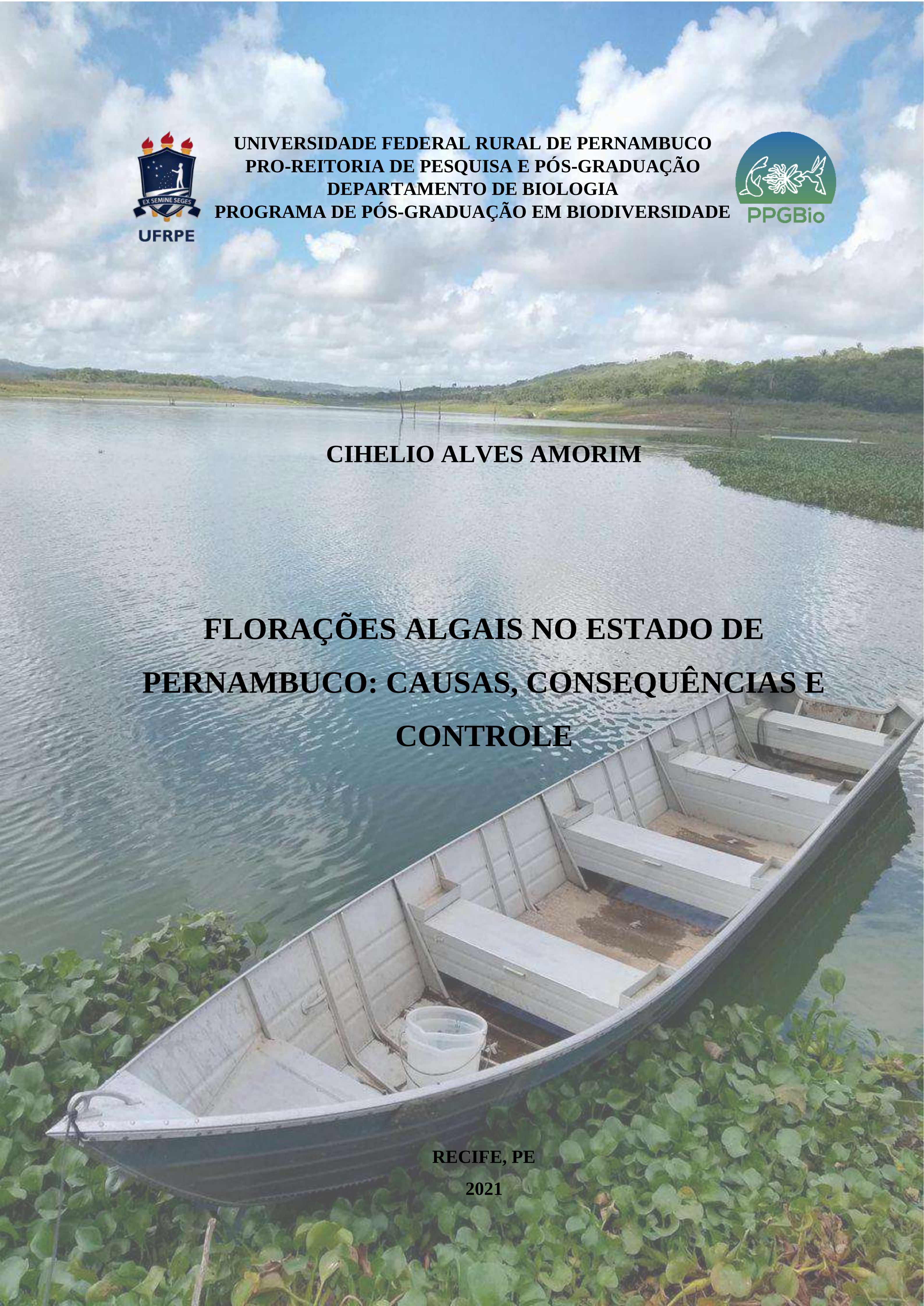

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DEPARTAMENTO DE BIOLOGIA
PROGRAMA DE PÓS-GRADUAÇÃO EM BIODIVERSIDADE

CIHELIO ALVES AMORIM

FLORAÇÕES ALGAIS NO ESTADO DE PERNAMBUCO: CAUSAS, CONSEQUÊNCIAS E CONTROLE

RECIFE, PE
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Tese apresentada ao Programa de Pós-Graduação em Biodiversidade da Universidade Federal Rural de Pernambuco – PPGBio/ UFRPE, como requisito para obtenção do título de Doutor em Biodiversidade.

Orientadora: Ariadne do Nascimento Moura
Coorientador: Ênio Wocyli Dantas

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Todo caminho apresenta obstáculos. O que fazer com eles? Vencer ou desistir? Sempre haverá estas opções.

Quando se vive em um ambiente sem oportunidades, ou até com condições precárias de subsistência, os obstáculos parecem dobrar ou triplicar de tamanho.

Nesse caso, a melhor opção seria desistir? Não! A melhor opção é lutar! Talvez recomeçar, mas jamais desistir.

Encarar os desafios representa resistir, adquirir resiliência, superar e vencer as adversidades.

Durante o percurso destes caminhos, desde muito cedo, são construídos inúmeros sonhos. Nem todos se realizam, ou demoram décadas para se concretizar.

Quem nunca pensou em ser astronauta quando criança? Seguir na carreira musical, talvez? Ou em descobrir um fóssil de dinossauro? As possibilidades são infinitas.

Ao longo da construção destes sonhos, muitas pessoas podem dificultar, dizer que não é possível, desestimular, ou até romper oportunidades.

Por outro lado, existem pessoas que apoiam, ajudam e abrem portas. Não é difícil lembrar de pessoas que já te incentivaram alguma vez.

Quando se vive em um cenário com poucas perspectivas, frases como: “você vai ser alguém na vida”, ditas pelos pais, ou professores do primário, marcam muito. Difícil de esquecer ao longo de toda a vida.

Escute estas pessoas! Acredite em você! Se apegue com seus sonhos! Nenhum deles é grande o suficiente a ponto de não ser alcançado.

Portanto, dedico a realização deste sonho, o de ser Doutor, a todas as pessoas que incentivaram e educaram. A todas as amizades que construí ao longo da vida.

Dedico aos meus pais, Jaceilde Alves Amorim e Raimundo Nonato Pereira Amorim, fonte de toda inspiração, amor e cuidado, exemplos de força, resistência e resiliência.

Dedico a todos os profissionais da educação que contribuíram na minha formação, desde os professores da alfabetização, ensino fundamental e médio, professores e orientadores da graduação e pós-graduação.

Dedico à ciência e cientistas do Brasil. Que nunca falte ânimo e incentivo para continuar pesquisando, ensinando e divulgando ciência. Tempos melhores virão!

"One day my father, he told me
Your wild heart will live for younger days
He said, one day you'll leave this world behind
So, live a life you will remember"
(The Nights)

"I can't tell where the journey will end
But I know where to start
They tell me I'm too young to understand
They say I'm caught up in a dream
So, wake me up when it's all over
When I'm wiser and I'm older"
(Wake Me Up)

"These are the days, we've been waiting for
On days like these, who could ask for more?
These are the days, we will never forget"
(The Days)

"Where there's a will, there's a way, kinda beautiful
And every night has its day, so magical
For every dreamer, a dream, we're unstoppable"
(Waiting for Love)

"This world can seem cold and grey
But you and I are here today
Nothing to fear but fear itself
We'll be okay just keep the faith"
(Fade into Darkness)

"We're going be birds and fly
We're gonna set the world alight
We're gonna lose ourselves tonight, oh
I think I just died, and went to heaven"
(Heaven)

"All the breath in your lungs
Is stronger than the tears in your eyes
It's do or die, we're alive
And while we're here, hold the line"
(Hold the Line)

"Cause it's not too late, and sometimes you lose it
Sometimes you're shooting broken arrows in the dark
But I, I see the hope in your heart"
(Broken Arrows)

"I'm a million miles smarter, but I ain't learn a thing
I've kept my word for whatever that's worth
Never been last but I've never been first
Oh, I may not be the best, but I'm far from the worst
Oh, I may not be perfect, but I'm loving this life
I'm a million miles ahead of where I'm from
But there's still another million miles to come"
(Trouble)

"My soul is trying to cross an ocean
Some nights I talk to myself
I said the words that I could say to no one else"
(Talk to Myself)

"What if I'm far from home?
Oh, if the sky comes falling down
There's nothing in this world I wouldn't do"
(Hey Brother)

"All of the troubled times that we have overcome
All of the trials to find somewhere that we belong
All of the tracks we traced, we raced to reach the sun
All of the lights to find a place where we belong"
(Fades Away)

"You must have saved me about a thousand times
I wouldn't be the one I am today
If you hadn't been a friend of mine"
(Friend of Mine)

"When you need a way to beat the pressure down
When you need to find a way to breathe
I could be the one to make you feel that way
I could be the one to set you free"
(I Could Be the One)

"I might hate myself tomorrow
But I'm on my way tonight"
(Lonely Together)

"Straight ahead on the path we have before us
Day by day, soon the change will come
Don't you know we took a big step forward?"
(Silhouettes)

"I've been some places, places I never should've been
I caught some changes, changes that made me who I am
When I get it, I ain't never going back again"
(Pure Grinding)

"But how you get there is the real test
Losing is only a sign that you really tried"
(What Would I Change It To)

"When you're feeling down in this place
Keep a vision on the one you've been chasing
We were living for the here and now"
(Can't Catch Me)

"I wasn't always the brightest
Life's what you make it, so here it comes, we go"
(True Believer)

"All my life I've been learning
I've always lived for today
And I don't know about tomorrow
But I can choose who I become"
(Faster than Light)

In loving memory of ***Tim Bergling – Avicii*** ◢◤
(1989 – 2018)
Your music is a source of inspiration and will be
forever in my soul.

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Eu não teria chegado até aqui sem o apoio e incentivo dos meus amados pais, Jaceilde Alves Amorim e Raimundo Nonato Pereira Amorim. Ambos vieram de famílias muito humildes, com muitas dificuldades. Tiveram que trabalhar desde criança para sustentar os oito ou nove irmãos e irmãs, sempre com muita garra e perseverança. Toda esta resiliência refletiu na criação dos filhos, educando pessoas com desejo de vencer na vida. Lembrarei eternamente de todas as vezes que estimularam os estudos. Além disso, meu pai também incentivou a pesquisa com cianobactérias, após uma intoxicação em um açude da região, desde então resolvi estudar as águas, mas só descobri as cianobactérias na graduação. Serei eternamente grato por tudo. Também agradeço imensamente aos meus irmãos José Francisco Alves Amorim e Cecília Alves Amorim, todo o convívio, brigas e abraços, foram importantes para o crescimento pessoal como ser humano. Toda minha família, amo vocês!

Novamente, mas desta vez não ficarei por último, agradeço imensamente a mim mesmo. Por favor, não entendam como egoísmo. Tese nenhuma seria construída sem todo o esforço, garra, perseverança, amor pelo que faz, dedicação, conhecimento, comprometimento, disciplina e paciência do ser humano que está por trás dela. Todo estudante de pós-graduação passa por momentos difíceis, brigas, relações mal resolvidas, noites sem dormir, incansáveis horas no laboratório, experimentos exaustivos, perrengues no campo, falta de financiamento e incentivo, o que muitas vezes desestimula, ou até desencadeia distúrbios psicológicos. Pior ainda para quem desenvolveu parte da tese durante uma pandemia global. Demonstrar ser forte, às vezes, é só uma máscara. Portanto, todo o esforço precisa ser valorizado!

O dicionário inteiro não teria palavras suficientes para demonstrar toda a minha gratidão à Profa. Ariadne Moura, minha orientadora e eterna mãe científica. Mesmo depois de dois “nãos” na seleção do mestrado, alguns atritos, que são normais na pós-graduação, nunca deixou de acreditar em mim. Serei eternamente grato por tudo, quando foi me buscar na rodoviária sem nem me conhecer, por me receber em sua casa, pelas caronas à universidade, todas as conversas, toda a orientação e por fornecer total suporte para a realização desta tese. Vir de outro estado é sempre muito difícil, e por muito tempo foi a única pessoa em que pude confiar, quem sempre se preocupou e cuidou de mim. Estou muito satisfeito com a relação científica e de amizade que construímos. O “menino metido” ou o “matuto do Crato” (no bom sentido) virou Doutor. Eu te amo Profa. Ariadne! PS. Agradecimento mais que especial à Ciara, muitas saudades.

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Em meio a uma análise sendo feita remotamente no LABFIC, quando um equipamento apresenta defeito, eis que conhecemos o Prof. Leucio Câmara. Mesmo em um laboratório de Doenças Parasitárias, sua colaboração foi essencial para a identificação e quantificação de cianotoxinas em amostras dos reservatórios. Além disso, juntamente com o Adriano, me ensinaram muito sobre biologia molecular e me deram suporte para analisar genótipos de cianobactérias potencialmente produtores de cianotoxinas. Agradeço também ao Prof. William Severi pela preciosa ajuda com a análise de nutrientes, sem sua colaboração nenhum trabalho poderia ser feito. Muitíssimo obrigado.

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Bom, nem só de trabalho vive o ser humano. Momentos alegres e de diversão são importantíssimos para a construção de uma boa tese. Ps. Queria colocar fotos.

Não tenho palavras para demonstrar a importância que outros dois "cearenses da cabeça redonda" tiveram no meu doutorado: Rayane Ribeiro e Diego Santos, vocês invadiram Recife e a UFRPE junto comigo. Rayane conheci logo de cara no início do Mestrado, acho que o alvoroço e a animação nos uniu. Parceria fortíssima! Te admiro muito amiga, sua energia, perseverança e dedicação me inspiram muito. Agradeço por todos os momentos felizes, todas as vezes que consolamos um ao outro, fofocamos, bebemos, comemos muito, fomos à praia, brincamos muitos carnavais e discutimos muita ciência. Sem isso, Recife seria um lugar triste. Eu te amo Rainha da Botânica, Diva das *Terminalia*. Quem era Diego antes do mestrado? Com certeza não a mesma pessoa de hoje. Obrigado por simplesmente ser quem é, exemplo de honestidade, bravura, dedicação e conhecimento. Com você dividi muitos momentos bons e felizes, conhecemos muitas pessoas especiais e aprendemos, juntos, a ser seres humanos melhores. Morar com você foi e continua sendo uma experiência incrível. Que jamais percamos toda a essência deste convívio, sempre com muito aprendizado, amor, diversão e compreensão. Te agradeço imensamente por todas as vezes que me ouviu, me compreendeu, me consolou e me acolheu. Eu te amo Chico. Também te desejo muito sucesso, Rei das Convolvulaceae.

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ARTIGO 1: Causas das Florações

Sap – *Sphaerospermopsis aphanizomenoides*, Saq – *Synechocystis aquatilis*, Sy1 – *Synechococcus* sp.1.

Fig. 5. Structural equation model evaluating the influence of physical (solar radiation, air temperature, and mixing zone depth), chemical (salinity and total dissolved phosphorus), and zooplankton (omnivorous and herbivorous crustaceans) variables on total cyanobacteria, colonies, heterocyted filaments, non-heterocyted filaments, and picocyanobacteria biomass in tropical reservoirs. Arrows between latent variables represent paths with a significant relationship ($p < 0.05$), which can be positive (green arrows) or negative (red arrows). The lines' thickness represents the intensity of the standard coefficients.

Fig. 6. Conceptual diagram showing the direct effects of physical (solar radiation, air temperature, and mixing zone depth), chemical (salinity and total dissolved phosphorus), and zooplankton (omnivorous and herbivorous crustaceans) variables on total cyanobacteria, colonies, heterocyted filaments, non-heterocyted filaments, and picocyanobacteria assessed through the structural equation model for tropical reservoirs. Green arrows represent positive effects and red arrows represent negative effects. As management strategies, we highlight the reduction of external sources of nutrients and salts, such as agriculture and domestic sewage, in addition to the removal of omnivorous, zooplanktivorous, and benthivorous fish, the introduction of non-invasive piscivorous fish and submerged macrophytes with allelopathic potential.

Fig. A.1. Geographic location of the ten tropical reservoirs studied distributed in the phytogeographic regions "Zona da Mata", "Agreste", and "Sertão" of Pernambuco, Brazil. See Table 1 for reservoir abbreviations.

Fig. A.2. Summary of all methodology steps applied to select the reservoirs, environmentally relevant variables, definition of the conceptual model, and the creation, calibration, and validation of the structural equation model. The a priori structural equation model (conceptual model) was used to assess the effects of physical (solar radiation, air temperature, and mixing zone depth), chemical (salinity and total dissolved phosphorus - TDP), and zooplankton (omnivorous and herbivorous crustaceans) variables on total cyanobacteria, colonies, heterocyted filaments, non-heterocyted filaments, and picocyanobacteria in tropical reservoirs.

Fig. A.3. Seasonal variation in the biomass of the cyanobacterial morphotypes (a) colonies, (b) heterocyted filaments, (c) non-heterocyted filaments, and (d) picocyanobacteria in the tropical reservoirs studied. See Table 1 for reservoir abbreviations.

