting under an inverted microscope (Olympus, CKX41). Whenever possible, 100 individuals (colonies, filaments and cells) of the most frequent species were listed in random fields (UHELINGER, 1964), with the error <20% (LUND et al., 1958). We do not consider individuals less than 3 mm in size.

Zooplankton density (ind. L^{-1}) was estimated through counts in Sedgewick-Rafter chambers with the aid of an optical microscope (Nikon Eclipse E200). The samples were concentrated in a 50 μm mesh network and sub-samples were made, totaling three sub-samples per sample. In each sub-sample, between 50-150 individuals from each taxon were counted (PREPAS, 1984).

1.2.4 Data analysis

The euphotic zone (Zeu) was estimated as 2.7 times Secchi disc extinction depth (COLE, 1994), and relative water column stability (RWCS) was estimated according to Padisák et al. (2003). Phytoplankton biovolume (mm^3 L^{-1}) was estimated by multiplying the density of each species (individuals/mL) times the average cell volume of each species (μm^3) from approximate geometric shapes (HILLEBRAND et al. 1999), based on at least 20 measurements. Phytoplankton biovolume was converted to carbon content (μg C L^{-1}) using specific equations for each taxonomic group (CARPENTER; SUBRAMANIAM; CAPONE, 2004; MENDEN-DEUER; LESSARD, 2000; MONTAGNES; FRANKLIM, 2001; VERITY et al., 1992).

Phytoplankton functional diversity was estimated based on six traits: maximum linear dimension (MLD, continuous); surface to volume ratio (SV, continuous); aerotopes (binary, presence/absence); flagella (binary, presence/absence); mucilage (binary, presence/absence); siliceous exoskeletal structures (binary, presence/absence). These traits were weighted equally and were chosen because they are related to resource acquisition, reproduction and predator avoidance (WEITHOFF, 2003; LITCHMAN; KLAUSMEIER, 2008). For instance, MLD provides information on phytoplankton size and physiological activities (e.g., growth) and can be correlated with bottom-up controls (light and nutrients). SV provides information on the ability of a phytoplankton species to intercept and assimilate resources (light and nutrients), as well as about their sinking rates (KIRK, 1996; PADISÁK et al. 2003; REYNOLDS, 1988). Aerotopes provide the ability to float, controlling the position of phytoplankton species in the water column (PADISÁK; SORÓCZKI-PINTÉR; REZNER, 2003). Flagella are related to locomotion and for some phytoplankton species it is considered a resource acquisition strategy (e.g., mixotrophy and phagotrophic) (GRAHAM; WILCOX, 2000). Mucilage production affects positively the acquisition of nutrients and buoyancy of phytoplankton species (REYNOLDS, 2006) and negatively the zooplankton grazing pressure (GER; LEITÃO; PANOSSO, 2016; GER, PANOSSO, LÜRLING, 2011). Lastly, silicon exoskeletal structures are related to the sink rate (KRUK et al., 2010) and resistance of phytoplankton to the water mixing in turbulent systems, as well as the resistance to zooplankton grazing (HAMM et al., 2003).

The biomass of rotifers and copepod cyclopoid nauplii were estimated by the proposed biovolume method of Ruttner-Kolisko (1977). This methodology uses mathematical formulas to calculate the volume of organisms, based on geometric shapes most similar to the shape of the body. Thirty individuals from each taxon were measured using a camera attached to the optical microscope (Nikon eclipse E200), using the BEL-VIEW software. The dry weight was estimated using the premise that 10^6 μm^3 is equivalent to 1 µg wet weight (BOTTRELL et al., 1976) and that dry weight is 10% of wet weight (PACE; ORCUTT, 1981). The total biomass was estimated using the number of individuals of each taxon and their average mass (dry weight), expressed in mg DW m^{-3}. Biomass of Cladocera was obtained from the literature due to low number of individuals (IDRIS; FERNANDO, 1981; MASUNDIRE, 1994; MATSUMURA-TUNDISI; RIETZLER TUNDISI, 1989; PANARELLI; CASANOVA; HENRY, 2010; GÜNTZEL; MATSUMURA-TUNDISI; ROCHA, 2004), while the cyclopoid copepods biomass (juvenile + adult) was obtained through dry weight. For this, 30 individuals of cyclopoid copepods ranging from juvenile to adult were washed in distilled water to remove all material adhered to the carapace without damaging them (CULVER et al., 1985). Before being weighed, the organisms were separated on glass microfiber filters previously dried at 60°C for two hours, then cooled in a desiccation chamber for 1 hour, and weighed on a microbalance (Mettler UMT MX5). The filters with the organisms were dried at 60°C for 24 hours, cooled in a desiccation chamber for 1 hour (MCCAULEY, 1984) and immediately weighed (BURNS, 1969; CULVER et al., 1985; EDMONDSON; WINBERG, 1971; HESSEN, 1989; MCCAULEY, 1984; PACE; ORCUTT, 1981; PERSSON; EKBOHM, 1980; ROSEN, 1981). The final biovolume of rotifers and nauplii as well as the biomass of cladocerans and cyclopoid copepods, were expressed in carbon content (µg C L^{-1}), being estimated as 50% of dry weight (LATJA; SALOMEN, 1978).

Zooplankton functional diversity was estimated based on four traits: adult body size (continuous), trophic group (herbivorous, carnivorous, detritivore, or omnivorous; categorical), feeding mode (raptorial, microphagous filter-feeders, *Bosminidae* filter-feeders, or stationary suspension-feeders; categorical) and reproduction form (sexual or asexual) (BARNETT; FINLAY; BEISNER, 2007). Zooplankton traits were selected based on laboratory and observational data on feeding and life history (KIØRBOE, 2011; LITCHMAN; OHMAN KIØRBOE, 2013; LOKKO; VIRRO; KOTTA, 2017; OBERTEGGER et al., 2011). Zooplankton body size was measured during sample counting, and a mean body size value was used to each zooplankton species.

Tree-way (ANOVA) analysis was performed to test whether the phytoplankton community would present a significant difference in its total biomass and MBFGs biomass as a function of seasonality (temporal variation: warmer/rainy and mild-cold/dry periods) and depth (variation vertical: surface and bottom). Two-way (ANOVA) was applied to check for the existence of a significant difference in the biomass of zooplankton community as a function of the sampled months and taxonomic groups. Pairwise multiple comparison procedures (Holm–Sidak method) were applied to distinguish means that were significantly different ($p<0.05$). All statistical tests were performed using the tool pack SigmaPlot12.5® (Systat Software, Inc).

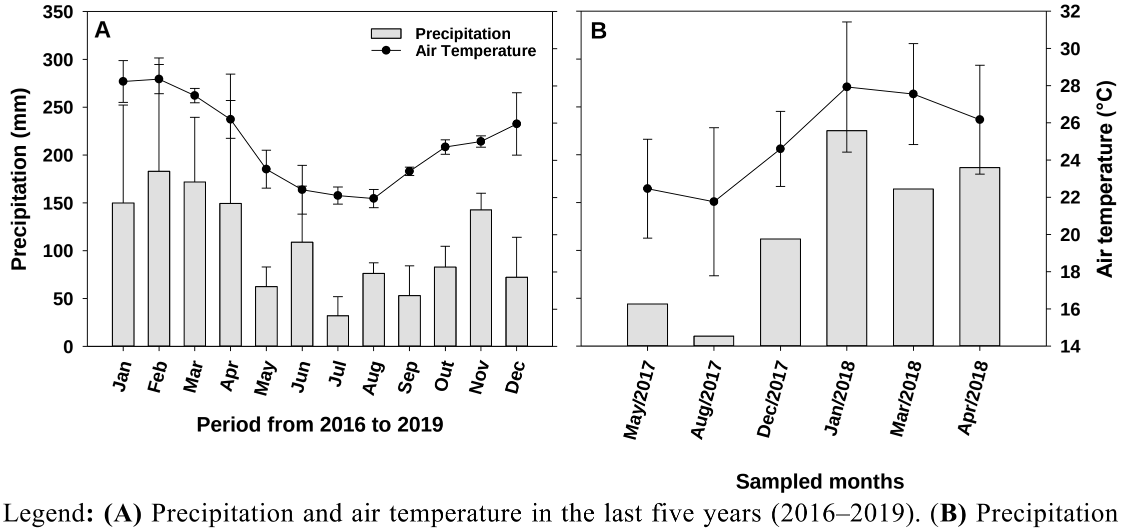
We applied the RLQ analysis (DOLÉDEC et al., 1996) to test the relationship between the functional traits of the species and the environmental variables, since when testing the co-inertia between the matrices of the environmental variables (R), species abundance (L) and species characteristics (Q) allows us to visualize the distribution of functional traits of species and their related ecological preferences. A Monte Carlo permutation test with 9999 permutations at $\alpha = 0.05$ was used to test the statistical significance of the RLQ axes. To complement the RLQ analysis, since it does not provide a significance test to identify which combination of environmental variables acts on which combination of functional traits, we used the fourth corner method and subsequently we used a method that combines the RLQ and the fourth corner (DRAY et al., 2014). The fourth corner method was applied to test the statistical significance of all pairs associations between functional characteristics of species and environmental variables. The strength of the associations was quantified through the Person r correlation coefficient and the F value of the global statistic (LEGENDRE; GALZIN; HARMELIN-VIVIEN, 1997). The p-values were corrected with 49999 model 6 permutations to correct type I errors, using the false discovery rate method to adjust the p-values to control the general error rate (see DRAY et al., 2014 for details). The combination between RLQ and the fourth corner analysis was given through the significance of the associations, tested by 99999 permutations of model 6 (DRAY; LEGENDRE, 2008) to correct type I errors, using the false discovery rate method to adjust p values to control the general error rate (DRAY et al., 2014). The analyzes were performed using the using the ade4 package (DRAY; DUFOUR, 2007) for the R software (R Core Team 2013).

1.3 Results

1.3.1 Regional climate, physical and chemical conditions

Along the study period, the air temperature ranged from 21.4 °C (August/2017) to 27.9 °C (January/2018) and total monthly precipitation from 44.0 mm (May/2017) to 274.8 mm (January/2018) (Figure 5 – B). Total precipitation and mean air temperature during the study (686.6 mm and 25.3 °C) (Figure 5 – B) were similar to the last five years (682.3 ± 82.0 mm and 25.5 ± 2.4 °C) (Figure 5 – A). We recognized May to August/2017 and April/2018 as mild-cold/dry season and December/2017, January and March/2018 as warmer/rainy season.

Figure 5 – Historical average of precipitation values and air temperature between 2016 and 2019 and precipitation values and average air temperature of the sampled months.



Legend: **(A)** Precipitation and air temperature in the last five years (2016–2019). (**B)** Precipitation and air temperature during the months sampled in the Camorim Resevoir.
Source: The author, 2020.

The maximum depth of the sampling station was 2.9 m in January/2018 (Figure 6). The euphotic zone (Zeu) reached the maximum depth of the water column in all sampled months, except for April/2018, although it represented 74% of water column (Figure 6). Because samplings were made in the morning (9:00-10:00 h), the vertical profiles of temperature are approximately related to the previous non-illuminated conditions (Figure 7). Therefore, we can assume that these profiles are not only a consequence of the diurnal warming. The relative stability of the water column (RWCS) reached maximum values in the months where the highest temperatures were recorded (warmer/rainy season) while lower values were observed in mild-cold/dry season (Table 1).

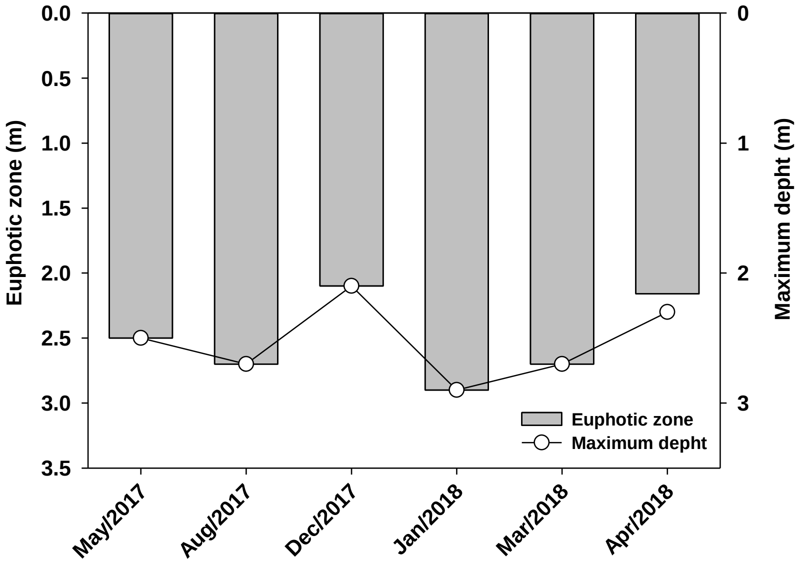
Table 1 – Values of relative stability of the water column (RWCS) in the sampling months in the Camorim Reservoir.

Months	**RWCS**
May/2017	5.37
August/2017	21.93
December/2017	63.62
January/2018	145.59
March/2018	100.70
April/2018	38.89

Source: The author, 2020.

The water temperature ranged from 17.4 ºC to 27.3 °C during the sampled months, with the highest temperatures in the warmer/rainy season and the lowest in mild-cold/dry season (Figure 7, Table 2). Vertical stratification was observed in the warmer/rainy season (Figure 7). pH values ranged from acid to circumneutral (5.6–7.5) without a seasonal pattern and, it was generally higher at the top 1 m (Figure 7, table). Dissolved oxygen (DO) always exhibited a clinograde profile, with highest values at the surface of the reservoir. Hypoxia conditions (< 3 mg L^{-1}) were recorded at the bottom of reservoir only in the months of March/2018 and April/2018 (Figure 7, Table 2). Electrical conductivity of water ranged between 40 and 58 µS cm^{-1}, with slightly higher values at the bottom and without seasonal pattern (Figure 7, Table 2).

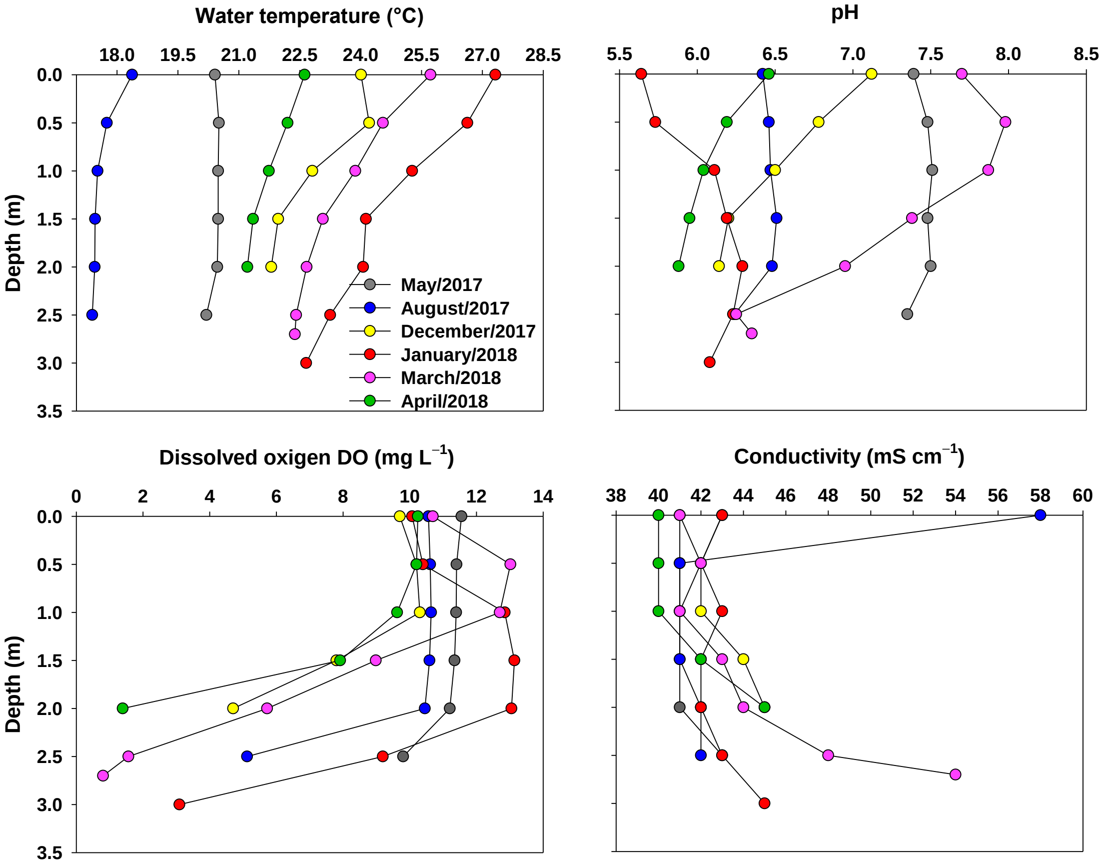
Figure 6 – Maximum depth and euphotic zone at the central station of the Camorim Reservoir during the sampled period (May/2017–April/2018).



Source: The author, 2020.

The soluble reactive phosphorus (SRP) values were below 10 µg L^{-1} at both surface and bottom (Table 2) during all sampled months. We observed that the bottom of reservoir always presented highest values of DIN when compared to the surface, which varied around 10–fold both for the surface and for the bottom (Table 2). The minimum values of DIN were below 100 µg L^{-1} and were observed on the surface (DIN = 46.7 µg L^{-1}) and bottom (DIN = 89.2 µg L^{-1}) of the reservoir in December/2017. Regarding the soluble reactive silica (SRSi), the lowest value was observed in April/2018 (surface – 39.6 µg L^{-1} and bottom – 8.6 µg L^{-1}), and there was a seasonal pattern where the highest values were observed in warmer/rainy period (Table 2). SRSi varied 21–fold at surface and 158–fold at the bottom of reservoir (Table 2). The higher values of molar ratio between dissolved inorganic nitrogen and soluble reactive phosphorus (DIN:SRP) was observed at the bottom of the reservoir, except in May/2017 (Table 2). The surface of reservoir exhibited a high variation of DIN:SRP when compared to the bottom, 11.9 and 8.4–fold, respectively (Table 2).

Figure 7 – Profile of temperature, pH, dissolved oxygen and conductivity in the water column at the sampling station in Camorim Reservoir.



Source: The author, 2020.

Table 2 – Minimum (min), maximum (max), median values and coefficient of variation (CV) of abiotic variables registered in Camorim Reservoir, during May/2017 to April/2018.

	Surface				Bottom			
	Min	Max	Median	CV	Min	Max	Median	CV
Water temperature (°C)	18.4	27.3	23.3	0.13	17.4	22.7	21.5	0.08
pH	5.6	7.7	6.7	0.10	50.9	7.4	6.2	0.07
Dissolved oxygen (mg L^{-1})	9.7	11.6	10.4	0.06	0.8	9.8	2.5	0.83
Conductivity (µS cm^{-1})	40	58	42	0.14	55	42	45	0.11
SRP (µg L^{-1})	3.5	6.2	4.8	0.19	3.6	5.3	4.8	0.12
DIN (µg L^{-1})	46.7	425	303.1	0.48	89.3	713.5	591.4	0.54
SRSi (µg L^{-1})	39.6	836.7	271.3	0.84	8.6	1368.2	153.8	1.38
DIN:SRP molar ratio	4.4	52.7	43.8	0.42	10.7	90.4	77.5	0.56

Legend: Soluble reactive phosphorus (SRP), Dissolved inorganic nitrogen (DIN), Soluble reactive silica (SRSi) and Dissolved inorganic nitrogen and soluble reactive phosphorus molar ratio (DIN:SRP molar ratio).

Source: The author, 2020.

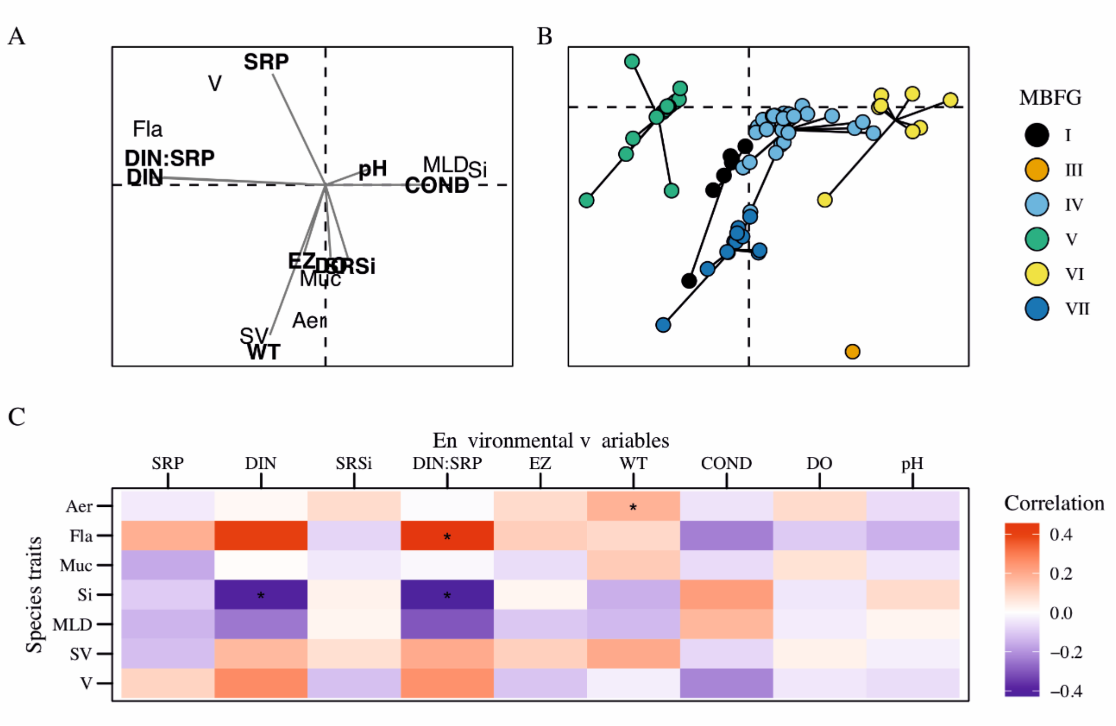
1.3.2 Phytoplankton community – taxonomic and functional diversity

During the study, 74 phytoplanktonic taxa were registered in Camorim Reservoir. Chlorophyceae showed the highest taxonomic richness (28 species), followed by Cyanobacteria (12 species), Cryptophyceae (11 species), Zygnematophyceae (9 species), Bacillariophyceae (Diatom) (8 species), Dinophyceae and Euglenophyceae (3 species each) (Anex A, Table 10). These species had a significant relationship between their functional traits and the environmental variables (Figure 8 – A). The first two axis of the RLQ analysis explained 93.56% of the variance of the data, mainly related to the water temperature, DIN, and DIN:SRP and its effects on the biomass of the species pursuing aerotopes, flagella or siliceous exoskeletal (Figure 8 – A). According to species traits we were able to identify six MBFGs, specifically MBFG **I**, **III**, **IV**, **V**, **VI** and **VII** (Figure 8 – B; Annex A, Table 11). In particular, there was a positive significant relationship between flagella and DIN: SRP, aerotopes and water temperature, but also negative relationship between siliceous exoskeletons and DIN and DIN:SRP (Figure 8 – C).

Regarding the dynamics of phytoplankton community, a fluctuation of biomass was observed over time. This fluctuation was more evident in March/2018, when there was a reduction in phytoplankton biomass, especially at the bottom of the reservoir (Figure 9). The most important contributors of the phytoplankton biomass during the sampled period were MBFG **IV** (Organisms of medium size lacking specialized traits) on the surface and MBFG **VI** (non-flagellated organisms with siliceous exoskeletons) on the bottom. The MBFG **V** (unicellular flagellates of medium to large size) was important for both depths (Figure 9 – Surface and Bottom). It is important to mention that MBFG **III** (large filaments with aerotopes) was only observed on the surface in the warmer/rainy, with low biomass, and MBFG **VII** (large mucilaginous colonies) exhibited high biomass at the surface when compared to the bottom, especially on January and April/2018 (Figure 9).

Tree-way ANOVA showed a significant difference in depth (F_1=6.299, p=0.016), in the biomass of MBFGs (F_5=9.907, p=0.001), and the interaction between deep and biomass of MBFGs (F_5=3.111, p=0.016). This analysis also showed that seasonality (F_1=2.654, p=0.110), as well as and the interactions between seasonality and depth (F_1=0.000, p=0.992), and seasonality and biomass of MBFGs (F_5=0.579, p=0.716) did not were statistically different. There was no significant difference in total phytoplankton biomass between the seasonality – warmer/rainy *vs* mild-cold/dry periods ($p>0.05$). The bottom of the reservoir showed phytoplankton biomass statistically higher when compared to the surface ($p <0.05$). Regarding the variation of the MBFGs biomass as a function of depth, only the bottom of the reservoir showed a significant difference, where the MBGFs **V** and **VI** had higher biomass when compared with the MBFGs **I** (small organisms with high S/V), **III** and **VII** ($p<0.05$), but did not differ statistically between them ($p>0.05$). In addition, MBFGs **V** and **VI** showed a significant difference in their biomass between surface and bottom, with the highest values in the bottom ($p<0.05$) (Figure 9).

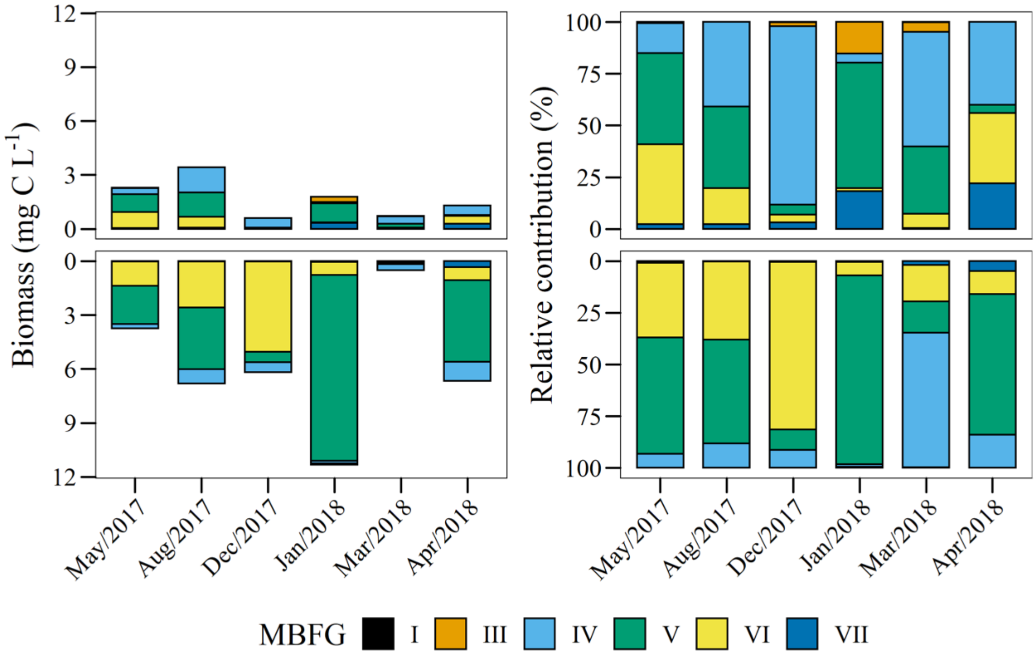
Figure 8 – Relationship between the functional traits of the phytoplankton community and environmental variables from Camorim Reservoir



Legend: (A) Ordination of the multiple relationships between functional traits and environmental variables. First and second axes summarized 64.9% and 20.7% of variation, respectively. (B) The six groups of species clustered into morphology-based functional groups according to their trait composition. Each point in the ordination plot represents the position of a species in the functional space modelled according to its traits. (C) The bivariate relationship between species traits and environmental variables. The point (*) inside the rectangles in figure C indicates a positive correlation. The functional traits are given as Si = Siliceous exoskeleton; MLD = Maximum linear dimension; SV = surface volume ratio; V = Individual volume; Fla = Flagella; Muc = Mucilage. The environmental variables are given as SRP = soluble reactive phosphorus; DIN = dissolved inorganic nitrogen; SrSi = soluble reactive silica; EZ = euphotic zone; °C = water temperature; Cond = conductivity; DO = dissolved oxygen.

Source: The author, 2020.

Figure 9 – Biomass (mg C L^{-1}) and relative biomass (mg C L^{-1}) contribution (%) of morphologically based functional group (MBFG) of phytoplankton in Camorim Reservoir (RJ) during the sampled period (May/2017 – April/2018).

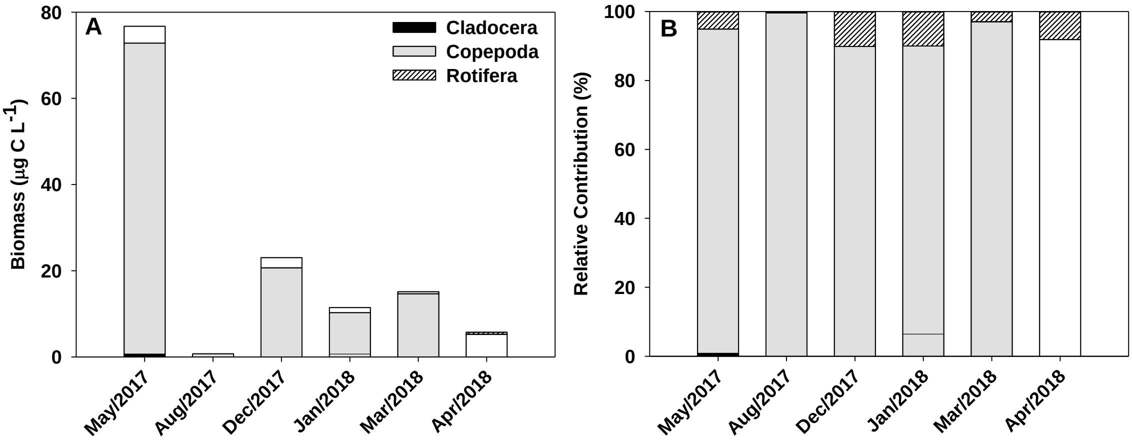


Source: The author, 2020.

1.3.3 Zooplankton community – taxonomic and functional diversity

We registered two cladocerans, one copepod cyclopoid and eight rotifers, totaling 11 taxa (Annex B, Table 12). Zooplankton biomass varied from 0.45 µg C L^{-1} to 72.21 µg C L^{-1}, and there was a strong reduction in biomass from May to August/2017. There was no temporal change in the zooplankton community, where cyclopoid copepod (*Thermocyclops* sp.) contributed with >83% of biomass during the study (Figure 10 – A). Rotifers were present in all sampled months, but in significantly less biomass when compared to cyclopoid copepod biomass. Cladocerans were observed only in March/2017 and January/2018 and, the increase in relative contribution in January/2018 was due to the change in species composition among *Diaphanosoma* sp. for *Macrothrix* sp. (Figure 10 – B).

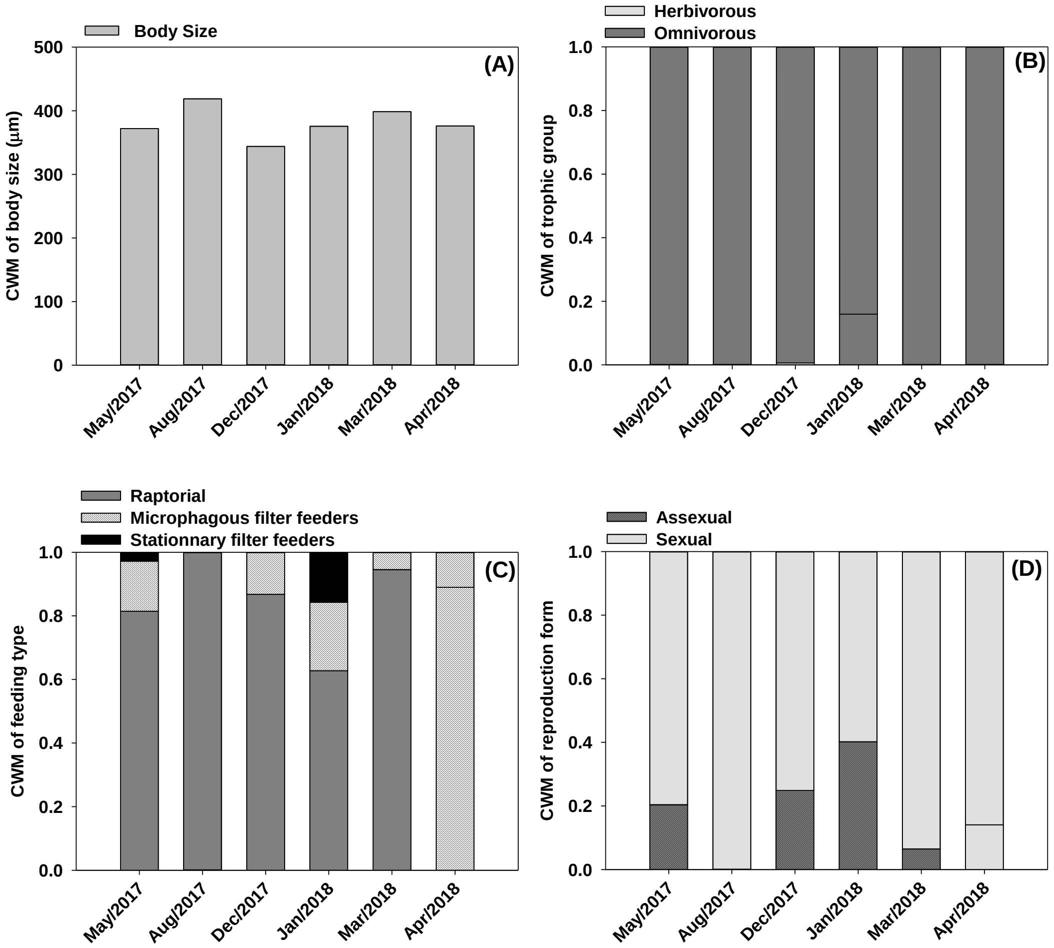
Figure 10 – Biomass (μg C L^{-1}) and relative contribution (%) of zooplankton community in Camorim Reservoir (RJ) during the sampled period (May/2017 – April/2018).