ARTIGO 2: Grupos Funcionais

Fig. 1 Total biomass of the phytoplankton assemblages and relative biomass of the Reynolds' functional groups (RFGs) with relative biomass >5% in the tropical reservoirs studied (n = 42). Diamonds are the mean biomass in each reservoir and error bars represent the standard error. Reservoirs: Tapacurá (TAP), Carpina (CAR), Goitá (GOI), Cursaí (CUR), Cajueiro (CAJ), Mundaú (MUN), Tabocas (TAB), Ipojuca (IPO), Cachoeira (CAC), and Serrinha (SER)

Fig. 2 Polar area charts showing the minimum and maximum values (limits of the red bars) of the 10 environmental axes where each Reynolds' functional group (RFG) can grow intensely and become abundant (relative biomass >5%) to represent the assembly rules (thresholds) of their occurrence as abundant in the tropical reservoirs studied (n = 42). Groups marked with an asterisk (*) represent those that did not reach relative biomass >5%, or only in one sample, so, the limits represent the thresholds of their occurrence. Variables: mixing zone depth, irradiance, water temperature, pH, the relative biomass of herbivorous zooplankton, soluble reactive phosphorus (SRP), dissolved inorganic nitrogen (DIN), trophic state index (TSI), water transparency, and salinity

Fig. 3 Non-metric multidimensional scaling (NMDS) ordination showing the clustering reservoirs based on environmental gradients (a) and the distribution of Reynolds' functional groups (RFG) and their relationships with the environmental axes in the tropical reservoirs studied (n = 42). Panels (c) and (d) show the distribution of each RFG along the gradients of resources (trophic state index) and energy (water transparency), respectively. Variables: mixing zone depth, irradiance, water temperature, pH, the relative biomass of herbivorous zooplankton, soluble reactive phosphorus (SRP), dissolved inorganic nitrogen (DIN), trophic state index (TSI), water transparency, and salinity. Red area clusters the Carpina (CAR) reservoir; yellow area represents Ipojuca (IPO) reservoir; purple area indicates Tapacurá (TAP), Mundaú (MUN), Serrinha (SER), and Cajueiro (CAJ) reservoirs; green area groups Goitá (GOI) and Cachoeira (CAC) reservoirs; and the blue area represents Cursaí (CUR) and Tabocas (TAB) reservoirs

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RESUMO

A presente tese visa compreender os fatores regulatórios das florações algais em reservatórios tropicais, considerando as características morfofuncionais das espécies, além de definir as causas, consequências sobre o fitoplâncton e zooplâncton e propor estratégias de controle destas florações. O estudo foi realizado em 10 reservatórios de abastecimento público com diferentes condições climáticas e de eutrofização no estado de Pernambuco, Brasil. Foi realizado um monitoramento trimestral, durante um ano em cada ambiente ($n = 42$), para entender o papel das variáveis limnológicas, climáticas e bióticas sobre a intensidade das florações e os efeitos delas nas comunidades planctônicas. Todos os ambientes apresentaram florações de cianobactérias classificadas no nível 1 (biomassa >0,2 e <10 mg L^{-1}) ou nível 2 (biomassa >10 mg L^{-1}) de alerta da Organização Mundial de Saúde. A partir daí, os dados foram distribuídos em cinco artigos. O primeiro artigo teve o objetivo de avaliar as influências das variáveis físico-químicas e interações tróficas com o zooplâncton sobre as florações de cianobactérias. A análise de correspondência canônica parcial revelou que as florações foram direcionadas principalmente pelas variáveis químicas, seguida das físicas e zooplâncton. Para atingir o objetivo, foi construído um modelo de equações estruturais, o qual demonstrou que a biomassa de cianobactérias foi favorecida principalmente pelo fósforo total dissolvido e presença de copépodos Cyclopoida. Por outro lado, a radiação solar, temperatura do ar, zona de mistura, salinidade, copépodos Calanoida e cladóceros foram importantes para explicar a biomassa dos morfotipos de cianobactérias. No segundo artigo, objetivou-se compreender as dinâmicas dos grupos funcionais do fitoplâncton de Reynolds para estabelecer as relações entre os fatores direcionadores da sua biomassa e distribuição. Foram criados os modelos de habitat e regras de assembleia para 20 grupos funcionais, integrando dados das preferências e tolerâncias dos grupos a 10 gradientes ambientais. Modelos generalizados aditivos revelaram que a salinidade, estado trófico, fósforo solúvel reativo, transparência da água, biomassa relativa do zooplâncton herbívoro e nitrogênio inorgânico dissolvido foram os principais preditores da biomassa dos grupos funcionais. O objetivo do terceiro artigo foi entender como as florações algais intensificam a deterioração da qualidade da água, alteram a diversidade e estrutura do fitoplâncton e zooplâncton e afetam o funcionamento do ecossistema. Os dados foram comparados com ambientes sem florações. Nesse estudo, florações de cianobactérias e mistas (cianobactérias, dinoflagelados, clorófitas e diatomáceas) estiveram associadas com uma intensa deterioração da qualidade da água, redução da riqueza e eficiência do uso do recurso do zooplâncton, enquanto estas florações aumentaram a riqueza de espécies e eficiência do uso do recurso fitoplâncton. Por outro lado, florações de dinoflagelados e clorófitas apresentaram menores impactos aos ambientes. Os outros dois artigos visaram analisar o potencial controle das florações de cianobactérias através da biomanipulação das macrófitas submersas e zooplâncton de grande tamanho (cladóceros), além de verificar os impactos dessas adições na estrutura do fitoplâncton e zooplâncton. Após 10 dias de experimento, todos os tratamentos com adição de macrófitas apresentaram uma redução de até 85% da biomassa total de cianobactérias e até 99% das filamentosas, mesmo com o incremento dos nutrientes. Além disso, as macrófitas reduziram a biomassa das cianobactérias e algas verdes, enquanto o zooplâncton consumiu as diatomáceas e fitoflagelados. Nesse sentido, as macrófitas constituíram o principal fator direcionador da diversidade, composição e estrutura do fitoplâncton e zooplâncton nativo. Portanto, a introdução de macrófitas submersas com potencial alelopático mostrou ser uma técnica eficiente para controlar florações de cianobactérias tropicais. De maneira geral, a presente tese contribui para o entendimento do papel das variáveis climáticas, eutrofização, salinidade e interações tróficas sobre a dominância de diversos grupos fitoplanctônicos, além de verificar como macrófitas submersas e grandes grupos zooplanctônicos podem controlar as florações. Os resultados desta tese destacam o potencial risco de incremento das florações algais nocivas em reservatórios tropicais, especialmente no semiárido brasileiro, considerando as previsões de aumento da temperatura, secas prolongadas, eutrofização e salinidade nos corpos hídricos, com sérios impactos na biodiversidade e funcionamento dos ecossistemas. Portanto, medidas de mitigação precisam ser adotadas para melhorar a eficiência e conservação dos recursos hídricos em reservatórios de abastecimento público com florações, garantindo uma maior oferta de água potável.

Palavras-chave: funcionamento do ecossistema, manejo da água, modelagem, reservatórios tropicais, semiárido brasileiro.

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ABSTRACT

This thesis aims to understand the regulatory factors of algal blooms in tropical reservoirs, considering the morphofunctional characteristics of the species, in addition to defining the causes and consequences on phytoplankton and zooplankton and proposing strategies to control these blooms. This study was performed in 10 public water supply reservoirs with different climatic and eutrophication conditions in Pernambuco state, Brazil. Samples were collected quarterly for one year in each environment ($n = 42$) to understand the role of limnological, climatic, and biotic variables on the intensity of blooms and their effects on planktonic communities. All reservoirs showed cyanobacterial blooms classified as level 1 (biomass >0.2 and <10 mg L^{-1}) or level 2 (biomass >10 mg L^{-1}) of World Health Organization alert. With that, data were distributed in five articles. The first article aimed to evaluate the influence of physicochemical variables and trophic interactions with zooplankton on cyanobacterial blooms. The partial canonical correspondence analysis revealed that blooms were driven mainly by chemical variables, followed by physical and zooplankton variables. To achieve the objective, a structural equation model was built, which demonstrated that the cyanobacterial biomass was mainly favored by the total dissolved phosphorus and the presence of cyclopoid copepods. On the other hand, solar radiation, air temperature, mixing zone, salinity, calanoid copepods, and cladocerans explained the biomass of cyanobacterial morphotypes. In the second article, the objective was to understand the dynamics of the functional groups of Reynolds phytoplankton to establish the relationships between the driving factors of its biomass and distribution. Habitat templates and assembly rules for 20 functional groups were created, integrating data on group preferences and tolerances to 10 environmental gradients. Additive generalized models revealed that salinity, trophic state, reactive soluble phosphorus, water transparency, relative biomass of herbivorous zooplankton, and dissolved inorganic nitrogen were the main predictors of functional group biomass. The third article aimed to understand how algal blooms intensify the deterioration of water quality, alter the diversity and structure of phytoplankton and zooplankton, and affect ecosystem functioning. To that, data were compared to environments without blooms. In this study, cyanobacterial and mixed blooms (cyanobacteria, dinoflagellates, chlorophytes, and diatoms) were associated with an intense deterioration of water quality, reduced richness, and zooplankton resource use efficiency, while these blooms increased phytoplankton species richness and resource use efficiency. On the other hand, blooms of dinoflagellates and chlorophytes had less impact on the environment. The other two articles aimed to analyze the potential control of cyanobacterial blooms through the biomanipulation of submerged macrophytes and large zooplankton (cladocerans), in addition to verifying the impacts of these additions on phytoplankton and zooplankton structures. After 10 days of the experiment, all treatments with macrophyte additions reduced up to 85% of the total biomass of cyanobacteria and up to 99% of filamentous cyanobacteria, even with nutrient additions. Furthermore, macrophytes reduced the biomass of cyanobacteria and green algae, while zooplankton consumed diatoms and phytoflagellates. In this sense, macrophytes were the main driver of the diversity, composition, and structure of native phytoplankton and zooplankton. Therefore, the introduction of submerged macrophytes with allelopathic potential proved to be an efficient technique to control tropical cyanobacterial blooms. In general, this thesis contributes to the understanding of the role of climatic variables, eutrophication, salinity, and trophic interactions on the dominance of several phytoplankton groups, in addition to verifying how submerged macrophytes and large zooplankton can control blooms. The results of this thesis highlight the potential risk of increasing harmful algal blooms in tropical reservoirs, especially in the Brazilian semiarid region, considering the forecasts of increased temperature, prolonged droughts, eutrophication, and salinity in water bodies, with serious impacts on biodiversity and ecosystem functioning. Therefore, mitigation measures need to be adopted to improve the efficiency and conservation of water resources in drinking water reservoirs with blooms, ensuring the supply of potable water.

Keywords: Brazilian semiarid, ecosystem functioning, modeling, tropical reservoirs, water management.

1 INTRODUÇÃO

Lagos e reservatórios são as principais fontes de serviços ecossistêmicos da Terra (HILT et al., 2017), apesar de serem os ecossistemas mais ameaçados (WWF, 2020). Eles estão expostos a uma variedade de estressores químicos, climáticos, biológicos e antrópicos, o que os torna sentinelas de mudanças locais e globais (ADRIAN et al., 2009; JENNY et al., 2020). Estes impactos estão ocasionando um declínio na biodiversidade dos ambientes de água doce (84% de redução das populações de seres vivos desde 1970) mais acentuado que nos ambientes terrestres e marinhos (WWF, 2020). Dentre as principais ameaças emergentes à biodiversidade de água doce, as florações de algas nocivas, em especial de cianobactérias, foram listadas como uma das mais problemáticas, podendo levar a perda de biodiversidade, além da redução do crescimento, sobrevivência e reprodução das espécies (REID et al., 2019).

Os primeiros estudos de modelagem fitoplanctônica mostravam que o incremento na concentração de nutrientes era o principal fator controlador da dominância de cianobactérias (PAERL et al., 2001; SCHINDLER et al., 2001). No entanto, pesquisas recentes sugerem que a elevação da temperatura também desempenha um importante papel na promoção de florações de cianobactérias, com efeitos sinérgicos com os nutrientes (PAERL; HUISMAN, 2008; CONLEY et al., 2009; KOSTEN et al., 2012; O'NEIL et al., 2012; RIGOSI et al., 2014, 2015; MARIANI et al., 2015; HO et al., 2019; GRIFFITH; GOBLER, 2020).

As florações de cianobactérias em regiões tropicais são perenes devido às características climáticas destas regiões, as quais favorecem a dominância destes organismos ao longo de todo o ano, tornando-as mais preocupantes (BOUVY et al., 2000; BITTENCOURT-OLIVEIRA et al., 2014). Por outro lado, as florações em regiões temperadas estão restritas a poucos períodos do ano, especialmente, no final da primavera e no verão (GER; HANSSON; LÜRLING, 2014). No entanto, com a elevação global da temperatura, muitas espécies estão ampliando sua distribuição para áreas de maiores latitudes, se tornando mundialmente distribuídas, como *Raphidiopsis* (*Cylindrospermopsis*) *raciborskii* (Woloszynska) Aguilera et al. (SINHA et al., 2012) e *Microcystis aeruginosa* (Kützing) Kützing (HARKE et al., 2016).

Para regiões subtropicais e temperadas, os efeitos da temperatura e nutrientes sobre a dominância de cianobactérias, variam conforme o estado trófico dos ambientes e a espécie formadora de florações (RIGOSI et al., 2014). Entretanto, existe uma lacuna sobre os nutrientes ou a temperatura controlarem isoladamente a dominância de cianobactérias em reservatórios tropicais, ou se existe uma interação destes fatores. Nesse sentido, algumas pesquisas foram desenvolvidas na região Nordeste para monitorar os efeitos de eventos climáticos (secas extremas e eventos de *El Niño*) ou eutrofização sobre a dominância de cianobactérias (BOUVY et al., 2000; LIRA et al., 2014; CÂMARA et al., 2015; BRASIL et al., 2016). Além do mais, é importante conhecer se estes processos também influenciam na substituição de espécies formadoras de florações de diferentes morfotipos, relacionando-se com características morfofuncionais das espécies, como tamanho celular, formação de colônias ou filamentos, presença de aerótopos, produção de toxinas e fixação de nitrogênio.

A resposta dos traços funcionais pode ser representada pelas classificações de grupos funcionais (p.e., REYNOLDS et al., 2002), as quais reduzem o número de espécies para um número menor de grupos sem perder informações ecológicas importantes (ABONYI et al., 2018), o que pode melhorar o entendimento dos efeitos dos filtros ambientais sobre a dinâmica do fitoplâncton (KRUK; SEGURA, 2012). Dentre as classificações, os grupos funcionais de Reynolds, definido com base nas sensibilidades e tolerâncias ambientais das espécies, pode ser útil em programas de monitoramento da qualidade da água, através da definição dos grupos nocivos (p.e., cianobactérias), seus efeitos negativos e respostas às condições ambientais (KRUK; DEVERCELLI; HUSZAR, 2021). Além disso, os traços morfológicos e ecofisiológicos das cianobactérias podem influenciar na tomada de decisões sobre o manejo dos lagos para controle das florações (VISSER et al., 1996; IBELINGS et al., 2016).

No Brasil, a região semiárida tropical apresenta uma maior intensidade nas florações de cianobactérias, por possuir condições favoráveis ao desenvolvimento destes organismos, como elevadas temperaturas e concentrações de nutrientes (BOUVY et al., 2000; BITTENCOURT-OLIVEIRA et al., 2014; BRASIL et al., 2016; MOURA et al., 2018). Esta região, especialmente o estado de Pernambuco, é conhecida pelo registro de um dos mais repercutidos casos de intoxicação humana por cianotoxinas no mundo, o qual ocorreu em 1996 em uma clínica de hemodiálise em Caruaru. Neste incidente, 76 pacientes renais morreram após a intoxicação com água contaminada com microcistinas e cilindrospermopsina, proveniente do reservatório de Tabocas (CARMICHAEL et al., 2001; AZEVEDO et al., 2002). A partir deste incidente, maior atenção tem sido dada aos reservatórios de abastecimento público, por serem mais vulneráveis à colonização pelas cianobactérias e oferecer riscos à saúde humana.

Além dos impactos socioeconômicos e nas populações humanas (OLOKOTUM et al., 2020), florações de cianobactérias também causam sérios impactos na biodiversidade (PAERL; OTTEN, 2013; AMORIM; ULISSES; MOURA, 2017) e ambientes aquáticos como um todo, reduzindo a provisão de serviços ecossistêmicos (SUKENIK; QUESADA; SALMASO, 2015). Dentre os principais impactos na qualidade da água, as florações podem aumentar a turbidez e pH (VISSER et al., 2016), produzir compostos causadores de mal gosto e odor, o que reduz o valor recreacional e potabilidade (JÜTTTNER; WATSON, 2007), causar anoxia e consequente morte de animais aquáticos (RABALAIAS et al., 2010), além de favorecer a liberação de nutrientes e gases do efeito estufa durante a decomposição da biomassa algal (YAN et al., 2017; BIŽIĆ et al., 2020).

Intensas florações de cianobactérias levam a um aumento na eficiência do recurso do fitoplâncton, porém, reduz a transferência de energia dos produtores primários para o zooplâncton (FILSTRUP et al., 2014; TIAN et al., 2017). Durante as florações de cianobactérias, podem ocorrer profundas modificações ecossistêmicas, alterando as interações tróficas entre as comunidades aquáticas. Nesse sentido, o zooplâncton é a comunidade diretamente afetada, pois, as defesas das cianobactérias, incluindo a produção de cianotoxinas, baixa qualidade nutricional e a formação de grandes colônias e filamentos reduzem o consumo pelo zooplâncton (MOUSTAKA-GOUNI; SOMMER, 2020). Portanto, a estrutura e dinâmica das teias tróficas são essenciais para o entendimento do funcionamento do ecossistema, uma vez que, as interações entre os predadores e as presas são mais importantes que as interações dentro do mesmo nível trófico (SCHAFFNER et al., 2019).

Duas outras teorias ecológicas, a de interações de cascata trófica (CARPENTER; KITCHELL; HODGSON, 1985) e a de estados alternativos dos lagos rasos (SCHEFFER et al., 1993), têm auxiliado no entendimento das interações ecológicas entre o fitoplâncton e os demais organismos. A teoria de cascata trófica prediz que a manipulação de diferentes níveis da cadeia alimentar tem efeitos diretos sobre as comunidades dos níveis inferiores, controlando, assim, a biomassa fitoplanctônica (CARPENTER; KITCHELL; HODGSON, 1985). Existem quatro níveis tróficos principais nos ecossistemas aquáticos, e a manipulação dos níveis superiores causa um efeito em cascata sobre os níveis inferiores. Nesse sentido, o aumento dos peixes piscívoros irá causar uma redução dos planctívoros filtradores, que consequentemente, provoca um incremento da biomassa zooplanctônica, aumentando sua capacidade de predação sobre algas e cianobactérias (BRETT; GOLDMAN, 1996).

A teoria de estados alternativos prediz que os ambientes aquáticos rasos apresentam dois estados que alternam entre si: um claro, dominado por macrófitas submersas; e outro túrbido, com elevada biomassa fitoplanctônica (SCHEFFER et al., 1993). Nesse sentido, as macrófitas são capazes de manter o estado claro através da liberação de aleloquímicos ou o consumo de nutrientes, os quais são eficientes na redução do fitoplâncton, seus principais competidores por luz e nutrientes (HILT; GROSS, 2008).

Como solução para o controle de florações algais e restauração dos ecossistemas aquáticos, a biomanipulação tem despertado cada vez mais o interesse dos pesquisadores

(TRIEST et al., 2016). A biomanipulação consiste na alteração de importantes componentes do ecossistema aquático para garantir a redução da eutrofização e, consequentemente, da biomassa fitoplanctônica (SHAPIRO et al., 1975). A eficiência da biomanipulação no controle de cianobactérias, seja diretamente, com o aumento da capacidade de filtração do zooplâncton, ou indiretamente, através de cascata trófica, dinâmica de nutrientes ou atuação das macrófitas, tem chamado a atenção dos gerenciadores de recursos hídricos (TRIEST et al., 2016).

Com base no exposto, a presente tese visa compreender os fatores regulatórios das florações algais em ambientes aquáticos tropicais, levando em consideração as características morfofuncionais das espécies dominantes. Com isso, foram definidas as causas e consequências das florações sobre o fitoplâncton e zooplâncton, além de propor estratégias de controle das florações tóxicas, através de amostragens e experimentos em reservatórios de abastecimento público com diferentes condições tróficas e climáticas no estado de Pernambuco.

1.1 Estrutura da tese

A presente tese está dividida em cinco artigos. No primeiro artigo (AMORIM; DANTAS; MOURA, 2020), foram estudadas as influências das variáveis físico-químicas e interações tróficas com o zooplâncton sobre as florações de cianobactérias em reservatórios tropicais. Duas hipóteses principais foram testadas: (1) os nutrientes e a temperatura são os principais fatores que favorecem o incremento da biomassa de cianobactérias em ecossistemas aquáticos tropicais; (2) a correlação entre a biomassa de cianobactérias e variáveis físico-químicas é intensificada pelo zooplâncton.

No segundo artigo, foram avaliadas as dinâmicas dos grupos funcionais do fitoplâncton de Reynolds para estabelecer as relações entre os fatores ambientais direcionadores da sua biomassa e distribuição. Neste trabalho, foram criados os modelos de habitat e regras de assembleias capazes de explicar e predizer a ocorrência dos grupos funcionais de Reynolds em reservatórios tropicais. Foram avaliadas as médias de 10 eixos ambientais ponderadas pela biomassa de cada grupo funcional (modelo de habitat e diferenciação de nicho), valores mínimos e máximos das variáveis que limitam a dominância dos grupos (regras de assembleia), análises de correlação para verificar a coexistência dos grupos e modelos generalizados aditivos para prever a biomassa dos grupos nos gradientes de 10 variáveis ambientais.

O terceiro artigo (AMORIM; MOURA, 2021) mostra como as florações algais, compostas por diferentes grupos fitoplanctônicos (cianobactérias, dinoflagelados, clorófitas e diatomáceas) intensificam a deterioração da qualidade da água, alteram a diversidade e estrutura do fitoplâncton e zooplâncton e afetam o funcionamento do ecossistema e a provisão de serviços ecossistêmicos. Para isso, buscou-se testar três hipóteses: (1) florações de cianobactérias apresentam uma maior deterioração da qualidade da água quando compradas com as demais categorias de florações algais, levando a uma reduzida transparência da água, elevado pH e condições hipereutróficas; (2) florações de cianobactérias e dinoflagelados impactam negativamente a riqueza de espécies, diversidade e equitabilidade do fitoplâncton e zooplâncton; (3) florações algais intensas, independente do grupo dominante, resultam em uma maior eficiência do uso do recurso pelo fitoplâncton e reduz esta eficiência para o zooplâncton.

No quarto artigo (AMORIM; MOURA, 2020), foi realizado um experimento de biomanipulação no qual a presença de macrófitas submersas, zooplâncton herbívoro de grande tamanho e o incremento dos nutrientes (duas vezes) foram manipulados (oito tratamentos e 32 unidades experimentais). Neste estudo, foi avaliado o potencial controle das florações de cianobactérias totais e de três morfotipos através das macrófitas submersas e zooplâncton de grande tamanho. Três hipóteses principais foram testadas: (1) a adição isolada de macrófitas submersas e zooplâncton herbívoro de grande tamanho é menos eficiente no controle das florações de cianobactérias que a adição combinada em um reservatório raso tropical; (2) a alelopatia, ao invés da competição por nutrientes, pode ser o principal modo de ação das macrófitas submersas no controle das florações de cianobactérias; (3) macrófitas submersas, mesmo em um cenário de incremento de nutrientes, são mais eficientes no controle de florações de cianobactérias de um reservatório raso tropical que a adição de zooplâncton.

No quinto artigo foram estudados os efeitos isolados e combinados da introdução de uma macrófita submersa e um grande cladócero sobre a diversidade, composição e estrutura do fitoplâncton e zooplâncton em um reservatório tropical raso, em dois cenários de eutrofização. Foram testadas três hipóteses: (1) as macrófitas submersas são mais eficientes no controle da biomassa de cianobactérias, enquanto grandes cladóceros podem reduzir eficientemente a biomassa de algas eucariontes; (2) o aumento da eutrofização reduz a eficiência dos controles *top-down* e *bottom-up* dos grandes cladóceros e macrófitas submersas sobre o fitoplâncton; e (3) a heterogeneidade ambiental, criada pela macrófita submersa, direciona a diversidade, composição e estrutura das comunidades nativas do fitoplâncton e zooplâncton.

Ao final da tese, são apresentadas as considerações finais, englobando todos os artigos da tese e com recomendações para estudos futuros, com foco especial ao semiárido brasileiro. Em seguida, são descritas outras contribuições adicionais relevantes ao tema da tese, incluindo ações de divulgação científica para disseminação do conhecimento sobre florações algais e outros quatro artigos publicados sobre o tema principal da tese.

2 REVISÃO BIBLIOGRÁFICA

2.1 Clima e hidrologia do semiárido brasileiro

A região Nordeste do Brasil é caracterizada por apresentar precipitação em geral inferior a 800 mm, elevada evaporação e risco de seca maior que 60%, abrangendo 1.133 municípios e uma população de 24 milhões de habitantes (BRASIL, 2005). Esta região sempre foi intensamente afetada por fenômenos climáticos que acarretam seca severa. Nesse sentido, os reservatórios, que são a principal fonte de água, constituem os ecossistemas mais impactados, tanto ecologicamente como socioeconomicamente (BOUVY et al., 1999). O principal problema enfrentado por esta região é a falta d'água para irrigação na agricultura e consumo humano (GARFÌ et al., 2011). Como a região apresenta uma baixa precipitação (500 - 800 mm anuais), concentrada em poucos meses do ano e aliada a uma elevada evaporação que vai até 2.200 mm por ano, a manutenção dos níveis dos reservatórios é um problema em todo o semiárido (BRASIL, 2004). Além disso, fenômenos climáticos como o *El Niño* e a *La Niña* criam um cenário de irregularidade pluviométrica na região, com anos extremamente secos e anos chuvosos (SUASSUNA, 2005).

A região semiárida brasileira, considerada a mais populosa do mundo (BARBOSA et al., 2012), é periodicamente afetada por secas severas, devido às características físicas desta região que a torna uma das áreas mais susceptíveis à desertificação no Brasil (SOUZA; OYAMA, 2011; VIEIRA et al., 2015). Entretanto, secas mais intensas (mais de 50 meses de duração) foram verificadas com maior frequência nos últimos 20 anos, sendo a seca de 2010-2016 a mais severa da história, afetando todos os estados e durando mais de 50 meses em mais da metade do semiárido brasileiro (MARENGO et al., 2018). Além disso, futuras projeções climáticas para a segunda metade do século 21 mostram que o semiárido será impactado por uma redução na precipitação pluviométrica e incremento da aridez do solo (MARENGO; BERNASCONI, 2015; VIEIRA et al., 2015). De fato, as mudanças climáticas afetam, especialmente as regiões semiáridas do mundo, as quais concentram cerca de 20% da população em um terço do território mundial. Estas regiões apresentam características de climas secos, como baixa precipitação de distribuição temporal- e espacialmente irregular. Com isso, o incremento da temperatura irá causar perdas na biodiversidade, degradação ambiental, secas prolongadas e redução na disponibilidade de água (GAISER; FERREIRA; STAHR, 2003).

Pernambuco, Ceará e Bahia foram os estados brasileiros mais impactados pelos eventos de seca severa que ocorreram nos últimos 40 anos (1981 a 2016) (BRITO et al., 2018), além do

Rio Grande do Norte e Piauí nos eventos mais recentes de seca (2010-2016) (MARENGO et al., 2018). Durante a seca de 1997-1998, na qual aproximadamente cinco milhões de pessoas foram afetadas, o estado de Pernambuco foi o mais impactado em termos de severidade e duração (BRITO et al., 2018). Durante esse período, mais de 60% das áreas agricultáveis foram perdidas, afetando o suprimento de alimentos, além disso, a escassez hídrica causou o maior racionamento no abastecimento de água registrado até então (DUARTE et al., 1999). Nesse período, a redução dos níveis dos reservatórios, elevadas temperaturas, salinidades e concentrações de nutrientes causaram uma intensa proliferação das florações de *Raphidiopsis raciborskii* no estado de Pernambuco (BOUVY et al., 2000).

Mais recentemente, a seca de 2010 a 2016 afetou a economia de todo o semiárido brasileiro, criando problemas sociais nas áreas urbanas e rurais de mais de 1.100 municípios. Este evento de seca impactou 33,4 milhões de pessoas no Nordeste do Brasil, com perdas estimadas em aproximadamente R$ 104 bilhões (MARENGO et al., 2018). Durante este período, as secas ocorreram em todo território nacional, porém, com maior severidade nas regiões mais pobres, especialmente no Nordeste do Brasil, o que criou sérios problemas ambientais, econômicos e sociais (CUNHA et al., 2019). Estes problemas também impactam significativamente a qualidade da água, com mudanças no estado trófico e turbidez provocadas pela redução no nível da água (BRAGA et al., 2015). Com isso, levando em consideração a previsão de incremento de 54% das áreas com déficit hídrico no Nordeste do Brasil até 2100 (MARENGO et al., 2020), é esperado que haja uma intensa deterioração da qualidade da água dos reservatórios desta região.

Pernambuco ocupa uma área de 98.938 km^2, dividido em três principais mesorregiões fisiográficas: Zona da Mata, Agreste e Sertão. Essa amplitude geográfica também proporciona uma ampla variabilidade interanual na precipitação, influenciada principalmente pelos fenômenos *El Niño*/Oscilação do Sul e Dipolo do Atlântico. Os valores anuais médios da precipitação se encontram entre 500 e 800 mm/ano no Sertão, de 700 a 800 mm/ano no Agreste e 700 a 2.200 mm/ano na Zona da Mata. No Sertão o período de chuvas vai de dezembro a maio, no Agreste de fevereiro a julho e na Zona da Mata de março a agosto (APAC, 2019).

Com exceção do Rio São Francisco, a hidrografia de Pernambuco é composta principalmente por rios de pequena extensão. Dentre os principais rios litorâneos destacam-se o Goiana, Capibaribe, Ipojuca, Sirinhaém, Una e Mundaú, enquanto os principais rios interiores são o Ipanema, Moxotó, Pajeú, Terra Nova, Brígida, Garças e Pontal. Pernambuco apresenta uma significativa infraestrutura hídrica, com 120 reservatórios com capacidade de acumulação superior 1.000.000 m^3, o que consiste na principal fonte de abastecimento público para os municípios. O estado possui 29 bacias hidrográficas, denominadas de Unidades de Planejamento, sendo 13 correspondentes aos principais rios, nove para os pequenos rios interiores, seis para pequenos rios litorâneos e uma para os pequenos corpos d'água do Arquipélago de Fernando de Noronha (APAC, 2019).

2.2 Histórico de pesquisas com cianobactérias no semiárido brasileiro

2.2.1 Histórico de pesquisas

Estudos com as cianobactérias desenvolvidos no Nordeste do Brasil se iniciaram na década de 1930, com trabalhos florísticos realizados por Drouet (1937; 1938) no estado do Ceará. No estado de Pernambuco, o primeiro estudo sobre cianobactérias foi o de Carvalho-de-La-Mora (1989), ainda envolvendo aspectos taxonômicos de cianobactérias planctônicas. Após este período, houve um incremento no número de publicações sobre cianobactérias no Nordeste do Brasil, especialmente após dois marcantes eventos de intoxicação humana por cianotoxinas.

O primeiro registro de intoxicação por cianobactérias na região foi em 1988 no Reservatório de Itaparica, no qual foram registrados mais de 2.000 casos de gastroenterites com 88 mortes. Apesar de não serem confirmadas as causas, os casos estiveram relacionados com florações de *Anabaena* e *Microcystis* no reservatório (TEIXEIRA et al., 1993). Anos mais tarde, em uma clínica de hemodiálise em Caruaru, Pernambuco, ocorreu um episódio de repercussão mundial de intoxicação por cianotoxinas, no qual 76 pacientes morreram após aplicação intravenosa de água contaminada com microcistinas do Reservatório de Tabocas, o caso ficou conhecido como a "Tragédia de Caruaru" (CARMICHAEL et al., 2001).

Após esses dois incidentes, se iniciaram os estudos com enfoque ecológico e toxicológico na região Nordeste. No final da década de 1990 e início dos anos 2000 foram publicados nos estados de Pernambuco e Rio Grande do Norte os primeiros trabalhos ecológicos do fitoplâncton com enfoque nas cianobactérias (p.e. COSTA; ARAÚJO; CHELLAPPA, 1998; BOUVY et al., 1999, 2000, 2003; ARAÚJO; COSTA; CHELLAPPA, 2000; HUSZAR et al., 2000; CHELLAPPA; COSTA, 2003), dois estados que sempre foram afetados pelas florações. Nesse período, também se iniciaram os primeiros estudos toxicológicos e de quantificação de cianotoxinas em Pernambuco (p.e. DOMINGOS et al., 1999; CARMICHAEL et al., 2001; MOLICA et al., 2002, 2005; BITTENCOURT-OLIVEIRA, 2003; BITTENCOURT-OLIVEIRA et al., 2014) e em seguida no Rio Grande do Norte (COSTA et al., 2006; CHELLAPPA; CHELLAPPA; CHELLAPPA, 2008; FONSECA et al., 2015). Os demais estados do Nordeste só iniciaram as pesquisas com cianobactérias anos mais tarde, como nos estados da Paraíba (p.e. VASCONCELOS et al., 2013; AZEVEDO et al., 2015; FELIX et al., 2015), Ceará (p.e. VON SPERLING et al., 2008; MOLISANI et al., 2010; FRANÇA et al., 2013; BARROS et al., 2019) e Bahia (p.e. FUENTES et al., 2010; MOURA et al., 2013).

Tem sido frequente o número de estudos reportando a dominância de cianobactérias tóxicas em ecossistemas aquáticos do Nordeste, com registro de hepatotoxinas, como microcistinas (BITTENCOURT-OLIVEIRA; PICCIN-SANTOS; GOUVÊA-BARROS, 2012) e cilindrospermopsina (BITTENCOURT-OLIVEIRA et al., 2014), e neurotoxinas, como guanitoxina (MOLICA et al., 2005) e saxitoxinas (MOLICA et al., 2002). Em decorrência do grande registro de florações de cianobactérias no Nordeste, muitas vezes com registro de produção de toxinas, o Brasil se tornou o primeiro país a adotar legislação específica para estabelecer limites máximos toleráveis de cianotoxinas em água para consumo humano, sendo 1,0 µg/L para microcistinas e cilindrospermopsina, e 3,0 µg/L para saxitoxinas, além de recomendar a análise de guanitoxina quando espécies potencialmente produtoras forem detectadas (BRASIL, 2011, 2017).

Recentes estudos demonstraram que a elevada incidência de florações de cianobactérias com maiores concentrações de saxitoxinas em reservatórios do Nordeste do Brasil esteve relacionada com um aumento nos casos de microcefalia em recém-nascidos com síndrome congênita do Zika vírus (PEDROSA et al., 2020). Através deste estudo, os autores demonstraram que a presença de saxitoxinas, em concentrações abaixo do limite máximo permitido em águas para abastecimento (BRASIL, 2017), foram suficientes para duplicar a quantidade de morte celular induzida pelo Zika vírus em organoides de cérebros humanos. Portanto, é importante fortalecer as regulamentações para o monitoramento das florações de cianobactérias e sua remoção durante o tratamento da água. Com isso, a redução dos valores máximos permitidos das concentrações de cianotoxinas em reservatórios de abastecimento do Nordeste pode minimizar os riscos de intensificação de problemas associados com as arboviroses (PEDROSA et al., 2020).