Legend: (**A)** Biomass of zooplankton community.; (**B)** relative contribution.
Source: The author, 2020.

The zooplankton community-weighted mean (CWM) values showed a dominance of species with small body sizes (Figure 11 – A), omnivores (Figure 11 – B) with sexual reproduction (Figure 11 – D). Although the omnivorous and sexual reproduction had higher CWM values during the sampled months, there was an increase in CWM values for herbivorous (Figure 11 – B) and asexual reproduction (Figure 11 – D) in the warmer/rainy season. Zooplankton feeding types were composed of microphage filter feeder species, stationary filter feeder species and raptorial species. The first two feeding types increase the CWM values in the warmer/rainy season, especially in January/2018, while the last one was dominant during the period (Figure 11 – C) (Annex B, Table 13).

Figure 11 – Total community-weighted mean trait values (CWM) of zooplankton community in Camorim Reservoir from May/2017 to April/2018.



Legend: (**A**) Body size; (**B**) Trophic group (herbivorous, carnivorous, detritivores, omnivorous); (**C**) Feeding type (raptorial, microphagous filter-feeders and stationary suspension-feeders) and (**D**) Reproduction form (asexual or sexual).

Source: The author, 2020.

1.4 Discussion

In shallow tropical ecosystems, the mixing regime is one main environmental driver that act on the dynamic of the planktonic community, especially for phytoplankton, as it promotes physical, chemical and biological changes in the water body (COSTA; ATAYDE; BECKER, 2016; DENG et al., 2018; FONSECA; BICUDO, 2008). Although these systems can undergo vertical mixtures practically all year round, relatively longer periods of thermal stratification of the water column can occur, especially in the summer where high temperatures are recorded (FONSECA; BICUDO, 2008; LOPES; BICUDO; FERRAGUT, 2005). Consequently, changes in structure of planktonic community (e.g., density and composition) could be observed (BECKER;

HUSZAR; CROSSETI, 2009; MA et al., 2019; RANGEL et al., 2016). We hypothesis that (H_1) stratification of water columns caused by temporal variation in environmental conditions would lead to an increase functional diversity of phytoplankton community. Consequently, the high functional diversity of phytoplankton (H_2) would increase the space-niche for zooplankton communities, which will allow species with different functional traits to co-exist. We observed that **MBFG IV**, **V** and **VI** were the ones that most contributed to the biomass of the phytoplankton community. Also, the biomass and diversity of that community were linked to environmental variables, especially in the warmer/rainy season, where the thermal stratification of the water column led to the appearance of **MBFG III** and the higher values of precipitation were decisive for the reduction of total biomass in March/2018. Hence, our first hypothesis cannot be rejected. Regarding the zooplankton community, the main contributor to total biomass were cyclopoid copepod and rotifers. We also observed that the zooplankton biomass did not change with the reduction of phytoplankton biomass. CWM approach showed the dominance of organisms with small body size, omnivorous, raptorial and sexual reproduction. Our results also showed that he emergence of **MBFG III** did not reflect an increase in niche-space for zooplankton community, rejecting our second hypothesis.

The Camorim Reservoir, in the last twelve years, experienced an increase in the degree of trophic state, changing from mesotrophic to eutrophic, based on the values of total phosphorus and nitrogen (unpublished data), according to Nürnberg (1996). Considering that Camorim Reservoir is in an area of environmental protection and its maximum depth is 3 m, we suggest that eutrophication of this water body can be considered as a natural process, due to the absence of direct anthropic sources that could result in an increase in the supply of nutrients. One of the possibilities to explain the increase in trophic status is from the continuous inflow of tributaries that drain an area of vegetation which can contribute to the increase in the external supply of nutrients (GÄCHTER et al., 2004; TOMBERG et al., 2014; SOROKOVIKOVA et al., 2019). For example, approximately 20% of the aquatic nitrogen comes from a natural source in agrarian regions of Switzerland (GÄCHTER et al., 2004), pointing out that external sources can contribute nutrients to the water body, even more those directed to cropland and grassland. Furthermore, the concentration of nutrients such as phosphorus, nitrogen and silica in the aquatic ecosystems can vary depending on water discharge and seasonality (GÄCHTER et al., 2004; TOMBERG et al., 2014; SOROKOVIKOVA et al., 2019).

In addition to external input of nutrients, internal source such as sediment can be an important contributor to the enhance of nutrients for the aquatic ecosystem, especially in shallow lakes, due the intense water–sediment interaction (DENG et al., 2018). In such waterbodies, the process of decomposition carried out in the sediment releases and makes nutrients available to the water column, being more diffused through the action of the winds that promote the mixing of the water column and the resuspension of the sediment. These mechanisms influence the chemical and biological processes (DA COSTA, ATTAYDE, BECKER, 2016; DENG et al., 2018; SØNDERGAARD; JENSEN; JEPPESEN, 2003; ZHU; QIN; GAO, 2005) and promoting an increase in internal nutrients loading (SØNDERGAARD; JENSEN; JEPPESEN, 2003; ZHU; QIN; GAO, 2005). The profile of water column allowed us to observe a reduction in the DO concentration at the bottom of the reservoir and a concomitant increase in electrical conductivity (March/2018), suggesting an intensification in the decomposition of organic matter, especially in a stratification period. These finding agree with the literature, where a recent study demonstrated that hypoxia resulting from decreased wind speed and increased stability of the water column, which are favorable conditions for the occurrence of thermal stratification, can promote the release of nutrients into the water column in shallow lakes (DENG et al., 2018). This process is facilitated in the Camorim Reservoir, as it is a shallow system. Therefore, the eutrophication of Camorim Reservoir can be explained by the input of external nutrients as well as the internal charge coming from the sediment.

Considering that the Camorim Reservoir is a eutrophic environment, it was not expected to observe low concentrations of nutrients. We emphasize that only the dissolved fraction of SRP, DIN and SRSi was analyzed. A possible limitation of phosphorus (P) throughout the period was observed, considering both the absolute concentrations of SRP (SRP concentrations $< 10\ \mu g\ L^{-1}$, SAS, 1989) and the molar ratio between NID:SRP (concentrations of NID:SRP > 50, KOSTEN et al., 2009). Phosphorus (P) is considered a naturally scarce resource in the environment and has already been identified as the main limiting factor for primary production in several ecosystems (RANGEL et al., 2012, 2016; SCHINDLER, 1978; TILZER, 1990; VOLLEMWEIDER; KEREKES, 1980). In other tropical reservoir, P limitation was associated with specific local factors, such as land use, capture characteristics, and hydrology (RANGEL et al., 2012). This explanation can also be applied to Camorim Reservoir, since there are no activities around the water body that could significantly increase the input of P (e.g., cropland, grassland and dwelling). We also observed low and potentially limiting values of DIN and SRSi for phytoplankton growth in December/2017 and April/2018, respectively. Here, we considered potential limiting values as DIN < 100 µg L^{-1}, (REYNOLDS, 1997) and SRSi < 50 µg L^{-1} (REYNOLDS, 2006). Contrary to our results, a previous study carried out in Camorim Reservoir between 2012-2013 demonstrated that there was no limitation of nitrogen (N) and SRSi (PEREIRA, 2018). This difference can be attributed to the lower precipitation values observed in this study, especially in December/2018. Overall, the external supply of nitrogen into aquatic ecosystems can occur through the atmospheric deposition, from the transport of allochthonous matter and acid rain (OBERGSTRÖM; JANSSON, 2006; HOBBS et al., 2000, PAERL, 1985; TOMBERG et al., 2014). On the other hand, silica is a key limiting element in aquatic ecosystems because it is mainly fixed in the form of silicate minerals and quartz fractions that cannot be taken up by aquatic organisms (ITTEKKOT; HUMBORG; SCHÄFER, 2000; GE et al. 2015). The dissolved fraction of silica used by aquatic organisms originates from the dissolution of phytolites and weathering of surface rocks (LAURELLE et al., 2009). Thus, the supply of N and dissolved silica to aquatic ecosystems such as lakes and reservoirs is given by water discharges or transported by rivers (MA et al., 2017; SOROKOVIKOVA et al., 2019; TOMBERG et al., 2014). Therefore, the rainy season is a determining factor for the entry of nutrients into aquatic ecosystems, especially those that are located within environmental preservation areas such as Camorim Reservoir. Moreover, the potential limiting concentration of SRSi did not correspond directly with higher biomass values of **MBFG VI (**functional group composed only of organisms with siliceous exoskeleton), but it may be the result of the rapid assimilation and proliferation of these organisms in earlier months (SOMMER; STABEL, 2014). Hence, it is possible that the low concentrations of these nutrients were the result of the fast incorporation by phytoplankton organisms, which would explain the high biomass of this community in almost sampled period (FEITOSA et al., 2019; RANGEL et al., 2016; SOMMER; STABEL, 2014; TOMBERG et al., 2014).

The phytoplankton community demonstrating a temporal and vertical variation in the total biomass and the composition of the **MBFGs.** Although a fluctuation in total phytoplankton biomass has been observed, there has been a drastic drop from January to March/2018. In January/2018 and February/2018, the precipitation was >250mm, above the average of the last 13 years, which was 150mm for January and <100mm for

February (Sistema Alerta Rio, 2020). High precipitation values increase the volume of the water body resulting in an increase in the rate of water runoff. This is one of the processes related to the loss of phytoplankton, especially in small and shallow lakes (REYNOLDS, 1997). The loss of phytoplankton through water runoff can be more pronounced in environments that have a high abundance of species with slow growth, as observed in the Camorim Reservoir, since it offers a competitive advantage for fast growing species (SCHEFFER, 1998). Consequently, the drop of phytoplankton biomass was associated with the high precipitation values combined with the composition of species' functional traits. Furthermore, when considering the eutrophic state of the reservoir and the relatively high biomass of phytoplankton community, it was expected that there would be a reduction in euphotic zone (TALLING, 2001). However, our results point to the opposite. The water column was almost always illuminated up to the bottom of the reservoir and this can be explained due to the shallow depth of this water body.

Inasmuch the abiotic limnological variables, mainly precipitation and air temperature showed a marked temporal variation, it was possible to separate into two climatological season (warmer/rainy and mild-cold/dry). The highest RWCS were observed in warmer/rainy season, indicating the existence of thermal stratification. This phenomenon occurs when an increase in air temperature which leads to the warming of the water, promoting a difference in the density of the water bodies (FONSECA; BICUDO, 2008). At the same time, we observed the appearance of **MBFG III** on the surface and a positive correlation between this functional group and water temperature. The **MBFG III** is composed by large filaments organisms with aerotopes (KRUK et al., 2010) which give them the ability to migrate and stay longer in the water column (PADISÁK; SORÓCZKI-PINTÉR; REZNER, 2003). Meanwhile, **MBFG VII** was observed in almost every month, although its high contribution to phytoplankton biomass was in January/2018 and April/2018. Corroborating with our data, a recent study demonstrated that stratification periods can favor **MBFGs III** and **VII**, being able to dominate the pelagic zone when environmental condition are favorable (e.g., high temperatures, water-column stability and light availability) (RANGEL et al., 2016). However, environmental conditions such as non-lasting stratification associated with persistent P limitation, mainly for **MBFG VII** (RANGEL et al., 2016), proved to be decisive for the low biomass of these **MBFGs** in Camorim Reservoir. In addition, **MBFGs III** and **VII** are not considered good food resources for zooplankton community, since large mucilaginous colonies (ROLLAND; HANS, 1987) and the capacity to produce toxins (KURMAYER; CHRISTIANSEN; CHORUS, 2003; WANG et al., 2013), which reduces the palatability for most zooplankton species leading the clearance and ingestion rates to values close to zero (COLINA et al., 2016). This means that the zooplankton community in Camorim Reservoir could favor the increase in the biomass of **MBFGs III** and **VII** by avoiding consuming them, but this did not happen. On the other hand, lower values of RWCS in the mild-cold/dry season demonstrated that the decrease in the air temperature leads to an increase in the density of the water allowing a complete water-column mixing (FONSECA; BICUDO, 2008). Consequently, higher biomass of the **MBFG VI** were observed, especially on the surface. **MBFG VI** is composed of organisms with siliceous skeleton (KRUK et al., 2010) whose silica walls protect cells against mechanical pressure, provide a barrier against zooplankton grazing (HAMM et al., 2003) and promotes rapid sedimentation rates (KRUK et al., 2010). Furthermore, other factors such as high concentrations of SRSi, water column always illuminated and limiting concentrations of SRP could explain the high abundance of **MBFG VI** in Camorim Reservoir, since the diatoms needs an amount of SRSi for their development and are considered as good competitors for phosphorus (MARINHO; AZEVEDO, 2007; TILMAN et al. 1982).

MBFGs IV and **V** were also important contributors to the total phytoplankton biomass. **MBFG IV** is composed by organisms of medium size lacking specialized traits, while **MBFG V** clusters the unicellular flagellates of medium to large size (KRUK et al., 2010). The dominance of these MBFGs over the sampled months can be explained by several factors. First, **MBFGs IV** and **V** can tolerate moderate to limiting resources (KRUK et al., 2010) combined with the ability of some species to execute mixotrophic strategies make these organisms good competitors for the environment with low or persistent nutrient limitations **(**BERGSTRÖM et al., 2003; GRAHAM; WILCOX, 2000; JONES, 1997, 2000) such as observed in Camorim Reservoir, since it is considered as a facultative ability to supplement nutrients other than carbon such as N or P (RIEMANN et al., 1995; LI; STOECKER; COATS, 2000). Secondly, the size of these organisms as prey which can hinder its consumption, even though they are considered a good food source for zooplankton (COLINA et al., 2016) due to the high content of fatty acids (AHLGREN et al., 1990; BOERSMA, 2000) and high-fat polyunsaturated acids (PUFAs) (BRETT et al., 2006). Lastly, the ability to reduce the sinking rates due to moderate size and surface/volume ratio and the presence of flagella

(**MBFG V**) (KRUK et al., 2010), which allows these organisms to spend more time suspended in the euphotic zone of the water column.

In addition, the biomass of **MBFG V** observed at the bottom of reservoir was statistically higher than the surface. Overall, flagellate organisms are associated with organically rich habitats due to the ability to assimilate organic carbon (REYNOLDS, 2006), but these organisms can also enhance their biomass through inorganic carbon dissolved (RANGEL et al., 2016). The supply of carbon (organic and inorganic) to aquatic ecosystems comes from: i) the solution of CO2 at the air–water interface; ii) the chemical weathering of carbonate rocks and debris in soils, including terrestrially sequestered atmospheric CO2, which is transported in run-off; and iii) the decomposition of organic matter (REYNOLDS, 2006). One of possibilities that may have contributed to the high biomass in the bottom was the non-limitation of light. Although mixotrophy is an important strategy in environments with low light availability (FEITOSA et al., 2019; GRAHAM; WILCOX, 2000; KATECHAKIS et al., 2005), the positive relationship between the availability of light and mixotrophic biomass indicates that mixotrophic flagellates may depend, to a large extent, on the energy and carbon derived from photosynthesis (BERGSTRÖM et al., 2003). Another possibility why **MBFG V** was dominant among phytoplankton can be given by bacterioplankton community, although it has not been evaluated here. The dominance of mixotrophic organisms over the obligate autotrophic is directly influenced by the abundance of bacterioplankton, as the autotrophic biomass decreased along the gradient of bacterial production (BERGSTRÖM et al., 2003; JANSSON et al., 2000). This is because bacteria can outcompete obligate autotrophs for inorganic nutrients by using allochthonous dissolved organic carbon (DOC) as an energy and carbon source (JANSSON, 1998), in conjunction with their innate superiority in taking up inorganic nutrients (CURRIE; KALFF, 1984; JANSSON, 1993). Thus, mixotrophic flagellates can become dominant over autotrophs, as phagotrophy allows them to use bacteria as a nutrient source (BERGSTRÖM et al., 2003; CARON, PORTER; SANDERS, 1990; JONES, 2000; SAAD et al., 2015). Lastly, the motility acquired by the presence of flagella allows these organisms to actively migrate up and down the water column being an advantageous strategy in heterogeneous environments (REYNOLDS, 2006). Also, the presence of flagella allows **MBFG V** to frequently change the direction of the swim which can decrease the rates of encounters with predators such as zooplankton (STOCKER; DURHAM, 2009).

MBFG I exhibited the lowest biomass during the period. This functional group is composed of small organisms with a high surface-to-volume ratio, fast growth rate (KRUK et al., 2010) and adapted to the rapid acquisition of resources (REYNOLDS, 1984). Such characteristics allow these organisms to be favored in low concentrations of nutrients due to a rapid exchange of nutrients across the cell surface (LITCHMAN et al., 2007), as well as for having lower sinking rates and dividing more quickly, which is favorable in reduced mixing conditions. In our system, conditions of nutrient limitation and stability of the water column would be favorable conditions for increasing the biomass of this **MBFG**. However, the constant mixing of the water column and a non-lasting stratification in the reservoir in warmer/rainy season may explain its low biomass. In addition, predation may have been another reason for the low biomass of MBFG **I** in the Camorim reservoir. This MBFG is highly ingested, especially by rotifers (COLINA et al., 2016).

The zooplankton community was dominated by cyclopoid copepods. Such dominance may explain why the drop in phytoplankton biomass in March/2018 did not negatively affect the biomass of the zooplankton community. Cyclopoid copepods are highly selective raptorial grazers known to graze on mobile heterotrophic prey (e.g., ciliates) (KIØRBOE, 2011). The contribution to total biomass of rotifers was minor when compared to copepods, but species had similar feeding habit – omnivorous, in which the main food resources are bacteria, flagellates and organic detritus (ARNDT, 1993). Meanwhile, cladocerans are known for their potential for top-down control due to the generalist habit (AMORIM et al., 2019; CARVALHO, 1994), in which there is no particle selectivity (HANSEN, 1994; REYNOLDS, 2006). Also, cladocerans presenting a wide spectrum of prey that allows predation medium to large size organisms (COLINA et al., 2016; HANSEN, 1994; REYNOLDS, 2006). However, these organisms were observed in just two months and in low biomass, being insignificant for top-down control in Camorim Reservoir. Phytoplankton and bacteria were the basis of the food web chains (FEITOSA et al., 2019; REYNOLDS, 2006), but the flows of matter and energy to the other trophic levels are quite different. Phytoplankton is directly consumed by mesozooplankton (e.g., rotifers, cladocerans and copepods), whereas bacteria due to their small size are generally consumed by the ciliate, which in turn, is consumed by mesozooplankton (BRETT et al., 2009). Taken together, cyclopoid copepods and rotifers suggest a strong consumption of heterotrophic prey such as bacteria and ciliate instead of phytoplankton and, combined with the low biomass of cladocerans indicates a weak relationship between zooplankton and phytoplankton in the Camorim Reservoir.

In addition, the consumption of phytoplankton and, therefore, the potential for top-down control of zooplankton depends on the functional traits of both communities involved (AMORIM et al., 2019; COLINA et al., 2016; KRZTOŃ; KOSIBA, 2020; KRZTOŃ et al., 2019; LÜRLING, 2020). CWM demonstrated the dominance of zooplankton species of small body size, omnivorous, raptorial, and sexual reproduction. Body size is inversely related to temperature in ectotherms, including zooplankton (ANGILETTA; DUNHAM, 2003; GILOOLEY et al., 2002; HAVENS et al., 2015). Thus, at higher temperature shortens generation time resulting in smaller adult body size (ANGILLETTA; DUNHAM, 2003). Furthermore, the body size is positively proportional to the prey size spectrum (BURNS, 1968), where rotifers due to small body size consume small-sized phytoplankton, cladocerans have a wider spectrum of prey sizes, while copepods usually feed on larger prey (HANSEN, 1994; LAMPERT; SOMMER, 2007; REYNOLDS, 2006). In this context, the small body size of the zooplankton community in Camorim Reservoir further reduced the prey size spectrum, since the dominant phytoplankton species were medium to large. In addition to the size of the prey, the consumption of phytoplankton by zooplankton is also linked to the quantity and quality of the resource (COLINA et al., 2016). Thus, the predominance of anti-grazing traits of phytoplankton species (e.g., size; motility; thick cell wall) in the Camorim Reservoir implies a reduction in grazing by the zooplankton.

The dominance of sexual reproduction was linked to the copepod's biomass. This type of reproduction result in a longer life cycle due to the larval and juvenile phases until reaching maturity and have a smaller number of offspring (REYNOLDS, 2006). The copepod's stage of development also reflects a difference in eating habits (BRUCET et al., 2008; REYNOLDS, 2006). For example, the larval (nauplii) stage prey small particles such as bacteria (FAITHFUL; GOETZE, 2017; TURNER; TESTER, 1992) and picoplankton (BRUCET et al., 2008) while the stages of juvenile (copepodites) and adults prefer large prey (e.g., micro- and nanoplankton) (BRUCET et al., 2008). As the juvenile and adult stages were predominant in relation to the nauplii in the Camorim Reservoir, a strong consumption of phytoplankton was expected, but the omnivorous and raptorial traits may have allowed this not to happen. In addition, studies have already shown that the high abundance of diatoms can have a negative impact on the life cycle of copepods, since they result a significant reduction in the incubation of eggs (IANORA et al., 2004; MIRALTO et al., 1999). Thus, the dominance of juveniles and adults may be related to the quality of the food in the Camorim reservoir, especially due to the high biomass of **MBFG VI**. Soon, the biomass of planktonic species and their respective functional traits are decisive factors for trophic transfer efficiency between phytoplankton and zooplankton.

1.5 Conclusion

The approach of functional diversity (MBFG and CWM) proved to be efficient for the study of planktonic communities in a shallow tropical reservoir. The major contributors for phytoplankton biomass were **MBFGs IV**, **V** and **VI**. Phytoplankton biomass and diversity were linked to environmental variables. In the warmer/rainy period, the thermal stratification of the water column led to the appearance of **MBFG III** and the rainy season were decisive for the reduction of total biomass in March/2018. Also, the availability of light to the bottom of the reservoir combined with low/limiting values of dissolved nutrients were determinant for the high biomass of **MBFG V** due to the ability to use photosynthesis and mixotrophy strategy for their development. Regarding the zooplankton community, it was dominated by copepod cyclopoid. The omnivorous functional trait observed in cyclopoid copepods and rotifers was responsible for maintaining the biomass of the zooplankton community even with the reduction of phytoplankton biomass due to the ability of organisms to feed on other organisms, such as ciliate and bacteria. The functional traits exhibited in the zooplankton (small body size, omnivorous, raptorial and sexual reproduction) and phytoplankton (medium to large size, siliceous skeleton and the presence of flagella) were determinant for the weak relationship between those communities and, consequently, inefficient trophic transfer. Hence, environmental conditions are the main driver to the phytoplankton community in shallow tropical eutrophic reservoir.

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2. COMBINED EFFECT OF LIGHT AND TEMPERATURE ON THE PRODUCTION OF SAXITOXINS IN *RAPHIDIOPSIS* (*CYLINDROSPERMOPSIS*) *RACIBORSKII* STRAINS.

(Artigo publicado na Toxins - final da tese)

Abstract

Raphidiopsis raciborskii is a potentially toxic freshwater cyanobacterium that can tolerate a wide range of light and temperature. Due to climatic changes, the interaction between light and temperature is studied in aquatic systems, but no study has addressed the effect of both variables on the saxitoxins production. This study evaluated the combined effect of light and temperature on saxitoxins production and cellular quota in *R. raciborskii*. Experiments were performed with three *R. raciborskii* strains in batch cultures under six light intensities (10, 40, 60, 100, 150, and 500 µmol of photons m^{-2} s^{-1}) and four temperatures (15, 20, 25, and 30°C). The growth of *R. raciborskii* strains was limited at lower temperatures and the maximum growth rates were obtained under higher light combined with temperatures equal or above 20°C, depending on the strain. In general, growth was highest at 30°C at the lower light intensities and equally high at 25°C and 30°C under higher light. Highest saxitoxins concentration and cell-quota occurred at 25°C under high light intensities, but were much lower at 30°C. Hence, increased temperatures combined with sufficient light will lead to higher *R. raciborskii* biomass, but blooms could become less toxic in tropical regions.

Key words: Cyanobacteria; Cyanotoxins; Saxitoxins, Intraspecific Variability.

2.1 Introduction

Raphidiopsis raciborskii is a freshwater cyanobacterium, which is widely distributed in tropical, subtropical, and temperate regions (ANTUNES, LEÃO, VASCONCELOS, 2015; BRIAND et al., 2004). It can tolerate a wide range of temperatures and light intensities (BITTENCOURT-OLIVEIRA et al., 2012; BONILLA et al., 2012, 2016) and is able to produce distinct cyanotoxins, like cylindrospermopsins (CYN) and saxitoxins (STXs) (DITTMANN, FEWER; NEILAN, 2013). The production of these cyanotoxins by *R. raciborskii* is related to its geographical distribution, where Brazilian strains produce STXs derivatives (CARNEIRO et al., 2009; MOLICA et al., 2002; LAGOS et al., 1999) and Australian strains can produce CYNs (ANTUNES, LEÃO, VASCONCELOS, 2015; CARNEIRO et al., 2013), while North American strains are still uncertain about the production of this toxin (BURNS, 2008; YILMAZ; PHILIPS, 2011), and European strains produce other, yet undescribed, neurotoxins (ANTUNES, LEÃO, VASCONCELOS, 2015).

The most common STXs are generally grouped according to their structural differences in the three main groups, namely the carbamoyl, dicarbamoyl, and sulfocarbamoyl derivatives (DITTMANN, FEWER; NEILAN, 2013). The carbamoyl group (STXs) includes STXs, neosaxitoxin (Neo) and the gonyatoxins (GTX 1–4), with STX the more studied (CARNEIRO et al., 2009, CARNEIRO; PACHECO; AZEVEDO, 2013; COSTA; FERRÃO-FILHO; AZEVEDO, 2013; LAGOS et al., 1999). The biosynthesis of STXs and the evolution of the genes responsible for their complex metabolism seem to indicate that these toxins are probably linked with ecological advantages for their producers (MURRAY, MIHALI, NEILAN, 2011; HACKETT et al., 2013; HOFF-RISSETI et al., 2013; WIESE et al., 2010). Environmental factors that have been found upregulating STXs biosynthesis are high light intensity (CARNEIRO et al., 2009), high and suboptimal temperature, extracellular salt (NaCl) (BOOPATHI; KI, 2014), conductivity, and dissolved inorganic nitrogen (DIN) (BRENTANO; GIEHL; PETRUCIO, 2016), whereas high nitrogen concentrations and darkness downregulated STX biosynthesis (BOOPATHI; KI, 2014; CARNEIRO et al., 2009).

In Brazil, higher *Raphidiopsis raciborskii* densities and higher STXs production were associated with lower temperatures in the field (CASALI et al., 2017). In contrast, few laboratory studies with isolated strains have observed increased production of STXs from low to high temperatures (CASTRO et al., 2004; RANGEL et al., 2016). In addition to the effect of temperature, light intensity also affects the production of STXs: High light intensity (100 µmol of photons $m–^{2}$ $s–^{1}$) promotes the increase in production of STXs (CARNEIRO et al., 2009).

The number of studies combining light and temperature in *R. raciborskii* has amplified (BITTENCOURT-OLIVEIRA et al., 2012; BONILLA et al., 2016; XIAO; WILLIS; BURFORD, 2017). In general, combined high light intensities at high temperatures yielded increased growth rates in *R. raciborskii* strains (BITTENCOURT-OLIVEIRA et al., 2012; BONILLA et al., 2016). However, the combined effect of light and temperature on STXs production has not been studied. Understanding how the interaction of these two variables may affect the production of STXs is crucial for the management of toxic *R. raciborskii* blooms. In this study, we tested the hypothesis that (1) *R. raciborskii* strains would be able to grow in a wide range of temperatures, and (2) combined high light intensity and high temperature would increase the production of STXs. To this end, we evaluated the combined effects of light and temperature on saxitoxins production in three *R. raciborskii* strains isolated from a naturally eutrophic reservoir.

2.2 Material and methods

Experiments were carried out with three strains of *Raphidiopsis raciborskii* (CYLCAM-01, CYLCAM-02 and CYLCAM-03). The strains were isolated between 2012 and 2013, from a tropical shallow reservoir located in a protected area (Pedra Branca State Park—22◦55 54.44 S/43◦28 22.44 W) in the western part of the municipality of Rio de Janeiro (Brazil). Currently the strains are maintained in the Culture Collection of the Laboratory of Ecology and Physiology of Phytoplankton, University of Rio de Janeiro State (UERJ) under the following conditions: WC medium (LÜRLING; BEEKMAN, 1999); temperature, 25°C; irradiance, 30 µmol photons m^{-2} s^{-1} provided by daylight fluorescent lamps (Sylvania T10–20 watts–5000 k) and measured with a Li-COR quanta meter sensor; photoperiod, 12–12h light-dark cycle. Cultures were not grown axenically, but regular microscopic inspection revealed that biomass of heterotrophic bacteria remained well under 1% of total biovolume.

Before starting the experiments, the inoculum was acclimatized for 10–15 days at different combinations of light intensity and temperature. Six light intensities (10, 40, 60, 100, 150, and 500 µmol of photons m^{-2} s^{-1}) and four water temperatures (15, 20, 25, and 30°C) were established. The light intensity was provided by daylight fluorescent lamps in a 12–12 h light-dark cycle. The flasks were agitated twice a day. The water temperatures tested were chosen based on the annual mean (25°C), winter mean (20°C), and the extreme temperatures (15°C and 30°C) observed in Camorim reservoir.

The combined effects of light and temperature on the *R. raciborskii* strains were studied in batch culture systems in triplicates. The experiments were setup in Erlenmeyer flasks of 300 mL, with 200 mL modified WC culture medium (LÜRLING; BEEKMAN, 1999). *Raphidiopsis raciborskii* strains were inoculated with initial biomass of 100 µg L^{-1} chlorophyll-a (Chl-a). The flasks were placed in incubators (SOLAB SL-224) under the 24 combinations of light and temperature and the growth was monitored for 10 days. Samples were collected daily for measurements of the Chl-a concentration and photosystem II efficiency (φPSII), analyzed with the phytoplankton analyzer Phyto-Pam (Heinz WalzGmbH, Effeltrich, Germany). The Phyto-PAM was calibrated against a spectrophotometric determination of Chl-a from the *R. raciborskii* strains, which was done with a 90% acetone extraction, based on Ritchie (2006) [36]. Phyto-Pam is a methodology that analyzes cells in vivo from fluorescence, with fluorescence being considered a rapid method for growth monitoring (BRIAND; GUILLARD; MURPHY, 1981).

Growth rates (µ) were calculated from the chlorophyll-a increase using a logistic curve model (Equation (1)) that was fitted iteratively in SigmaPlot 12.5® software.

$$N_t = \frac{\mathrm{N}_o * \mathrm{k}}{(\mathrm{N}_o + (k - \mathrm{N}_o) * \exp(-\mu * t))} \qquad (1)$$

Where Nt = chlorophyll-a; t = time (days); N0 = initial value of chlorophyll-a; k = support capacity; µ = intrinsic rate of population increases (day^{-1}).

Saxitoxins (STXs) were analyzed in samples taken at the end of the experiment (day 10). Depending on cell density, 5–15 mL culture volume was filtered through a 25 mm diameter glass fiber filter (GF1, Sartorius, pore size: 0.7 µm) and filters were immediately stored in a freezer (−20°C) until analysis. For extraction of PSP, the filters were soaked with 2 mL of acetic acid 30 mM and, for 1 min, sonicated at 30% of potency in falcon tubes placed in an ice bath. After this step, the samples were centrifuged at

4°C for 15 min. The supernatant was collected, filtered through 0.22 µm PVDF filters (13 mm diameter—Analitica) and transferred into HPLC vials for subsequent analysis according to Diener et al. 2006 and 2007. STXs were analyzed using a post-column oxidation method with fluorescence detection (HPLC-FD), according to Diener et al. 2006 and 2007 based on hydrophilic interaction liquid chromatography coupled fluorescence detector. Briefly, STXs and its analogues were separated on a Shimadzu Prominence (Kyoto, Japan) liquid chromatography system equipped with a post-column reaction oven and a fluorescence detector (RF10AX). Commercially available standards of STXs derivatives (National Research Council/Institute for Marine Biosciences, Halifax, NS, Canada) were employed in HPLC-FD experiments for compound identification. Toxins were detected using a fluorometric detector, with excitation at 330 nm and emission at 390 nm. Toxins were identified and quantified by comparison with known retention time and integrated areas of analytical standards. Calibration standards for all analyzed toxins (STX, dcSTX, NEO, GTX-2,3 dcGTX-2,3) were obtained from the Institute of Marine Bioscience, National Research Council of Canada (Halifax, NS, Canada).