2.2.2 Estudos de caso mais abrangentes

O aumento das florações de cianobactérias foi associado com as intensas secas que ocorreram em Pernambuco durante o *El-Niño* de 1996-1997, com predominância de *Raphidiopsis raciborskii* (BOUVY et al., 2000). Nesse estado, também foi constatada a presença de florações tóxicas (microcistinas e cilindrospermopsinas) em 10 reservatórios eutróficos, compostas principalmente por *R. raciborskii*, *Microcystis aeruginosa*, *M. panniformis* Komárek et al., *Planktothrix agardhii* (Gomont) Anagnostidis & Komárek, *P. isothrix* (Skuja) Komárek & Komárková, *Sphaerospermopsis aphanizomenoides* (Forti) Zapomelová et al., *Geitlerinema amphibium* (Gomont) Anagnostidis e *Merismopedia tenuissima* Lemmermann (BITTENCOURT-OLIVEIRA et al., 2014).

Para o estado da Paraíba, florações de cianobactérias, compostas principalmente por *M. aeruginosa*, *R. raciborskii*, *P. agardhii* e *Oscillatoria tenuissima* Gomont, foram estudadas em 20 reservatórios, as quais apresentaram maiores valores de biomassa no período de estiagem. Desses, 11 apresentaram concentrações de microcistinas que chegaram até 5 µg/L, e em três reservatórios foi detectada a presença desta cianotoxina em tecidos musculares de peixes (VASCONCELOS et al., 2011). No Rio Grande do Norte, foram estudados 40 lagos rasos com florações de cianobactérias, cujo incremento de sua biomassa foi relacionado com a ocorrência de secas prolongadas no semiárido e consequente redução dos níveis de água (BRASIL et al., 2016). No Ceará, florações de cianobactérias foram registradas em 20 reservatórios de abastecimento público, com ocorrência de microcistinas em 13, saxitoxinas em nove e cilindrospermopsinas em cinco. Nesses ambientes, a eutrofização foi o principal fator que favoreceu as florações, que foram intensificadas pelo clima quente e seco da região (BARROS et al., 2019).

Em reservatórios do semiárido pernambucano, diversos tipos de cianotoxinas foram detectadas e quantificadas em 11 reservatórios com florações de cianobactérias, em um dos estudos toxicológicos mais abrangentes do Nordeste do Brasil (LORENZI et al., 2018). As microcistinas foram as cianotoxinas mais frequentes e registradas em maiores quantidades, seguidas da neo-saxitoxina, saxitoxinas, cilindrospermopsinas e anatoxina-a. As elevadas concentrações destas cianotoxinas nos reservatórios estudados põem em risco a biota aquática e a saúde das pessoas que se abastecem destes mananciais, alertando para a necessidade de um monitoramento contínuo e uma maior eficiência no gerenciamento dos recursos hídricos da região (LORENZI et al., 2018).

Como reportado anteriormente, diversos estudos mostram que a intensificação das secas no semiárido brasileiro pode favorecer o incremento das florações de cianobactérias (p.e., BOUVY et al., 2000; BRASIL et al., 2016; BARROS et al., 2019; REGO; RANGEL-JUNIOR; COSTA, 2020). No entanto, em condições de seca extrema, o baixo volume de água dos reservatórios podem favorecer outros grupos do fitoplâncton, como diatomáceas e criptofíceas, pois apresentam menores requerimentos por recursos, incluindo luz e nutrientes inorgânicos (MEDEIROS et al., 2015).

Em uma importante revisão sobre os casos de dominância de cianobactérias para reservatórios do Nordeste do Brasil ao longo de 80 anos, 102 trabalhos de 79 ambientes foram revisados para os estados de Pernambuco, Rio Grande do Norte, Paraíba, Ceará e Bahia (MOURA; ARAGÃO-TAVARES; AMORIM, 2018). Nesse trabalho, foi verificado um aumento na frequência e intensidade das florações nas duas últimas décadas, sendo acompanhado de elevadas concentrações de cianotoxinas. Com isso, o crescimento de cianobactérias nessa região foi favorecido pela elevada eutrofização e mudanças no clima dos últimos anos, alertando para um possível incremento das florações tóxicas nos próximos anos (MOURA; ARAGÃO-TAVARES; AMORIM, 2018).

No semiárido brasileiro, especialmente nos estados de Pernambuco, Rio Grande do Norte e Paraíba, as florações de cianobactérias são compostas por 49 espécies, distribuídas nas ordens Chroococcales, Nostocales e Oscillatoriales. Dentre as espécies mais frequentes, destacam-se *R. raciborskii* (Nostocales), *M. aeruginosa* (Chroococcales) e *P. agardhii* (Oscillatoriales) (MOURA; ARAGÃO-TAVARES; AMORIM, 2018).

2.3 Causas das florações: aprendizados com as regiões temperada e subtropical e relevância para os trópicos

2.3.1 Fatores regulatórios das florações

As florações de cianobactérias estão se tornando cada vez mais frequentes em ecossistemas aquáticos do mundo todo, especialmente em regiões tropicais (GER; HANSSON; LÜRLING, 2014). Estas florações estão relacionadas, principalmente, com o incremento nas concentrações de nutrientes e elevação da temperatura nos corpos hídricos (PAERL; OTTEN, 2013; MARIANI et al., 2015; RIGOSI et al., 2015). Com estas características, a distribuição e biomassa das cianobactérias têm sido elevadas, fazendo com que elas se tornem cada vez mais invasivas, tanto em regiões tropicais como temperadas (PADISÁK, 1997; BRIAND et al., 2004; HARKE et al., 2016).

Nos trópicos, devido às características climáticas dessa região, as florações de cianobactérias são consideradas um problema de saúde pública, pois podem persistir durante todo o ano (BOUVY et al., 2000; BITTENCOURT-OLIVEIRA et al., 2014). Entretanto, com o atual cenário de mudanças climáticas, muitas cianobactérias estão se expandindo para regiões de maiores latitudes, como é o caso de *R. raciborskii* (SINHA et al., 2012) e *M. aeruginosa* (HARKE et al., 2016), as quais são as espécies mais problemáticas para o manejo dos recursos hídricos.

As condições climáticas e de eutrofização atuais condicionam um aumento da frequência e intensidade das florações, seja diretamente, com o aumento da temperatura e concentrações de nutrientes, ou indiretamente, com a redução do volume da água dos reservatórios e estratificação (PAERL; OTTEN, 2013). A redução dos níveis de água dos reservatórios, muitas vezes provocada pela seca, também provocam um aumento na biomassa das cianobactérias (NASELI-FLORES; BARONE, 2005; BAKKER; HILT, 2016; YANG et al., 2016).

Esses fatores também são importantes na alternância de genótipos tóxicos e não tóxicos de cianobactérias, especialmente os produtores de microcistinas. Dentre os principais preditores da razão entre linhagens tóxicas e não tóxicas, destacam-se a temperatura (DZIALLAS; GROSSART, 2011), nutrientes (VÉZIE et al., 2002), luz (KARDINÄAL et al., 2007) e concentrações de CO_2 (VAN DE WAAL et al., 2011). Nesse sentido, linhagens tóxicas de *Microcystis* crescem mais rapidamente que as não tóxicas em condições de elevadas temperaturas e concentrações de nutrientes (DAVIS et al., 2009). Essa maior taxa de crescimento foi influenciada pela interação entre o incremento dos nutrientes e da temperatura, o que pode levar a ocorrência de florações mais intensas e com maior produção de cianotoxinas (DAVIS et al., 2009).

As preocupações aumentam quando se analisam os cenários futuros de incremento da temperatura de até 7 °C (CORLETT, 2012) e aumento das secas (DAI, 2012) até o final do século 21. Além disso, o Painel Intergovernamental de Mudanças Climáticas – IPCC prevê um aumento entre 1 (0,3-1,7 - RCP 2.6) e 3,7 °C (2,6-4,8 - RCP 8.5) na temperatura global média até 2100, incrementando os riscos de eutrofização e florações algais nos ambientes de água doce (IPCC, 2014). Estas mudanças também aumentam os riscos de aridez e secas prolongadas, fazendo com que o Nordeste brasileiro se torne um dos “hotspots” com maior risco de seca no mundo, além da região mediterrânea e o sul do continente africano (IPCC, 2019). Previsões mais específicas para o Nordeste brasileiro alertam para um aumento de 4 °C na temperatura e 54% nas áreas com déficit hídrico até o final do século 21 (MARENGO et al., 2020).

2.3.2 Alguns estudos de caso utilizando modelagem estatística

Diante disso, uma das formas de predizer a ocorrência e dominância de um determinado grupo fitoplanctônico é através da modelagem determinística, utilizando-se de variáveis que possam explicar a ocorrência e intensidade das florações. Com relação a isso, diversos modelos foram propostos para explicar a participação da eutrofização e mudanças climáticas sobre a dominância de algas planctônicas. Dentre os principais modelos, como apontado por Elliott (2012a), destacam-se o Phytoplankton RespOnses To Environmental CHange (PROTECH) (REYNOLDS et al., 2001; ELLIOTT et al., 2005, 2006; ELLIOTT; MAY, 2008; ELLIOTT, 2010, 2012b), PROgram for Boundary layers in the Environment & BIOgeochemical LAke model (PROBE & BIOLA) (ARHEIMER et al., 2005), PROTech Based Algal Simulations (PROTBAS) (MARKENSTEN et al., 2010) e PCLake (MOOIJ et al., 2007).

Diversos modelos podem ser usados para prever as florações algais em respostas às mudanças ambientais a curto e longo prazo. Dentre as principais variáveis preditoras utilizadas nos modelos, destacam-se a temperatura da água, fósforo e nitrogênio. Entretanto, de uma maneira geral, a maioria dos modelos é restrita para uma única localidade ou espécie, especialmente nas regiões temperadas, o que limita seu poder preditivo em outras regiões com condições e espécies dominantes distintas. Portanto, é importante que os gerenciadores de recursos hídricos usem modelos mais gerais e com características similares ao ambiente de interesse (ROUSSO et al., 2020).

A criação de Modelos de Equações Estruturais tem se mostrado eficiente na predição de como as variáveis ambientais regulam a dominância das cianobactérias (SHAN et al., 2019). Esta abordagem baseia-se na modelagem determinística para explicar as relações existentes entre diferentes variáveis. O uso destes modelos tem emergido nos últimos anos em diversas áreas da ecologia, entretanto poucos estudos o utilizam para explicar a ocorrência e intensidade das florações de cianobactérias, e mesmo assim restritos aos ambientes temperados e subtropicais (p.e. YANG et al., 2016; SHAN et al., 2019).

Ao longo de um gradiente longitudinal com dados de 143 lagos da América do Sul e Europa, apesar de a temperatura não ter um efeito significativo no incremento da biomassa fitoplanctônica, foram observadas maiores contribuições relativas das cianobactérias em lagos de regiões mais quentes, sugerindo relações sinérgicas com os nutrientes (KOSTEN et al., 2012). Por outro lado, para um conjunto de mais de 1.000 lagos norte-americanos, a interação entre temperatura e nutrientes não é sinérgica, sendo os nutrientes o principal fator que favorece as florações de cianobactérias. Nesse conjunto de lagos, os nutrientes desempenharam um importante papel em lagos oligotróficos, enquanto a temperatura foi mais importante para os mesotróficos e a interação entre estes fatores foi significativa para lagos eutróficos e hipereutróficos (RIGOSI et al., 2014). Além disso, diversas espécies de cianobactérias apresentaram sensibilidades distintas à atuação da temperatura da água, nutrientes e a interação entre esses fatores (RIGOSI et al., 2014).

A construção de Redes de Estrutura Bayesiana foi importante para avaliar a probabilidade de ocorrência de florações de cianobactérias em 20 lagos subtropicais e temperados. Através deste método, foi possível verificar que a intensidade das florações de cianobactérias é mais sensível à temperatura que ao fósforo total (RIGOSI et al., 2015). Ao testar cenários futuros, os autores destacam que um aumento de apenas 0,8 ºC na temperatura da água e de 0,01 mg/L nas concentrações de fósforo total seria suficiente para aumentar a probabilidade de ocorrência das florações de cianobactérias de maior intensidade em 5%, chegando a 20% no final do século 21 (RIGOSI et al., 2015).

Um importante estudo desenvolvido no semiárido brasileiro, utilizando modelos de regressão linear, revelou que que a biomassa de cianobactérias foi positivamente relacionada com a estabilidade da coluna d’água, pH e nitrogênio total, e negativamente com a transparência da água e sólidos suspensos totais. Os autores mostraram que a elevação da temperatura, prevista para o final do século 21, poderá reduzir a qualidade e quantidade de água no semiárido brasileiro, e consequentemente, aumentando os riscos de salinização, anóxia, eutrofização e florações de cianobactérias (BRASIL et al., 2016).

Em escala mundial, uma análise temporal a longo prazo das florações algais, principalmente de cianobactérias, durante o verão em 71 grandes lagos evidenciou que as florações de cianobactérias aumentaram a intensidade a partir 1985, contradizendo a hipótese de que este resultado é devido a um maior esforço de pesquisa (HO et al., 2019). A maioria desses lagos apresentou um padrão de deterioração da qualidade da água com o aumento das florações e à medida que se aumenta a temperatura, enquanto lagos com tendência de decréscimo nas florações foram raros e restritos às regiões mais frias. Entre os lagos com tendência de deterioração, o incremento das florações esteve relacionado com o aumento do uso de fertilizantes agrícolas. No entanto, fatores climáticos, como a temperatura e precipitação, também influenciaram positivamente no incremento das florações algais (HO et al., 2019).

A utilização de Modelos de Equações Estruturais para predição das florações de cianobactérias mostrou que um incremento no nível da água de 2 m em quatro lagos subtropicais na China seria suficiente para reduzir a biomassa de cianobactérias através da diluição ou modificação das variáveis limnológicas (YANG et al., 2016). Em outros lagos subtropicais chineses (Taihu, Chaohu e Dianchi), a biomassa de *Microcystis* foi influenciada principalmente pela temperatura, seguida do fósforo total e macrozooplâncton, enquanto as biomassas de *Dolichospermum* e *Aphanizomenon* apresentaram uma menor dependência das variáveis limnológicas, com respostas específicas para cada lago (SHAN et al., 2019).

Como esperado, a maioria dos estudos foi desenvolvida em lagos de regiões temperadas e subtropicais, mostrando que de fato os nutrientes e a temperatura têm um maior peso na explicação das florações de cianobactérias. Essa maior explicação pode ser atribuída às amplitudes térmicas anuais registradas nessas regiões, ao contrário das regiões tropicais, que apresentam temperaturas mais uniformes ao longo do ano. Nesse sentido, é importante que se desenvolvam mais estudos de modelagem utilizando corpos d'água tropicais em diferentes gradientes climáticos (precipitação, temperatura e secas) e de eutrofização, para um melhor entendimento do papel das variáveis ambientais sobre a biomassa total de cianobactérias e dos diferentes morfotipos, como as picocianobactérias, cianobactérias cocoides, filamentosas heterocitadas e filamentosas não heterocitadas. Por exemplo, estudos realizados no Nordeste do Brasil detectaram diferenças nos padrões de distribuição das florações algais e sua dependência das variáveis preditoras entre regiões semiáridas e úmidas, com forte influência da precipitação em áreas mais secas (p.e., BRASIL et al., 2016; OLIVEIRA; MOURA; DANTAS, 2018).

2.4 Grupos funcionais de Reynolds como indicadores das florações algais

O fitoplâncton é um grupo extremamente diverso de organismos, com cerca de 10.000 a 25.000 espécies nos ambientes de água doce e marinhos (FALKOWSKI et al., 2004; VARGAS et al., 2015; BORICS et al., 2021). A coexistência entre estas espécies é direcionada por diversos fatores bióticos e abióticos (BORICS et al., 2020), os quais influenciam mais os traços das espécies, do que as espécies propriamente ditas, criando diferentes padrões de dominância (REYNOLDS et al., 2002). Portanto, uma importante questão em ecologia não é quem são as espécies (identidade), e sim o que elas fazem no ambiente (papel funcional) (SUDING et al., 2008).

Os traços funcionais de cada organismo podem representar a resposta das espécies às condições ambientais ou seus efeitos no funcionamento do ecossistema, além de aumentar a previsibilidade das respostas da comunidade fitoplanctônica às mudanças ambientais (KRUK; DEVERCELLI; HUSZAR, 2021). Neste sentido, diversas classificações funcionais, baseadas em traços morfo e ecofisiológicos foram desenvolvidas (p.e., REYNOLDS et al., 2002; REYNOLDS, 2006; SALMASO; PADISÁK, 2007; LITCHMAN; KLAUSMEIER, 2008;

KRUK et al., 2010; FRAISE; BORMANS; LAGADEUC, 2013; CAREY et al., 2012), as quais podem ser úteis no estudo da dinâmica do fitoplâncton, sem perder importantes informações das espécies e suas respostas ao ambiente.