The concentrations of gonyatoxins GTX2 + GTX3 (only GTX 2 and GTX3 were detected) were used to assess the cellular quota. The cell quota of STXs was determined by dividing the STXs concentration (µg mL^{-1}) by cell density. Cell density was calculated from the sample counts of the last day (t10) fixed in lugol 2% in a Neubauer chamber through the optical microscope (Nikon Eclipse E-200 LED MV R, Nikon Corporation, Tokyo, Japan).

2.2.1 Statistical Analysis

In order to verify the combined effect of light and temperature on the growth, saxitoxins production and saxitoxins cellular quota in the three strains of *Raphidiopsis raciborskii*, two-way ANOVA with temperature and light as fixed factors were run. Prior to analysis, normality was tested using a Shapiro-Wilk test, whereas homogeneity of variance was tested by Levene's Equal Variance Test. When normality and variance failed, the data were log-transformed. To detect differences between groups, Holm-Sidak post-hoc comparisons were carried out. In all analyses, the level of significance was set at $p < 0.05$. All statistical tests were performed using the SigmaPlot® program version 12.5

2.3 Results

2.3.1 Growth Rates

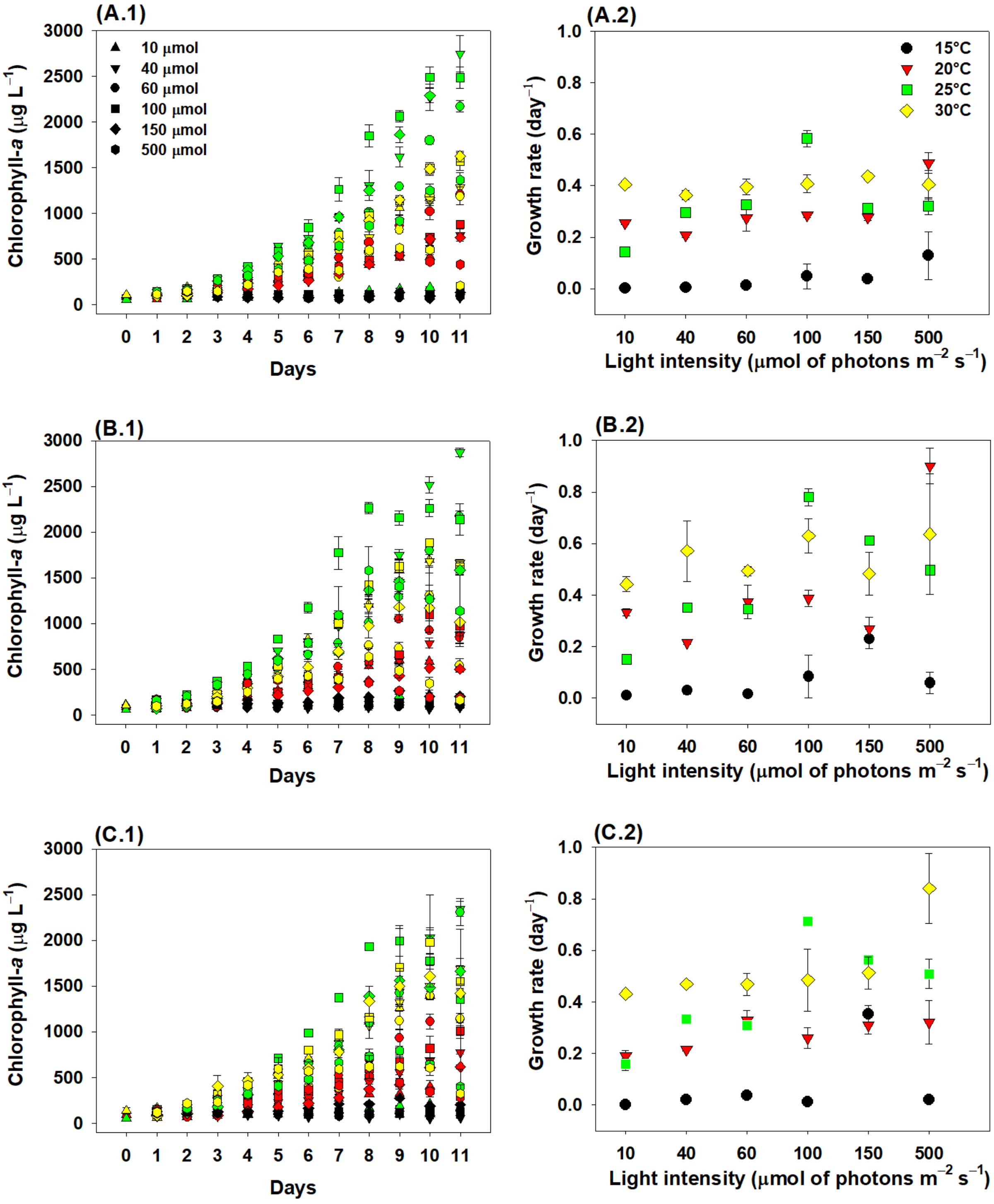
Light, temperature, and light × temperature interaction had significant effects on growth rates of *R. raciborskii* strains (Table 3). All strains showed the lowest growth rates ($p<0.05$) at 15°C, combined with low light intensities (≤ 60 µmol of photons m^{-2} s^{-1}) (Figure 12).

Table 3 – Two-way ANOVA table for effects of temperature and light intensity on growth rates of *Raphidiopsis raciborskii* strains.

Source of Variation	**DF**	**F**	**P**
CYLCAM-01			
Light	5	15.93	<0.001
Temperature	3	270.40	<0.001
Light × Temperature	15	5.18	<0.001
Residual	48		
Total	71		
CYLCAM-02			
Light	5	22.84	<0.001
Temperature	3	116.17	<0.001
Light × Temperature	15	11.18	<0.001
Residual	48		
Total	71		
CYLCAM-03			
Light	5	29.68	<0.001
Temperature	3	195.63	<0.001
Light × Temperature	15	13.72	<0.001
Residual	48		
Total	71		

Legend: DF = Degree of freedom; F = statistic values; P = significance level.

Figure 12 – Growth based on chlorophyll-*a* (day^{-1}) and calculated growth rates (μ) of *Raphidiopsis raciborskii* strains under the combinations of six light intensities and four water temperatures.



Legend: Letters indicated *R. raciborskii* strains: CYLCAM-01 (A); CYLCAM-02 (B) and CYLCAM-03 (C). Symbols indicate light intensities: Up triangles = 10 μmol photons m^{-2} s^{-1}; down triangles = 40 μmol photons m^{-2} s^{-1}; circles = 60 μmol photons m^{-2} s^{-1}; squares = 100 μmol photons m^{-2} s^{-1}; diamonds = 150 μmol photons m^{-2} s^{-1}; hexagons = 500 μmol photons m^{-2} s^{-1}; Colors indicate temperatures: black = 15 °C; red = 20 °C; green = 25 °C; yellow = 30 °C. Bars are standard deviations (n=3).

Source: The author, 2020.

The increase in temperature was an important factor for the growth rate of CYLCAM-01 strain when combined with light intensities of 10 and 100 µmol photons m^{-2} s^{-1}. CYLCAM-01 strain exhibited the highest growth rates ($p<0.05$) at 25°C when combined with 100 µmol photons m^{-2} s^{-1}, reaching 0.58 ± 0.03 day^{-1} (Figure 12 – A1, A2).

At light intensities of 40, 100, and 500 µmol photons m^{-2} s^{-1}, the increase in temperature was a significant factor for the growth of CYLCAM-02 (Figure 14 – B1, B2). This strain showed the highest growth rate of the study (0.90 ± 0.06 day^{-1}) when combining 500 µmol photons m^{-2} s^{-1} at 20°C ($p<0.05$). This strain exhibited an increase in the growth rate with increasing light intensity of up to 100 µmol photons m^{-2} s^{-1} at 25°C, and reduction occurred when combined with the highest light intensities ($p<0.05$). At 30°C, the increase in light intensity did not affect the growth rate ($p>0.05$) (Figure 12 – B2).

The CYLCAM-03 showed a significant increase in growth rate in 150 µmol of photons m^{-2} s^{-1} at 15°C (Figure 12 – C1, C2). Likewise observed for CYLCAM-02, the increase in temperature was an important factor for the growth rate of CYLCAM-03 strain when combined with light intensities of 40, 100 and 500 µmol photons m^{-2} s^{-1}. At 30°C, only CYLCAM-03 strain showed a significant difference in growth rates, with an increase when combined with 500 µmol of photons m^{-2} s^{-1}, reaching its highest growth rate (0.84 ± 0.13 day^{-1}, $p<0.005$).

2.3.2 Saxitoxins (STXs) concentrations

Typical chromatograms of CYLCAM-01, 02, 03 strains, as well as PSP analytical standards, can be seen in Figure 13, where we only found gonyatoxins (GTX-2 and GTX-3). The saxitoxins concentration was made by the sum of GTX-2 and GTX-3 (Figure 13). Although some variation occurred, on average, each variant represented 50% of the total STXs. Two-way ANOVA revealed a significant temperature and light intensity effect, and a significant interaction between the two factors on STXs concentrations in the cultures (Table 4).

Table 4 – Two-way ANOVA table for effects of temperature and light intensity on saxitoxins (STXs) production of *Raphidiopsis raciborskii* strains**.**

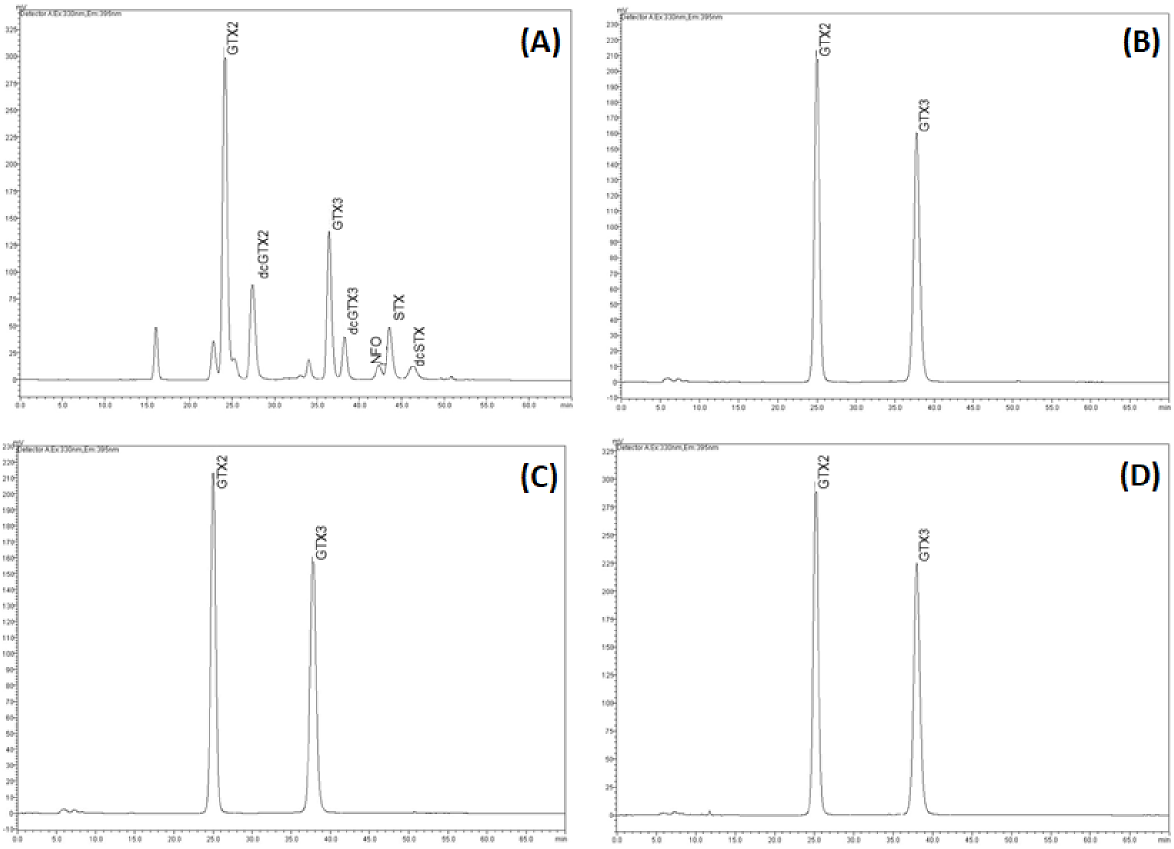
Source of Variation	**DF**	**F**	**P**
CYLCAM-01			
Light	5	5.583	<0.001
Temperature	3	27.787	<0.001
Light × Temperature	15	5.292	<0.001
Residual	48		
Total	71		
CYLCAM-02			
Light	5	2.546	<0.041
Temperature	3	24.874	<0.001
Light × Temperature	15	5.162	<0.001
Residual	46		
Total	69		
CYLCAM-03			
Light	5	0.726	0.607
Temperature	3	7.644	<0.001
Light × Temperature	15	2.798	0.004
Residual	47		
Total	70		

Legend: DF = Degree of freedom; F = statistic values; *P* = significance level.

The three strains of *R. raciborskii* showed the lowest STXs concentrations ($p<0.05$) at 15°C, regardless of light intensity (Figure 16). The higher STXs values were recorded at 25°C under high light (≥ 100 μmol of photons m^{-2} s^{-1}) for all strains and at 30°C for CYLCAM-03 in combination with low light (≤ 60 μmol of photons m^{-2} s^{-1}) (Figure 14 – C) ($p<0.05$).

The CYLCAM-01 strain showed no significant difference in STX production, regardless of tested temperature when combined with low light intensities (10 and 40 μmol of photons m^{-2} s^{-1}) ($p>0.05$). However, there was an increase of STXs when combining high light intensities (≥ 100 μmol of photons m^{-2} s^{-1}) at 25°C (Figure 14 – A) ($p<0.05$).

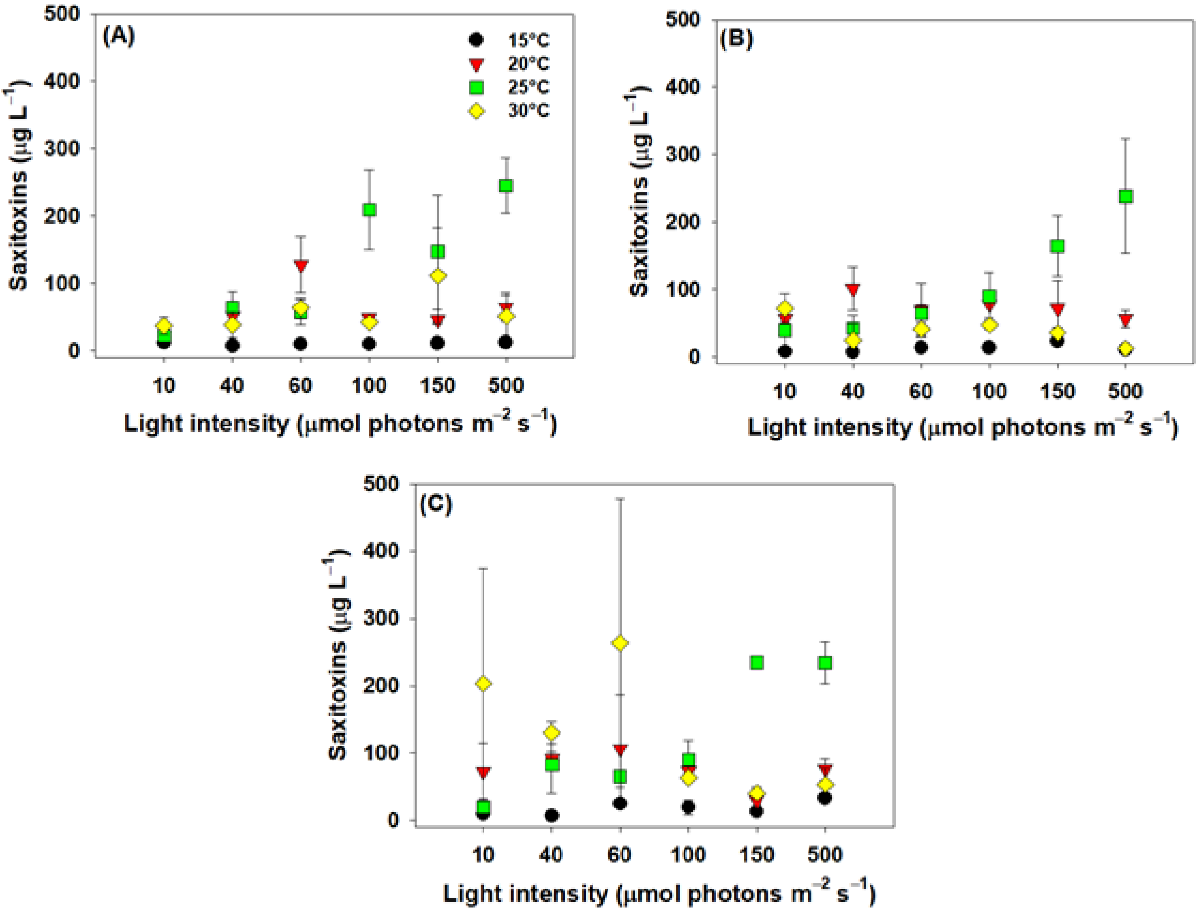
Figure 13 – Typical HPLC-FD (HILIC) chromatograms of saxitoxins variants in *Raphidiopsis raciborskii* (CYLCAM-01, CYLCAM-02 and CYLCAM-03) strains.



Legend: Commercial standards of saxitoxins (A); *Raphidiopsis raciborskii* CYLCAM-01 (B); *R. raciborskii* CYLCAM-02 (C); *R. raciborskii* CYLCAM-03 (D). Chromatograms were acquired as described in Material and Methods section.
Source: The author, 2020

The CYLCAM-02 strain when cultivated at 10, 60 and 100 µmol of photons m^{-2} s^{-1} showed no significant difference in STXs production, regardless of the combined temperature (Figure 14 – B) ($p>0.05$). This strain also did not show differences in STX production when submitted to 15, 20 and 30°C, regardless of the combined light intensity ($p>0.05$). CYLCAM-03, when combined with 10 and 100 µmol of photons m^{-2} s^{-1}, showed no significant difference in STX production, regardless of the combined temperature (Figure 14 – C) ($p>0.05$). At 25°C in combination with light intensities ≥ 150 µmol of photons m^{-2} s^{-1} CYLCAM-03 showed an increase in STXs production, but a decrease was observed at 30°C when combined with light intensities ≥ 100 µmol of photons m^{-2} s^{-1} at 30°C ($p<0.05$).

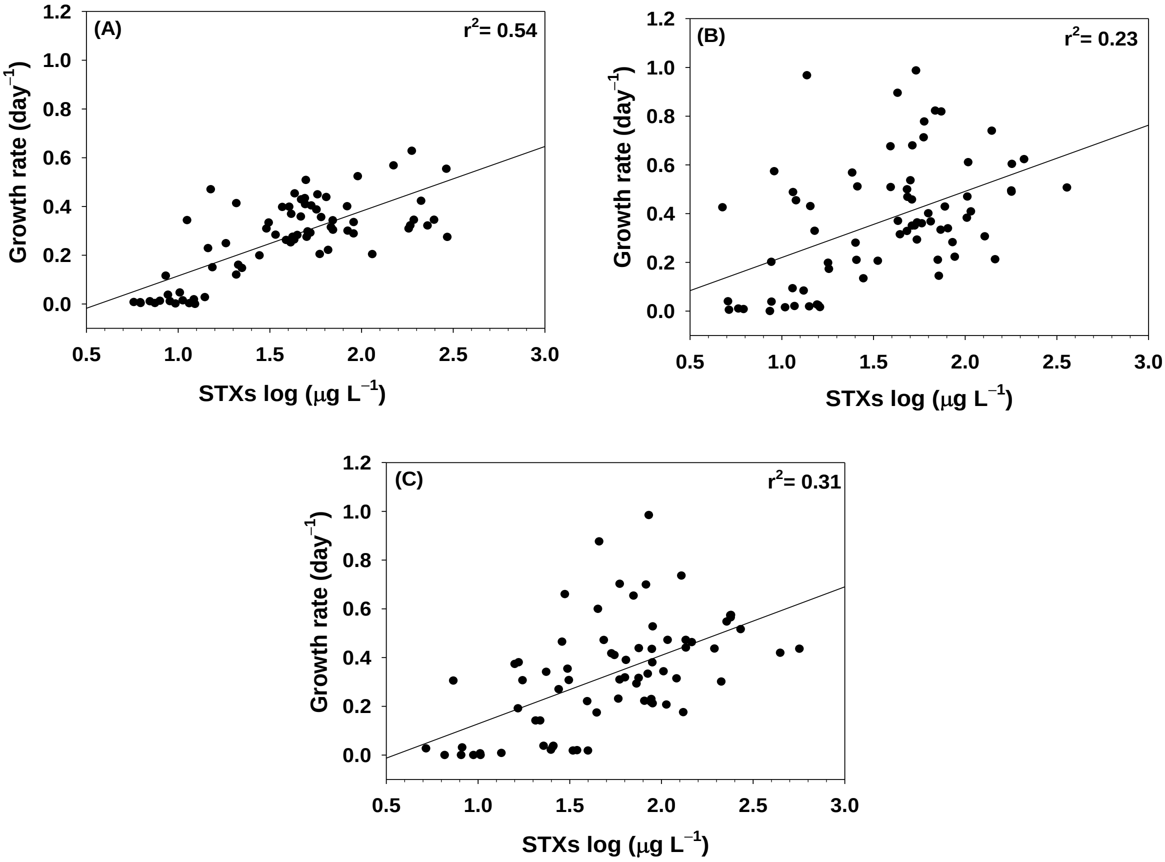
Figure 14 – Production of saxitoxins by three strains of *Raphidiopsis raciborskii* under the combinations of the six light intensities and four water temperatures.



Legend: CYLCAM-01 (**A**); CYLCAM-02 (**B**) and CYLCAM-03 (**C**). Black circles = 15 ◦C; down red triangles = 20°C; green squares = 25°C; yellow diamonds = 30°C. Bar on vertical lines are standard deviations (n=3).

Positive linear relations between growth rates and STXs concentrations for the three *R. raciborskii* strains were found (Figure 15). CYLCAM-01 strain showed a high correlation ($r^2 = 0.54$) (Figure 15 – A) in relation with others (Figure 15 – B and C).

Figure 15 – Relation between growth rate and saxitoxins production of three strains of *Raphidiopsis raciborskii* under the combinations of the six light intensities and four water temperatures



Legend: CYLCAM-01 (A); CYLCAM-02 (B) and CYLCAM-03 (C). Linear regression is indicated by black line.

2.3.3 Saxitoxins cellular quota

Two-way ANOVA indicated no effect of light, temperature, or light x temperature interaction on STXs cellular quota of CYLCAM-03 (Table 5, Figure 16 – C). Conversely, CYLCAM-02 STXs cellular quotas were influenced by light, temperature or light x temperature interaction (Table 5). The highest STXs cellular quota was observed when combining 500 μmol of photons m^{-2} s^{-1} at 25°C (Figure 16 – B) ($p<0.05$). There was a reduction when this strain was combined with light intensities ≥ 40 μmol of photons m^{-2} s^{-1} at 30°C ($p<0.05$). CYLCAM-02 when combined at 15°C and 20°C did not exhibit a significant difference in the STXs cellular quota, regardless of tested light intensity ($p>0.05$).

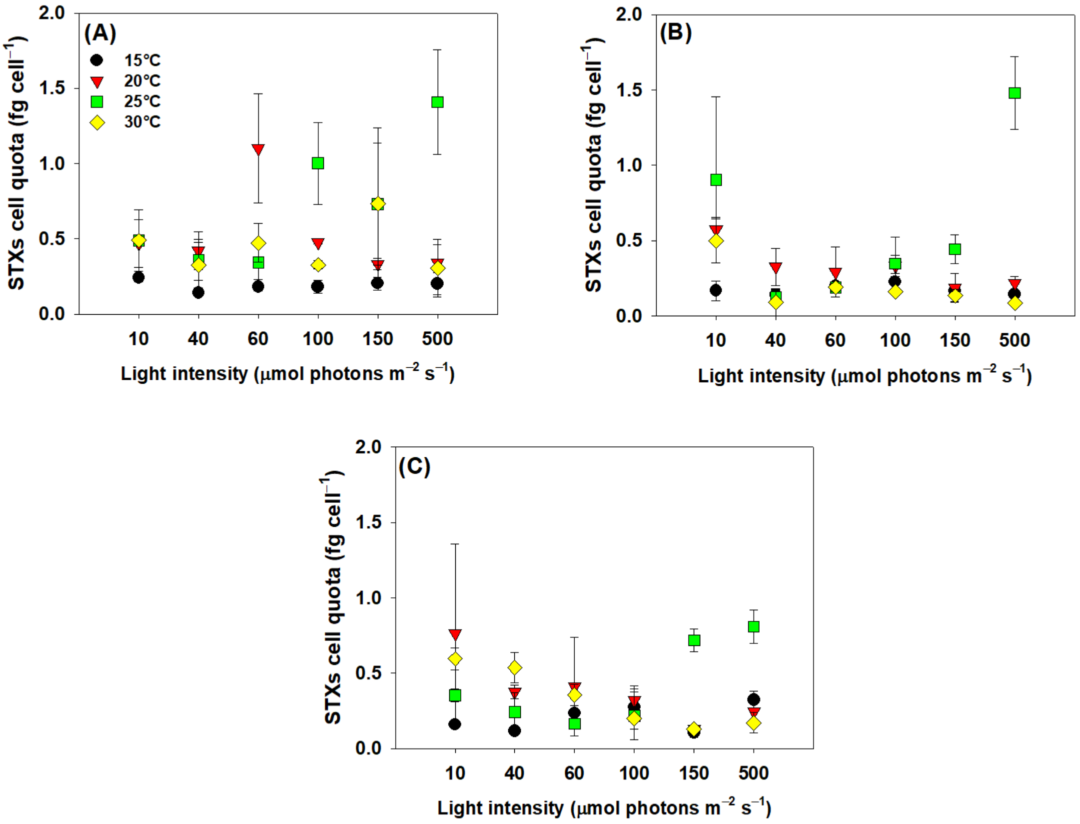
Table 5 – Two-way ANOVA table for effects of temperature and light intensity on saxitoxins (STXs) cellular quota of *Raphidiopsis raciborskii* strains.

Source of Variation	**DF**	**F**	**P**
CYLCAM-01			
Light	5	1.42	0.232
Temperature	3	18.03	<0.001
Light × Temperature	15	3.58	<0.001
Residual	48		
Total	71		
CYLCAM-02			
Light	5	8.70	<0.001
Temperature	3	19.87	<0.001
Light × Temperature	15	6.87	<0.001
Residual	48		
Total	71		
CYLCAM-03			
Light	5	1.83	0.123
Temperature	3	2.35	0.083
Light × Temperature	15	1.82	0.058
Residual	48		
Total	71		

DF = Degree of freedom; F = statistic values; *P* = significance level.

The STXs cellular quota of CYLCAM-01 was not influenced by light, but significantly affected by temperature, and two-way ANOVA indicated a temperature × light interaction (Table 5). The CYLCAM-01 strain showed the highest STXs cellular quota when combined 60 µmol of photons m^{-2} s^{-1} at 20°C and 100 and 500 µmol of photons m^{-2} s^{-1} at 25°C (Figure 16 – A). When cultivated under light intensities of 10, 40, and 150 µmol of photons m^{-2} s^{-1}, CYLCAM-01 did not show a significant difference in STXs cellular quota regardless of the combined temperature ($p>0.05$). At the extreme temperatures tested (15°C and 30°C), the increase in light intensity did not resulted in significant difference of the STXs cellular quota ($p < 0.05$) (Figure 16 – A).

Figure 1146 – Saxitoxins cellular quota of three strains of *Raphidiopsis raciborskii* under the combinations of the six light intensities and four water temperatures



Legend: CYLCAM-01 (A); CYLCAM-02 (B) and CYLCAM-03 (C). Black circles = 15°C; down red triangles = 20°C; green squares = 25°C; yellow diamonds = 30°C. Bars on the vertical lines are standard deviations (n = 3).

2.4 **Discussion**

In this study, we tested the hypothesis that *Raphidiopsis raciborskii* was able to grow in a wide range of temperatures and that *R. raciborskii* would increase the production of STXs when a high temperature was combined with high light intensity. Our results are in line with the first hypothesis, as strains expressed growth over a wide range of temperatures and when higher growth rate with increasing light intensity at temperatures equal to or greater than 20°C was found. The highest STXs concentrations and cellular quota were not obtained under extreme conditions where both light and temperature were high. Hence, we reject our second hypothesis. In addition, our results showed that the strains exhibited different responses when submitted to different combinations of light and temperature tested for all parameters, reflecting intraspecific variability.

Raphidiopsis raciborskii can be considered a shadow species due to its low light requirement (Ik) (PADISÁK; REYNOLDS, 1998), despite being a species of tropical origin, which global distribution is currently linked with increased temperature (ANTUNES, LEÃO, VASCONCELOS, 2015; BRIAND et al., 2004). Most studies have shown that *R. raciborskii* strains require high light intensities to achieve maximum growth (BITTENCOURT-OLIVEIRA et al.,2012; BONILLA ET AL., 2016; PIERANGELINI et al., 2014) and that optimum growth rates occur at a relatively elevated temperature (25–31°C) (BONILLA et al., 2016; SOARES; LÜRLING; HUSZAR, 2013). In this study, the range of light intensity and temperature used encompassed those that have been reported for blooms of *R. raciborskii* in Brazil (BOUVY et al., 1999), respectively, 14 to 830 µmol photons m^{-2} s^{-1} of light (BOUVY et al., 1999) and high temperature, such as 27°C (SOARES et al., 2009) Our results emphasized the ability of *R. raciborskii* to show optimal growth rates covering a wide range of light intensity and temperature.

Considering the effects of climatic changes in aquatic systems, the interaction between light and temperature on growth has been studied for *R. raciborskii* strains (BITTENCOURT-OLIVEIRA et al.,2012; BONILLA et al., 2012, 2016). Controlled experiments revealed that higher light intensity (135 compared to 60 µmol photons m^{-2} s^{-1}) and higher temperature (25°C compared to 20°C and 15°C) promoted the growth of *R. raciborskii* (BONILLA et al., 2016). Where, in the study of Bonilla et al. (2016), no difference was found in the growth of *R. raciborskii* at 15°C and 20°C, in our study 15°C clearly was the least favorable growth temperature. The *R. raciborskii* strains in this study demonstrated a great tolerance to grow over a wide range of light and temperature, where they obtained maximum growth rates in high light intensities combined with temperatures ≥20°C. This is in agreement with *R. raciborskii* strains isolated from different regions of South America exhibiting maximum growth rates when high light intensities (≥90 µmol of photons m^{-2} s^{-1}) were combined with high temperatures (25°C and 31°C) (BITTENCOURT-OLIVEIRA et al.,2012; BONILLA et al., 2016), regardless of the straight or spiral morphotype of the strains (BITTENCOURT-OLIVEIRA et al.,2012). Although *R. raciborskii* evidently grows faster at higher light and higher temperatures, our study, as well as others (e.g., BITTENCOURT-OLIVEIRA et al., 2012; BONILLA et al., 2012, 2016), show that *R. raciborskii* has the capacity to also grow under different combinations of light and temperature, emphasizing the physiological flexibility of *R. raciborskii*, which would be a favorable factor to explain the current distribution of this species worldwide.

Raphidiopsis raciborskii is a potential cyanotoxin-producing species (ANTUNES; LEÃO; VASCONCELOS, 2015; RANGEL et al., 2016). One interesting aspect is that strains of this species from Oceania and Asia might produce cylindrospermospsins, while those in Brazil might produce saxitoxins, whereas also numerous strains there and elsewhere produce none of these toxins (ANTUNES; LEÃO; VASCONCELOS, 2015). The biological reasons why Brazilian *R. raciborskii* strains produce STX variants instead of cylinsdrospermopsin are still not clear. However, based on genomic and target metabolomics approaches, HOFF-RISSETI et al. (2013) suggested that, besides the presence of *cyr* genes in Brazilian strains, some crucial steps are missing in the cluster, and only STX genes and variants were evidenced. The strains used in this study originated from Brazil and are STXs producers, which the production or/and increased concentration of this cyanotoxin has already been associated with some environmental factors like suboptimal temperature, light (intensity, quality, and dark cycles), high conductivity, dissolved inorganic nitrogen, and water hardness (BRENTANO; GIEHL; PETRUCIO, 2016; CARNEIRO et al., 2009; CARNEIRO; PACHECO; AZEVEDO, 2013; CASALI et al., 2017; CASTRO et al., 2004; RANGEL et al., 2016). Clearly, the effects of individual environmental factors, such as (e.g., light and temperature) on STX concentrations have already been previously studied, but our study is the first paper that combined the effects of light and temperature in STXs concentrations in *R. raciborskii* strains. At 25°C and in 150 and 500 µmol photons m^{-2} s^{-1}, the highest STXs concentrations were measured in each strain.