Baseado nisso, os grupos funcionais do fitoplâncton foram criados para agrupar espécies com preferências e tolerâncias similares e coocorrem no mesmo ambiente e ao mesmo tempo (REYNOLDS, 1984, 1998, 2006). Portanto, elas compartilham o mesmo modelo de habitat (*habitat template*), que é definido como o conjunto de condições ambientais favoráveis ao desenvolvimento dos grupos (REYNOLDS, 1998). Para a descrição original dos grupos funcionais, Reynolds (2006) utilizou oito eixos ambientais: profundidade da zona de mistura, irradiância, temperatura da água, concentrações de CO_2, taxa de filtração do zooplâncton herbívoro, fósforo solúvel reativo, nitrogênio inorgânico dissolvido e sílica solúvel reativa. A partir de então, esta classificação funcional se tornou uma das principais e mais utilizadas na ecologia do fitoplâncton (PADISÁK; CROSSETTI; NASELLI-FLORES, 2009; SALMASO; NASELLI-FLORES; PÁDISAK, 2015; KRUK; DEVERCELLI; HUSZAR, 2021).

Conhecendo o melhor modelo de habitat de um determinado grupo funcional, é possível estabelecer as regras de assembleia, que representa os valores limites das condições ambientais nos quais este grupo ocorre e pode se tornar abundante (KEDDY; WEIHER, 1999). O estabelecimento das regras de assembleia é um processo difícil e que exige um profundo conhecimento da ecologia das espécies e de como os fatores ambientais afetam a estrutura e padrões espaço-temporais das assembleias fitoplanctônicas (ROJO, 2021). Além disso, as regras devem ser valores quantitativos explícitos de variáveis ambientais relevantes para a ecologia do grupo, ao invés de apenas descrever um padrão (KEDDY; WEIHER, 1999).

Existem diversas aplicabilidades da abordagem de grupos funcionais de Reynolds, dentre elas, a predição das florações de cianobactérias. A descrição dos grupos dominantes, bem como seus efeitos negativos e respostas às condições ambientais, podem ser úteis para programas de monitoramento da qualidade da água, especialmente se tratando de espécies nocivas, como as cianobactérias (KRUK; DEVERCELLI; HUSZAR, 2021).

Dentre os principais traços ecofisiológicos das cianobactérias, destacam-se: formação de grandes colônias e filamentos, produção de aerótopos, presença de pigmentos acessórios, fixação de nitrogênio, produção de acinetos, crescimento em elevadas temperaturas, liberação de cianotoxinas e a dinâmica entre linhagens tóxicas e não tóxicas. As combinações destes traços determinam as respostas das cianobactérias ao ambiente (MANTZOUKI et al., 2016).

As cianobactérias fazem parte de alguns dos principais grupos funcionais de Reynolds de importância ecológica e toxicológica, dentre eles: S_1/S_2 – *Planktothrix agardhii*; S_N –

Raphidiopsis raciborskii; H_1/H_2 – *Dolichospermum flos-aquae*; M/L_M – *Microcystis aeruginosa*. Portanto, estratégias de controle de florações de cianobactérias devem levar em consideração as características funcionais dos grupos predominantes (MANTZOUKI et al., 2016), uma vez que, a dominância destes grupos está associada com determinados fatores ambientais que selecionam traços específicos favoráveis ao seu crescimento.

Com isso, alguns potenciais métodos de controle das florações de cianobactérias foram criados baseados nos grupos funcionais dominantes, informações do estado trófico, padrões de estratificação e desestratificação e tempo de retenção dos ambientes estudados (IBELINGS et al., 2016). Portanto, estas estratégias podem ser empregadas para a prevenção, manejo e mitigação das florações nocivas, evitando riscos ecológicos e à saúde pública. Além disso, o índice Q do fitoplâncton é usado pela *European Water Framework Directive* para avaliar a qualidade da água e estado ecológico dos ambientes aquáticos, o qual leva em consideração a abundância relativa de determinados grupos funcionais, incluindo cianobactérias, seus potenciais efeitos e preferências pelas condições ambientais, tanto em lagos (PÁDISAK et al., 2006), como em rios (BORICS et al., 2007).

Os grupos funcionais de Reynolds também podem ser empregados na avaliação dos impactos antrópicos de ambientes aquáticos, considerando que a diversidade funcional, representada pelo número de grupos funcionais, ilustrou melhor os impactos que a riqueza de espécies (ABONYI et al., 2012). De forma similar, a riqueza de grupos funcionais é o melhor preditor do funcionamento do ecossistema (eficiência do uso do recurso do fitoplâncton), quando comparada com a riqueza de espécies ou de traços funcionais (ABONYI et al., 2018).

Para o semiárido brasileiro, diversos estudos demonstram a aplicabilidade dos grupos funcionais de Reynolds para descrever a qualidade da água em reservatórios de abastecimento (p.e., MOURA et al., 2007; MOLISANI et al., 2010). Além disso, esta classificação revelou importantes padrões espaço-temporais do fitoplâncton em resposta aos gradientes rio-barragem (MOURA et al., 2013), cobertura de macrófitas aquáticas (SILVA et al., 2018), condições ambientais em tanques de pedra (SILVA et al., 2020), presença de piscicultura (MOURA; NASCIMENTO; DANTAS, 2012; LINS et al., 2017), temperatura e nutrientes (SOUZA; CROSSETTI; BECKER, 2018). A classificação funcional de Reynolds também foi útil para esclarecer as relações das assembleias fitoplanctônicas com as condições ambientais em reservatórios rasos e profundos do semiárido brasileiro, com fortes influências da sazonalidade, profundidade, padrões térmicos e disponibilidade de luz (DANTAS; BITTENCOURT-OLIVEIRA; MOURA, 2012).

Outra importante aplicação dos grupos funcionais de Reynolds é no entendimento dos efeitos das secas prolongadas na região semiárida (p.e., BARROSO et al., 2018; REGO; RANGEL-JUNIOR; COSTA, 2020). Nesse sentido, esta classificação é a que fornece mais informações sobre as assembleias fitoplanctônicas, permitindo descrever os efeitos das alterações ambientais provocadas pelas secas na dominância de determinados grupos algais, especialmente cianobactérias (BRAGA; BECKER, 2020). Entretanto, apesar das secas favorecerem grupos funcionais que incluem cianobactérias em lagos profundos do semiárido, lagos rasos podem apresentar uma redução no nível da água mais intensa, favorecendo a ressuspensão do sedimento e incrementando a turbidez, o que pode substituir as cianobactérias por grupos funcionais de algas mixotróficas, como as criptofíceas (COSTA; ATTAYDE; BECKER, 2016).

2.5 Consequências das florações algais na qualidade da água, biodiversidade e funcionamento do ecossistema

2.5.1 Qualidade da água

As cianobactérias são componentes naturais dos lagos e reservatórios, participando de processos importantes nestes ecossistemas, como ciclagem de nutrientes, participação nas cadeias tróficas, produção de oxigênio, dentre outros (WETZEL, 2001; REYNOLDS, 2006). No entanto, a partir do momento que estes organismos passam a se desenvolver exageradamente, muitos problemas podem aparecer em decorrência deste massivo crescimento, especialmente, quando relacionados com a presença de cianotoxinas (WIEGAND; PFLUGMACHER, 2005).

Um dos principais problemas relacionados com o manejo da qualidade da água dos reservatórios é a ocorrência e proliferação de cianobactérias potencialmente tóxicas (CARMICHAEL; BOYER, 2016), nas quais podem estar presentes linhagens produtoras de cianotoxinas, como microcistinas, saxitoxinas, cilindrospermopsinas e anatoxinas (WIEGAND; PFLUGMACHER, 2005; PAERL; OTTEN, 2013), podendo causar sérios problemas de saúde pública, incluindo irritação e morte de populações que entram em contato com a água contaminada (CARMICHAEL; BOYER, 2016). Dentre estas toxinas, as microcistinas são as mais amplamente distribuídas e letais nos ecossistemas aquáticos (HARKE et al., 2016), as quais podem afetar negativamente o desenvolvimento das demais comunidades aquáticas, como peixes (BOARU; DRAGOŞ; SCHIRMER, 2006), zooplâncton (LIANG et al., 2017) e macrófitas (AMORIM; ULISSES; MOURA, 2017).

Diversos fatores relacionados à reduzida qualidade da água podem ser intensificados com as florações de cianobactérias, especialmente a turbidez, através do acúmulo de biomassa na superfície, podendo causar sérias consequências para os animais aquáticos, como redução da captura visual das presas e forçar os organismos a migrarem para áreas mais claras (SUKENIK; QUESADA; SALMASO, 2015). Em resposta às elevadas taxas fotossintéticas, as florações de cianobactérias também elevam o pH da água, prejudicando o desenvolvimento de espécies sensíveis (VERSPAGEM et al., 2014). Apesar das cianobactérias serem positivamente influenciadas pela temperatura e estratificação da coluna d’água (PAERL; HUISMAN, 2008), as florações também podem aumentar a atenuação de luz e temperatura e, consequentemente, intensificar a estratificação (RINKE et al., 2010). Isto se deve ao acúmulo de grandes colônias e filamentos, os quais podem absorver mais energia e aquecer mais rapidamente a camada superficial, favorecendo a estratificação térmica (KUMAGAI et al., 2000).

A decomposição das florações algais pode causar um rápido declínio nas concentrações de oxigênio dissolvido e no pH, acompanhado do incremento da condutividade elétrica. Essa decomposição também é acompanhada de uma alta taxa de liberação de nutrientes, CO_2 e CH_4, o que favorece o desenvolvimento das futuras florações após a decomposição da atual (YAN et al., 2017). Com isso, é possível observar dois círculos viciosos: (1) a liberação de nutrientes aumenta a eutrofização dos lagos e promove novas florações; (2) a decomposição potencializa a liberação de gases do efeito estufa (CO_2 e CH_4), contribuindo para o aquecimento global, outro importante fator influenciador das florações (YAN et al., 2017).

2.5.2 Biodiversidade e funcionamento do ecossistema

Florações de cianobactérias apresentam sérios impactos à biodiversidade aquática, especialmente do zooplâncton (KRZTON et al., 2019). A reduzida riqueza de espécies durante as florações pode estar relacionada à presença de espécies mais adaptadas (GER; HANSSON; LÜRLING, 2014). Por outro lado, enquanto alguns estudos reportam uma redução da riqueza de espécies do fitoplâncton durante as florações de cianobactérias (p.e., ESCALAS et al., 2019), outros demonstram que as cianobactérias podem beneficiar ou estabilizar a diversidade do fitoplâncton durante as florações (p.e., CAREY et al., 2014; YANG et al., 2017; LIU et al., 2019).

Além do mais, o fitoplâncton pode apresentar uma maior resiliência durante as florações, recuperando sua biomassa mesmo após longos períodos de florações (YANG et al., 2017). Nesse sentido, uma maior diversidade também resulta em um aumento no funcionamento do ecossistema, favorecendo uma maior estabilidade temporal e a manutenção da riqueza de espécies (ALLAN et al., 2011). Consequentemente, um maior número de espécies coexistindo, com diferentes requerimentos nutricionais, aumenta a produtividade da comunidade e influencia no funcionamento do ecossistema (TILMAN et al., 1997).

O principal mecanismo responsável pela maior riqueza do fitoplâncton é a liberação de nutrientes durante a decomposição algal, o que promove a coexistência de um maior número de espécies nas florações de cianobactérias (CAREY et al., 2014). A elevada biomassa, aliada a uma maior disponibilidade de nutrientes, pode favorecer o surgimento de organismos decompositores e mixotróficos, as quais são favorecidas em ambientes eutróficos devido ao elevado desenvolvimento de bactérias, servindo de alimento para algas mixotróficas e ciliados (FLYNN et al., 2018). Estes fatores impactam significativamente na composição do zooplâncton, selecionando pequenos organismos especializados no consumo destas fontes alternativas de alimento (WILSON; SARNELLE; TILLMANNS, 2006).

Uma das formas de estudar as modificações na biodiversidade e interações tróficas é através da teoria de biodiversidade-funcionamento do ecossistema (FILSTRUP et al., 2014). Dentre as métricas utilizadas, a eficiência do uso do recurso é capaz de explicar as relações de troca de energia entre as teias tróficas (POISOT; MOUQUET; GRAVEL, 2013), especialmente durante as florações algais (TIAN et al., 2017). Para a relação fitoplâncton-zooplâncton, as florações de cianobactérias bloqueiam a assimilação de carbono pelo zooplâncton, levando a uma comunidade limitada (GREY; JONES; SLEEP, 2000). Além do mais, as florações induzem uma mudança na dieta do zooplâncton para a assimilação de pequenas partículas (MAJOR et al., 2017), especialmente de fontes microbianas (KLUIJVER et al., 2012).

Portanto, as florações podem limitar o crescimento do zooplâncton através de inúmeros fatores, incluindo morfológicos, fisiológicos e nutricionais, reduzindo a reprodução e tamanho corporal (GHANDOUANI et al., 2003; GER et al., 2019). Por outro lado, espécies de menor tamanho podem ser selecionadas, como rotíferos, náuplios e copépodos Cyclopoida, as quais podem coexistir com as cianobactérias (WILSON; SARNELLE; TILLMANNS, 2006). Estes pequenos organismos podem ser beneficiados através do consumo de bactérias heterotróficas e pequenos protozoários durante as florações (ENGSTRÖM-ÖST et al., 2013)

Intensas florações algais provocam uma redução da equitabilidade do fitoplâncton, o que pode aumentar a eficiência do uso do recurso do fitoplâncton, porém pode reduzir para o zooplâncton, indicando que a energia não é transferida de forma eficiente para os demais níveis tróficos (FILSTRUP et al., 2014). As florações de *Microcystis* spp. estiveram relacionadas com o reduzido turnover intra- e interanual do fitoplâncton, demonstrando uma reduzida substituição de espécies durante as florações. Portanto, é importante associar dados do funcionamento do ecossistema (produção de biomassa e ciclagem de nutrientes) com atributos estruturais das comunidades (riqueza, composição e equitabilidade), além de integrar as relações horizontais (intra-comunidades) e verticais (inter-comunidades) para entender os efeitos da biodiversidade sobre o ecossistema (FILSTRUP et al., 2014).

A biomassa de cianobactérias é capaz de influenciar negativamente a riqueza de espécies do zooplâncton em lagos com florações de cianobactérias de longa duração, além da riqueza funcional nos lagos com florações de curta duração. Além disso, florações de cianobactérias são responsáveis pela limitação do papel funcional do zooplâncton e afetam o uso de recursos, além de enfraquecer os processos ecológicos e a funcionalidade dos ecossistemas (KRZTON et al., 2019). De maneira similar, o fitoplâncton apresenta uma menor riqueza de espécies durante a dominância das cianobactérias, dinoflagelados e outras algas. De uma maneira geral, as florações de cianobactérias apresentam um maior impacto negativo na estrutura da comunidade, portanto, constituem um desafio para o funcionamento do fitoplâncton, a qual cria condições favoráveis às florações (ESCALAS et al., 2019).

Por outro lado, florações de cianobactérias podem ser fortemente correlacionadas com as redes de co-ocorrência do plâncton eucarionte (LIU et al., 2019). Nesse sentido, é possível observar um maior número de correlações positivas que correlações negativas na estrutura da comunidade, indicando um padrão de coexistência entre as espécies, dependendo das flutuações ambientais causadas pelas florações (LIU et al., 2019). Para o zooplâncton, também é possível observar efeitos positivos das florações de cianobactérias na evolução adaptativa, a qual ocorre de forma rápida suficiente para afetar a dinâmica sazonal dos consumidores e presas (SCHAFFNER et al., 2019). Com isso, as florações podem causar um incremento marcante na biomassa de *Daphnia* no verão, sugerindo que estes cladóceros podem adquirir resistência às cianobactérias. Experimentos de adaptação revelaram que a rápida evolução do zooplâncton se deu em decorrência dos ciclos sazonais contrastantes (primavera e verão) do fitoplâncton, reiniciando a cada ano com as florações de cianobactérias. Portanto, a dinâmica evolutiva tem importantes consequências para processos ecológicos (SCHAFFNER et al., 2019).

2.6 Biomanipulação no controle das florações algais

2.6.1 Teoria de interações de cascata trófica e as razões para sua ineficiência em regiões (sub)tropicais

A biomanipulação tem sido amplamente utilizada para restauração de lagos temperados, seja pela retirada dos peixes zooplanctívoros e bentívoros, ou pela adição dos piscívoros, promovendo uma melhoria da qualidade da água e aumento na transparência (JEPPESEN et al., 2012; LIU et al., 2018; DANTAS et al., 2019; PENG et al., 2021). Essa manipulação se dá através da interação de cascata trófica, na qual a remoção dos peixes zooplanctívoros favorece o zooplâncton, e consequentemente a redução do fitoplâncton (TRIEST et al., 2016).

A remoção de peixes planctívoros e bentônicos foi usada para a restauração de 36 lagos rasos e eutróficos na Dinamarca. Nestes ambientes, houve uma redução nos nutrientes, sólidos suspensos e biomassa fitoplanctônica entre 50-70% após a remoção dos peixes, seguido do aumento da pressão de predação pelo zooplâncton, transparência da água e colonização de macrófitas. Entretanto, os efeitos da biomanipulação só foram persistentes até cerca de 6 a 10 anos, sugerindo que sucessivas remoções de peixes são necessárias para o controle eficiente das cianobactérias (SØNDERGAARD et al., 2008). Nesse sentido, os mesmos autores demonstraram que após dois períodos de remoção de peixes, com predominância de espécies zooplanctívoras, foi notória a redução da clorofila *a*, mudança de um estado túrbido dominado pelo fitoplâncton para um estado claro dominado pelas macrófitas (*Elodea canadensis*) e redução nas concentrações de nitrogênio e fósforo (SØNDERGAARD et al., 2017).