Light and temperature are environmental variables that affect the photosynthetic apparatus and metabolism in phytoplankton species (COLES; JONES, 2000; PIERANGELINI et al., 2014; REYNOLDS, 2006) and consequently affects the metabolic via to STX production (KELLMAN; NEILAN, 2007). Laboratory experiments demonstrate that STX production can be affected by light intensity. For example, *R. raciborskii* T3 strain showed greatest concentration of STX and NEO at 100 µmol photons m^{-2} s^{-1}, compared with 50 and 150 µmol photons m^{-2} s^{-1} (CARNEIRO et al., 2009). Our results showed the highest production of STXs at light intensities equal to or greater than 100 µmol photons m^{-2} s^{-1}.

Besides light, also temperature may influence the STXs content in *R. raciborskii*, where, in general, higher temperatures yield higher STXs concentrations (CASTRO et al., 2004; RANGEL et al., 2016). In our study, high amounts of STXs (GTX2 + GTX3) were found in the *R. raciborskii* strains reared at high light intensity at 25°C. Interestingly, in our study, STX concentrations dropped sharply when *R. raciborskii* strains were cultured at 30°C, compared with STX concentrations found in strains reared at 25°C. This finding deviates from the increase in STX concentrations with temperatures up to 32°C observed in another study (RANGEL et al., 2016). However, where we detected GTX-2 and GTX-3, Rangel et al. (2016) measured STX, dcSTX, NEO, and dcNEO in their strain. Although temperature can influence the composition of the STXs (CASTRO et al., 2004; RANGEL et al., 2016), as dcSTX was not detected in *R. raciborskii* strains at low temperature (17°C and 19°C), but it was present in cultures reared at 25°C and 32°C (CASTRO et al., 2004; RANGEL et al., 2016), no such shift in saxitoxin/gonyautoxin variants was measured in our study. Assuming that as a consequence of climate change the temperature will increases by 2–5°C (IPCC, 2014), the annual average in the tropical will be over 25°C. This implies that, based on our results, strains and blooms of saxitoxin producing *R. raciborskii* might become less toxic under these future climate scenarios. In addition to saxitoxin production, it is important to know how the behavior of *R. raciborskii* is in relation to STXs cellular quota, since there are few studies about it (CASALI et al., 2017; RANGEL et al., 2016). It was demonstrated with other cyanotoxins, such as cylindrospermopsins (CYN), that CYN cell quota of two *R. raciborskii* strains were not affected by the intensity of the surrounding light during growth (CARNEIRO et al., 2013), while another study with microcystin verified that an increase in temperature (18–30°C) can diminish the microcystin cell quota (PENG et al., 2018).

In relation with STXs cell quota, a field study observed the highest saxitoxin quota per trichome in the periods with the lowest *R. raciborskii* density and conclude that the production of this toxin or the selection of toxic strains may be an adaptation to the stress condition (CASALI et al., 2017). In laboratory experiments, the *R. raciborskii* strain LETC CYRF-01 increased toxicity, since the increase in saxitoxin production was related not only to the increase in density but to the amount of toxins per unit volume increased as well (RANGEL et al., 2016). Our results of the STXs cellular quota were similar to those of STXs production, where *R. raciborskii* strains accumulated more intracellular STXs when they were combined with high light intensities at 25°C and decreased by 30°C. Therefore, our results contrast with idea that the STXs cellular quota is related to the less ideal conditions (CASALI et al., 2017), since combinations of high light intensity at 25°C promoted higher levels of STXs and high growth rate.

2.5 Conclusion

The growth of *Raphidiopsis raciborskii* strains isolated from a natural tropical eutrophic reservoir was limited by lower temperature in our laboratory experiments. The maximum growth rates were obtained in higher light intensity combined with temperatures equal to or above 20°C, depending on the strain. Highest STX concentration and cell-quota occurred at 25°C under high light intensities, but were much lower at 30°C. Hence, increased temperatures combined with sufficient light will lead to higher *R. raciborskii* biomass, but blooms could become less toxic in tropical regions.

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3 COMBINED EFFECT OF LIGHT AND TEMPERATURE ON ECOPHYSIOLOGY RESPONSE IN TROPICAL STRAINS OF AULACOSEIRA AMBIGUA

3.1 **Introduction**

3.1.1 General aspects about the ecology of diatoms

Diatoms are autotrophic protists that exhibit wide morphological variability, with shapes that vary between spherical and cylindrical or centric and pennate (ROUND; CRAWFORD; MAN, 1990), and their cells can remain solitary or group into colonies. The main characteristic is the highly silicified porous cell wall (frustule), whose ornamentation follows a species-specific pattern that is preserved and faithfully reproduced between generations (ROUND; CRAWFORD; MAN, 1990). Variation in shape and size is important for diatom fitness in nature because it relates to suspension in the water column, nutrient uptake, and absorption of light (JIANG; SCHOFIELD; FALKOWSKI, 2005).

Diatoms are one of the most important components of pelagic food networks, tending to dominate in low temperatures associated with mixed water columns and high nutrient concentrations of (BORGES; TRAIN; RODRIGUES, 2008; FONSECA; BICUDO, 2008; SOMMER et al., 1986). However, diatoms can be found in a wide range of habitats (e.g., rivers, lakes and reservoirs) from tropical to polar regions (FIALA; ORIOL, 1990; JEWSON; GRANIN, 2015; POISTER et al., 2012; ZNACHOR et al., 2013). Also, they are considered indicators of water quality due to their high abundance, specific ecological preferences and a short lifespan, which allows them to respond quickly to environmental changes at the levels of genera and species (JUGGINS et al., 2016; HEINO; SOININEN, 2007; TEITTINEN et al., 2015; VAN VUURE; SABER; CANTONATI, 2018).

Among the genera of diatoms, *Aulacoseira* Thwaites has 70 described species to date (current and fossil) (BICUDO; MENEZES, 2017), found exclusively in freshwaters. The individuals belonging to this genus have as main characteristics the presence of cylindrical frustules with a diameter, generally smaller than the height, joined in chains through the connection of spines of varying size and number (ROUND; CRAWFORD; MANN, 1990). The distinction of species and varieties is made based on taxonomic characters such as the height of the mantle, the shape of spines, density, size and distribution pattern of the areolas (EDGAR; THERIOT, 2003; HOUK; KLEE, 2007; POTAPOVA et al., 2008; SIVER; KLING, 1997). Regarding ecology, *Aulacoseira* has a worldwide distribution and generally represents an important component of phytoplankton in eutrophic lakes, ponds and rivers, and may also be present in oligotrophic waters (DENYS et al., 2003). Also, *Aulacoseira* is generally associated with turbulent environments (e.g., water column mixing), high availability of nutrients and waters with low alkalinity (HUBBLE, 2000). In addition, this genus has high rates of predation compared to other phytoplankton species (e.g., *Scenedesmus* sp. and *Microcystis* sp.) because it does not have "anti-grazer" structures, such as spines or algae sheaths that are found in other genera (MCNAUGHT; GRIESMER, KENNEDY, 1980), being considered as a good source of food for the zooplanktonic community.

The species - *Aulacoseira ambigua* was described by Grunow in Van Heurck (1882), with cells in valves that are often longer than broad, spiral rows of areolas in the mantle and have a relatively smooth valve face. The connecting spines have a triangular shape while the separation valves generally have a single areola ring on the margin of the valve face (SIVER; KLING, 1997). Furthermore, *A. ambigua* is one of the most common species of the genus and is characterized by presenting filamentous chains of the straight morphotype (TREMARIM, LUDWIG, TORGAN, 2013). However, variations in taxonomic structures between tropical and temperate individuals have already been observed (TREMARIM, LUDWIG, TORGAN, 2013), as well as variation of the straight to spiral morphotype in natural samples, being called *A. ambigua f. japonica* (VAN VUUREN et al., 2018; VAN VUUREN; TAYLOR, 2016).

Regarding the distribution and ecology of *A. ambigua*, it can be found in lotic and lentic environments in various regions of the world, including having a wide distribution in Brazil (CAVALCANTE; TREMARIN; LUDWIG, 2013; DUNK; NOGUEIRA; MACHADO, 2012; GUIRY; GUIRY, 2014; HENRY; USHINOHAMA; FERREIRA, 2006). While this species occurs in waters ranging from oligotrophic to eutrophic (VAN DAM; MERTENS; SINKELDAM, 1994, STENGER-KOVACS et al. 2007), prefers those with high concentrations of nutrients (HOUK, 2003; TAYLOR; HARDING; ARCHIBALD, 2007). Also, *A. ambigua* shows high abundance in turbulent waters (e.g., mixed water columns) with relative low temperatures, low light conditions and slightly acidic pH (BICUDO et al., 2016; FONSECA; BICUDO, 2008; HOUK, 2003; TAYLOR; HARDING; ARCHIBALD, 2007).

3.1.2 Ecophysiological responses of diatoms in relation to temperature and light intensity

Light and temperature are extremely impacting environmental factors in the physiology of phytoplankton species because they are related to cellular metabolism (COLES; JONES, 2000; MESQUITA et al., 2019; REYNOLDS, 2006). In the aquatic ecosystem, light is attenuated by the conditions of the environment, resulting in a vertical gradient in intensity and spectral distribution (KIRK, 1994). Thus, phytoplankton species are subject to daily fluctuations in the availability of light due to the transitions between night and day and to less regular changes in light intensity and quality of the spectrum (MANN, 2002). Phytoplankton species have a group of functional traits that allow the use of different wavelengths and intensity to perform photosynthesis and, for this reason, may exhibited different preferences in relation to the use of light, being distribute heterogeneously along the water column. On the other hand, temperature can directly or indirectly influence the growth of phytoplankton (LÜRLING et al., 2013; MESQUITA et al., 2020). Temperature directly affects cell metabolism and can stimulate, reduce and even inhibit the photosynthetic process (COLES; JONES, 2000; GOMES; LÜRLING; AZEVEDO, 2015; MESQUITA et al., 2020), while indirect effects can be observed on the mixing regime of the water column, promoting stratifications which can benefit the growth of specific groups phytoplankton (e.g., cyanobacteria) to the detriment of others (e.g., diatoms).

In the literature, there are numerous studies that have indicated the availability of light and temperature variation as the main regulators of the phytoplankton community (DE SENERPONT DOMIS et al., 2013; FONSECA; BICUDO, 2008; RANGEL et al., 2016; TUCCI; SANT'ANNA, 2003), especially for diatoms (BORGES; TRAIN; RODRIGUES, 2008; FONSECA; BICUDO, 2008; POISTER et al., 2012). In addition, there is a considerable amount of laboratory studies that addressed the ecological preferences of diatoms in relation to light and temperature alone (GOMES; LÜRLING; AZEVEDO, 2015; JAVAHERI et al., 2015; MONTAGNES; FRANKLIN, 2001; MESQUITA et al., 2020). However, changes in environmental conditions can occur over time and space and, therefore, diatoms can experience different light and temperature conditions in a short period of time (DE SENERPONT DOMIS et al., 2013; REYNOLDS, 2006). For this reason, researchers began to study and deepen their knowledge about the possible effects caused by the combination of different light and temperature conditions on the ecophysiological responses of phytoplankton species (FIALA; ORIOL, 1990; NISHIKAWA; YAMAGUCHI, 2006). However, to the author's knowledge, only one study evaluated the combined effect of light and temperature on the growth of *Aulacoseira* sp. (SHEAR; NALEWAJKO; BACCHUS, 1976). Therefore, there is still a gap in the literature on the combined effects of light and temperature, not only on the growth of *Aulacoseira* sp., but also on other ecophysiological parameters, such as morphology, photosynthetic capacity and lipid production.

3.1.2.1 Growth

The growth of phytoplankton individuals in natural conditions is predominantly determined by physical-chemical interactions (YAMAGUCHI et al., 1997; YAMAGUCHI; IMAI; HONJO, 1991; YAMAGUCHI; HONJO, 1989). Many environmental factors control the distribution of freshwater diatoms (e.g., nutrients, light, temperature), among them, temperature have been mentioned as a less limiting factor for the structure of the community, since diatoms are cosmopolitan (ANDERSON, 2000). Although diatoms can dominate the coldest waterbodies, with temperatures varying between 4 – 15.2°C (BORGES; TRAIN; RODRIGUES, 2008; JEWSON et al., 2008; ZNACHOR et al., 2013), they are important contributors to phytoplankton biomass in tropical aquatic ecosystems (FONSECA; BICUDO, 2008; LOPES; BICUDO; FERRAGUT, 2005; RANGEL et al., 2016). Moreover, laboratory studies have already shown that some species of diatoms are capable of growing at moderated to high temperatures (17–30°C) (GOMES; AZEVEDO; LÜRLING, 2015; MARINHO; AZEVEDO, 2007; NISHIKAWA; YAMAGUCHI, 2006) and some of them enhanced the growth rates with increasing temperature (MONTAGNES; FRANKLIN, 2001).

Light intensity has also been identified as an important environmental variable for diatoms, since affect the photosynthetic efficiency, regulating the intrinsic growth rate (YODER, 1979). Diatoms can grow in a wide range of light intensities (5 – 450 µmol of photons m^{-2} s^{-1}) (MONTAGNES; FRANKLIN, 2001; NISHIKAWAA; YAMAGUCHI, 2006; SPILLING et al., 2015), showing a linear relationship between the growth rate and increasing light intensity (NISHIKAWAA; YAMAGUCHI, 2006; YODER, 1979).

In the last two decades, the number of studies that address the possible effects of climate change has stood out in limnology (ADRIAN et al., 2009; GLIBERT, 2019; JEPPESEN et al., 2014; MESQUITA et al., 2019, 2020; MOSS et al., 2011; PAERL; HUISMAN, 2008, 2009; RICHARDON, 2008). Among the main effects, the increase in temperature and its consequences have been widely studied, since temperature directly affects the cellular metabolism of aquatic organisms (LÜRLING et al., 2013; MESQUITA et al., 2019, 2020; RICHARDON, 2008), as well as acting on the mixing regime of aquatic ecosystems promoting thermal stratifications and de-stratifications of water column (FONSECA; BICUDO, 2008; PAERL; HUISMAN, 2008; PAERL; PAUL, 2012). Furthermore, the increase in temperature may change the quality and quantity of light in aquatic ecosystems, mainly affecting phytoplanktonic species (DE SERNAPOINT-DOMIS et al., 2013; HÄDER et al., 2007, 2011). In some regions, for example, the increase in temperature may reduce precipitation leading to more prolonged droughts and, with this, stratifications of the water column that are more lasting may be observed. Consequently, a reduction in the depth of the surface mixture may occur, which may result in a greater exposure of aquatic organisms that live in the upper layers to ultraviolet radiation (DE SERNAPOINT-DOMIS et al., 2013; HÄDER et al., 2007, 2011; JANKOWSKI et al., 2006). For these reasons, there has been an enhance in studies that focus on the effects of light and temperature on the ecophysiology of planktonic organisms (BONILLA et al., 2016; GOMES; AZEVEDO; LÜRLING, 2015; MESQUITA et al., 2019). Recently, there has been an increase in the number of studies that evaluated the combined effect of these two variables on ecophysiological responses, using cyanobacterial species (BONILLA et al., 2016; MESQUITA et al., 2019; XIAO; WILLIS; BURFORD, 2017) while few studies have evaluated the growth of diatoms under the combined effect of light and temperature (NISHIKAWA; YAMAGUCHI, 2006; SPILLING et al., 2015), especially in *A. ambigua* (SHEAR; NALEWAJKO; BACCHUS, 1976).

3.1.2.2 Morphology

Diatoms can phenotypically adapt their morphology in response to environmental changes, especially those of a cylindrical shape (e.g., *Aulacoseira* and *Cyclotella*) whose rigid silica cell wall allows only two possibilities for adaptation - varying in length or diameter (JEWSON et al., 2010; MALIK; SAROS, 2016). Among them, the variation in length is the main option since changes in diameter are related to the time of events during the life cycle of a diatom, which can occur regardless of seasonal changes (JEWSON, 1992; CRAWFORD, 1995). On the other hand, there are two options for variations in length: i) change the length of individual cells or ii) change the number of cells per filament (JEWSON et al., 2010). This last option is closely related to the ability of diatoms to form special valves with smooth separation spines that allow the separation of cells (BABANAZAROVA; LIKOSHWAY; SHERBAKOV, 1996; JEWSON et al., 2010; ROUND; CRAWFORD; MANN, 1990). The filamentous shape in phytoplanktonic species, and more specifically the formation of chains in diatom species, may result in a high ratio of surface to volume that promotes a longer stay in the water column, better exchange of nutrients and a large surface to intercept light energy (LEWIS, 1976), conferring competitive advantages over other phytoplankton forms.

Change in the morphology of diatoms, including *A. ambigua*, have already been reported due to variations in environmental conditions (e.g., light, temperature and concentration of nutrients) in the field or in laboratory experiments (ASLAMOV; JEWSON, 2009; POISTER et al., 2012). Temperature affects the process of intracellular silicification, inducing changes in morphology (JAVAHERI et al., 2015; HANSEN et al., 2011), and temperature-induced variation in diatoms is strongly associated with their survival strategy, whereby a reduction in cell size confers a competitive advantage over other larger phytoplankton species, which face strong competition for limited resources (JEWSON, 1992). Also, studies have already shown that some species of diatoms decrease the size and volume of cells with increasing temperature (JUNG et al., 2013; MONTAGNES; FRANKLIN, 2001) while others increase the size of cells (THOMPSON; GUO; HARRISON, 1992). These studies

Light is another environmental variable important for the morphology in diatom species such as wavelength, intensity and exposure time can affecting the cell length and width, the diameter and pore density (ASLAMOV; JEWSON, 2009; GUAN, PENG; LU, 2016; THOMPSON; HARRISON; PARSLOW, 1991; SU; LUNDHOLM; ELLEGAARD, 2018). For instance, a study showed that longer cells were formed when the light period was reduced (ASLAMOV; JEWSON, 2009), pointing out the influence of light on morphology. Also, diatoms under chronic exposure to UV radiation can increase cell volume and decrease growth rates due to a decoupling between photosynthetic processes and cell division under UV stress conditions (HESSEN; DE LANGE; VAN DONK, 1997; KARENTZ; CLEAVER; MITCHELL, 1991).

In addition to the individual effect of environmental variables (abiotic factors), such as light and temperature, some studies have shown that abiotic factors can interact, interfering with diatom morphology, such as light intensity and CO_2 concentration (HOOGSTRATEN; TIMMERMANS; BAAR, 2012) as well as temperature and nutrients (JAVAHERI et al., 2015). However, to our knowledge, there is no study evaluating the combined effect of light and temperature on morphology in *A. ambigua*.

3.1.2.3 Photosynthetic capacity

Photosynthesis is a highly regulated process, where several mechanisms help to protect photosystems against damage induced by light (photoinhibition), especially when the density of the photon fluxes exceeds the photosynthetic capacity. Excessive energy that cannot be used to conduct photosynthesis increases the production of reactive oxygen species (ROS) and induces photooxidative damage (SEIBERT et al., 1988). The efficiency of photosynthesis is affected by specific environmental conditions, such as high salt concentration, high levels of reactive oxygen species, high or low temperatures and quality and intensity of light (ALLAKHVERDIEV et al., 1999, 2001; GOESSLING; CARTAXANA; KÜHL, 2016; OGREN, 1988; OQUIST; HUNER, 1991; POWLES, 1994; WACKER; PIEPHO; SPIJKERMAN, 2015), which can inactivate the photosynthetic machinery (ARO; VIRGIN; ANDERSSON, 1993; POWLES, 1994).

Decrease in temperature has already been shown to reduce enzyme activity, membrane fluidity and electron chain transfer, restricting photosynthesis, respiration, nutrient uptake and the subsequent growth of algae cells (FALKOWSKI; RAVEN 1997; RAVEN; GEIDER 1988). In addition, low temperatures can result in a reduction in chlorophyll content and in the proportion of photosynthetic pigments compared to photoprotective pigments, as well as reducing the rate of light supply to the photosynthetic apparatus, negatively affecting photosynthesis. While it can promote an increase in cell volume and cell carboxylation activities (DAVIDSON, 1991; BERNER; DUBINSKY 1989; FALKOWSKI; LAROCHE; 1991; STRAMSKI; SCIANDRA; CLAUSTRE, 2002; YODER, 1979). On the other hand, as the temperature increases, the restrictions on photosynthesis (capture of light, nutrients, and transport of activation energy) gradually decrease, causing a strong stimulus to carbon fixation, biosynthesis and, finally, cell growth. However, under high temperature conditions, the performance of algal cells may be impaired due to marked protein inactivation or denaturation (RAVEN; GEIDER, 1988).

Besides temperature, light intensity can affect the photosynthetic capacity. In the natural environment, phytoplankton species undergo daily fluctuations in light intensity, where the upper layer has high light intensities that are generally detrimental to ideal photosynthesis, while the deeper layers may have low light availability and may limit photosynthesis and growth. In this context, typical species acclimated to high light intensities inhabit the superficial layers, while acclimated species or specialists in low light intensity inhabit the deeper layers (GERLOFF-ELIAS; SPIJKERMAN; SCHUBERT, 2005). Also, under high irradiance, the excess energy is quickly dissipated as heat or changing the distribution of excitation energy between the photosystems. Consequently, systems to repair damage to photosystem II (PSII) or to eliminate reactive oxygen species (ROS) are activated (EBERHARD; FINAZZI; WOLLMAN, 2008). However, in low light condition, the main need is to sustain photocapture and therefore there are changes in the size or number of photosynthetic units (EBERHARD FINAZZI; WOLLMAN, 2008, PIERANGELINI, 2015).

Diatoms are generally good competitors for light, because they often inhabit deeper layers due to the density of silica structure and found in high abundance in turbulent environments, where the mixture of water promotes the resuspension of matter which ends up reducing the light penetration (FONSECA; BICUDO, 2008; HOUK, 2003; MARQUARDT et al., 2018; TAYLOR; HARDING; ARCHIBALD, 2007). Moreover, the ability of diatoms to adapt to a variable light environment is thought to be closely related to their high photosynthetic productivity and evolutionary success (MOCK; VALENTIN, 2004). To avoid excessive reduction of the photosynthetic electron transport chain, diatoms use effective energy dissipation mechanisms, such as the xanthophyll cycle, which is controlled by blue light photoreceptors (SCHUMANN et al., 2007) and have the ability to move chloroplasts (DEPAUW et al., 2012), reducing light-induced stress and optimizing photosynthetic performance under floating light regimes (HAUPT, 1973).

The photosynthetic capacity in phytoplankton organisms can be access from some approaches such as the productivity rate and the use of rapid light curves (RLC) (PIERANGELINI et al., 2014, 2015). This last approach provides us with an *in vivo* assessment of photosynthetic activity, indicating the ability of the photosynthetic organism to tolerate immediate fluctuations in light intensity (FALKOWSKI; RAVEN, 2007). From the RLC, some photosynthetic parameters are obtained, such as maximum relative electron transport rate ($r\mathrm{ETR}_{\mathrm{max}}$), photosynthetic light saturating (EK) and light harvesting efficiency (α) (KRAUSE; WEIS, 1991), providing information in real time of photosynthetic apparatus. However, these parameters are still poorly studied in diatoms (COLES; JONES, 2000; WACKER; PIEPHO; SPIJKERMAN, 2015).

In natural ecosystems, photosynthetic organisms are exposed to combinations of various environmental factors (e.g., light, temperature, pH), and it is possible the combination of two or more of them inactive to a considerable degree with photosynthetic machinery (ALLAKHVERDIEV et al., 2002; NISHIYAMA et al., 2001). Therefore, studies that evaluate the combined effect of two or more environmental variables are extremely important for a better understanding of the physiology of photosynthetic organisms. In the literature, several studies can be found that evaluated the photosynthetic response of diatoms under different light conditions (quality or intensity) (BAILLEUL et al., 2010; GOESSLING; CARTAXANA; KÜHL, 2016; NYMARK et al., 2009, WACKER; PIEPHO; SPIJKERMAN, 2015) and temperatures (DAVIDSON, 1991; FALKOWSKI; LAROCHE; 1991; STRAMSKI; SCIANDRA; CLAUSTRE, 2002). However, as far as we know, rare are the studies that demonstrated the combined effect of light and temperature on diatoms (EL-SABAAWI; HARRISON, 2006; SPILLING et al., 2015), pointing out the need to broaden our understanding.

3.1.2.4 Lipid production

The capacity of phytoplankton species to survive or proliferate in a wide range of environmental conditions can be reflected in the huge diversity of cellular lipids (e.g., polar and neutral lipids, sterols, prenyl derivatives and phytylated pyrrole derivatives), as well as in the ability to efficiently modify lipid metabolism in response to changes in environmental conditions (GUSCHINA; HARWOOD, 2006; THOMPSON, 1996; WADA; MURATA, 1998). Under favorable growth conditions, phytoplanktonic organisms synthesize fatty acids mainly by esterification into glycerol-based membrane lipids (5–20% dry cell weight (DCW)) that plays a structural role. However, under unfavorable or stressful conditions, several species alter their biosynthetic lipid pathways towards the formation and accumulation of neutral lipids (20-50% DCW), mainly in the form of triacylglycerol (TAG) and hydrocarbons (HU et al., 2008).

The composition and amount of neutral lipids in phytoplankton species **c**an be influenced by a number of abiotic factors, such as salinity, temperature, light intensity and nutrient concentration (ARAÚJO; GARCIA, 2005; CHEN; JIANG; CHEN, 2008; MCGINNIS; DEMPSTER; SOMMERFELD, 1997; MORTENSEN et al., 1988). Temperature and light intensity play an important role in phytoplankton species due to direct effects on growth and metabolism (COLES; JONES, 2000; MESQUITA et al., 2019, 2020) which, in turn, ends up influencing the production and composition of neutral lipids (CONVERTI et al., 2009; GUIHÉNEUF et al., 2009; LIU et al., 2012). For example, increase in the amount and change in composition of neutral lipids occur over a wide range of temperature (15-30°C) (CONVERTI et al., 2009; MORTENSEN et al., 1988; RENAUD et al., 2002; ZHU; LEE, CHAO, 1997) and light intensity (20-400 μmol photons m^{-2} s^{-1}) (GUIHÉNEUF et al., 2009; LIU et al., 2012). However, there is still a lack of studies in the literature that combine more than one abiotic factor in neutral lipid content (GUIHÉNEUF et al., 2009), such as the interaction between temperature and light intensity.

Some groups of phytoplankton (e.g., Diatom, Chlorophyceae and Eustigmatophyceae) have been considered good sources of neutral lipids (CHEN et al., 2009; COOKSEY et al., 1987; ELSEY et al., 2007; LIU et al., 2012). Indeed, diatoms (ARAÚJO; GARCIA, 2005; COOKSEY et al., 1987; MCGINNIS et al., 1997) have a wide variety of lipids (including polar membrane-bound lipids, triglycerides and free fatty acids) (DUNSTAN et al., 1994; LEE; NEVENZEL; PAFFENHO, 1971) and are capable of combining high growth rates with significant lipid productivity (SATO et al., 2014).

3.1.3 Goal and hypotheses

The strains of *A. ambigua* have been isolated from a tropical system, where high light intensities and high temperatures are recorded during the year. Such conditions can be stressful, even considering that these strains are adapted to these conditions. Hence, we expected that the combination of high light intensity and high temperatures would (H1a) reduce the growth rate, photosynthetic capacity, trichome size and surface volume ratio of *A. ambigua* strains and (H1b) would result in an increase in the volume cell and neutral lipid content. The goal of this chapter was evaluated the combined effect of light and temperature on the growth, morphology, photosynthetic capacity and neutral lipid content of four tropical strains of *Aulacoseira ambigua.*

3.2 **Material and methods**

3.2.1 Isolation and growth of *Aulacoseira ambigua* strains

Experiments were carried out with four strains of *Aulacoseira ambigua* (AULA-01, AULA-03, AULA-05 and AULA-06), which were isolated between 2012 and 2013, from Camorim Reservoir, a tropical shallow reservoir located in a protected area (Pedra Branca State Park—22º55 54.44 S/43º28 22.44 W) in the western part of the municipality of Rio de Janeiro (Brazil). Currently the strains are maintained in the Culture Collection of the Laboratory of Ecology and Physiology of Phytoplankton, University of Rio de Janeiro State (UERJ) under the following conditions: WC medium (LÜRLING; BEEKMAN, 1999); temperature, 25 °C; irradiance, 30 μmol photons m^{-2} s^{-1} provided by daylight fluorescent lamps (Sylvania T10–20 watts–5000 k) and measured with a Li-COR quanta meter sensor; photoperiod, 12–12h light-dark cycle. Cultures were not grown axenically, but regular microscopic inspection revealed that biomass of heterotrophic bacteria remained well under 1% of total biovolume.

Before starting the experiments, the inoculum was acclimatized for 10–15 days at different combinations of light intensity and temperature tested. Three light intensities (10 – L10, 100 – L100 and 500 – L500 μmol of photons m^{-2} s^{-1}) and four water temperatures (15, 20, 25, and 30 °C) were established. The light intensities were based on the intensity variation observed in the literature for laboratory experiments, including from low to high light intensity (NISHIKAWA; YAMAGUCHI, 2006; SPILLING et al., 2015), and the illumination was provided by daylight fluorescent lamps in a 12–12 h light-dark cycle. The water temperatures tested were chosen based on the annual mean (25 °C), winter mean (20 °C), and the extreme temperatures (15 and 30 °C) observed in Camorim reservoir, site where the strains were isolated. After acclimatization of the cultures, new inoculums were made for the beginning of the experiment.

The combined effects of light and temperature on the *A. ambigua* strains were studied in batch culture systems in triplicates. The experiments were setup in

Erlenmeyer flasks of 300 mL, with 300 mL modified WC culture medium (LÜRLING; BEEKMAN, 1999). *Aulacoseira ambigua* strains were inoculated with initial biomass of 100 µg L^{-1} chlorophyll-a (Chl-a). The flasks were placed in incubators (SOLAB SL-224) under the 12 combinations of light and temperature. The growth was monitored for 18 days, enough time for the cultures to reach the stationary phase. The flasks were agitated twice a day to avoid self-shading. Samples were collected daily for measurements of the Chl-a concentration and photosystem II efficiency (φPSII), analyzed with the phytoplankton analyzer Phyto-Pam (Heinz WalzGmbH, Effeltrich, Germany). The chlorophyll-a is used as a "proxy" of biomass, while the photosynthetic efficiency is an important parameter to analyze the physiological state of algae. The Phyto-PAM was calibrated against a spectrophotometric determination of Chl-a from the *A. ambigua* strains, which was done with a 90% acetone extraction, based on Ritchie (2006). Phyto-Pam is a methodology that analyzes cells in vivo from fluorescence, with fluorescence being considered a rapid method for growth monitoring (BRIAND; GUILLARD; MURPHY, 1981).

Growth rates (µ) were calculated from the chlorophyll-a increase using a logistic curve model (Equation (1)) that was fitted iteratively in SigmaPlot 12.5® software. The growth rates were obtained in the time interval between the beginning of the experiment (day 0) until the beginning of the stationary phase, which varied depending on the strain and the combinations of light and temperature. Optimum combination of light and temperature for each *A. ambigua* strain was defined based on the incubation that produced the highest growth rate (LÜRLING et al., 2013).

$$N_t = \frac{\mathrm{N0}*\mathrm{k}}{N0+(K-N0)^{-rt}} \qquad (1)$$

Where N_t = final value of chlorophyll-a; t = time (days); N_0 = initial value of chlorophyll-a; k = carrying capacity (µg L^{-1}), μ = intrinsic growth rate (day^{-1}).

3.2.2 Morphology

The morphology of *A. ambigua* strains was evaluated by measuring filament size, cell volume and surface-volume ratio (S/V). These parameters were measured using a camera attached to the optical microscope (Nikon Eclipse 200) with the BELView-7 software package, totaling 45 individuals and 45 cells for each strain in each treatment. The samples were preserved in 2% lugol and measurements were taken only one day (day 10), corresponding to the middle of the growth curve.

To verify the existence of the combined effect of light and temperature on the morphology in *A. ambigua* strains, mixed linear model analysis was performed. Associated with this model, a two-way analysis of variance (Two-Way ANOVA) was performed. Multiple pair comparison (t-test) was applied to distinguish significantly different means ($p < 0.05$). For this statistical test, light and temperature were considered as fixed variables and the strains were selected as a random variable. We performed this statistical analysis using the available packages in R 2.6.1 (R Core Team 2015).

3.2.3 Photosynthetic activity

The combined effects of light and temperature on photosynthetic activity were evaluated using rapid light curves (RLC) estimated by electron transport rates (ETR) and analyzed with the phytoplankton analyzer Phyto-Pam (Heinz WalzGmbH, Effeltrich, Germany). The Phyto-PAM was calibrated against a spectrophotometric determination of chlorophyll-a (Chl-a) from the *A. ambigua* strains, which was done with a 90% acetone extraction, based on Ritchie (2006). The Phyto-Pam is a methodology that analyzes cells in vivo from fluorescence, with fluorescence being considered a rapid method for growth monitoring (BRIAND; GUILLARD; MURPHY, 1981). Besides, this methodology uses chlorophyll fluorescence to provide rapid, real-time information of the photosynthetic apparatus in relation to photosynthesis (KRAUSE; WEIS, 1991).