Em uma outra abordagem, a introdução de peixes piscívoros, juntamente com a adição de *Daphnia*, foram eficientes no controle das cianobactérias no lago eutrófico Shirakaba, Japão. Nesse estudo, a adição de peixes piscívoros causou uma redução dos zooplanctívoros e, consequentemente, incremento na biomassa zooplanctônica, redução da biomassa fitoplanctônica, desaparecimento das florações de *Microcystis* e recolonização da macrófita *Elodea nuttalli* (HA et al., 2013).

A biomanipulação no Lago Ringsjön na Suécia, através da remoção de peixes bentívoros, foi eficiente na melhoria da qualidade da água, aumento da proporção relativa de *Daphnia* e consequente redução da dominância de cianobactérias e concentrações de microcistinas (EKVALL; URRUTIA-CORDERO; HANSSON, 2014). No mesmo lago, os copépodos Cyclopoida e pequenos cladóceros suprimiram o crescimento de cianobactérias potencialmente tóxicas durante as florações (URRUTIA-CORDERO; EKVALL; HANSSON, 2015). Esses resultados também foram verificados em experimentos de predação *in situ*, nos quais o incremento da densidade e tamanho corporal dos copépodos Cyclopoida, associado ao aumento no tamanho de *Daphnia*, contribuíram para as reduções na biomassa das cianobactérias formadoras de florações, especialmente *Microcystis* spp. (URRUTIA-CORDERO; EKVALL; HANSSON, 2015).

Para as regiões tropicais os efeitos da cascata trófica em lagos naturais são menos evidentes que em zonas temperadas, devido principalmente à predominância de peixes onívoros de rápida reprodução, grandes quantidades de peixes jovens e baixa abundância dos piscívoros (JEPPESEN et al., 2007, 2012). Com isso, a efetividade da biomanipulação é dependente da remoção massiva de peixes planctívoros e onívoros, para que se consiga alcançar um efeito em cascata trófica e o controle da biomassa fitoplanctônica (OKUN et al., 2008).

Outra problemática da biomanipulação em lagos tropicais é a predominância de pequenos organismos zooplanctônicos, como rotíferos, copépodos Cyclopoida e pequenos cladóceros (JEPPESEN et al., 2007), ao contrário das regiões temperadas, onde predominam grandes cladóceros, como *Daphnia* (JEPPESEN et al., 2012; GER et al., 2016). Além do mais, as cianobactérias formadoras de florações possuem adaptações que podem prevenir a predação pelo zooplâncton, como deficiência nutricional, formação de grandes colônias e filamentos e a capacidade de produzir cianotoxinas (GER et al., 2016), enfraquecendo a cascata trófica.

Nesse sentido, o zooplâncton não foi capaz de reduzir o fitoplâncton mesmo após o incremento de 10 vezes na densidade zooplanctônica ou a adição de *Daphnia* em dois experimentos em mesocosmos em um lago hipereutrófico subtropical (LACEROT et al., 2013). A adição do peixe, mesmo em pequenas densidades, foi suficiente para reduzir os grandes organismos zooplanctônicos, sendo assim, drásticas reduções na biomassa dos peixes onívoros seriam necessárias para se obter um incremento do zooplâncton. Além disso, para um efetivo controle *top-down* do fitoplâncton seria necessária uma mudança na composição do zooplâncton para espécies maiores e adaptadas às defesas do fitoplâncton contra a predação, como tamanho, impalatabilidade e toxicidade (LACEROT et al., 2013).

Alguns estudos laboratoriais mostram que o zooplâncton tropical é capaz de controlar as cianobactérias. Por exemplo, pequenos cladóceros (*Moina micrura* e *Ceriodaphnia cornuta*) consumiram eficientemente pequenos filamentos de cianobactérias, enquanto os rotíferos (*Brachionus angularis*, *B. falcatus* e *Keratella* sp.) e o copépodo *Pseudodiaptomus hessei* consumiram um amplo espectro de tamanho de cianobactérias, no entanto, nenhuma espécie zooplanctônica foi eficiente no consumo de *Microcystis aeruginosa* (KÂ et al., 2012). Similarmente, a adição do cladócero de médio tamanho *Macrothrix spinosa* foi eficiente na redução de florações algais compostas por diferentes grupos morfofuncionais, incluindo cianobactérias coloniais (grupo VII) e filamentosas (grupo III) quando em menores biomassas (AMORIM et al., 2019b). Durante as florações de cianobactérias filamentosas, apesar do cladócero não reduzir as biomassas, ele foi eficiente na redução da taxa de crescimento do fitoplâncton e do tamanho médio dos filamentos, sugerindo que ele é capaz de cortar e consumir as partes menores (AMORIM et al., 2019b).

Em campo, as relações entre o zooplâncton e as cianobactérias podem ser mais complexas, considerando que algumas espécies zooplanctônicas apresentam seletividade alimentar, como os copépodos, sendo capazes de identificar suas presas através de mecanorreceptores e selecionar apenas as palatáveis (HEUSCHELE; SELANDER, 2014; COLINA et al., 2016). Essa seletividade foi demonstrada para o copépodo Calanoida *Notodiaptomus iheringi*, que favoreceu a dominância de *M. aeruginosa* ao consumir apenas criptófitas palatáveis (LEITÃO; GER; PANOSSO, 2018), e para o copépodo Cyclopoida *Thermocyclops decipiens* que favoreceu a dominância de *R. raciborskii* ao preferir predar uma diatomácea (GEBREHIWOT; KIFLE; TRIEST, 2019). Portanto, a pastagem seletiva do zooplâncton pode favorecer a dominância de cianobactérias, que terão vantagem competitiva através da redução dos organismos palatáveis (GER et al., 2016). O incremento da frequência e duração das florações de cianobactérias também pode levar a uma adaptação do zooplâncton, selecionando espécies que coexistam com as florações ao invés de consumir as cianobactérias (WILSON et al., 2006).

Apesar dos recentes avanços no campo da biomanipulação, ainda pouco se sabe sobre como a alteração das comunidades aquáticas pode controlar a eutrofização e o desenvolvimento de florações algais em regiões tropicais (JEPPESEN et al., 2007, 2012). Dentre as pesquisas de biomanipulação já desenvolvidas na região tropical, a introdução da tilápia do Nilo (peixe onívoro), causou uma redução dos cladóceros. No entanto, para um eficiente controle da biomassa fitoplanctônica, seria necessária uma remoção de peixes em larga escala (OKUN et al., 2008). A adição da tilápia em um lago do Rio Grande do Norte não foi capaz de reduzir o fitoplâncton, havendo um incremento na biomassa de *Raphidiopsis*, redução da maior parte do zooplâncton e da transparência da água (MENEZES; ATTAYDE; VASCONCELOS, 2010).

Em relação à biomanipulação do zooplâncton, o incremento destes organismos provocou uma redução do fitoplâncton do açude de Apipucos, um reservatório eutrófico de Pernambuco, mesmo em condições de limitação por nitrogênio e fósforo ou ausência de limitação. Por outro lado, houve um incremento na biomassa da cianobactéria potencialmente tóxica *R. raciborskii* (SEVERIANO et al., 2017). Além do mais, o incremento da biomassa zooplanctônica foi capaz de reduzir a biomassa de algumas cianobactérias em um reservatório eutrófico de Pernambuco, como *Aphanocapsa* sp., *Chroococcus* sp., *Dolichospermum* sp., *Merismopedia tenuissima*, *Microcystis aeruginosa* e *Pseudanabaena* sp., além da diatomácea *Cyclotella meneghiniana* e da criptofícea *Cryptomonas* sp. No entanto, nenhum efeito foi observado sobre as espécies de maior biomassa, como *R. raciborskii* e *Planktothrix agardhii*, sugerindo que a biomanipulação do zooplâncton pode não ser efetiva no controle de cianobactérias filamentosas (SEVERIANO et al., 2018).

Em um lago raso no Rio Grande do Norte, os peixes bentívoros foram capazes de ressuspender o sedimento e liberar o fósforo para coluna d'água e, consequentemente, provocar um aumento na clorofila *a*, enquanto peixes planctívoros favorecem o desenvolvimento do fitoplâncton através do consumo do zooplâncton. Esses resultados sugerem que a remoção de ambos os tipos de peixes é necessária para se obter a melhoria na qualidade da água de lagos rasos tropicais (DANTAS et al., 2019). Similarmente, mesmo a remoção de peixes bentívoros isoladamente ser um método eficiente na melhoria da qualidade da água, melhores resultados podem ser obtidos em combinação com a adição de policloreto de alumínio (ARAÚJO; BECKER; ATTAYDE, 2016).

2.6.2 Macrófitas submersas e seu potencial para controle das florações

Os ambientes aquáticos rasos podem apresentar dois estados alternativos, um de águas claras, sendo dominado por macrófitas submersas, e outro túrbido, caracterizado principalmente pela elevada biomassa fitoplanctônica, especialmente de cianobactérias (SCHEFFER et al., 1993; 2001). A alternância entre o fitoplâncton e macrófitas submersas é baseada em três princípios: (1) a turbidez aumenta com o incremento da biomassa algal em função da eutrofização; (2) a vegetação submersa reduz a turbidez; e (3) a vegetação submersa desaparece quando um nível crítico de turbidez é alcançado (SCHEFFER; VAN NES, 2007). Baseado nisso, para competir por luz e manter o estado claro dos lagos rasos, as plantas submersas podem liberar aleloquímicos que inibem o fitoplâncton (HILT; GROSS, 2008; BLINDOW; HARGEBY; HILT, 2014).

Outro fator que pode ser atribuído à atuação das macrófitas é a sua capacidade de reduzir a ressuspensão do sedimento e níveis de nutrientes da coluna d'água, além de fornecer refúgio ao zooplâncton, que atua na herbivoria do fitoplâncton (JEPPESEN et al., 1998; VAN DONK; VAN DE BUND, 2002; CAZZANELLI; WARMING; CHRISTOFFERSEN, 2008). No entanto, a elevada turbidez da água, proporcionada pelo crescimento fitoplanctônico, reduz o desenvolvimento das macrófitas devido à menor disponibilidade de luz na coluna d'água (TAKAMURA et al., 2003).

Diversos estudos mostram o potencial alelopático das macrófitas aquáticas sobre cianobactérias e microalgas em estudos laboratoriais, sendo que, até o momento, já se conhecem mais de 40 espécies de plantas aquáticas com potencial de suprimir espécies fitoplanctônicas, sendo as submersas mais eficientes (MOHAMED, 2017). No entanto, poucos estudos relatam os efeitos alelopáticos de macrófitas sobre o fitoplâncton em ambientes naturais, devido à atuação de outros fatores, como limitação por luz e nutrientes, competição e herbivoria.

O primeiro estudo a comprovar a presença de respostas alelopáticas de macrófitas sobre o fitoplâncton *in situ* foi o de Hilt, Ghobrial e Gross (2006), mostrando que *Myriophyllum verticillatum* inibiu o fitoplâncton, principalmente as cianobactérias. Os efeitos foram atribuídos à alelopatia por não haver diferença entre os outros parâmetros ambientais nos tratamentos controle e de coexistência com a macrófita. Em seguida, outro estudo revelou que a taxa de crescimento da comunidade fitoplanctônica foi significativamente inibida quando em contato com a macrófita submersa *Stratiotes aloides* (MULDERIJ; SMOLDERS; VAN DONK, 2006).

A introdução de *Ceratophyllum demersum* L. em um lago eutrófico subtropical inibiu o crescimento da cianobactéria *M. aeruginosa*, beneficiando na restauração do lago (DONG et al., 2014). Além disso, efeitos negativos sobre o fitoplâncton foram observados para as macrófitas *Egeria densa* Planch., *Potamogeton illinoensis* Morong e *Elodea nuttallii* (Planch.) St. John. Essas plantas inibiram o fitoplâncton pela competição por nutrientes, porém outros experimentos mostraram que a alelopatia também pode ter sido responsável pela redução do crescimento algal (VANDERSTUKKEN et al., 2011, 2014). A macrófita *Myriophyllum spicatum* é capaz de controlar mais eficientemente as populações de cianobactérias, sendo que cepas tóxicas e não tóxicas de *M. aeruginosa* são igualmente inibidas, causando uma maior taxa de produção de microcistinas (ŠVANYS; PAŠKAUSKAS; HILT, 2014).

Em outro estudo, cepas não tóxicas são mais sensíveis ao ácido tânico, mostrando que algo relacionado com a síntese das microcistinas confere uma maior tolerância aos aleloquímicos para as cepas tóxicas, uma vez que uma cepa deficiente para a síntese destas toxinas também se mostrou tolerante (ŠVANYS et al., 2016). Entretanto, uma cepa tóxica de *M. aeruginosa* foi mais sensível aos aleloquímicos de *S. aloides* que uma linhagem não tóxica (MULDERIJ et al., 2005). Com isso, os estudos de controle de cianobactérias que avaliam os efeitos das macrófitas sobre a razão entre cepas tóxicas e não tóxicas são escassos e controversos, demonstrando a necessidade da realização de mais estudos que esclareçam como se dá a relação entre cianobactérias tóxicas e não tóxicas com os demais organismos, e quais as implicações para o gerenciamento das florações.

No entanto, em coexistência, *Egeria densa* inibe significativamente cianobactérias tóxicas, enquanto estimula as não tóxicas, mostrando que a planta libera os aleloquímicos apenas sob estresse (AMORIM, 2017). Entretanto, nesse estudo, a cepa não tóxica foi mais sensível aos efeitos do extrato metanólico da macrófita, demonstrando seu potencial de utilização em estratégias de biomanipulação para controle de florações de cianobactérias tóxicas e não tóxicas. Subsequentemente, foi demonstrado que a macrófita submersa *Ceratophyllum demersum* é capaz de inibir mais significativamente o crescimento de uma cepa tóxica de *M. aeruginosa*, seguida de uma não tóxica de *M. panniformis*, através da liberação de substâncias alelopáticas, enquanto cepas das clorófitas *Ankistrodesmus falcatus* e *Raphidocelis subcapitata* foram menos afetadas (AMORIM et al., 2019a).

2.6.3 Estudos utilizando biomanipulação dos peixes e introdução de macrófitas submersas in situ para controle das florações subtropicais e tropicais

Estudos de biomanipulação ou de interação entre peixes, zooplâncton, macrófitas submersas e fitoplâncton são escassos em regiões subtropicais e tropicais. A princípio, sabe-se que nessas regiões as macrófitas são consideradas um pobre refúgio para o zooplâncton contra a predação por peixes, uma vez que peixes jovens podem se agregar densamente em todas as formas biológicas de macrófitas (MEERHOFF et al., 2006). Em lagos subtropicais da China (DONG et al., 2014) e do Uruguai (VANDERSTUKKEN et al., 2014), as macrófitas submersas foram responsáveis pela redução da biomassa fitoplanctônica, em especial cianobactérias, sendo a alelopatia considerada o principal mecanismo de ação responsável pelo controle algal.

Portanto, para uma maior eficiência da biomanipulação em regiões tropicais é necessário que sejam adotadas estratégias múltiplas, combinando diferentes manipulações nas comunidades aquáticas, como por exemplo a remoção de peixes e a introdução de macrófitas submersas. Uma maior eficiência dessa ação conjunta foi verificada em lagos que passaram pela biomanipulação na Dinamarca (SØNDERGAARD et al., 2017) e Japão (HA et al., 2013), onde a remoção dos peixes zooplanctívoros favoreceu o reaparecimento das macrófitas submersas, auxiliando na melhoria da qualidade da água.

Em lagos na China, a introdução de macrófitas submersas, associada com a introdução de peixes piscívoros e remoção dos planctívoros, contribuíram para a melhoria da qualidade da água, redução das concentrações de nutrientes e clorofila *a*, além de aumentar da transparência da água. Os efeitos *bottom-up*, mediados pela macrófita submersa, foram mais expressivos que os efeitos *top-down*, uma vez que a biomassa dos crustáceos planctônicos permaneceu inalterada e os peixes planctívoros voltaram a ser dominantes no ambiente. Entretanto, os autores não atribuíram os efeitos das macrófitas à alelopatia, uma vez que a razão entre a clorofila *a* e fósforo total permaneceu inalterada (YU et al., 2016; LIU et al., 2018).

Ainda existe um conhecimento limitado sobre o papel da alelopatia sobre a comunidade fitoplanctônica de ambientes naturais, e mesmo assim, nenhum estudo que comprove a alelopatia foi desenvolvido em regiões tropicais. Além disso, ainda não se conhecem quais os efeitos alelopáticos das macrófitas aquáticas sobre diferentes cenários de dominância de cianobactérias, como as cocoides, filamentosas heterocitadas e filamentosas não heterocitadas. Nesse sentido, se torna importante entender qual o papel da vegetação submersa sobre a dominância de diferentes espécies de cianobactérias, além de esclarecer qual o seu papel na dinâmica das interações tróficas de ambientes tropicais. Além disso, é necessário avaliar se a interação entre os efeitos alelopáticos das macrófitas submersas e da predação do zooplâncton é sinérgica, antagônica ou aditiva.