The samples of *A. ambigua* strains were analyzed shortly after measuring chlorophyll-a and photosystem II efficiency for all light and temperature combinations. For the analysis of the photosynthetic parameters obtained from the rapid light curve, three measurements were taken at different times of the growth curve, beginning (day 1), middle (day 8) and ending (day 17), respectively. These samples were exposed for 20 seconds to 21 light intensities ranging from 16 to 1864 μmol photons m^{-2} s^{-1}. The ETR parameter was calculated using equation 2 (modified), where PAR corresponds to the photosynthetic active radiation (16 to 1864 μmol photons m^{-2} s^{-1}) multiplied by the photosynthetic efficiency values of photosystem II (ϕPSII). The experimental data acquired in PHYTO-PAM were exported to the SigmaPlot 12.5® program and the RLC's were adjusted by equation 3 (RALPH; GADEMANN, 2005), in which the maximum photosynthesis value (Pm) was replaced by relative maximum electron transport rate ($r\text{ETR}_{\text{max}}$). The photosynthetic saturation intensity (EK) was calculated from equation 4, in which the $r\text{ETR}_{\text{max}}$ value is divided by the slope (light harvesting efficiency - α) of the RLC (FALKOWSKI, 1994).

At first, we performed an analysis of variance of two-way repeated measurements (RmANOVA), using strains and light intensity as fixed factors, to verify differences in the photosynthetic capacity of *A. ambigua* in the four temperatures tested from the beginning of logarithmic phase (day 1) to the end of growth (day 17). Pairwise multiple comparison procedures (Bonferroni method) were applied to distinguish means that were significantly different ($p < 0.05$), using the IBM SPSS 12.5 software. Then, we performed a test T in order to verify if the parameters of photosynthetic capacity (maximum relative transport rate, light harvesting efficiency and photosynthetic light saturating) would change from the beginning of logarithmic phase (day 1) to the end of growth (day 17) for each *A. ambigua* strain and in each combination of light and temperature tested. Data normality and heteroscedasticity were checked by Normality Test (Shapiro-Wilk) and Equal Variance test in SigmaPlot prior to running the test, using the SigmaPlot 12.5® (Systat Software, Inc). To distinguish significantly different means, was considered $p<0.05$.

$$\text{ETR} = \phi\text{PSII x PAR} \tag{2}$$

$$\text{P} = P_m\left(1 - \text{e}^{-\left(\frac{\alpha\text{Ed}}{\text{Pm}}\right)}\right) \tag{3}$$

$$\text{EK} = \frac{\text{rETRmax}}{\alpha} \tag{4}$$

3.2.4 Neutral lipid content

The combined effect of light and temperature on neutral lipid production was analyzed every three days, totaling seven points on the growth curve (0, 3, 6, 9, 12 and 18 days). The neutral lipid protocol was based on those available in the literature (CHEN et al. 2009), but none of them performed this methodology with *A. ambigua* species.

Therefore, the protocol in this study must be modified to optimize neutral lipid analysis in *A. ambigua* strains.

We used the technique Nile red dye + fluorescence to analyze the content of neutral lipids in *A. ambigua*, since it has been shown to be effective for the analysis of neutral lipids in phytoplankton species (CHEN et al., 2009; ELSEY et al., 2007; ORR; REHMANN, 2015). For this, a small aliquot (5 mL) was taken from the cultivation flasks and transferred to a falcon tube (final volume of 15 mL) to analyze the chlorophyll-a concentration trough phytoplankton the analyzer – PhytoPam. Due to sensitivity of the spectrofluorimeter (Varian Carey Eclipse) used for the analysis of neutral lipids, concentrations values above 100 µg Chl-a L^{-1} were diluted with a modified WC culture medium (the same culture medium used in the cultures) (LÜRLING; BEEKMAN, 2006). This procedure was done for each triplicate of each *A. ambigua* strain, in different combinations of light and temperature tested. After this step, the aliquot (5 mL) diluted or not was separated into two subsamples in eppendorfs, each of them with a final volume of 2 mL. One of these subsamples was treated with Nile red dye, while the other was used as a control (without Nile red dye). The subsample treated with Nile red dye were prepared in a volume of 2 mL with a final Nile red concentration of 0.5 µg / mL. To this was added 10 µL of solution 0.1 mg / mL of Nile red. After adding the Nile red dye, the subsample was homogenized and incubated at 40°C for 7 minutes in the dark, before analyzing on the quartz cuvette in spectrofluorimeter. Both subsamples were analyzed in excitation and emission wavelengths 525 *nm* and 657 *nm*, respectively, with 10 *nm* excitation and emission slit, open excitation and automatic excitation filter, average voltage (PMT) of 600. The final fluorescence result (arbitrary units) was obtained by subtracting the fluorescence of subsample with Nile Red dye from the fluorescence of the control.

To verify the existence of the combined effect of light and temperature on values of relative fluorescence (arbitrary units) in *A. ambigua* strains, a Three-way repeated-measures analysis of variance (RmANOVA) was performed, with light, temperature and strains as factors. We also calculate ratio of relative fluorescence values to chlorophyll-a, to know if the increase in the relative fluorescence values was due to the increase in neutral lipid instead of the increase in biomass, and we performed Three-way repeated analysis of variance (RmANOVA). The paired comparison procedures (Bonferroni method) were used to distinguish between different media of both analysis ($p<0.05$) using the software IBM SPSS 12.5.

3.3 **Results**

3.3.1 Combined effect of light and temperature on the growth

Aulacoseira ambigua exhibited relatively high growth rates in almost combinations of light and temperature tested, according to the strains (Table 6, Annex C). The A. ambigua strains showed distinct growth rates, ranging from 0.15 to 1.08 day^{-1}, where the optimal growth rates were observed by combining low light intensity (L10) at high temperatures (25 and 30°C) and moderate light intensity (L100) at 20 and 25°C (Table 6).

Table 6 – Mean growth rates (day^{-1}, ± one standard error, n = 3) for four *Aulacoseira ambigua* strains combined at three light intensities and four different temperatures.

Light	Temperature	AULA-01	AULA-03	AULA-05	AULA-06
L10	15°C	0.38 ± 0.03	-	-	-
	20°C	0.51 ± 0.05	0.66 ± 0.00	0.15 ± 0.01	-
	25°C	0.52 ± 0.10	0.27 ± 0.00	0.38 ± 0.06	**0.54** ± 0.00
	30°C	0.58 ± 0.10	**0.96** ± 0.09	0.52 ± 0.13	**0.54** ± 0.14
L100	15°C	0.31 ± 0.03	0.16 ± 0.03	0.36 ± 0.02	0.18± 0.03
	20°C	0.54 ± 0.06	0.46 ± 0.23	**0.64** ± 0.00	-
	25°C	**1.08** ± 0.16	-	-	-
	30°C	0.59 ± 0.08	0.43 ± 0.04	-	-
L500	15°C	-	-	-	-
	20°C	0.83 ± 0.00	-	-	-
	25°C	-	-	-	-
	30°C	-	-	-	-

Legend: (-) growth rate not determined. Light intensities: L10 = 10 µmol photons m^{-2} s^{-1}; L100 = 100 µmol photons m^{-2} s^{-1} and L500 = 500 µmol photons m^{-2} s^{-1}. Temperatures: 15°C, 20°C, 25°C and 30°C. Optimum growth rate of specified strains indicated in bold.
Source: The author, 2020.

With the increase in light intensity L10 to L100 at 15°C, all *A. ambigua* strains were able to grow and exhibited growth (Table 6). These strains also showed growth rates combining low light intensity (L10) at the highest temperature tested (30°C). However, the combination of low light and low temperature (L10 at 15°C) and high light intensity regardless of temperature (Table 6) were limiting conditions for the growth of *A. ambigua*, where only AULA-01 strain was able to grow. The AULA-01 strain demonstrated wide phenotypic plasticity, being able to grow in nine of the twelve light and temperature conditions (Table 6) when comparing with others.

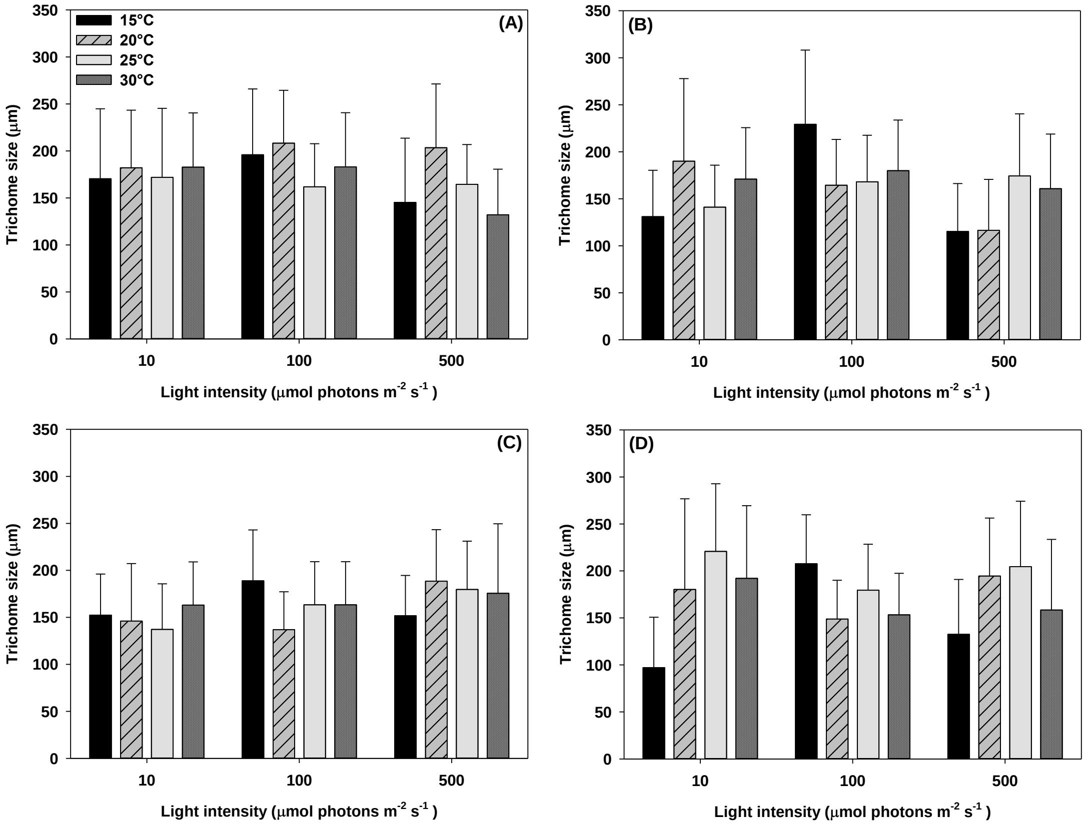
3.3.2 Combined effect of light and temperature on the morphology

3.3.2.1 Trichome size

Aulacoseira ambigua strains isolated from Camorim Reservoir have a straight morphotype. However, a slight curvature of the filaments was observed depending on the combination of light and temperature. All strains exhibited a wide phenotypic plasticity in L500, showing different trichome sizes at the temperatures tested (Figure 17).

The strains AULA-01 and AULA-05 showed no significant variation in the trichome size in low light (L10) with increasing temperature, as well as for AULA-03 at temperatures ≥ 25°C, regardless of the combined light intensity (Figure 17, Table 7) ($p > 0.05$). Significantly longer trichomes were observed by increasing the light intensity from L10 to L100 at 15°C for AULA-03; when combining L500 at low temperatures (15 and 20°C) for AULA-05, and at 25°C for AULA-06 ($p<0.05$) (Figure 17, Table 7). On the other hand, significantly shorter trichomes were observed when increasing the light intensity from L10 to L500 at 20°C and 30°C for AULA-03 and AULA-01, respectively; by increasing the temperature from 15 to 20°C at L100 for AULA-05; and for AULA-06 when comparing L10 at 15°C with the other temperatures ($p<0.05$) (Figure 17, Table 7).

Figure 17 – Combined effect of light and temperature on the trichome size of four strains of *Aulacoseira ambigua*



Legend: *Aulacoseira ambigua* strains: AULA-01 (A), AULA-03 (B), AULA-05 (C) and AULA-06 (D). Vertical bars indicate standard desviation (n=3).

Source: The author, 2020.

Table 7 – Table with data from Two-way ANOVA for the effects of light and temperature on the size of the trichome of the four strains of *Aulacoseira ambigua***.**

Strains	**Temperature**	**Light intensities**		
		10	100	500
AULA-01	15°C	BCD	CD	AB
	20°C	BCD	D	CD
	25°C	BCD	ABC	ABC
	30°C	BCD	BCD	A
AULA-03	15°C	AB	E	A
	20°C	DE	BCD	A
	25°C	ABC	BCD	CD
	30°C	BCD	CD	BCD
AULA-05	15°C	AB	C	AB
	20°C	AB	A	C
	25°C	A	ABC	BC
	30°C	ABC	ABC	BC
AULA-06	15°C	A	E	AB
	20°C	CDE	BCD	BCD
	25°C	E	CDE	E
	30°C	CDE	BCD	BCD

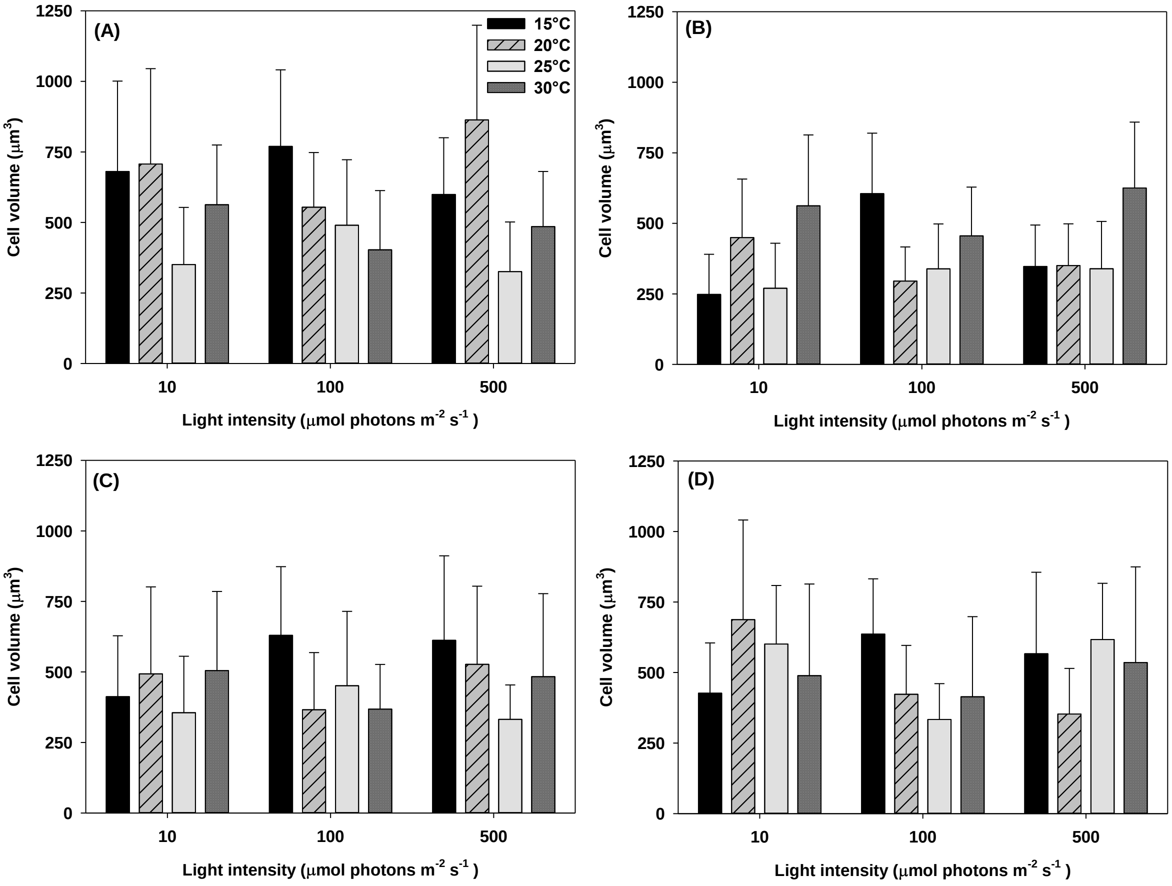
3.3.2.2 Cell volume

The strains of *Aulacoseira ambigua* showed differences in cell volume, depending on the combinations of light and temperature (Figure 18). AULA-01 exhibited a tendency to reduce cell volume when subjected to high temperatures (25 and 30°C), regardless of light intensity (Figure 18). Significantly higher cell volumes were observed when combining L100 at 15°C for AULA-01; when combining L10 and L500 at 30°C and L100 at 15°C for AULA-03, and when comparing L100 at 15°C with the other temperatures tested for AULA-06 ($p<0.05$) (Figure 18, Table 8). The lowest cell volumes were displayed when combining L10 and L500 at 25°C for AULA-01; by combining L100 at temperatures ≥ 20°C and L500 at 25°C for AULA-05 ($p<0.05$) (Figure 18, Table 8).

Table 8 – Table with post-hoc data from Two-way ANOVA for the effects of light and temperature on the cell volume of the four strains of *Aulacoseira ambigua.*

Strains	**Temperatures**	**Light intensities**		
		10	100	500
AULA-01	15°C	DEF	FG	CDEF
	20°C	EFG	BCDE	G
	25°C	A	ABC	A
	30°C	BCDE	AB	ABCD
AULA-03	15°C	A	D	AB
	20°C	AB	A	AB
	25°C	A	AB	AB
	30°C	CD	BC	D
AULA-05	15°C	ABC	E	DE
	20°C	ABCDE	ABC	CDE
	25°C	AB	ABCD	A
	30°C	BCDE	ABC	ABCDE
AULA-06	15°C	AB	CD	BCD
	20°C	D	AB	A
	25°C	CD	A	CD
	30°C	ABC	AB	BCD

Figure 18 – Combined effect of light and temperature on the cell volume of four strains of *Aulacoseira ambigua*.

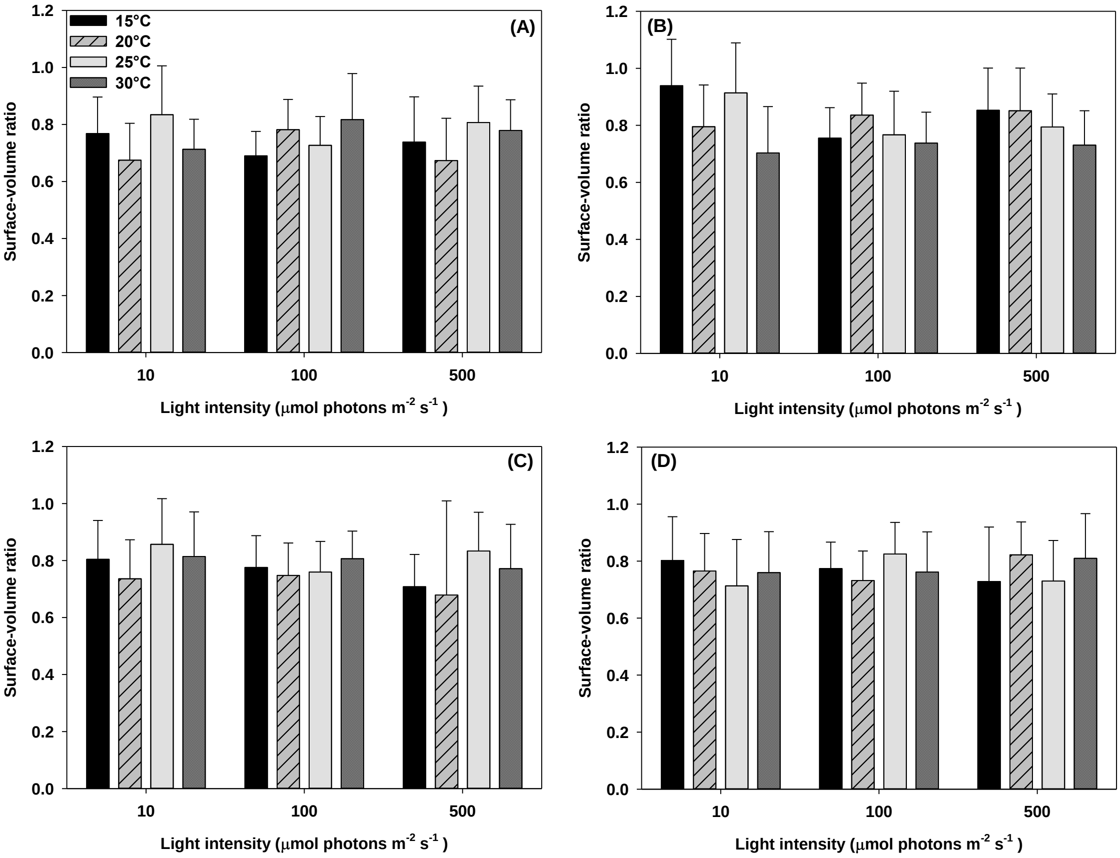


Legend: *Aulacoseira ambigua* strains: AULA-01 (A), AULA-03 (B), AULA-05 (C) and AULA-06 (D). Vertical bars indicate standard deviation (n=3).
Source: The author, 2020.

3.3.2.3 Surface-volume ratio (S/V)

Aulacoseira ambigua exhibited S/V values ranging from 0.67 – 0.94 and a tendency to show higher S/V when combining L10 at 15 and 25°C (Figure 19). The significantly higher S/V values were observed for AULA-01 when combining L10 at 15 and 25°C, L100 at 20 and 30°C and L500 at higher temperatures ($p<0.05$). AULA-03 exhibited higher S/V in L10 at 15°C when compared to 20 and 30°C combining L100 regardless of temperature, and L500 at higher temperatures ($p<0.05$). For AULA-05, significantly higher S/V values were exhibited in L10 at 25°C when compared to 20°C, and when combining L500 at 15°C. Unlike the other strains that showed a significant increase in the S/V values in different combinations of light and temperature, AULA-06 showed significantly lower S/V values at L10 at 25°C, when compared at L100 at 25°C and L500 at 20°C (Figure 19, Table 9).

Figure 19 – Combined effect of light and temperature on the surface-volume ratio of four strains of *Aulacoseira ambigua*



Legend: *Aulacoseira ambigua* strains: AULA-01 (A), AULA-03 (B), AULA-05 (C) and AULA-06 (D). Vertical bars indicate standard deviation (n=3).

Source: The author, 2020.

Table 9 – Table with post-hoc data from Two-way ANOVA for the effects of light and temperature on the ratio between surface to volume (S/V) of the four strains of *Aulacoseira ambigua*.

Strains	Temperature	Light intensities		
		10	100	500
AULA-01	15°C	BCDEF	AB	ABCDE
	20°C	A	CDEF	A
	25°C	F	ABCD	DEF
	30°C	ABC	EF	BCDEF
AULA-03	15°C	E	ABC	CDE
	20°C	ABC	BCD	CDE
	25°C	DE	ABC	ABC
	30°C	A	AB	A
AULA-05	15°C	ABC	ABC	A
	20°C	AB	ABC	ABC
	25°C	C	ABC	BC
	30°C	ABC	ABC	ABC
AULA-06	15°C	AB	AB	AB
	20°C	AB	AB	B
	25°C	A	B	AB
	30C	AB	AB	AB

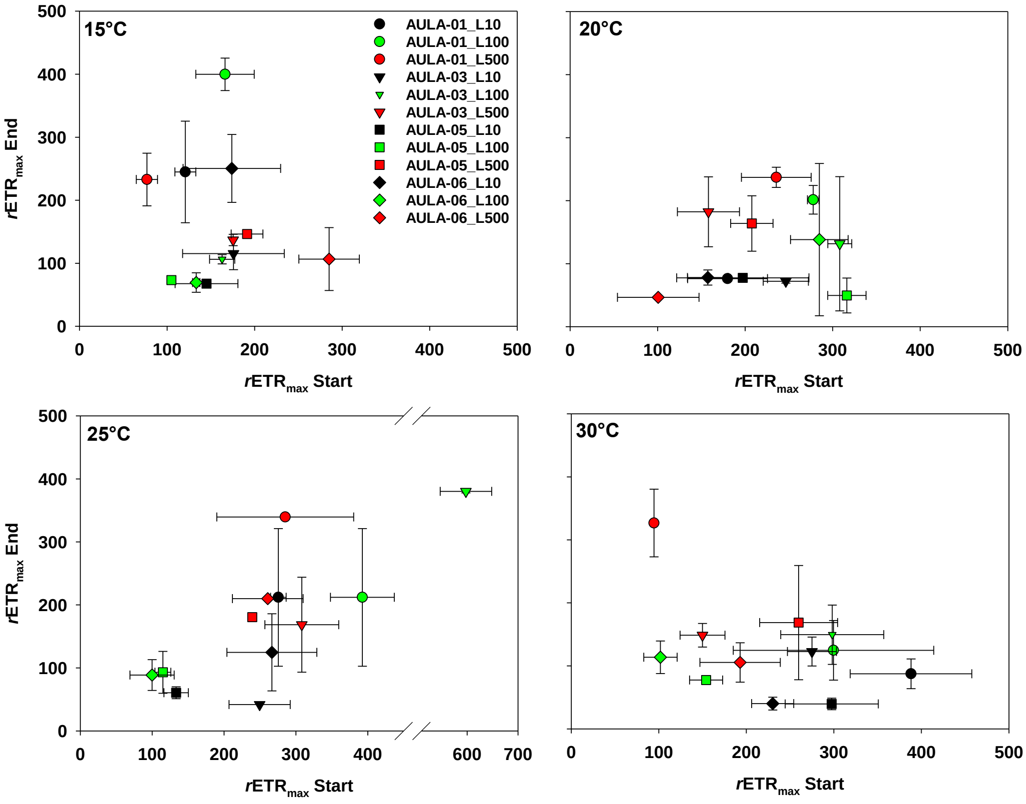
3.3.3 Combined effect of light and temperature on photosynthetic capacity

As expected, *Aulacoseira ambigua* strains showed difference in photosynthetic capacity, when combined with different light intensities and temperatures conditions, demonstrating a phenotypic plasticity and intraspecific variability (Figure 20, 21, 22 and 23. We also observed that all strains majority reduced their photosynthetic capacity when comparing the beginning of the exponential phase (day 1) and the end of growth (day 17).

3.3.3.1 Relative maximum electron transport rate (*r*ETR$_{max}$)

Aulacoseira ambigua showed *r*ETR$_{max}$ values ranging from 39.60 to 666.81 μmol electron m^{-2} s^{-1}, depending on the strain and the combination of light and temperature (Figure 20).

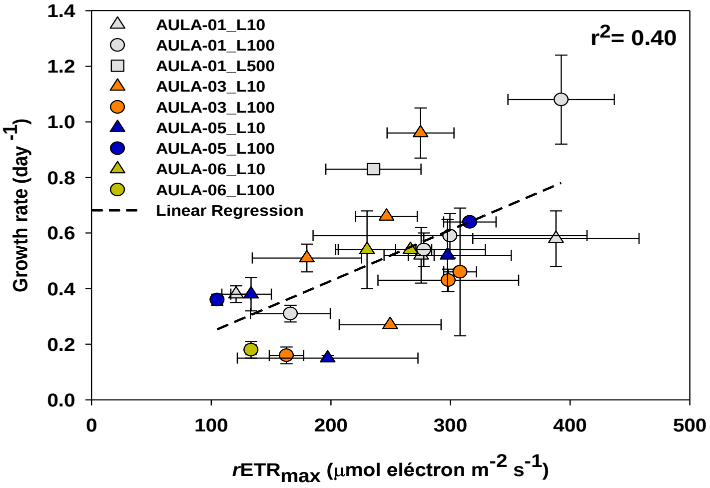
Figure 20 – Combined effect of light and temperature on maximum relative transport rate (*r*ETR$_{max}$) of four strains of *Aulacoseira ambigua* at the start (day 1) and in the end (day 17) of the experiment.



Legend: Geometric forms indicate *Aulacoseira ambigua* strains: circle = AULA-01, inverted triangle = AULA-03, = square = AULA-05 and diamond = AULA-06. Colors indicate light intensity: black = 10 μmol photons m^{-2} s^{-1} (L10) , green = 100 μmol photons m^{-2} s^{-1} (L100) and red = 500 μmol photons m^{-2} s^{-1} (L500). Vertical and horizontal bars indicate standard desviation (n=3).
Source: The author, 2020.

The Two-Way repeated measurement using strains and light intensity as fixed factors demonstrated that there was no significant difference between strains at each temperature ($p>0.05$). And, in relation to the light intensity there was only significant difference between L10 and L100 at 20°C (Figure 20) ($p<0.05$). When comparing the $r\mathrm{ETR}_{max}$ values displayed for each strain of *A. ambigua* between the beginning of the exponential (day 1) and the end of growth (day 17), in each combination of light and temperature tested, all strains showed a significant reduction in the $r\mathrm{ETR}_{\mathbf{max}}$, when combining L10 and L100 at temperatures ≥20°C and ≥25°C, respectively ($p<0.05$). AULA-01 was the only strain that showed a significant increase in the $r\mathrm{ETR}_{\mathbf{max}}$ values by combining L100 and L500 at 15°C ($p<0.05$) (Figure 20). In addition, we also observed a positive correlation between $r\mathrm{ETR}_{max}$ and growth rate ($r^2 = 0.40$) (Figure 21).

Figure 21 – Regression between growth rate (day^{-1}) and the relative maximum transport rate ($r\mathrm{ETR}_{max}$) on *Aulacoseira ambigua* strains under different combinations of light and temperature.



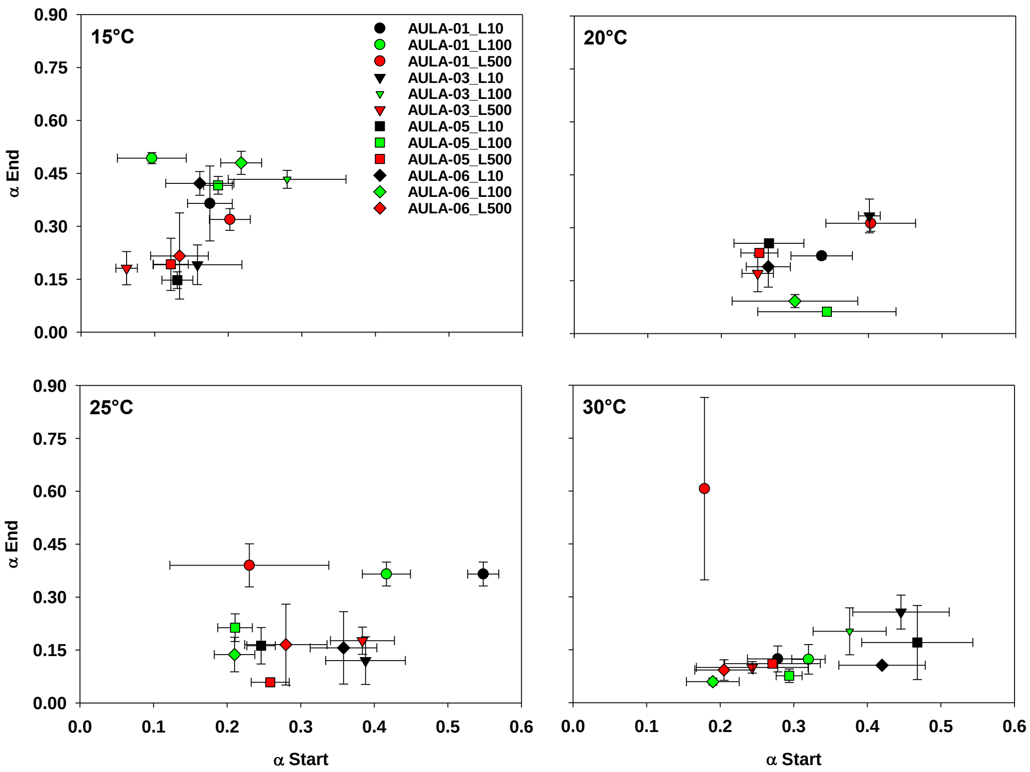
Legend: Colors indicate Aulacoseira ambigua strains: gray = AULA-01, orange = AULA-03, blue = AULA-05 and green = AULA-06. Geometric forms indicate light intensity: triangle = 10 µmol photons m^{-2} s^{-1} (L10) circle = 100 µmol photons m^{-2} s^{-1} (L100), and square = 500 µmol photons m^{-2} s^{-1} (L500). Dotted line indicates linear regression ($r^2 = 0.40$). Vertical and horizontal bars indicate standard desviation (n=3).

Source: Author, 2020.

3.3.3.2 Light harvesting efficiency (α)

Aulacoseira ambigua strains showed α values ranging from 0.06 to 0.62 µmol photons m^{-2} s^{-1}, depending on the strain and the combination of light and temperature (Figure 22)**.** The two-way repeated measurement using strains and light intensity as fixed factors demonstrated that for all temperatures tested, there was a significant difference in time and in the interaction between time x light ($p<0.05$). At 15, 25 and 30°C, there were significant differences in the interactions between time x strain, and time x light x strain ($p <0.05$) (Figure 22).