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ARTIGO 1:

Causas das Florações

3 ARTIGO 1

Modeling cyanobacterial blooms in tropical reservoirs: the role of physicochemical variables and trophic interactions

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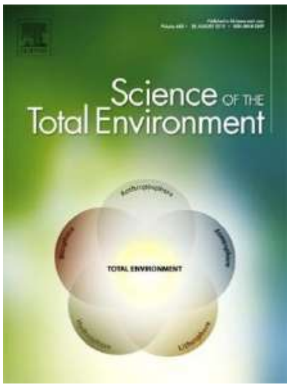
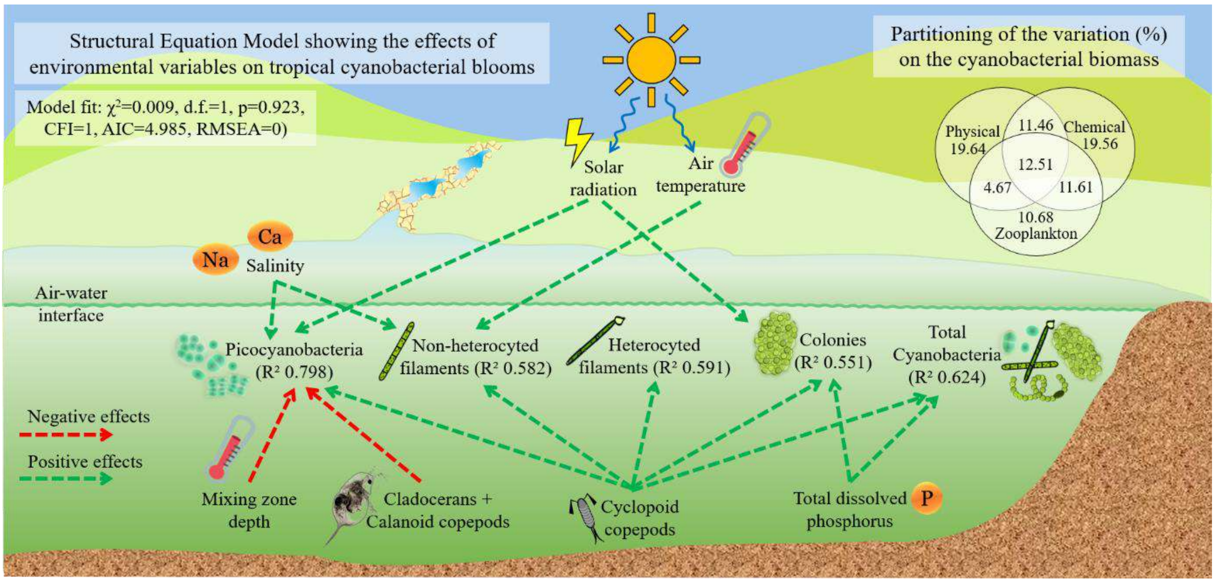


Graphical Abstract



Highlights

- Chemical and physical factors were the main drivers of tropical cyanobacterial blooms
- Omnivorous zooplankton and phosphorus better predicted the cyanobacterial blooms
- Eutrophication favored omnivorous zooplankton that coexists with tropical blooms
- Temperature and salinity hurt zooplankton with direct and indirect effects on blooms
- Our model will benefit in the management of tropical drinking water reservoirs

Abstract: Understanding the importance of environmental variables on the dominance of cyanobacteria is crucial for appropriately managing water resources. Although studies about temperate and subtropical regions show a high influence of nutrients and temperature on blooms, this relationship is still unclear for the tropics. Accordingly, we hypothesized that nutrients and temperature are the main factors driving cyanobacterial blooms in tropical reservoirs, and those relationships are intensified by the zooplankton. To test these hypotheses, we constructed a structural equation model based on the monitoring of ten reservoirs from Northeast Brazil. We analyzed the effects of physicochemical variables and zooplankton on cyanobacterial blooms and the biomass of four morphotypes. Cyanobacterial biomass varied within the reservoirs, with bloom records (0.2 - 268.4 mg L^{-1}) in all of them, primarily constituted by the colonial morphotype, followed by picocyanobacteria, heterocyted, and non-heterocyted filaments. The cyanobacterial community was driven mainly by chemical variables (55.14% of the variation), followed by physical (48.28%), and zooplankton (39.47%). Through the structural equation model, we demonstrated that total cyanobacterial biomass, as well as the morphotypes, were mainly influenced by omnivorous crustaceans and total dissolved phosphorus. Solar radiation, air temperature, mixing zone, and salinity were important to explain the biomass of the morphotypes. The model explained most of the variation in the picocyanobacterial blooms (79.8%), followed by total cyanobacteria (62.4%), heterocyted filaments (59.1%), non-heterocyted filaments (58.2%), and coccoids (55.1%). Zooplankton groups were also influenced by the physicochemical variables, which presented direct and indirect effects on cyanobacteria. Given the predictions of increased eutrophication, warming, and salinization, cyanobacterial blooms will be more intense in tropical reservoirs. Thus, restoring measures must be adopted to reduce bloom development, such as external phosphorus and salt loadings, and biomanipulation.

Keywords: Brazilian semiarid; Cyanobacterial morphotypes; Phosphorus; Salinity; Structural Equation Modeling; Zooplankton.

1. Introduction

The eutrophication process of aquatic systems, besides increasing temperature, results in a higher occurrence of potentially toxic cyanobacterial blooms, generating serious ecological and health problems (Pearl and Huisman, 2008). The extended proliferation of cyanobacteria induces severe consequences for the utilization of water resources, threatening other aquatic communities and human health through the release of cyanotoxins, including irritation and death of populations exposed to contaminated water (Carmichael and Boyer, 2016; Amorim et al., 2017).

Pioneering studies on phytoplankton modeling have shown that nutrients were the main factor driving cyanobacterial biomass (Paerl et al., 2001; Schindler, 2001). However, recent studies have shown increasing participation of temperature in predicting blooms (e.g. Zhang et al., 2018). The rising of nutrients and temperature interact concurrently to boost cyanobacterial growth (Kosten et al., 2012; Rigosi et al., 2015). In this sense, some authors proposed that climate change can worsen the effects of eutrophication on aquatic ecosystems (Moss et al., 2011; Jeppesen et al., 2020). Moreover, temperature can intensify cyanobacterial blooms directly by boosting cyanobacterial growth rates, or indirectly through thermal stratification and increased phosphorus release from sediments (Paerl and Huisman, 2008; Wagner and Adrian, 2009). Thus, understanding these forces and their consequences on the dominance of cyanobacteria is useful for determining which management strategies should be applied to each bloom-forming species, especially aiming to diminish the impacts of eutrophication and warming (Mantzouki et al., 2016; Shan et al., 2019).

Another regulating factor of cyanobacterial biomass is zooplankton grazing; however, its effects can vary depending on the climatic region. For example, in temperate regions, where zooplankton is represented mainly by large cladocerans (Jeppesen et al., 2012), cyanobacteria can be efficiently consumed (e.g. Ha et al., 2013; Ekvall et al., 2014). In tropical regions, zooplankton has little effect on cyanobacteria (Amorim and Moura, 2020), since this community is frequently composed of small rotifers and microcrustaceans (Ger et al., 2016; Amorim et al., 2019b). Moreover, some zooplankton groups present food selectivity and avoid consuming cyanobacteria (Leitão et al., 2018). Omnivorous zooplankton, such as cyclopoid copepods, can also significantly reduce microzooplankton biomass (e.g. rotifers, copepod nauplii, and ciliates), that could potentially graze on cyanobacteria during blooms (Rose et al., 2017). Furthermore, some morphological and physiological traits of cyanobacteria, including the formation of large colonies and filaments, nutritional deficiency, and production of toxins, can prevent from being consumed by zooplankton (Wilson et al., 2006).

In the tropics, cyanobacterial blooms are more problematic, persisting throughout the year due to the higher temperatures and irregular rainfall (Bouvy et al., 2000; Bittencourt-Oliveira et al., 2014). Meanwhile, blooms are restricted to a few times of the year in temperate regions, particularly in the summer (Harke et al., 2016). In Brazil, the Northeastern region presents more intense blooms, as this region has favorable conditions for the development of cyanobacteria, i.e., higher temperatures, salinity, and nutrients (Bouvy et al., 2000; Moura et al., 2018). These conditions are strengthened by the frequent drought events in the region (Marengo et al., 2017). Moreover, drought-induced water level reductions in Northeast Brazil significantly alter phytoplankton dynamics in lakes and reservoirs, increasing the dominance of cyanobacteria (Brasil et al., 2016).

Water level reductions and high evaporation rates, as consequences of droughts, can intensify the risk of salinization of aquatic ecosystems (Paerl and Paul, 2012). Salinization of freshwaters in drylands is a serious environmental problem, which is intensified by the agriculture and the increased demand for irrigation (Cañedo-Argüelles et al., 2013). Salinization can severely threaten freshwater ecosystems, reducing local biodiversity, multiple uses (Jeppesen et al., 2015), and zooplankton grazing on phytoplankton, including cyanobacteria (Beklioğlu et al., 2011). In Brazilian drylands, most of the inland waters are getting saltier, due to the high evaporation rates of surface waters, desertification process, the predominance (80%) of crystalline soils with high concentrations of salts, and the absence of soil drainage (Cirilo, 2008).

Given the influence of changing environmental parameters on cyanobacterial blooms, modeling approaches have been applied to predict and anticipate bloom occurrence. Recently, Rousso et al. (2020) reviewed the models applied to cyanobacterial blooms and showed that most of them are site-specific and without standardization, hindering generalizations. For instance, there are only six modeling studies for tropical aquatic ecosystems, most of them are lake-specific or taxon-specific (Recknagel et al., 2015; Bui et al., 2017; Magalhães et al., 2019; McHau et al., 2019). In the tropics, only Brasil et al. (2016) assessed the influence of environmental parameters on total cyanobacterial biomass in a wide geographical range (40 lakes in Northeast Brazil), through linear regression models.

Accordingly, this study aims to model the influence of physicochemical variables and trophic interactions with zooplankton on cyanobacterial blooms in tropical reservoirs. For that, we constructed a structural equation model to predict the influence of physical (solar radiation, air temperature, and mixing zone depth), chemical (total dissolved phosphorus, and salinity), and zooplankton (omnivorous and herbivorous crustaceans) variables on cyanobacterial blooms and the biomass of the picocyanobacterial, colonial, heterocyted, and non-heterocyted filament morphotypes. Furthermore, we tested two hypotheses: (i) nutrients and temperature are the main factors that favor increased cyanobacterial biomass in tropical aquatic ecosystems; (ii) the correlation between cyanobacterial biomass and physicochemical variables is intensified by the zooplankton. Therefore, we modeled for the first time the isolated and interactive role of meteorological, physical, chemical, and zooplankton variables on cyanobacterial blooms, composed of different potentially toxic bloom-forming taxa, in reservoirs exclusively from tropical regions.

2. Material and Methods

2.1. Study area

Limnological, meteorological, and biological data were collected from ten reservoirs of Pernambuco state, Northeast Brazil. The reservoirs are distributed in the phytogeographic regions "Zona da Mata" (4), "Agreste" (4), and "Sertão" (2) (Fig. A.1), most of them are inserted in the semiarid or dry sub-humid region. These environments were selected based on the trophic state, being classified as oligotrophic (1), mesotrophic (2), eutrophic (2), and hypereutrophic (5) (Table 1). All reservoirs are used for public supply, irrigation for agriculture, livestock, fishing, among other uses. All of them are inserted in agricultural landscapes.

2.2. Sampling and analyses

Samples were collected quarterly for seasonal monitoring of physicochemical variables, zooplankton, and cyanobacterial biomass between October 2017 and January 2019, comprising an annual cycle, with four or five samples collected in the dry and rainy periods, in each reservoir. Samples were collected at a point close to the dam and the deepest location, always in the morning (08:00 to 12:00). Depth was checked with an echo sounder, water transparency with a Secchi disk, and light intensity with a photometer (LI-COR model LI-250) on the subsurface (20 cm). Water temperature, salinity, and pH were analyzed in situ with a HANNA multiparametric probe. Water temperature was checked every 0.5 m in the water column to estimate the mixing zone depth, established when there was a difference greater than 0.5 °C at each 0.5 m depth. Daytime averages of air temperature and solar radiation were obtained from the nearest weather stations (located less than 30 km from the reservoirs), from the databases of the National Institute of Meteorology, Brazil (INMET, 2019).

Table 1. List of the ten tropical reservoirs selected for this study, their phytogeographical regions in Northeast Brazil, geographical coordinates, storage capacity, and trophic state.

Reservoir - abbreviation	**Phytogeographical region**	**Geographical location**	**Storage (10^3 m^3)[a]**	**Trophic state[b]**
Carpina-CAR	Zona da Mata	7°53'43.3"S; 35°20'26.2"W	270,000	Hypereutrophic
Cursaí-CUR	Zona da Mata	7°52'36.6"S; 35°10'45.8"W	13,034	Oligotrophic
Goitá-GOI	Zona da Mata	7°58'14.5"S; 35°07'00.8"W	52,536	Eutrophic
Tapacurá-TAP	Zona da Mata	8°02'39.4"S; 35°11'52.0"W	104,871	Hypereutrophic
Cajueiro-CAJ	Agreste	8°58'09.2"S; 36°28'48.7"W	19,283	Mesotrophic
Ipojuca-IPO	Agreste	8°20'49.4"S; 36°22'35.2"W	29,336	Hypereutrophic
Mundaú-MUN	Agreste	8°56'33.7"S; 36°29'23.7"W	1,969	Hypereutrophic
Tabocas-TAB	Agreste	8°14'54.9"S; 36°22'38.4"W	1,168	Mesotrophic
Cachoeira-CAC	Sertão	7°58'18.9"S; 38°19'25.8"W	21,031	Eutrophic
Serrinha-SER	Sertão	8°11'41.0"S; 38°31'19.6"W	311,080	Hypereutrophic

[a]Source: APAC (2019); [b]Data from the present study.

The concentrations of nutrients, such as nitrite (NO_2), nitrate (NO_3), ammonia (NH_4), dissolved inorganic nitrogen (DIN), orthophosphate (PO_4), total dissolved phosphorus (TDP), and total phosphorus (TP), were determined according to specific methods (Strickland and Parsons, 1965; Golterman et al., 1971; Valderrama, 1981). The DIN:TDP ratio was calculated based on the molar concentrations of dissolved inorganic nitrogen and total dissolved phosphorus. Samples used for chlorophyll-*a* quantification were filtered through glass fiber filters with 0.45 µm porosity and analyzed according to Chorus and Bartram (1999). The trophic state index of each reservoir was estimated using the annual geometric means of chlorophyll-*a* and total phosphorus (Cunha et al., 2013).

Samples collected through surface trawls with a 25 µm mesh-size plankton net were fixed with 4% formaldehyde and analyzed to identify cyanobacteria, following specialized literature. Samples used to quantify cyanobacteria were collected from the subsurface, preserved with acetic lugol, and analyzed under an inverted microscope (Utermöhl, 1958). The species' biovolume was determined according to Hillebrand et al. (1999) for later conversion to biomass (mg L^{-1}). Cyanobacterial communities were classified according to the presence and intensity of blooms following the criteria of the World Health Organization (WHO) in surveillance level (absence of blooms, biomass below 0.2 mg L^{-1}), alert level 1 (biomass between 0.2 and 10 mg L^{-1}), and alert level 2 (biomass greater than 10 mg L^{-1}) (Chorus and Bartram, 1999). Species with a relative abundance greater than 50% of total cyanobacterial biomass were classified as dominant, and those with relative biomass greater than 20% were codominant. Cyanobacterial species were grouped into four morphotypes: colonies, heterocyted filaments, non-heterocyted filaments, and picocyanobacteria.

For identification and quantification of zooplankton, 100 L of water from the reservoirs were filtered with a 60 µm mesh-size plankton net. The samples were then fixed in 4% formaldehyde and analyzed in a 1 mL Sedgewick-Rafter chamber (three subsamples per sample) under an optical microscope. Biomass (µg L^{-1}) was estimated according to Ruttner-Kolisko (1977) for rotifers and Dumont et al. (1975) for microcrustaceans. The biomasses were grouped into rotifers, copepod nauplii, calanoid copepods, cyclopoid copepods, and cladocerans.

2.3. Statistical analyses

All statistical analyses were performed using the statistical program R version 3.5.2, with a significance level of 5% ($p < 0.05$) (R Core Team, 2019). Spearman correlation was used to verify the relationships between the environmental variables with total cyanobacterial biomass, and the biomass of the four morphotypes. Partial Canonical Correspondence Analysis (pCCA) and Canonical Correspondence Analysis (CCA) were performed using the vegan package, that has tools for descriptive and multivariate analyses in community ecology (Oksanen et al., 2018). Environmental variables were standardized using the range function, while cyanobacterial biomass was transformed ($\log(x+1)$). Only species with total biomass greater than 5 mg L^{-1} in all occurrences were selected.

The variables were divided into four matrices: physical (depth, water transparency, light intensity, water temperature, mixing zone to depth ratio, air temperature, and solar radiation), chemical (pH, salinity, nitrite, nitrate, ammonia, dissolved inorganic nitrogen, orthophosphate, total dissolved phosphorus, total phosphorus, and DIN:TDP ratio), zooplankton (rotifers, copepod nauplii, cyclopoid copepods, calanoid copepods, and cladocerans), and cyanobacterial biomass. Mixing zone to depth ratio was used as a proxy of thermal stratification. Thereafter, a pCCA was performed to define the explanation of each set in the variation of cyanobacteria, considering that the length of the first axis of the Detrended Correspondence Analysis (DCA) was greater than 3. A CCA was performed to explore the relationships between cyanobacteria and environmental factors, selected with the stepwise function and with inflation below 20 to avoid collinearity, verified with the vif.cca function. The significance of the models, as well as variables and axes, was tested with anova.cca function.

2.4. Structural equation modeling

A structural equation model was generated to investigate the direct and indirect relationships between cyanobacterial biomass and the four morphotypes with the environmental variables, based on a conceptual model. For that, we used the statistical program R version 3.5.2, with a significance level set at $p < 0.05$ (R Core Team, 2019). Additionally, we used the packages lavaan.survey (Oberski, 2014), to create the model, and semPlot (Epskamp, 2019), to graphically represent the main paths in the model.