Figure 22 – Combined effect of light and temperature on light harvesting efficiency (α) of four strains of *Aulacoseira ambigua* at the beginning (day 1) and the end (day 17) of the experiment.



Legend: Geometric forms indicate *Aulacoseira ambigua* strains: circle = AULA-01, inverted triangle = AULA-03, = square = AULA-05 and diamond = AULA-06. Colors indicate light intensity: black = 10 µmol photons m^{-2} s^{-1} (L10), green = 100 µmol photons m^{-2} s^{-1} (L100) and red = 500 µmol photons m^{-2} s^{-1} (L500). Vertical and horizontal bars indicate standard deviation (n=3).

Source: The author, 2020.

At the lowest temperature tested (15°C), the strain AULA-05 showed significantly lower values of **α,** when comparing with AULA-01 and AULA-06 ($p<0.05$). At temperatures 20 and 25°C, the statistical analysis was able to separate into two groups – AULA-01 + AULA-03 and AULA-05 + AULA-06, where the strains of each group were statistically similar to each other ($p> 0.05$), but different between group ($p <0.05$). At the highest temperature tested (30°C), AULA-03 showed a significant difference in relation to AULA-06 ($p <0.05$). We also observed that α values were also significantly different depending on the combination of light at each temperature tested, where L100 differed significantly from the other light intensities at 15, 20 and 25°C, while L10 differed at 30°C (Figure 22).

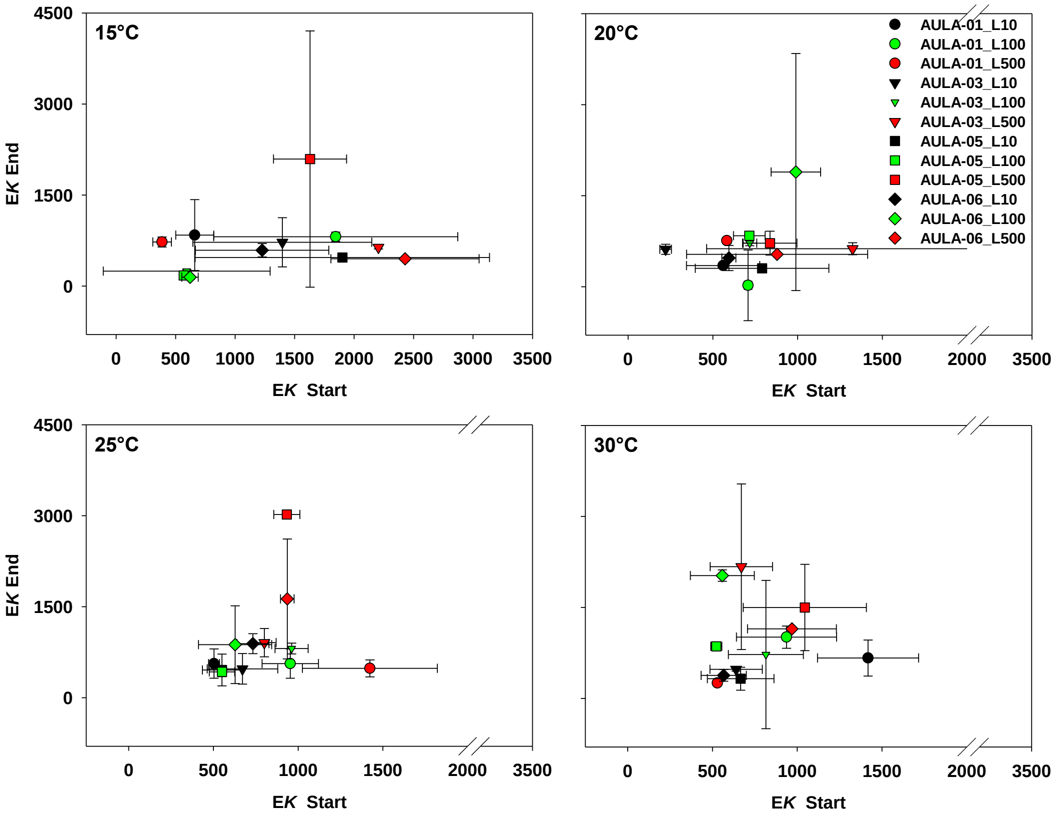
When comparing the α values displayed for each strain of *A. ambigua* between the beginning of the exponential (day 1) and the end of growth (day 17), in each combination of light and temperature tested, all strains significantly reduced the α value by combining high temperatures (≥ 25°C) regardless of the light intensity tested. AULA-01 and AULA-03 showed an increase in the α value by combining 100 and 500 µmol photons m^{-2} s^{-1} at 15°C while AULA-06 increased when combining 10 and 100 µmol photons m^{-2} s^{-1} at that same temperature ($p <0.05$) (Figure 22).

3.3.3.3 Photosynthetic light saturating (E*K*)

Aulacoseira ambigua showed E*K* values ranging from 145.48 to 2427.83 µmol photons m^{-2} s^{-1}, depending on the strain and the combination of light and temperature (Figure 23). The Two-Way repeated measurement demonstrated that the strains showed no significant difference between them at all temperatures tested ($p>0.05$). Regarding the light intensity tested, we observed only a significant difference in E*K* values when comparing L10 to L100 at 20°C (Figure 23) ($p<0.05$).

When comparing the E*K* values displayed for each strain of *A. ambigua* between the beginning of the exponential (day 1) and the end of growth (day 17), in each combination of light and temperature tested, the strains AULA-03, AULA-05 and AULA-06 showed a significant reduction in E*K* values when combining L100 at 15°C ($p<0.05$). We also observed a significant reduction by combining L10 at 25°C L500 30°C for AULA-01, while AULA-03 reduced the E*K* values by combining L10 at 20°C. On the other hand, AULA-01 was the only strain that showed a significant increase in EK values by combining L500 at low temperatures (15 and 20°C) ($p<0.05$) (Figure 23).

Figure 23 – Combined effect of light and temperature on photosynthetic light saturating (E*K*) of four strains of *Aulacoseira ambigua* at the beginning (day 1) and the end (day 17) of the experiment



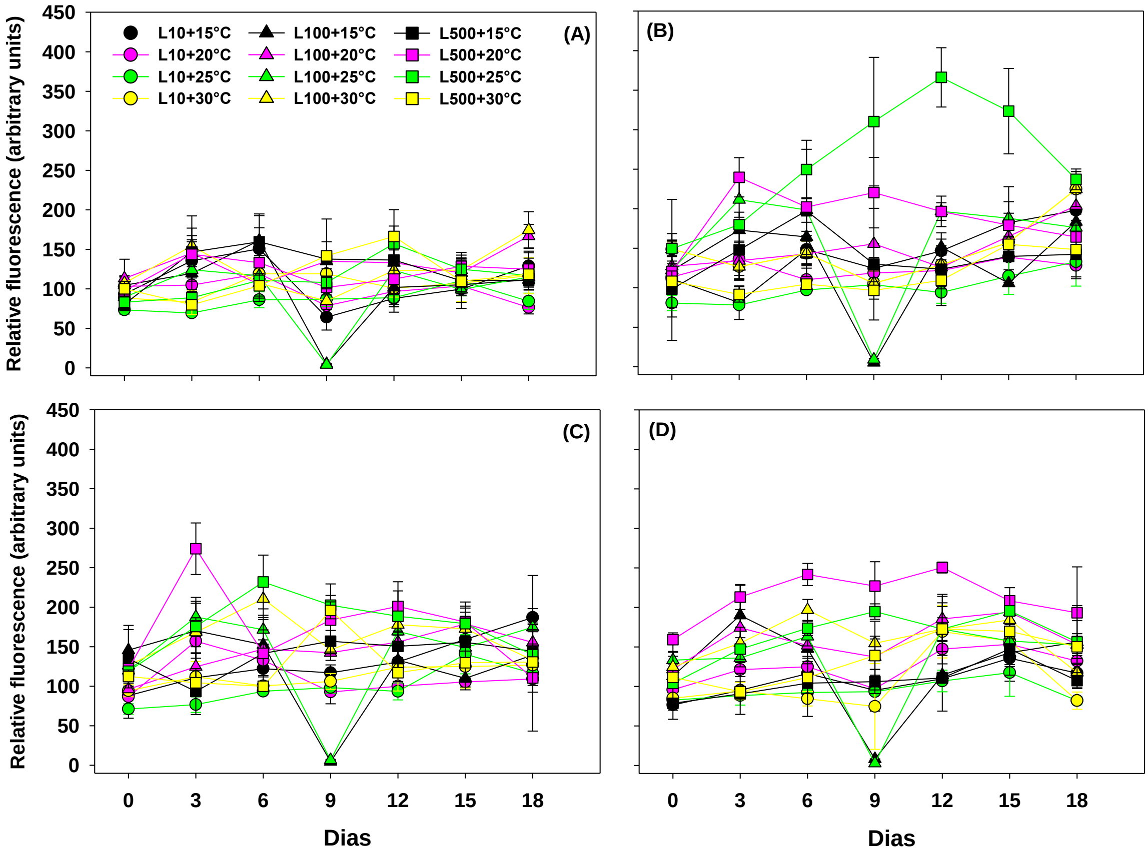
Legend: Geometric forms indicate *Aulacoseira ambigua* strains: circle = AULA-01, inverted triangle = AULA-03, = square = AULA-05 and diamond = AULA-06. Colors indicate light intensity: black = 10 µmol photons m^{-2} s^{-1} (L10) , green = 100 µmol photons m^{-2} s^{-1} (L100) and red = 500 µmol photons m^{-2} s^{-1} (L500). Vertical and horizontal bars indicate standard deviation (n=3).

Source: The author, 2020.

3.3.4 Neutral lipid content

The three-way repeat measurements of *A. ambigua* strains in neutral lipid content by the relative fluorescence of the samples showed a significant difference in time (F_6=46.581, p=0.000) and for all interactions tested ($p<0.05$). The analysis also showed that light intensity and temperature were determinant variables for the relative fluorescence, where the light intensities tested differed significantly from each other ($p<0.05$), and 20 and 25°C were different between themselves and in relation to the other temperatures ($p<0.05$) (Figure 24).

Figure 24 – Combined effect of light and temperature on neutral lipid production in relative fluorescence (arbitrary units) of four strains of *Aulacoseira ambigua.*

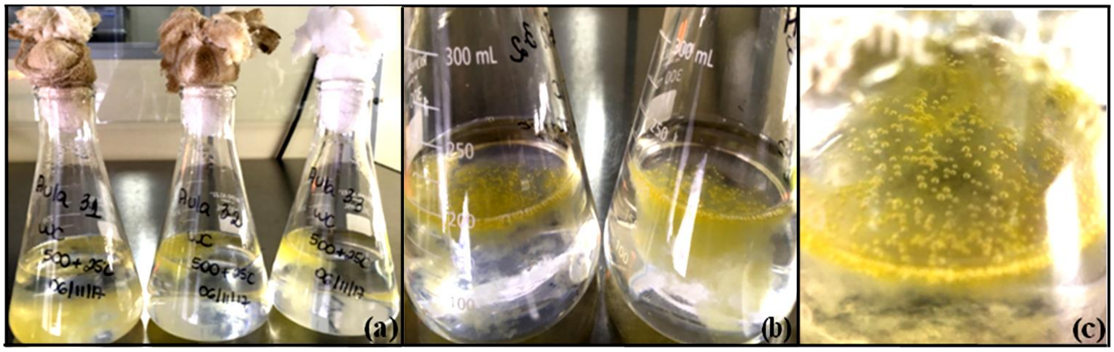


Legend: AULA-01 (A), AULA-03 (B), AULA-05 (C) and AULA-06 (D). Geometric forms indicate light intensity: circle = 10 µmol photons m^{-2} s^{-1}, triangle = 100 µmol photons m^{-2} s^{-1} and square = 500 µmol photons m^{-2} s^{-1}. Colors indicate temperature: black = 15°C, pink - 20°C, green = 25°C and yellow = 30°C. Vertical bars indicate standard deviation (n=3).

Source: The author, 2020.

Among the strains, AULA-03 had the highest relative fluorescence while AULA-01 had the lowest ($p<0.05$) (Figure 24 – D). We also observed that AULA-03 and AULA-06 showed higher values of relative fluorescence, when they were subjected to the highest light intensity tested L500 at 25°C and 20°C, respectively. (Figure 24 – B and C, Figure 25). The *A. ambigua* strains also showed an increase of neutral lipid content between days 6 and 18, demonstrating that high values were exhibited when the strains were the stationary or senescence growth phase.

Figure 25 – Combined effect of light and temperature on neutral lipid production in relative fluorescence (arbitrary units) of *Aulacoseira ambigua* - AULA-03 strain.

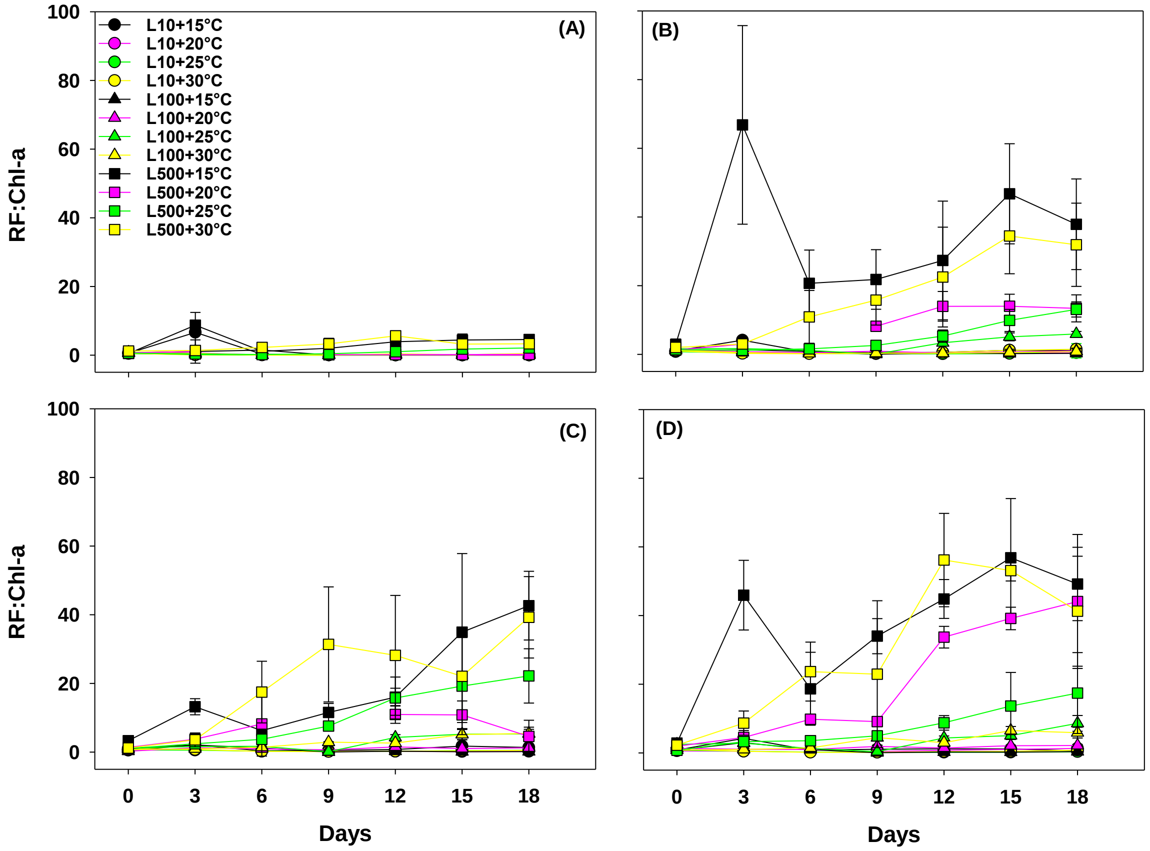


Legend: The pictures show the three replicas of AULA-03 (a) and in detail the strain floating in the culture flasks (b and c).
Source: The author, 2020.

The ratio between values of relative fluorescence and chlorophyll-a (RF:Chl-a) was applied to indicate that the increase in the relative fluorescence values was due to the increase in neutral lipid instead of the increase in biomass. The RF:Chl-a values ranged from 0.01 to 66.82 depending on the strain and the combination of light and temperature tested (Figure 26). The Three-Way repeat measurements of RF:Chl-a showed a significant difference in time (F_6=28.969, p=0.000) and for all interactions tested (p<0.05), except for the interaction between time x strains x temperature (F54=1.334 p=0.063). This analysis also showed that highest light intensity (L500) tested was significant difference from others (p<0.05), and temperatures were separated into 2 statistically different groups: 15 + 30°C and 20 + 25°C (p<0.05).

Higher values of RF:Chl-a were observed for AULA-03, AULA-05 and AULA-06 in L500. The strains AULA-01 and AULA-06 were significantly different between themselves and from the other strains, where AULA-01 showed the lowest RF:Chl-a for all combinations of light intensity and temperate while AULA-06 exhibited higher values in L500 at three temperatures tested (15, 20 and 30°C) (p<0.05). On the other hand, the strains AULA-03 and AULA-05 were statistically similar (p>0.05), with higher values by combining L500 at 15 and 30°C. Moreover, we also observed a relationship between neutral lipid content and growth rate where AULA-01 showed high growth rates and lower neutral lipid content. While AULA-03, AULA-05 and AULA-06 exhibited high neutral lipid content without growing (e.g., 500 µmol photons m^{-2} s^{-1}) (Figure 26).

Figure 26 – Combined effect of light and temperature on the ratio between neutral lipid production in relative fluorescence (arbitrary units) and chlorophyll-a of four strains of *Aulacoseira ambigua*



Legend: RF= relative fluorescence in (arbitrary units) and Chl-a = chlorophyll-a (µg L^{-1}). Strains: AULA-01 (A), AULA-03 (B), AULA-05 (C) and AULA-06 (D). Geometric forms indicate light intensity: circle = 10 µmol photons m^{-2} s^{-1}, triangle = 100 µmol photons m^{-2} s^{-1} and square = 500 µmol photons m^{-2} s^{-1}. Colors indicate temperature: black = 15°C, pink - 20°C, green = 25°C and yellow = 30°C. Vertical bars indicate standard deviation (n=3).

Source: The author, 2020.

3.4 Discussion

3.4.1. Growth

In our study, we tested the hypothesis that high light intensity combined at high temperatures would reduce the growth rate of *Aulacoseira ambigua* strains. Our results showed that the combination of high light intensity (L500) regardless of temperature reduced the growth rates and, therefore, our hypothesis has not been totally refuted. We also observed that the combination of low light intensity and low temperature was a limiting factor for the growth of *A. ambigua*. The optimum growth rates were exhibited by combining low light intensity at high temperatures (L10 at 25 and 30°C) and moderate light intensity (L100) at 20 and 25°C, depending on the strain. Among strains, AULA-01 showed a wide phenotypic plasticity, growing in nine from twelve conditions of ligh and temperature tested. Therefore, our hypothesis has not been refuted.

Diatoms are known to be more abundant in natural systems at low temperatures (4–15.2°C) (BORGES; TRAIN; RODRIGUES, 2008; JEWSON et al., 2008; ZNACHOR et al., 2013) and low light intensity (MARQUARDT et al., 2018). Growing at low temperature allows diatoms to benefit in relation to other phytoplankton groups, giving them a competitive advantage over cyanobacteria and chlorophytes, which showed higher growth rates at higher temperatures (GOMES; AZEVEDO; LÜRLING, 2015; MESQUITA et al., 2020) and minimize grazing losses, since zooplankton is usually present in low numbers in cold periods (ANDERSON, 2000). While the preference for low light can be an adaptive strategy for diatoms due to the high sedimentation rates in relation to other phytoplankton species, as well as being important components of phytoplankton in turbulent environments, where there is a large amount of suspended material which leads to a decrease in the availability of light (BORGES; TRAIN; RODRIGUES, 2008; FONSECA; BICUDO, 2008; NARDELI et al., 2016; REYNOLDS, 1997; SMOL; STOERMER, 2010).

In tropical regions, the difference in temperature between field and laboratory experiments are usually smaller when compared to the temperate region, as can be seen in the Camorim Reservoir where temperatures ranged from 17.4 to 27.3°C. Likewise, the highest biomass of diatom (represented by functional group based on morphology – **MBFG VI** in chapter I of this thesis) were observed in the mild-cold/dry period, when the water temperature varied from 17.4 to 22.6°C. Corroborating our findings, another study also carried out at the Camorim Reservoir demonstrated that diatoms are more abundant in the cold period (winter season), however, no significant difference was observed when purchased with the other seasons (PEREIRA, 2018). According to Anderson (2000), temperature is considered a less limiting variable for the growth of diatoms since they have a worldwide distribution. Our laboratory results showed the ability of *Aulacoseira ambigua* to growth at different temperatures, emphasizing phenotypic plasticity in relation to temperature. Regarding to light, both studies in Camorim Reservoir only estimated whether the light intensity was sufficient or not for photosynthesis through the euphotic zone, but there was no information on the light intensity effectively. Therefore, our study aims to fill a gap on the physiology of *A.*

ambigua, since some strains of this species has shown to be able to grow in a wide range of temperatures, but there is no report of the preference for light intensity.

Laboratory experiments evaluating only one environmental variable indicate that diatoms are capable of growing at moderated to high temperatures (17–30°C) (GOMES; AZEVEDO; LÜRLING, 2015; MARINHO; AZEVEDO, 2007; NISHIKAWA; YAMAGUCHI, 2006) and some of them enhanced the growth rates with increasing temperature (MONTAGNES; FRANKLIN, 2001). This can be explained due to the exclusion of competitive situations added to a large amount of nutrients in the medium of culture (ANDERSON, 2000; SHEAR; NALEWAJKO; BACCHUS, 1976). In relation to light, studies have shown that diatoms species were able to grow in a wide range of light intensities (5–450 μmol of photons m^{-2} s^{-1}) (MONTAGNES; FRANKLIN, 2001; NISHIKAWAA; YAMAGUCHI, 2006; SPILLING et al., 2015).

Experiments combining light and temperature on the growth rate had already been conducted on a variety of diatom species (FIALA; ORIOL, 1990; NISHIKAWAA; YAMAGUCHI, 2006; SPILLING et al., 2015), including *A. ambigua* (SHEAR; NALEWAJKO; BACCHUS, 1976). Our results demonstrated that *A. ambigua* strains exhibited optimum growth rates under conditions of low light at high temperatures (25 and 30°C) and moderate light intensity at 20 and 25°C. In contrast with our findings, diatoms have demonstrated that growth rate increased with the temperature regardless of light intensity (NISHIKAWAA; YAMAGUCHI, 2006; FIALA; ORIOL, 1990); the maximum growth rate was exhibited by combining high light intensity at low temperature (450 μmol of photons m^{-2} s^{-1} at 11°C) (SPILLING et al., 2015); and especially for *A. ambigua*, preference to growth with high temperatures and high light intensity (SHEAR; NALEWAJKO; BACCHUS, 1976). Considering our results and those of Shear et al. (1976), we concluded that *A. ambigua* is able to grow under different light and temperature conditions, demonstrating a wide phenotypic plasticity and intraspecific variability which would explain its occurrence in different regions of the world.

We also observed that under high light intensity regardless of temperature, the *A. ambigua* strains were unable to grow. In addition, *A. ambigua* strains tend to reduce photosynthetic capacity at high light intensity, demonstrating that high irradiance can cause damages to the photosynthetic apparatus and consequently cannot grow. The excess of light energy absorbed by photosynthetic pigments accelerates photoinhibition by suppressing the repair of photodamaged in photosystem II (MURATA et al., 2007;

NISHIYAMA; ALLAKHVERDIEV; MURATA, 2006, 2005; TAKAHASHI; MURATA, 2008), and the repair rate also depends on the intensity of the incident light where it peaks in relatively weak light (ALLAKHVERDIEC; MURATA, 2004), pointing out that the high light intensity negatively affects the photosynthetic apparatus and consequently the growth rate, even for tropical *A. ambigua* strains where high temperatures and light intensities could occur throughout the year.

3.4.2 Morphology

In our study, we tested the hypotheses that (H_{1a}) the combined effect of low light intensity and low temperature would result in longer filaments, higher S/V and lower cell volumes of *A. ambigua* strains, due to the need to intercept more light energy and the lower stimulus for replication; while (H_{1b}) high light intensity combined with high temperature would result in shorter filaments, lower S/V and higher cell volumes. Our results did not support our hypothesis, where longer trichomes were observed by combining L100 or L500 at temperatures ≤25°C, depending on the strain while S/V and cell volume did not show a patterns where higher values of these parameters were exhibited in several combinations of light and temperature.

We observed that depending on the combination of light and temperature, the strains of *A. ambigua* showed a slight curvature in the trichome of the straight morphotype in which we consider it to be an expression of them for experimental conditions. However, more abrupt changes in the morphotype, such as the change from the straight to spiral morphotype, configured a variation of this species – *A. ambigua* f. *japonica* (TUJI; WILLIAMS, 2007; VAN VUUREN et al., 2018). Although there is no report of change in the trichome morphotype in *A. ambigua* due to environmental conditions, such change has already been observed in the field for another species of *Aulacoseira* – *A. granulata*, where the curvature in their filaments may be the result of the impact of an external force (e.g., turbulence), an adaptation to the continuous flow of water in large rivers, or it may be an indicator of increased load of nutrients (WANG et al., 2017). Therefore, our results from laboratory experiment showed that the combination of certain light intensities and temperatures can lead to the curvature of the filaments, being and adaptative strategy for *A. ambigua* in these conditions.

The *A. ambigua* strains showed different morphological responses to the combined effect of light and temperature, demonstrating a wide phenotypic plasticity and a wide intraspecific variability. To our knowledge, the present study represents the first report of the interaction between light and temperature on some parameters of *A. ambigua* morphology. Therefore, more studies need to be carried out to improve our understanding of the possible effects caused by the combination of these variables, not only in *A. ambigua* morphology, but also in other diatom species, as well as in other physiological parameters of diatoms.

Despite this, there are numerous reports in the literature about the effect of only one environmental variable (e.g., light, temperature or nutrients) on morphology of diatoms species, including observations made in the field or in laboratory experiments (CHARALAMPOUS; MATTHIESSEN; SOMMER, 2018; POISTER et al., 2012; TREMARIM et al., 2013; TURKIA; LEPITÖ, 1999). For instance, some species of diatoms decrease cell size and volume or increase cell size with increasing temperature (JUNG et al., 2013; MONTAGNES; FRANKLIN 2001; SU; LUNDHOLM; ELLEGAARD; 2018; THOMPSON; GUO; HARRISON, 1992). Other studies showed that high light intensities induce a reduction in cell size or an increase in cell volume and length and width of the frustule (ASLAMOV; JEWSON, 2009; SU; LUNDHOLM; ELLEGAARD, 2018; THOMPSON; HARRISON; PARSLOW, 1991).

We observed that *A. ambigua* strains showed an increase in cell volume when combining moderate light intensity at low temperature (L100 at 15°C) and in conditions of low or high light intensity at the highest temperature tested (L10 and L500 at 30°C). While the lowest cell volume was observed when combining high temperatures (25 and 30°C) regardless of light intensity, in conditions of moderate light intensity (L100) at ≥ 20°C and in conditions of high light intensity (L500) at 20 and 25°C. Studies have indicated that diatoms reduce cell volume with increasing temperature (JUNG et al. 2013; MONTAGNES; FRANKLIN, 2001). Others have shown that an increase in cell volume with decreasing temperature because such conditions lead to a reduction in efficiency of photosynthetic, as well as reducing the assimilation of nutrients and the subsequent growth of algal cells (FALKOWSKI; RAVEN 1997; RAVEN; GEIDER 1988). Hence, the change in cell volume in relation to temperature is a species-specific and moreover depends on the analyzed cells (MONTAGNES; FRANKLIN, 2001). For example, *Coscinodiscus* sp. (diatom) showed an increase in cell volume with the increasing temperature when all cells were considered, but there was no significant relationship when the large cells were excluded (MONTAGNES; FRANKLIN, 2001). Although *A. ambigua* strains did not show similar responses that could reflect a pattern, we suggest that changes in cell volume may be an attempt by *A. ambigua* to maintain its homeostatic balance under different light and temperature conditions.

Regarding in trichome size, *A. ambigua* strains showed longer trichomes when combining moderate light intensity at low temperature (L100 at 15°C) and high light intensity at temperatures ≤ 25°C. Temperature is an environmental variable that modulates cellular metabolism, directly influencing metabolic processes related to photosynthesis and biosynthesis (e.g., growth rate) (COLES; JONES, 2000). This means that lower temperatures can result in longer filaments due to less cellular metabolism and consequently less stimulus for replication, corroborating part of our results. On the other hand, the increase in light intensity or the increase in the period of exposure to light resulted in shorter filaments, which can be considered a stressful condition for the physiology of some phytoplankton organisms (ASLAMOV; JEWSON, 2009). Although our study evaluated the combined effect of light and temperature, *A. ambigua* exhibited longer trichomes in high light intensity (L500), even under high temperature condition. This can be explained by the growth rates, where *A. ambigua* did not show growth rate under high light intensity, resulting in longer trichomes.

Previous studies with several phytoplankton species have shown that longer filaments or trichomes, higher S/V values and lower cell volumes tend to be observed under conditions of low light intensity or at low temperatures while high light intensity or high temperature are associated with shorter filaments, lower S/V values and higher cell volumes (ASLAMOV; JEWSON, 2009; CHARALAMPOUS; MATTHIESSEN; SOMMER, 2018; SOARES; LÜRLING; HUSZAR, 2013). This is because phytoplankton species generally tend to increase cell metabolism when exposed to conditions of high light intensity or high temperature, leading to a higher growth rate and consequently a reduction in filament size and S/V, as well as an increase in cell volume.

The ability of *Aulacoseira ambigua* strains has changed the filament size and the surface-to-volume ratio (S/V) is a physiological response, in an attempt to acclimate to different light and temperature conditions. Both morphology parameters are important for the ecology of phytoplankton species because they are related to the ability to intercept light, assimilate nutrients, in addition to helping to keep species longer suspended in water column (KIRK, 1996; LEWIS, 1976; REYNOLDS, 1988). In addition, filamentous phytoplankton species can be considered good competitors for light, since they have a greater contact surface area for the capture of light energy.

(KIRK, 1996; LEWIS, 1976; REYNOLDS, 1988). This competitive advantage has already been observed for two species of *Aulacoseira* (*A. ambigua, A. baicalensis*), where they showed increase in abundance or longer filaments in situations of low light availability (ASLAMOV; JEWSON, 2009; MARQUARDT et al., 2018). On the other hand, longer filaments in *A. ambigua* can result in rapid sedimentation rates (POISTER et al., 2012). Therefore, the size of the filament is an important characteristic for *A. ambigua*, in which it is associated with capturing light energy for photosynthesis, staying longer suspended in the water column and even an indicator of its metabolism.

3.4.3 Photosynthetic capacity

In our study, we hypothesized (H1a) high light intensity combined at high temperature would reduce the photosynthetic capacity of *Aulacoseira ambigua*. We observed that the strains of *A. ambigua* reduced mainly the photosynthetic capacity under high light combined with high temperature, but it was also possible to observe a reduction in other combinations of light and temperature. Therefore, our hypothesis has not been refuted.

Our results demonstrated that the interaction between different light intensities and temperatures affect the photosynthetic capacity of *A. ambigua*, as well as the duration of the experiment (day 1 vs. 17). Although our experiments were carried out in batch cultures that could justify the reduction in photosynthetic capacity, a recent study demonstrated that even under conditions of semi-continuous culture, a species of diatom showed a reduction in photosynthetic capacity (TRAMPE; HANSEN; KÜHL, 2015). This leads us to believe that the reduction in the photosynthetic capacity of *A. ambigua* strains was directly influenced by the experimental conditions (e.g., light, temperature and the time of the experiment).

All strains of *Aulacoseira ambigua* demonstrated to alter their photosynthetic capacity even in those combinations of light and temperature that did not show growth rate, demonstrating that this species first modifies its photosynthetic apparatus to survive and maybe later it can invest in growth. Contrasting with our findings, a study evaluated the growth and photosynthetic capacity of *Coscinodiscus granii* (diatom) under 5 light intensities (50, 150, 235, 332, 450 μmol of photons m^{-2} s^{-1}) and demonstrated that this diatom modified its chlorophyll-a content and growth rate at different light intensities, but under high light intensities the cell division was prioritized above the synthesis of photosynthetic pigments (TRAMPE; HANSEN; KÜHL, 2015). Another study demonstrated that *A. granulata* (diatom) did not alter its photosynthetic rate when combined with 40 µmol of photons m^{-2} s^{-1} at temperatures above 20°C (COLES; JONES, 2000), which also contrasts with our results. Under conditions of lower light intensity (10 µmol of photons m^{-2} s^{-1}) and combined at high temperature (30°C), *A. ambigua* was able to alter its photosynthetic response (e.g., $r\mathrm{ETR}_{\mathrm{max}}$). Therefore, *A. ambigua* exhibited a wide phenotypic plasticity, changing its photosynthetic capacity in different combinations of light and temperature, even in conditions that are not favorable for its growth.

The parameter $r\mathrm{ETR}_{\mathrm{max}}$ was the only one that showed a positive linear correlation with the growth rate. Although this photosynthetic parameter is an estimate of photosynthesis, not effectively expressing the amount of carbon fixed by an organism, it has been seen that an increase in ETR would result in increased demand for ATP to support carbon fixation and other associated ATP-dependent cellular processes at higher growth rates (THAMATRAKOLN et al., 2013). Another study showed that relative electron transport rates were similar between -1 and 7°C, but growth rates were significantly higher at 7°C, suggesting that at low temperatures enzyme activity is usually decreased, which could account for the slower growth rates (MOCK; HOCH, 2005). In the same way, *A. ambigua* strains showed different values of growth rate and $r\mathrm{ETR}_{\mathrm{max}}$ in different combinations of light and temperature tested, indicating the increased of the $r\mathrm{ETR}_{\mathrm{max}}$ was to support higher growth rates.

In our methodology, *A. ambigua* strains were subjected to a variation of 16 to 1864 µmol photons m^{-2} s^{-1} to obtain photosynthetic parameters. Unexpectedly, three of the four strains (AULA-01, AULA-03 and AULA-06) when combined with moderate (L100) and high light (L500) at 20°C did not show the values of the parameters analyzed on day 17, pointing out that such conditions can be considered as stressful for its photosynthetic machinery. For another species of diatom - *Aulacoseira granulata*, an increase in the photosynthetic rate was observed with an increase in the density of the photon flow until the levels reached 210 to 550 µmol of photons m^{-2} s^{-1}, which represents about 10 at 25% of the total sun, and even at levels above irradiance, photoinhibition was not observed (COLES; JONES, 2000), demonstrating that the *Aulacoseira* presents different photosynthetic responses. Furthermore, studies have shown that low temperature inhibits PSII repair (MURATA et al., 2007), as well as reducing photosynthetic efficiency (DAVIDSON, 1991; FALKOWSKI; LAROCHE; 1991;

FALKOWSKI; RAVEN; 1997; STRAMSKI; SCIANDRA; CLAUSTRE, 2002). Our results add that the high light intensity combined with low temperature, for a relatively long period, affected the photosynthetic efficiency of *A. ambigua*, suggesting that under these conditions the repair rates may have been low or even inhibited, being unable to supply the damage caused by high light intensities and probably causing a photoinhibition (ALLAKHVERDIEC; MURATA, 2004; MURATA et al., 2007; NISHIYAMA; ALLAKHVERDIEV; MURATA, 2006 and 2005; TAKAHASHI; MURATA, 2008).

One of the parameters evaluated in photosynthetic capacity was $r\mathrm{ETR}_{\mathrm{max}}$, which allows us to measure the speed at which photosynthetic electrons in PSII are transported and consequently allows us to infer the conditions of photosynthetic machinery. We observed that *A. ambigua* could change its $r\mathrm{ETR}_{\mathrm{max}}$ depending on the combinations of light and temperature, demonstrating a wide phenotypic plasticity and intraspecific variability. In addition, temperature proved to be a determining factor for $r\mathrm{ETR}_{\mathrm{max}}$ in this species, since the strains reduced $r\mathrm{ETR}_{\mathrm{max}}$ were subjected to temperatures ≥20°C. Corroborating with our data, a study evaluated the $r\mathrm{ETR}_{\mathrm{max}}$ of a marine benthic diatom (*Cylindrotheca closterium)* at low light intensity with the increase in temperature (5, 10, 15, 20, 25, 30, 35 and 40ºC) and showed that this parameter constantly increased with increasing temperature, reaching an optimum at 30-35°C, but at 40ºC it was not possible to measure photosynthesis, indicating that $\mathrm{ETR}_{\mathrm{max}}$ was extremely sensitive to temperature (MORRIS; KROMKAMP, 2003).

Another photosynthetic parameter analyzed in this study was the light harvesting efficiency (**α**). This parameter is related to the efficiency of light capture and the conversion efficiency of photosynthetic energy (HENLEY, 1993). The strains of *A. ambigua* increased α in conditions of low and moderate light intensity at low temperatures. Corroborating our findings, it has already been demonstrated that the increase in the efficiency of light capture is considered a mechanism used to deal with sub-ideal intensities of light and can manifest itself as an increase in **α** (HOLLAND et al., 2012). Furthermore, several studies indicate that low temperatures lead to a reduction in photosynthetic capacity by reducing a number of factors related to photosynthesis (enzyme activity, membrane fluidity and electron chain transfer and cell chlorophyll content) (BERNER; DUBINSKY 1989; DAVIDSON, 1991; FALKOWSKI; LAROCHE, 1991; FALKOWSKI; RAVEN; 1997; RAVEN; GEIDER 1988; STRAMSKI; SCIANDRA; CLAUSTRE, 2002; YODER, 1979). Hence, ours results indicated that the low temperature was a favorable factor in the **α** of *A. ambigua* when they are exposed for a relatively long period (18 days) and suggests that the increase in this parameter may be a compensatory mechanism when conditions are unfavorable for photosynthesis.

Lastly, we measured the photosynthetic saturation light (E*K*) which is widely used to characterize the state of microalgae photoaclimation. This parameter allows us to infer if the species or strain is adapted to low or high intensities (BEHRENFELD et al., 2004; BEHRENFELD; HALSEY; MILLIGAN, 2008). In the literature, there are different methods for obtaining the photosynthetic saturation intensity, using since primary productivity (PALMISANO; BEELER; SULLIVAN, 1987) or the maximum growth rate (BONILLA et al., 2012) up to the maximum relative transport rate (IHNKEN; EGGERT; BEARDALL, 2010; PIERANGELINI et al., 2014; WHITE; ANANDRAJ; BUX, 2011; WU; SHI; LI, 2009). It is important to know how this parameter was obtained, since the values can be discrepant between each methodology, which can cause false comparisons.

We observed that *Aulacoseira ambigua* showed the highest values of E*K* were obtained by combining 100 µmol photons m^{-2} s^{-1} at 25°C and there was a reduction at high light intensity, demonstrating that *A. ambigua* was sensitive to high light intensity tested, therefore, we can consider that *A. ambigua* is a kind of shadow species - adapted to low light intensities. In addition, *A. ambigua* was able to increase or decrease its E*K* values depending on the combination of light and temperature. In nature, phytoplankton organisms are often subject to large irradiance gradients and such variations can determine the composition of the phytoplanktonic community, demonstrating that *A. ambigua* has an extensive phenotypic plasticity and intraspecific variability which may be a favorable factor for the survival of this species and explain its occurrence in different environments around the world.

3.4.4 Neutral lipid content

Phytoplankton species synthesize and accumulate neutral lipids generally in unfavorable or stressful conditions (HU et al., 2008; SUKENIK; WAHNON, 1991). High temperatures and high light intensities can be considered stressful conditions for diatoms, even those originating in tropical environments. In this way, we hypothesized that the combination of high light intensity and high temperature would increase a neutral lipid content in *Aulacoseira ambigua* strains. Our results demonstrated that the combination of high light intensity at 20 and 25°C resulted in an increase in the relative fluorescence values for AULA-03 (L500 at 25°C) and AULA06 (L500 at 20°C). Also, we observed the increase in RF:Chl-a under conditions of high light intensity at different temperatures tested, depending on the strain. Therefore, our hypothesis was not totally rejected.

In the literature, there are several methodologies for extracting neutral lipids or for obtaining some related parameters (e.g., relative fluorescence) that include the use of solvents, gravimetric determination and currently using spectrofluorimetric with the Nile Red dye (BLIGH; DYER, 1959; ELSEY et al., 2007). In the latter technique, a wide range of excitation and emission wavelengths have already been used, such as 480/575 nm (CHEN et al., 2009), 486/576 nm (ELSEY et al., 2007), respectively. The protocol developed in this study for obtaining neutral lipids for *A. ambigua* strains exhibited optimal excitation lengths of 525 nm and emission of 657 nm, demonstrating that the wavelengths can vary depending on the species analyzed. In addition, the fluorescence of Nile red dye is strongly influenced by temperature and the duration of staining with high temperatures or long periods of staining leads to shortening of fluorescence (DEYE; BERGER; ANDERSON, 1990). Under our experimental conditions, the Nile red dye was used at 40°C for 7 minutes. These conditions were ideal for producing maximum fluorescence in *A. ambigua* strains, similarly to that used in another study (CHEN et al., 2009).

Regarding the neutral lipid content in *A. ambigua* strains, our results showed that the growth phase was an important factor for obtaining higher values, where it was possible to observe an increase in both relative fluorescence and RF:Chl-a among the day 6 and 18, which corresponded to the stationary and senescence phases, depending on the strain and the combination of temperature at high light intensity. Corroborating with our findings, studies have shown that culture aging and stationary or senescent phases can affect the content and composition of lipids and fatty acids in some phytoplankton species (BIGOGNO et al., 2002; COOKSEY et al., 1987; MANSOUR; VOLKMAN; BLACKBURN, 2003). For example, *Parietochloris incise* (Chlorophyte) and *Gymnodinium sp.* (Dinoflagellate) increased their production of neutral lipid as triacylglycerol (TAG) in the stationary phase, reaching 1,79 to 3,75 folders compared to the logarithmic phase, respectively (BIGOGNO et al., 2002; MANSOUR; VOLKMAN; BLACKBURN, 2003). In the same way, two strains of *Navicula* sp.

(diatom) increased their neutral lipid content 1.90 and 2.43 folders in stationary phase (COOKSEY et al., 1987). Therefore, *A. ambigua* strains started to accumulate neutral lipids when they are not investing in growth.

The neutral lipid content was not only influenced by the growth phase, but also in relation of light and temperature combination. The highest values of relative fluoresce were observed by combining high light intensity at 20 and 25°C, reaching 250.50 ± 6.92 a.u and 366.19 ± 37.57 a.u, respectively. Another study looked at fluorescence emission with Nile red in nine phytoplankton species (including one diatom) and demonstrated that the intensity of fluorescence emission is species-specific (CHEN et al., 2009). Under the same experimental conditions, two species (*Rhodomonas salina* and *Nannochloropsis* sp.) reached high fluorescence intensities (5013 a.u), two other species (*Chlorella vulgaris* and *Pseudochlorococcum* sp.) produced low fluorescence intensity (37-80 a.u) while *Chlorella zofingiensi* showed no fluorescence intensity (CHEN et al., 2009). Although the highest relative fluorescence values of *A. ambigua* were lower than those exhibited in Chen et al. (2009), we emphasize that the experimental conditions and the studied species were different, justifying the differences in values and proving the efficiency of our methodology.

Considering that the increase in the relative fluorescence could reflect the increase in algal biomass instead of neutral lipid content, we did the ratio between relative fluorescence and chlorophyll-a (RF:Chl-a). The highest values of RF:Chl-a were observed when combining high light intensity at 15, 20 and 30°C, depending on the strain. Like most strains of *A. ambigua* exhibited increase in neutral lipid content in a wide temperature range, we considered that high light intensity was the main variable for the increase in neutral lipid content, being considered a stressful light condition for *A. ambigua* strains.

In the literature, temperature and light have been found to have a major effect on the lipid production and composition of algae, since the fluidity and stability of the membranes may be related to the temperature range in which organisms grow (KLEINSCHMIDT; MCMAHON, 1970; ZHU; LEE, CHAO et al., 1997) while range of light intensities exhibit remarkable changes in their gross chemical composition, pigment content and photosynthetic activity (LEPETIT et al., 2012; FALKOWSKI; OWENS, 1980; POST et al., 1985; SUKENIK et al., 1987). Studies that analyzed these two variables separately demonstrated that low temperature or high light intensities favor the formation of polyunsaturated fatty acids and increase in the amount of neutral storage lipids (e.g. triacylglycerol – TAG), while high temperatures and low light intensities induces the formation of polar lipids, particularly the membrane polar lipids associated with the chloroplast (BROWN et al., 1996; KHOTIMCHENKO; YAKOVLEVA, 2005; MORTENSEN et al. 1988; RENAUD et al., 2002). We suggest that the difference between our results regarding the literature is not only related to the combined effect of light and temperature, but also because *A. ambigua* did not growth at high light intensity which allowed to increase its neutral lipid content.

Extrapolating our results in the laboratory to the natural system, the increase in the neutral lipids content in *A. ambigua* strains could result in competitive advantages due to the ability to float (e.g., AULA-03, Figure 25) and consequently stay longer in the water column. Diatoms have a relatively heavy cell structure due to the presence of silica which consequently provides high sedimentation rates (KRUK et al., 2010). This means that, in a natural system, diatoms depend on environmental factors (for example, mixing the water column by the wind, turbulence of the currents) (FONSECA; BICUDO, 2008; MARQUARDT et al., 2018) remaining longer suspended in the water column or they can accumulate lipids (HILDEBRAND et al., 2012). This competitive advantage can be even more trivial in systems that have long-lasting stratification of the water column or in turbid systems where the availability of light does not reach the bottom of water body. Therefore, the increase in the neutral lipid content is one of the factors that can favor buoyancy, allowing *A. ambigua* to experience different environmental conditions to which it was subjected.

3.5 **Conclusion**

Aulacoseira ambigua showed wide intraspecific variability and wide phenotypic plasticity in relation to the parameters analyzed (growth rate, morphology, photosynthetic capacity and production of neutral lipids), demonstrating being able to change its physiology under different light and temperature conditions. However, this species was sensitive to high light intensity, as it did not show growth rate, reduced its photosynthetic capacity and increased its production of neutral lipids. For these reasons, *A. ambigua* can be considered as a species adapted to low light intensity. The increase in neutral lipids as well as different changes in their morphology may favor their buoyancy, allowing this species to remain suspended longer in the water column.

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DISCUSSÃO GERAL

Ao longo dos últimos 12 anos, o Reservatório do Camorim tem passado por um aumento do grau de trofia, modificando seu estado de mesotrófico para eutrófico, baseados nos valores de fósforo total e nitrogênio total (dados não publicados), de acordo com Nürnberg (1996). Considerando que esse reservatório está localizado numa área de proteção ambiental e por ser raso (profundidade máxima = 3 m), sugerimos que a eutrofização seja resultado de um processo natural devido à ausência de fontes antrópicas diretas que poderiam ocasionar o aumento do suprimento de nutrientes. Desse modo, a eutrofização do Reservatório do Camorim pode ser resultado do fluxo contínuo de afluentes que drenam uma grande área de vegetação, caracterizando um aporte externo de nutrientes para esse corpo hídrico, assim como pelo aporte interno através do sedimento (ex. decomposição, ressuspensão do sedimento).

Embora o Reservatório do Camorim já tenha sido objeto de estudo, poucos foram os trabalhos que abordaram a comunidade planctônica (fito e zooplanctônica) (MENEZES, 1996; MENEZES; BICUDO, 2008; PEREIRA, 2018). Estes, por sua vez, analisaram a taxonomia de um determinado grupo fitoplanctônico como, por exemplo, os fitoflagelados (MENEZES, 1996; MENEZES; BICUDO, 2008) e apenas um abordou sobre a ecologia dos organismos planctônicos (PEREIRA, 2018). Esse reservatório era uma das principais fontes de água para a população local, no entanto, a distribuição de água foi interrompida devido à intensa proliferação de cianobactérias (CEDAE, 2010). Logo, estudos que busquem uma melhor compreensão sobre a dinâmica das comunidades aquáticas são de grande relevância, principalmente àquelas que podem ocasionar consequências negativas ao suprimento de água, servindo de embasamento para o gerenciamento destes sistemas. Outro fator a se considerar é que mesmo um sistema artificial localizado numa área de proteção ambiental, como é o caso do Reservatório do Camorim, está sujeito ao processo de eutrofização, o que é pouco usual e pode trazer conhecimentos importantes para outros sistemas semelhantes a ele.

A tese objetivou avaliar a dinâmica da comunidade planctônica (capítulo I) no Reservatório do Camorim, buscando esclarecer os principais fatores responsáveis em sua estruturação. Além disso, experimentos laboratoriais foram realizados com o intuito de aprofundar o conhecimento sobre a fisiologia de duas espécies fitoplanctônicas presentes no reservatório e ainda que esses resultados possam ajudar a explicar a ocorrência delas no Reservatório do Camorim (capítulo II e III), assim como nos demais sistemas ao redor do mundo.

Considerando que o Reservatório de Camorim é um ambiente eutrófico, não se esperava observar baixas concentrações de nutrientes. Uma possível limitação do P ao longo do período foi observada, considerando tanto as concentrações absolutas de SRP (concentrações de SRP <10 µg L-1, SAS, 1989) quanto a razão molar entre NID: SRP (concentrações de NID: SRP> 50, KOSTEN et al., 2009). O fósforo (P) é considerado um recurso naturalmente escasso no meio ambiente e já foi identificado como o principal fator limitante da produção primária em diversos ecossistemas (RANGEL et al., 2012, 2016a; SCHINDLER, 1978; TILZER, 1990; VOLLEMWEIDER; KEREKES, 1980). Em outro reservatório tropical, a limitação de P foi associada a fatores locais específicos, como uso do solo, características de captura e hidrologia (RANGEL et al., 2012). Essa explicação também pode ser aplicada ao reservatório de Camorim, uma vez que não há atividades ao redor do corpo d'água que poderiam aumentar significativamente a entrada de P (por exemplo, terras agrícolas, pastagens e habitação). Também observamos valores baixos e potencialmente limitantes de DIN e SRSi para o crescimento do fitoplâncton em dezembro/2017 e abril/2018, respectivamente. Aqui, consideramos os valores limites potenciais como DIN <100 µg L-1, (REYNOLDS, 1997) e SRSi <50 µg L-1 (REYNOLDS, 2006). Contrariamente aos nossos resultados, um estudo anterior realizado no reservatório de Camorim entre 2012-2013 demonstrou que não houve limitação de nitrogênio (N) e SRSi (PEREIRA, 2018). Essa diferença pode ser atribuída aos menores valores de precipitação observados neste estudo, principalmente em dezembro/2018. De maneira geral, o aporte externo de nitrogênio aos ecossistemas aquáticos pode ocorrer por meio da deposição atmosférica, do transporte de matéria alóctone e chuva ácida (OBERGSTRÖM; JANSSON, 2006; HOBBS et al., 2000, PAERL, 1985; TOMBERG et al., 2014) Por outro lado, a sílica é um elemento limitante chave em ecossistemas aquáticos porque é fixada principalmente na forma de minerais silicatos e frações de quartzo que não podem ser absorvidos por organismos aquáticos (ITTEKKOT; HUMBORG; SCHÄFER, 2000; GE et al. 2015) A fração dissolvida da sílica utilizada pelos organismos aquáticos tem origem na dissolução de fitólitos e intemperismo de rochas superficiais (LAURELLE et al., 2009). Assim, o fornecimento de N e sílica dissolvida para ecossistemas aquáticos, como lagos e reservatórios, é dado por descargas de água ou transportado por rios (MA et al., 2017; SOROKOVIKOVA et al., 2019; TOMBERG et al., 2014). Portanto, a estação chuvosa é um fator determinante para a entrada de nutrientes nos ecossistemas aquáticos, principalmente aqueles que estão localizados em áreas de preservação ambiental, como o reservatório de Camorim. Além disso, a concentração limite potencial de SRSi não correspondeu diretamente a valores mais elevados de biomassa de MBFG **VI** (grupo funcional composto apenas por organismos com exoesqueleto silicioso), mas pode ser o resultado da rápida assimilação e proliferação desses organismos nos meses anteriores (SOMMER; STABEL, 2014). Ressaltamos que apenas a fração dissolvida de SRP, DIN e SRSi foi analisada. Assim, é possível que as baixas concentrações desses nutrientes sejam decorrentes da rápida incorporação por organismos fitoplanctônicos, o que explicaria a elevada biomassa dessa comunidade no período quase amostrado (FEITOSA et al., 2019; RANGEL et al., 2016a; SOMMER; STABEL, 2014; TOMBERG et al., 2014).

A comunidade fitoplanctônica apresentou variação temporal e vertical na biomassa total e na composição dos MBFGs. Embora tenha sido observada uma flutuação na biomassa fitoplanctônica total, houve uma queda drástica da biomassa total no mês de janeiro a março/2018. Nos meses de janeiro/2018 e fevereiro/2018, a precipitação foi> 250mm, acima da média dos últimos 13 anos, que foi de 150mm para janeiro e <100mm para fevereiro (Sistema Alerta Rio, 2020). Valores altos de precipitação aumentam o volume do corpo d'água, resultando em um aumento na taxa de escoamento da água. Este é um dos processos relacionados à perda de fitoplâncton, principalmente em lagos pequenos e rasos (REYNOLDS, 1997). A saída de água (descarga) pode ser mais pronunciada em ambientes com alta abundância de espécies de crescimento lento, como observado no reservatório de Camorim, uma vez que oferece uma vantagem competitiva para espécies de rápido crescimento (SCHEFFER, 1998). Consequentemente, a queda da biomassa do fitoplâncton foi associada aos altos valores de precipitação combinados com a composição das características funcionais das espécies. Além disso, ao se considerar o estado eutrófico do reservatório e a biomassa relativamente elevada da comunidade fitoplanctônica, era de se esperar que ocorresse uma redução da zona eufótica (TALLING, 2001). No entanto, nossos resultados apontam para o oposto. A coluna d'água estava quase sempre iluminada até o fundo do reservatório e isso pode ser explicado pela pouca profundidade desse corpo d'água.

A partir das variáveis limnológicas abióticas, principalmente precipitação e temperatura do ar apresentaram uma variação temporal acentuada, foi possível separar em duas estações climatológicas (quente/chuvoso e fria/seca). Os maiores RWCS foram observados na estação quente/chuvosa, indicando a existência de estratificação térmica. Esse fenômeno ocorre quando ocorre um aumento da temperatura do ar que leva ao aquecimento da água, promovendo uma diferença na densidade dos corpos d'água (FONSECA; BICUDO, 2008). Ao mesmo tempo, observamos o aparecimento de **MBFG III** na superfície e uma correlação positiva entre esse grupo funcional e a temperatura da água. O **MBFG III** é composto por organismos de grandes filamentos com aerótopos (KRUK et al., 2010) que lhes conferem a capacidade de migrar e permanecer mais tempo na coluna d'água (PADISÁK; SORÓCZKI-PINTÉR; REZNER, 2003). Já o MBFG VII foi observado em quase todos os meses, embora sua alta contribuição para a biomassa fitoplanctônica tenha ocorrido em janeiro / 2018 e abril / 2018. Corroborando com nossos dados, um estudo recente demonstrou que os períodos de estratificação podem favorecer os **MBFGs III** e **VII**, podendo dominar a zona pelágica quando as condições ambientais são favoráveis (ex. altas temperaturas, estabilidade da coluna d'água e disponibilidade de luz) (RANGEL et al., 2016a). No entanto, condições ambientais como estratificação não duradoura associada à limitação persistente de P, principalmente para MBFG VII (RANGEL et al., 2016a), mostraram-se decisivas para a baixa biomassa desses MBFGs no reservatório de Camorim. Além disso, **MBFGs III** e **VII** não são considerados bons recursos alimentares para a comunidade zooplanctônica, visto que grandes colônias mucilaginosas (ROLLAND; HANS, 1987) e a capacidade de produzir toxinas (KURMAYER; CHRISTIANSEN; CHORUS, 2003; WANG et al., 2013), o que reduz a palatabilidade para a maioria das espécies de zooplâncton levando as taxas de eliminação e ingestão a valores próximos a zero (COLINA et al., 2016). Isso significa que a comunidade zooplanctônica do reservatório de Camorim poderia favorecer o aumento da biomassa dos **MBFGs III** e **VII** evitando consumi-los, mas isso não aconteceu.

Os valores mais baixos de RWCS na estação fria/seca demonstraram que a diminuição da temperatura do ar leva a um aumento na densidade da água permitindo uma mistura completa da coluna d'água (FONSECA; BICUDO, 2008). Consequentemente, maior biomassa do **MBFG VI** foi observada, principalmente na superfície. O **MBFG VI** é composto por organismos com esqueleto silicioso (KRUK et al., 2010) cujas paredes de sílica protegem as células contra a pressão mecânica, fornecem uma barreira contra o pastejo do zooplâncton (HAMM et al., 2003) e promove taxas de sedimentação rápidas (KRUK et al., 2010). Além disso, outros fatores como altas concentrações de SRSi, coluna de água sempre iluminada e concentrações limitantes de SRP poderiam explicar a alta abundância de **MBFG VI** no reservatório de Camorim, uma vez que as diatomáceas precisam de uma quantidade de SRSi para seu desenvolvimento e são consideradas bons competidores para fósforo (MARINHO; AZEVEDO, 2007; TILMAN et al. 1982).

Outros contribuintes importantes para a biomassa fitoplanctônica total foram **MBFGs IV** e **V**. O **MBFG IV** é composto por organismos de tamanho médio sem características especializadas, enquanto o **MBFG V** agrupa os flagelados unicelulares de médio a grande porte (KRUK et al., 2010). A predominância desses MBFGs ao longo dos meses da amostra pode ser explicada por vários fatores. Em primeiro lugar, **MBFGs IV** e **V** podem tolerar recursos moderados a limitados (KRUK et al., 2010) combinados com a capacidade de algumas espécies de executar estratégias de mixotrofia tornam esses organismos bons competidores para o ambiente com limitações de nutrientes baixas ou persistentes (BERGSTRÖM et al. , 2003; GRAHAM; WILCOX, 2000; JONES, 1997, 2000), uma vez que é considerada uma capacidade facultativa de suplementar nutrientes diferentes de carbono, como N ou P (RIEMANN et al., 1995; LI; STOECKER; COATS, 2000) tal como observado no reservatório de Camorim. Em segundo lugar, o tamanho desses organismos como presas pode dificultar o seu consumo, embora sejam considerados uma boa fonte alimentar para o zooplâncton (COLINA et al., 2016) devido ao alto teor de ácidos graxos (AHLGREN et al., 1990; BOERSMA, 2000) e ácidos poli-insaturados com alto teor de gordura (PUFAs) (BRETT et al., 2006). Por último, a capacidade de reduzir as taxas de afundamento devido ao tamanho moderado e relação superfície: volume e presença de flagelos (MBFG **V**) (KRUK et al., 2010), o que permite que esses organismos fiquem mais tempo suspensos na zona eufótica de a coluna de água.

MBFG I exibiu a menor biomassa durante o período. Este grupo funcional é composto por pequenos organismos com alta relação superfície-volume, rápida taxa de crescimento (KRUK et al., 2010) e adaptados à rápida aquisição de recursos (REYNOLDS, 1984). Tais características permitem que esses organismos sejam favorecidos em baixas concentrações de nutrientes devido a uma rápida troca de nutrientes pela superfície celular (LITCHMAN et al., 2007), bem como por terem menores taxas de afundamento e divisão mais rápida, o que é favorável em condições de mistura reduzidas. Em nosso sistema, as condições de limitação de nutrientes e estabilidade da coluna d'água seriam condições favoráveis para o aumento da biomassa deste MBFG. No entanto, a constante mistura da coluna d'água e uma estratificação não duradoura no reservatório na estação quente/chuvosa podem explicar sua baixa biomassa. Além disso, a predação pode ter sido outra razão para a baixa biomassa de MBFG I no reservatório de Camorim. Este MBFG é altamente ingerido, principalmente por rotíferos (COLINA et al., 2016).

A comunidade zooplanctônica do Reservatório do Camorim apresentou baixa biomassa de herbívoros e rotíferos sendo, portanto, dominada pelos copépodos ciclopóides. Tal dominância pode explicar por que da queda da biomassa fitoplanctônica em março/2018 não ter afetado negativamente a biomassa da comunidade zooplanctônica. Os copépodos ciclopóides são animais raptoriais altamente seletivos que predam presas heterotróficas móveis (por exemplo, ciliados) (KIØRBOE, 2011). Apesar da baixa biomassa de rotíferos, as espécies observadas tinham hábito alimentar semelhante aos copépodos ciclopóides - onívoro, em que os principais recursos alimentares são bactérias, flagelados e detritos orgânicos (ARNDT, 1993). Tomados em conjunto, os copépodos ciclopóides e rotíferos sugerem um forte consumo de presas heterotróficas, como bactérias e ciliados em vez de fitoplâncton e, combinado com a baixa biomassa de cladóceros, indica uma relação fraca entre zooplâncton e fitoplâncton no reservatório de Camorim.

Além disso, o consumo de fitoplâncton e, portanto, o potencial de controle de cima para baixo do zooplâncton depende das características funcionais de ambas as comunidades envolvidas (AMORIM et al., 2019; COLINA et al., 2016; KRZTOŃ; KOSIBA, 2020; KRZTOŃ et al., 2019; LÜRLING, 2020). A abordagem por CWM demonstrou a dominância de espécies zooplanctônicas de pequeno porte, onívora, raptorial e reprodução sexuada. O tamanho do corpo está inversamente relacionado à temperatura em ectotérmicos, incluindo zooplâncton (ANGILETTA; DUNHAM, 2003; GILOOLEY et al., 2002; HAVENS et al., 2015). Assim, em temperaturas mais altas, o tempo de geração encurta-se resultando em menor tamanho corporal do adulto (ANGILLETTA; DUNHAM, 2003). Além disso, o tamanho do corpo é positivamente proporcional ao espectro de tamanho da presa (BURNS, 1968), onde os rotíferos, devido ao pequeno tamanho do corpo, consomem fitoplâncton de pequeno porte, os cladóceros têm um espectro mais amplo de tamanhos de presas, enquanto os copépodos geralmente se alimentam de presas maiores (HANSEN, 1994; LAMPERT; SOMMER, 2007; REYNOLDS, 2006). Nesse contexto, o pequeno tamanho do corpo da comunidade zooplanctônica no reservatório de Camorim reduziu ainda mais o espectro de tamanho das presas, uma vez que as espécies fitoplanctônicas dominantes eram de médio a grande porte. Além do tamanho da presa, o consumo do fitoplâncton pelo zooplâncton também está ligado à quantidade e qualidade do recurso (COLINA et al., 2016). Assim, a predominância de características anti-pastejo de espécies de fitoplâncton (por exemplo, tamanho; motilidade; parede celular espessa) no reservatório de Camorim implica uma redução no pastejo pelo zooplâncton.

A dominância da reprodução sexuada foi associada à biomassa dos copépodos ciclopóides. Este tipo de reprodução resulta em um ciclo de vida mais longo devido às fases larval e juvenil até atingir a maturidade e ter um menor número de descendentes (REYNOLDS, 2006). O estágio de desenvolvimento dos copépodos também reflete uma diferença nos hábitos alimentares (BRUCET et al., 2008; REYNOLDS, 2006). Por exemplo, as larvas de copépodos, conhecidas como náuplios, consomem pequenas partículas como bactérias (FAITHFUL; GOETZE, 2017; TURNER; TESTER, 1992) e picoplâncton (BRUCET et al., 2008), enquanto os estágios juvenis (copepoditos) e adultos preferem presas a maiores (ex. micro e nanoplâncton) (BRUCET et al., 2008).

Assumindo que os estágios juvenil e adulto foram predominantes em relação aos náuplios no reservatório de Camorim, era esperado um forte consumo de fitoplâncton, mas as características onívoras e raptoriais podem ter impedido que isso acontecesse. Além disso, estudos já demonstraram que a alta abundância de diatomáceas pode ter um impacto negativo no ciclo de vida dos copépodos, uma vez que resultam em uma redução significativa na incubação de ovos (IANORA et al., 2004; MIRALTO et al., 1999) Assim, a dominância de juvenis e adultos pode estar relacionada à qualidade do alimento no reservatório de Camorim, principalmente devido à alta biomassa de **MBFG VI**. Logo, a biomassa das espécies planctônicas e suas respectivas características funcionais são fatores decisivos para a eficiência da transferência trófica entre o fitoplâncton e o zooplâncton.

Devido à dominância do **MBFG VI** e pelo aparecimento do **MBFG III** em condições ambientais específicas, experimentos laboratoriais utilizando uma espécie de cada MBFG foram realizados, a fim de verificar as possíveis adaptações fisiológicas que pudessem explicar a dominância e/ou ocorrência desses grupos no Reservatório do Camorim. Os experimentos foram realizados com diferentes combinações de luz e temperatura, considerando algumas razões, como i) luz e temperatura são variáveis ambientais que atuam diretamente no metabolismo celular (COLES; JONES, 2000; MESQUITA et al., 2019; NISHIKAWAA; YAMAGUCHI, 2006); ii) as espécies fitoplanctônicas experimentam flutuações de luz e temperatura diariamente, assim como sazonalmente (FONSECA; BICUDO, 2008; MARQUARDT et al., 2018) e iii) incremento de luz e temperatura podem ser resultado das mudanças climáticas (IPCC, 2014).

Dentre as espécies pertencentes ao **MBFG III**, *Raphidiopsis (Cylindrospermosis) raciborskii*, é uma cianobactéria potencialmente produtora de cianotoxinas e sua expansão ao redor do mundo tem motivado inúmeros estudos ecofisiológicos (ANTUNES, LEÃO, VASCONCELOS, 2015; BONILLA et al., 2012, 2016; BRIAND et al., 2004; DITTMAN; FEWER; NEILAN, 2013). Outro fato a ser considerado é que essa espécie tem aumentado sua ocorrência e frequência em reservatórios de abastecimento (PEREIRA, 2018; SOARES et al., 2012), causando preocupação aos gestores desses recursos. Embora o foco do nosso primeiro capítulo tenha sido em grupos funcionais, *R. raciborskii* foi a única representante do **MBFG III** e sua ocorrência já tinha sido observada anos atrás no Reservatório do Camorim (PEREIRA, 2018). Por outro lado, *Aulacoseira ambigua* foi uma das espécies de diatomáceas mais abundantes do **MBFG VI** e por isso foi selecionada para os experimentos laboratoriais. Apesar dessa espécie não produzir toxinas e nem estar relacionada com problemas de manejo e de saúde pública no Reservatório do Camorim, este gênero já foi responsável pela interrupção da distribuição de água devido à sua rápida proliferação em determinadas condições ambientais o que levou ao entupimento das turbinas (SILVA; MARTI; IMBERGER, 2014).

O primeiro experimento (capítulo 2) foi realizado com três cepas de *Raphidiopsis raciborskii* isoladas do Reservatório do Camorim com o objetivo de analisar o efeito combinado de luz e temperatura sobre o crescimento e na produção de saxitoxinas. Nós observamos que o crescimento das cepas de *R. raciborskii* foi limitado quando estava submetida à baixa temperatura e as maiores taxas de crescimento foram obtidas sob luz mais alta combinado com temperaturas iguais ou superiores a 20°C, dependendo da cepa. E, em geral, o crescimento foi maior a 30°C nas intensidades de luz mais baixas e igualmente alto a 25°C e 30°C sob luz mais alta. Estudos anteriores demonstraram que cepas subtropicais de *R. raciborskii* conseguem crescer mesmo em condições de baixa temperatura (BONILLA et al., 2012), enquanto cepas tropicais apresentam incremento do crescimento em condições de temperatura mais elevada, corroborando com nossos resultados (SOARES, LÜRLING; HUSZAR, 2013; BITTENCOURT-OLIVEIRA et al., 2012).

Embora *Raphidiopsis raciborskii* tenha sido descrita como espécie de sombra devido aos baixos valores de saturação de luz (Ik) durante a fotossíntese (PADISÁK; REYNOLDS, 1998), nossos resultados demonstraram que essa espécie apresentou taxas ótimas de crescimento em condições de alta intensidade luminosa. Estudos utilizando cepas de diferentes localidades (ex. Austrália, Alemanha e Hungria) também indicaram que *R. raciborskii* é capaz de tolerar e até mesmo incrementar seu crescimento sob alta luz (BRIAND et al., 2004; DOKULIL; MAYER, 1996; PIERANGELINI et al., 2014), demonstrando que essa espécie apresenta ampla plasticidade fenotípica em relação a luz. Nós observamos que a interação entre luz e temperatura afetou as taxas de crescimento, incrementando ao combinar alta luz e temperaturas ≥20°C. Outros estudos, também demonstraram que essa espécie incrementa seu crescimento em condições de alta intensidade luminosa e temperatura (BITTENCOURT-OLIVEIRA et al., 2012; BONILLA et al., 2016; XIAO et al., 2016). No entanto, esses estudos não haviam utilizado intensidades luminosas tão altas quanto a nossa (500 µmol fótons m^{-2} s^{-1}). A capacidade de crescer em diferentes condições de luz e temperatura já era considerada uma das possíveis razões para sua ampla distribuição geográfica (ANTUNES; LEÃO, VASCONCELOS, 2015). Mais recentemente, os resultados obtidos ao combinar diferentes condições de luz e temperatura sugerem que *R. raciborskii* possa ser beneficiada com as mudanças climáticas.

Devido à grande expansão de *Raphidiopsis raciborskii* somada à capacidade de produzir toxinas, inúmeros estudos ecofisiológicos começaram a ser realizados a fim de entender as principais variáveis ambientais que poderiam propiciar a produção de cianotoxinas em *R. raciborskii* (CARNEIRO et al., 2009; CASALI et al., 2017; CASTRO, 2004; RANGEL et al., 2016b). Dentre eles, estudos utilizando apenas uma variável, como por exemplo, luz (intensidade, tempo de exposição e qualidade de luz) e temperatura demonstraram ser capazes de alterar a produção de cianotoxinas em *R. raciborskii* (CASALI et al., 2017; CARNEIRO et al., 2009; CASTRO, 2004; RANGEL et al., 2016b). No entanto, até a nossa publicação, o efeito combinado de luz e temperatura sobre a produção de saxitoxinas ainda não tinha sido realizada. Essa espécie de cianobactéria é potencialmente produtora de cianotoxinas, sendo capaz de produzir diferentes tipos dependendo da região geográfica de origem, como por exemplo, cepas australianas podem produzir cilindrospermopsina (ANTUNES, LEÃO, VASCONCELOS, 2015; CARNEIRO et al., 2013) e cepas brasileiras podem produzem derivados de saxitoxinas (STX) (CARNEIRO et al., 2009; MOLICA et al., 2002;

LAGOS et al., 1999). Corroborando com a literatura, nós identificamos que as cepas de *R. raiborskii* produziram derivado de saxitoxinas, mais precisamente GTX 2 e 3, nas diferentes condições de luz e temperatura testadas. Nossos resultados também demonstraram que as maiores concentrações de saxitoxinas e quota celular de saxitoxinas ocorreram a 25°C sob altas intensidades de luz, mas foram muito menores a 30°C. Considerando que uma das consequências das mudanças climáticas é o aumento da temperatura (IPCC, 2014), a partir dos nossos resultados, concluímos que o aumento da temperatura combinado com luz suficiente leva a maior biomassa de *R. raciborskii*, mas as florações podem se tornar menos tóxicas nas regiões tropicais.

O segundo experimento (capítulo 3) foi realizado com cepas de *Aulacoseira ambigua,* também isoladas do Reservatório do Camorim, com objetivo de analisar o efeito combinado de luz e temperatura nas respostas ecofisiológicas. Inúmeros estudos tratam das respostas ecofisiológicas de diatomáceas (GOMES; AZEVEDO; LÜRLING, 2015; NISHIKAWAA; YAMAGUCHI, 2006; ZHANG et al., 2016), porém, pouquíssimos utilizam *A. ambigua*, mesmo sendo uma espécie cosmopolita e considerada um importante componente em sistemas com moderada produtividade (CAMBURN; KINGSTON 1986, FRITZ; KINGSTON; ENGSTROM, 1993). Portanto, o estudo ecofisiológico dessa espécie, apresentada no capítulo 3 dessa tese, preencheu uma lacuna na literatura.

Nossos resultados demonstraram que as cepas de *A. ambigua* alteraram suas respostas ecofisiológicas dependendo das combinações de luz e temperatura testadas. No entanto, esta espécie demonstrou ser sensível à alta intensidade luminosa (500 µmol fótons m^{-2} s^{-1}), uma vez que não apresentou crescimento e reduziu sua capacidade fotossintética. Por outro lado, tal intensidade luminosa propiciou o aumento do conteúdo de lipídios neutros, uma vez que a biossíntese e incremento do conteúdo de lipídios neutros, em organismos fitoplanctônicos, estão associados às condições ambientais desfavoráveis ou estressantes (HU et al., 2008). Além disso, nós observamos que o incremento do conteúdo de lipídios neutros em *A. ambigua* ocorreu quando as cepas estavam na fase estacionária ou de senescência, demonstrando que a fase de crescimento é outra variável que interferiu no conteúdo de lipídios neutros.

Embora o incremento do conteúdo de lipídio neutro esteja associado a condições desfavoráveis para *A. ambigua*, tal resultado pode ter um viés ecológico vantajoso, permitindo que essa espécie possa flutuar e consequentemente permanecer mais tempo suspensa na coluna d`água. As diatomáceas têm uma estrutura celular relativamente pesada devido à presença de sílica, o que propicia altas taxas de sedimentação (KRUK et al., 2010). Isso significa que, em um sistema natural, as diatomáceas dependem de fatores ambientais (por exemplo, mistura da coluna de d'água ou turbulência das correntes) (FONSECA; BICUDO, 2008; MARQUARDT et al., 2018) para permanecerem mais tempo suspensas na coluna d`água ou podem acumular lipídios (HILDEBRAND et al., 2012). Essa vantagem competitiva pode ser ainda mais trivial em sistemas que apresentam estratificação duradoura da coluna d'água ou em sistemas muito túrbidos, onde a disponibilidade de luz não atinge o fundo do corpo d'água. Portanto, o incremento de lipídios neutros parece ser um fator favorável para a sobrevivência de *A. ambigua,* onde pode experimentar diferentes condições ambientais das quais estava submetida.

As cepas de *A. ambigua* isoladas do Reservatório do Camorim exibiram o morfotipo reto, mas dependendo da combinação de luz e temperatura houve uma leve curvatura do tricoma. Embora não haja relato do efeito das condições ambientais atuando sobre o morfotipo do tricoma de *A. ambigua*, tal alteração já foi observada em campo para outra espécie de *Aulacoseira* - *A. granulata*, podendo ser resultado do i) impacto de uma força externa (por exemplo, turbulência), ii) uma adaptação ao fluxo contínuo de água em grandes rios, ou iii) pode ser um indicador de aumento da carga de nutrientes (WANG et al., 2017). Portanto, a leve curvatura observada no tricoma de *A. ambigua* foi considerada como uma resposta às condições nas quais *A. ambigua* estava submetida.

Além do morfotipo, outros parâmetros morfológicos foram observados, como por exemplo, tamanho do tricoma, volume celular e razão superfície-volume (S/V). O tamanho do filamento e a relação superfície/volume (S/V) são atributos importantes para a ecologia das espécies de fitoplâncton, incluindo diatomáceas, pois eles estão relacionados à capacidade de interceptar luz, assimilar nutrientes, além de ajudar a manter espécies mais suspensas na coluna d'água (KIRK, 1996; LEWIS, 1976; REYNOLDS, 1988). Embora as cepas de *A. ambigua* não tenham mostrado respostas semelhantes que pudessem refletir num padrão, nossos resultados demonstraram que *A. ambigua* apresentou grande variabilidade intraespecífica e ampla plasticidade fenotípica, sendo, portanto, capazes de alterar sua morfologia sob diferentes condições de luz e temperatura.

As espécies *Raphidiopsis raciborskii* e *Aulacoseira ambigua* apresentaram diferentes requisitos de luz e temperatura, corroborando com o que foi observado no

Reservatório do Camorim. *Aulacoseira ambigua* foi sensível à alta intensidade luminosa, mas tolera diferentes temperaturas, por isso pode ser observada em todos os meses no Reservatório do Camorim, especialmente em maior biomassa no fundo do reservatório na estação fria/seca. Por outro lado, *R. raciborskii* apresentou incremento do crescimento em condições de alta intensidade luminosa e temperatura. Essa espécie foi observada apenas na estação quente/chuvosa do reservatório. A partir das observações de campo e dos resultados experimentais, as duas espécies estudadas não compartilham o mesmo nicho ecológico e, consequentemente, não competem pelos mesmos recursos, o que justificam a coexistência delas no Reservatório do Camorim. Além disso, a grande variabilidade intraespecífica observada é um fator fundamental para a manutenção dessas duas espécies no ambiente, o que não apenas justifica suas ocorrências no Reservatório do Camorim, mas também em diferentes regiões ao redor do mundo.

CONCLUSÕES GERAIS

A abordagem da diversidade funcional (MBFG e CWM) provou ser eficiente para o estudo de comunidades planctônicas em um reservatório tropical raso. Os principais contribuintes para a biomassa do fitoplâncton foram **MBFG IV**, **V** e **VI**. A biomassa e a diversidade fitoplanctônica foram associadas a variáveis ambientais. No período quente/chuvoso, a estratificação térmica da coluna d'água levou ao surgimento do **MBFG III** e o período chuvoso foi decisivo para a redução da biomassa total em março/2018. Além disso, a disponibilidade de luz até o fundo do reservatório combinada com valores baixos e/ou limitantes de nutrientes dissolvidos foram determinantes para a alta biomassa de MBFG **V** devido à capacidade de usar estratégias de fotossíntese e mixotrofia para seu desenvolvimento. Em relação à comunidade zooplanctônica, esta foi dominada por copépodos ciclopóides. O traço funcional onívoro observado nos copépodos ciclopóides e rotíferos foi responsável pela manutenção da biomassa da comunidade zooplanctônica mesmo com a redução da biomassa fitoplanctônica devido à capacidade dos organismos de se alimentar de outros organismos, como ciliados e bactérias. As características funcionais exibidas no zooplâncton (tamanho corporal pequeno, onívoro, raptorial e reprodução sexuada) e no fitoplâncton (tamanho médio a grande, esqueleto silicioso e presença de flagelos) foram determinantes para a fraca relação entre essas comunidades, sugerindo uma ineficiente transferência energética. Assim, as condições ambientais foram os principais fatores direcionadores para a comunidade fitoplanctônica de um reservatório eutrófico tropical raso.

O crescimento de *Raphidiopsis raciborskii* (cianobactéria) foi limitado pela baixa temperatura e as maiores taxas de crescimento ocorreram sob alta intensidade de luz combinada com temperaturas iguais ou superiores a 20°C, dependendo da cepa. As maiores concentrações de saxitoxinas e quota celular de saxitoxinas ocorreram a 25°C sob altas intensidades de luz, mas foram muito menores a 30°C. O aumento da temperatura combinado com luz suficiente levará a maior biomassa de *R. raciborskii*, mas as florações podem se tornar menos tóxicas nas regiões tropicais.

Aulacoseira ambigua demonstrou ser sensível a alta intensidade luminosa, onde não apresentou taxa de crescimento e reduziu sua capacidade fotossintética, sendo considerada uma espécie de sombra. Por outro lado, alta intensidade luminosa favoreceu o incremento do conteúdo de lipídios neutros em *A. ambigua*, podendo ajudar sua flutuabilidade.

As duas espécies fitoplanctônicas estudadas – *Aulacoseira ambigua* e *Raphidiopsis raciborskii,* apresentaram diferentes requisitos de luz e temperatura, demonstrando que não compartilham nicho e consequentemente não competem pelos mesmos recursos o que justifica a coexistência delas no Reservatório do Camorim. Além disso, a grande variabilidade intraespecífica observada nos experimentos laboratoriais pode ser considerado um fator fundamental para a manutenção dessas duas espécies no ambiente, explicando suas ocorrências no Reservatório do Camorim e em diferentes regiões ao redor do mundo.

PERSPECTIVAS

Esta tese buscou responder um amplo número de perguntas, principalmente relacionadas à fisiologia de organismos fitoplanctônicos. No entanto, novas ideias e perguntas foram geradas ao longo de sua execução. Apesar de muitos estudos abordarem o efeito das variáveis ambientais na fisiologia dos organismos fitoplanctônicos, especialmente em cianobactérias, ainda existe uma lacuna a ser preenchida no que diz respeito ao efeito combinado de luz e temperatura em diatomáceas e algas verdes, bem como a influência da interação entre essas variáveis na competição interespecífica. Um dos principais objetivos futuros é acompanhar o efeito combinado de luz e temperatura na competição interespecífica de espécies fitoplanctônicas em quimiostatos. Além disso, ao considerar os efeitos das mudanças climáticas, uma série de novas condições ainda precisam ser testadas para se avaliar melhor a fisiologia de diferentes espécies do fitoplâncton, tais como variações nas condições de nutrientes e pH em condições extremas de luz e temperatura

Outro importante aspecto que deve ser aprofundado futuramente é a interação fito-zooplâncton. Estudos utilizando organismos tropicais da comunidade zooplantônica ainda são escassos. Portanto, experimentos que abordem as comunidades naturais ou que utilizem cepas tropicais são de extrema relevância. Tais experimentos podem indicar se os organismos zooplanctônicos tropicais serão capazes de controlar ou facilitar florações de cianobactérias ou demais organismos fitoplanctônicos. Além disso, a grande variabilidade de traços funcionais das espécies fitoplanctônicos (toxinas, produção de lipídio, morfotipo), como foi observado nesta tese, pode dificultar a interação entre essas comunidades. Logo, experimentos que abordem o comportamento do zooplâncton frente a diferentes traços funcionais do fitoplâncton podem esclarecer algumas relações que são observadas em campo, assim como trazer novas respostas à literatura.

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ANEX A – Phytoplankton community in camorim reservoir during may/2017 to march/2018.

Table 10 – List of species and their respective taxonomic groups observed in the Camorim Reservoir during May/2017 to March/2018.

Taxonomic group	**Species**
Bacillariophyceae	*Aulacoseira ambigua*
	Centrophycidae 1
	Centrophycidae 2
	Centrophycidae 3
	Pennatophycidae 1
	Nitzschia sp 1
	Synedra sp 1
	Synedra sp 2
Chlorophyceae	*Actinastrum* sp.
	Acutodesmus acuminatus
	Chlorella minutissima
	Chlorella sp.
	Closteriopsis sp.
	Coelastrum astroideum
	Coelastrum indicum
	Desmodesmus armatus var. bicaudatus
	Desmodesmus communis
	Desmodesmus protuberans
	Dictyosphaerium sp
	Eudorina sp.
	Golenkinia cf radiata
	Golenkiniopsis solitaria
	Kirchneriella sp
	Lagerheimia sp.
	Micractinium pussillum
	Monoraphidium arcuatum
	Monoraphidium circinale
	Monoraphidium contortum
	Monoraphidium griffithii
	Monoraphidium minutum
	Monoraphidium nanum
	Oocystis sp.
	Radiococus sp
	Tetraedrom sp.
	Chlorophyceae 1
	Chlorophyceae 2 (colonial)
Cryptophyceae	Cryptophyceae 1
	Cryptophyceae 2
	Cryptophyceae 3
	Cryptophyceae 4
	Cryptophyceae 5
	Cryptophyceae 6
	Cryptophyceae 7
	Cryptophyceae 8
	Cryptophyceae 9
	Cryptophyceae 10
	Cryptophyceae 11
Cyanobacteria	*Aphanocapsa elachista*
	Aphanotece minutissima
	Aphanocapsa sp.
	Chrooccocus minor
	Chroococcus minutus
	Raphidiopsis raciborskii
	Jaaginema metaphyticum
	Planktolyngbya subtilis
	Pseudoanabaena limnetica
	Cyanobacteria 1 (colonial)
	Cyanobacteria 2 (colonial)
	Cyanobacteria 3
Dinophyceae	*cf Gymnodinium* sp
	Peridinium cf cinctum
	Peridinium umbonatum
Euglenophyceae	*Trachelomonas cf intermedia var intermedia*
	Trachelomonas sp 1
	Trachelomonas sp 2
Zygnematophyceae	*Cosmarium sphagnicola*
	Cosmarium sp 1
	Cosmarium tumidum var. tumidum f. minus
	Cosmarium cf contractum var minutum
	Mougeotia sp.
	Staurastrum cf tetracerum
	Staurastrum sp 1

Table 11 – Morphological description and representative taxa of the six phytoplankton morphology-based functional groups (MBFG) found in Camorim Reservoir.

MBFG	Description	Taxa
MBFG **I**	Small organisms with high surface/volume	*Chrooccocus minor; C. minutus; Chlorella minutíssima;* Chlorophyceae 2 (colonial); *Dictyosphaerium* sp.; *Kirchneriella* sp; *Micractinium pussillum; M. contortum; M. minutum; Radiococus* sp.; *Tetraedrom* sp.
MBFG **III**	Large filaments with aerotopes	*Raphidiopsis raciborskii*
MBFG **IV**	Organisms of medium size lacking specialized traits	*Actinastrum* sp.; *Acutodesmus acuminatus; Chlorella* sp.*;* Chlorophyceae 1; *Closteriopsis* sp.; *Cosmarium* sp 1; *Cosmarium cf contractum var minutum; C. sphagnicola; C. tumidum var. tumidum f. minus, Desmodesmus armatus var. bicaudatus, D. communis; D. protuberans; Golenkinia cf radiata; Golenkiniopsis solitária; Jaaginema metaphyticum; Lagerheimia* sp.; *Monoraphidium arcuatum; M. circinale; M. griffithii; M. nanum; Mougeotia* sp.; *Planktolyngbya subtilis; Pseudoanabaena limnetica; Staurastrum cf tetracerum; Staurastrum* sp 1; *Staurastrum* sp 2; *Staurastrum* sp 3
MBFG **V**	Organisms of medium size lacking specialized traits	Cryptophyceae 1; Cryptophyceae 2; Cryptophyceae 3; Cryptophyceae 4; Cryptophyceae 5; Cryptophyceae 6; Cryptophyceae 7; Cryptophyceae 8; Cryptophyceae 9; Cryptophyceae 10; Cryptophyceae 11; *Eudorina* sp., *Gymnodinium* sp.; *Peridinium cf cinctum; Peridinium umbonatum; Trachelomonas cf intermedia var intermedia; Trachelomonas* sp 1; *Trachelomonas* sp 2
MBFG **VI**	Non-flagellated organisms with siliceous exoskeletons	*Aulacoseira ambigua;* Centrophycidae 1; Centrophycidae 2; Centrophycidae 3; *Nitzschia* sp 1; Pennatophycidae 1; *Synedra* sp 1; *Synedra* sp 2
MBFG **VII**	Large mucilaginous colonies	*Aphanocapsa elachista; Aphanotece minutíssima; Aphanocapsa* sp.*;* Cyanobacteria 1 (colonial); Cyanobacteria 2 (colonial); Cyanobacteria 3; *Oocystis* sp.

ANEX B – Zooplankton community in camorim reservoir during may/2017 to march/2018.

Table 12 – List of zooplankton taxa and representative groups found in the Camorim reservoir.

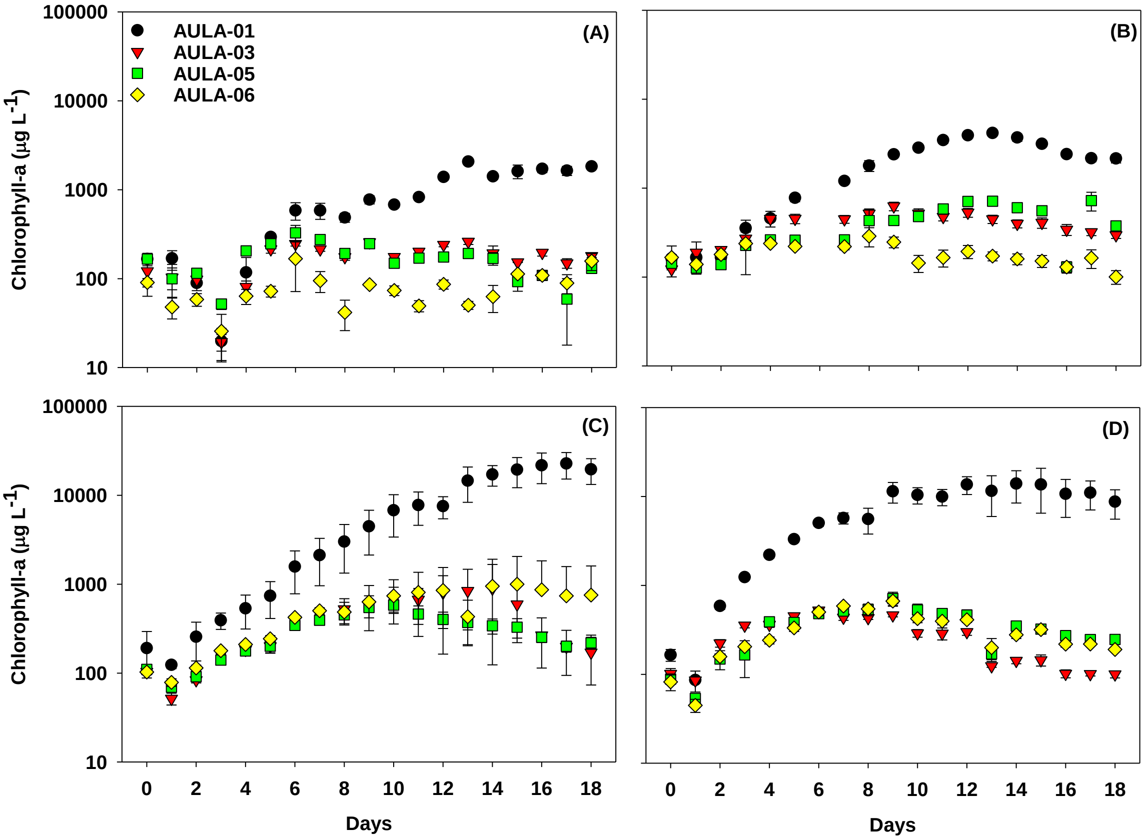
Group	**Taxa**
Rotifera	*Aplanchna* sp.; *Brachionus calyciflorus; Brachionus falcatus; Brachionus havanaensis; Dicranophorus* sp.; *Filinia novaezelandiae; Keratella americana; Trichocera* cf *mus*
Cladocera	*Diaphanosoma* sp.; *Macrothrix* sp.
Copepoda	*Thermocyclops* sp.

Table 13 – Functional traits of the zooplankton taxa found in Camorim Reservoir.

Functional traits		**Species**
Trophic group	Omnivorous	*Aplanchna* sp.; *Brachionus calyciflorus*; B. *falcatus; B. havanaensis*; *Diaphanosoma* sp.; *Filinia novaezelandiae; Keratella americana; Thermocyclops* sp.
	Herbivorous	*Dicranophorus sp.; Trichocera* cf *mus; Macrothrix* sp.
Feeding type	Microphagus filter feeders	*Brachionus calyciflorus; B. falcatus; B. havanaensis; Filinia novaezelandiae; Keratella americana*
	Raptorial	*Aplanchna* sp.; *Dicranophorus* sp.; T*richocera* cf *mus; Thermocyclops* sp.
	Stationnary filter feeders	*Diaphanosoma* sp.; *Macrothrix* sp.
Reproduction form	Assexual	*Aplanchna* sp.; *Brachionus calyciflorus; B. falcatus; B. havan*aensis; *Dicranophorus* sp.; *Filinia novaezelandiae; Keratella americana; Trichocera* cf *mus*; D*iaphanosoma* sp.; *Macrothrix* sp.
	Sexual	*Thermocyclops* sp.

ANEX C – Growth curves of the four strains of aulacoseira ambigua under different combinations of light and temperatures

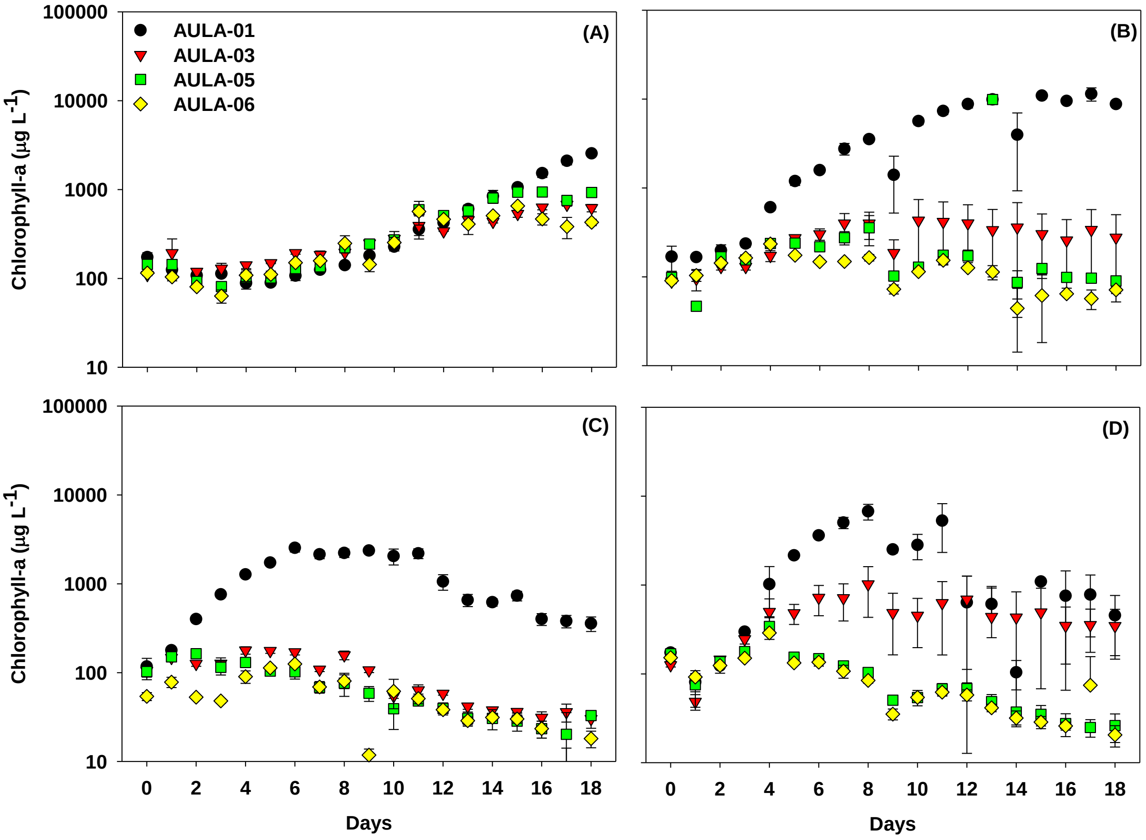
Figure 27 – Growth curve of four strains of *Aulacoseira ambigua* in 10 µmol photons m^{-2} s^{-1} at different temperatures.



Legend: Temperatures: 15°C (A), 20°C (B), 25°C (C) and 30°C (D). The y-axis scale is logarithmic. Vertical lines indicate standard deviation (n=3).

Source: The author, 2020.

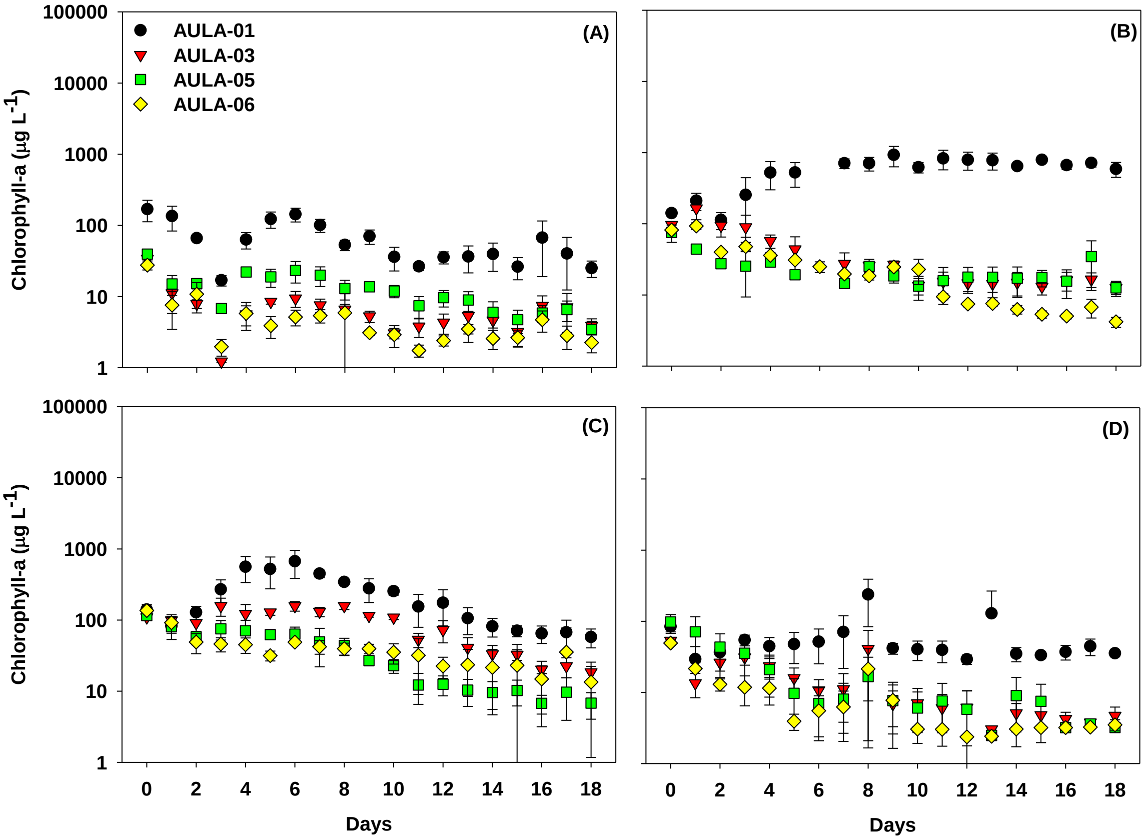
Figure 2815 – Growth curve of four strains of *Aulacoseira ambigua* in 100 µmol photons m^{-2} s^{-1} at different temperatures.



Legend: Temperatures: 15°C (A), 20°C (B), 25°C (C) and 30°C (D). The y-axis scale is logarithmic. Vertical lines indicate standard deviation (n=3).

Source: The author, 2020.

Figure 29 16 – Growth curve of four strains of *Aulacoseira ambigua* in 500 µmol photons m^{-2} s^{-1} at different temperatures.



Legend: Temperatures: 15°C (A), 20°C (B), 25°C (C) and 30°C (D). The y-axis scale is logarithmic. Vertical lines indicate standard deviation (n=3).

Source: The author, 2020.