For the conceptual model, solar radiation, air temperature, mixing zone depth, salinity, total dissolved phosphorus, omnivorous crustaceans (cyclopoid copepods), and herbivorous crustaceans (the sum of nauplii, calanoid copepods, and cladocerans) were considered as latent variables. We assumed that besides influencing cyanobacterial biomass, zooplankton groups were also influenced by the other predictor variables. These variables were selected considering that photothermal processes (Paerl and Huisman, 2008; Zhang et al., 2018), eutrophication (Qin et al., 2019), and zooplankton (Ger et al., 2016) are among the most important variables explaining cyanobacterial blooms, in addition to salinity that plays a significant role in dryland regions (Jeppesen et al., 2015) and agriculture landscapes (Cañedo-Argüelles et al., 2013) (Fig. A.2).

For the structural equation model, all variables were transformed ($\log(x+1)$) to meet the normality criteria. The Satorra-Bentler index was used to calibrate the model with several permutations, recommended for when sampling effort is small (Satorra and Bentler, 2001). Model fit and validation (goodness-of-fit) were assessed using the chi-square (χ^2) and p-value ($p > 0.05$). Lower χ^2 and higher p-values indicate that the model does not differ from the proposed conceptual model, therefore, it fits with reality and data collected in nature (Hopcraft et al., 2012). We also used the Comparative Fit Index (CFI) and the Akaike Information Criteria (AIC) to assess model fit (Hu and Bentler, 1995). The Root Mean Square Error of

Approximation (RMSEA) was used to evaluate the model error or potential bias (Rousso et al., 2020). In the final model, the values of standard coefficients between explanatory and dependent variables, R^2 of each dependent variable, and correlations between variables are displayed. A summary of all methodology steps applied to select the reservoirs, environmentally relevant variables, definition of the conceptual model, and the creation, calibration, and validation of the structural equation model is shown in Fig. A.2.

3. Results

3.1. Physicochemical variables, zooplankton, and cyanobacterial biomass

The studied reservoirs presented greater variability in physicochemical variables, the concentration of nutrients, and biomass of zooplankton groups (Table A.1). Cyanobacterial biomass varied from 0.02 mg L^{-1}, in Goitá reservoir, to 268.4 mg L^{-1}, in Tapacurá. Cyanobacterial blooms classified at WHO level 2 (biomass greater than 10 mg L^{-1}) were recorded in five reservoirs, while the others presented blooms at WHO level 1 (biomass between 0.2 and 10 mg L^{-1}) or at surveillance level (Fig. 1a; Table 2).

Most of the reservoirs (Carpina, Cursaí, Tapacurá, and Cajueiro) presented the dominance of colonies, while Ipojuca reservoir presented the dominance of picocyanobacteria with some periods of codominance with colonies. Cyanobacterial blooms at Serrinha, Mundaú, and Goitá were dominated by the colonies, with periods of codominance with heterocyted filaments, non-heterocyted filaments, and picocyanobacteria. Tabocas reservoir presented the dominance of picocyanobacteria and the codominance of colonies, while Cachoeira showed a codominance between picocyanobacteria and heterocyted filaments, or the isolated dominance of colonies and heterocyted filaments. (Fig. 1b, Fig. A.3). Dominant cyanobacterial species from each reservoir are described in Table 2.

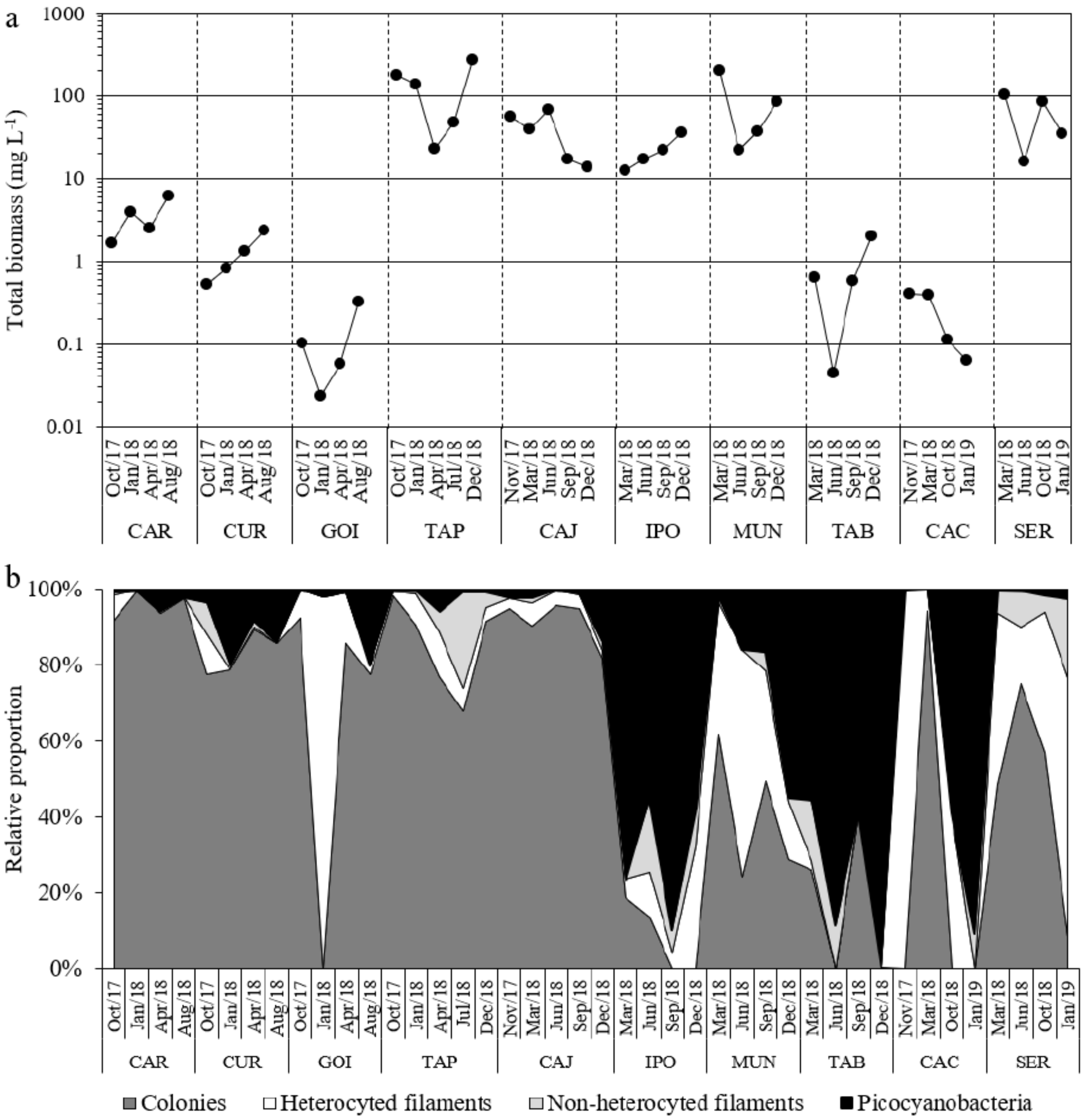


Fig. 1. Seasonal variation in cyanobacterial biomass (a) and the relative contribution of the colonial, heterocyted filament, non-heterocyted filament, and picocyanobacteria morphotypes (b) in the tropical reservoirs studied. See Table 1 for reservoir abbreviations.

Table 2. Classification of cyanobacterial blooms based on World Health Organization - WHO criteria and dominant cyanobacteria species registered in each reservoir studied. SL: WHO surveillance level; L1: WHO alert level 1; L2: WHO alert level 2.

Reservoir	**WHO level**	**Dominant species**
Carpina	L1	*Microcystis protocystis* W.B.Crow
Cursaí	L1	*Microcystis protocystis*
Goitá	L1; SL	*Microcystis aeruginosa* (Kützing) Kützing; *Radiocystis fernandoi* Komárek & Komárková-Legnerová; *Raphidiopsis raciborskii* (Woloszynska) Aguilera et al.
Tapacurá	L2	*Microcystis panniformis* Komárek et al.
Cajueiro	L2	*Microcystis aeruginosa*
Ipojuca	L2	*Aphanocapsa incerta* (Lemmermann) G.Cronberg & Komárek
Mundaú	L2	*Microcystis flosaquae* (Wittrock) Kirchner; *Merismopedia tenuissima* Lemmermann
Tabocas	L1; SL	*Aphanocapsa delicatissima* West & G.S.West; *Aphanocapsa holsatica* (Lemmermann) G.Cronberg & Komárek
Cachoeira	L1; SL	*Aphanocapsa delicatissima*; *Microcystis aeruginosa*; *Sphaerospermopsis aphanizomenoides* (Forti) Zapomelová et al.
Serrinha	L2	*Microcystis panniformis*; *Raphidiopsis raciborskii*

3.2. Drivers of cyanobacterial biomass

Through the Spearman correlation, we verified that water transparency negatively influenced total cyanobacteria and the four morphotypes, while pH, total phosphorus, and cyclopoid copepods positively impacted them. Depth and rotifers negatively and positively correlated with total cyanobacteria and all morphotypes except colonies, respectively. Copepod nauplii, total zooplankton, orthophosphate, and total dissolved phosphorus positively affected total cyanobacteria, colonies, and heterocyted filaments, the latter variable also positively influenced the non-heterocyted filaments. Salinity showed a positive relationship with total cyanobacteria, heterocyted filaments, and picocyanobacteria, while DIN:TDP ratio was negatively associated with total cyanobacteria, heterocyted, and non-heterocyted filaments.

Moreover, mixing zone depth, calanoid copepods, and cladocerans presented a negative correlation with picocyanobacteria (Fig. 2).

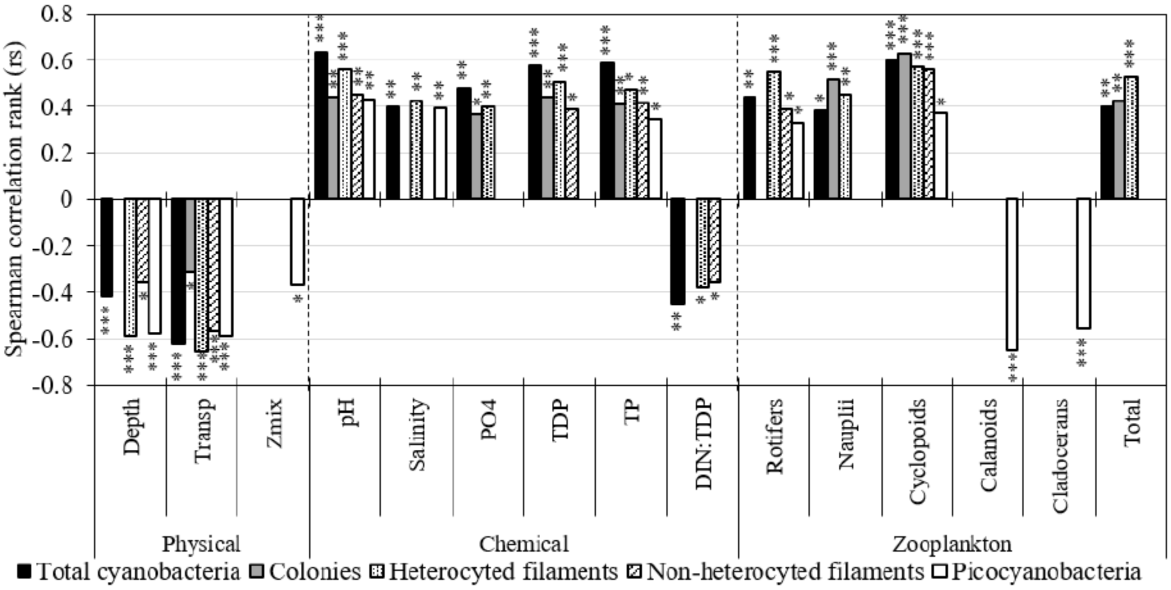


Fig. 2. Spearman correlation rank (rs) between physical (depth, water transparency - Transp, and mixing zone depth - Zmix), chemical (pH, salinity, orthophosphate - PO4, total dissolved phosphorus - TDP, total phosphorus - TP, and DIN:TDP ratio), and zooplankton (rotifers, copepod nauplii, cyclopoid copepods, calanoid copepods, cladocerans, and total zooplankton) variables with total cyanobacteria, colonies, heterocyted filaments, non-heterocyted filaments, and picocyanobacteria in the tropical reservoirs studied. Only significant correlations are shown (*: p <0.05; **: p <0.01; and ***: p <0.001).

Partial canonical correspondence analysis revealed that the chemical variables (55.14%) better explained cyanobacterial biomass, followed by the physical (48.28%), and zooplankton (39.47%). The shared explanation of the three sets of variables was greater than all other interactions and the isolated explanation of zooplankton, suggesting synergistic relationships between variables. Among data sets, greater interactions were found between chemical variables and zooplankton, followed by physical and chemical variables (Fig. 3).

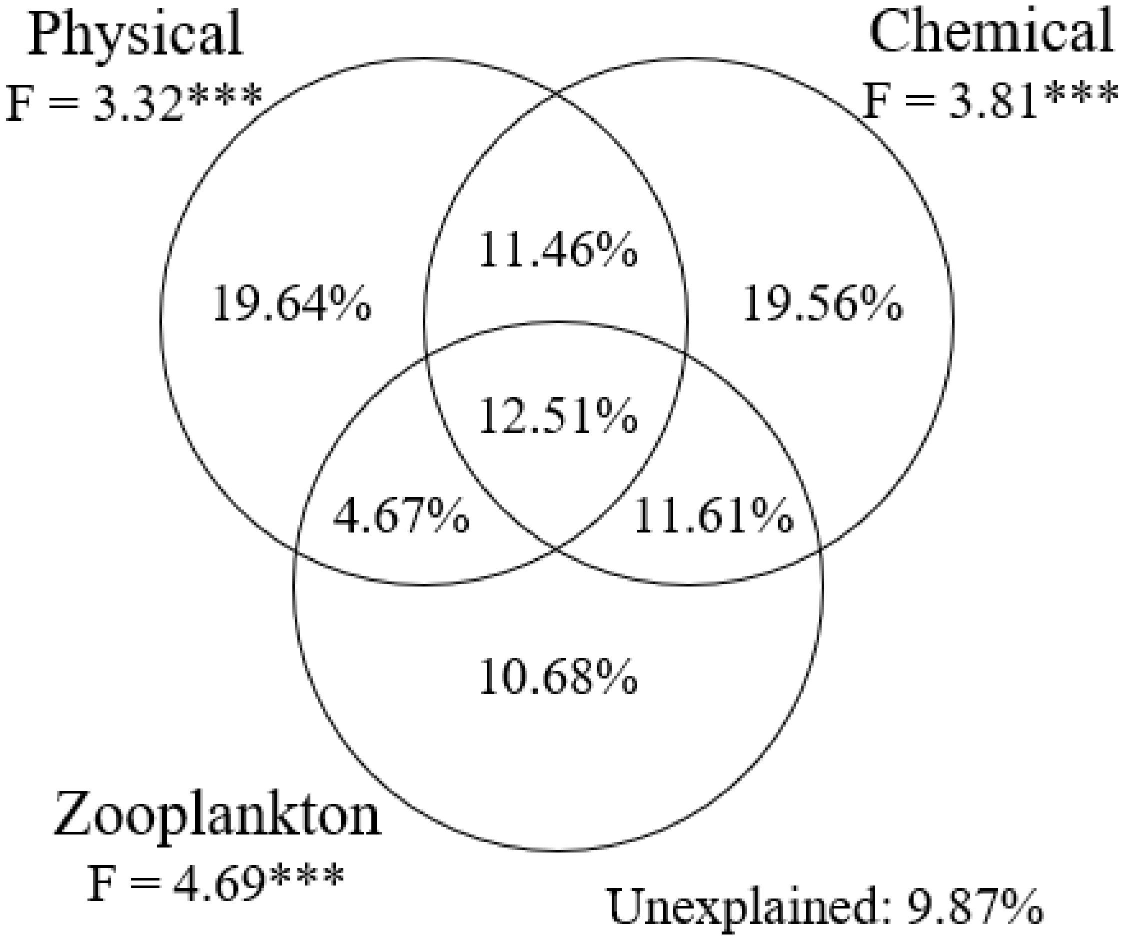


Fig. 3. Partial Canonical Correspondence Analysis (pCCA) showing the partitioning of the variation (%) of the cyanobacterial biomass explained by the sets of physical, chemical, and zooplankton variables, in the tropical reservoirs studied. The overlapping areas represent the interaction between the data sets. Asterisks represent significant effects verified with the anova.cca function (***: $p < 0.001$).

Canonical correspondence analysis generated a significant model (Inertia = 2.669; F = 6.67; $p < 0.001$) capable of explaining 80.32% of the cyanobacteria distribution. The model presented six significant axes ($p < 0.01$), with most of the variation explained by the first (23.04%) and second (17.63%) axes. Fifteen variables significantly influenced the structure of cyanobacteria. In axis 1, salinity ($r = 0.86$) influenced the cyanobacterial structure on the positive side, while depth ($r = -0.71$), cladocerans ($r = -0.51$), light intensity ($r = -0.42$), cyclopoid copepods ($r = -0.47$), mixing zone to depth ratio ($r = -0.31$), and calanoid copepods (r = -0.53) influenced on the negative side. For axis 2, nitrate (r = 0.27), DIN:TDP ratio (r = 0.46), and copepod nauplii (r = 0.45) showed a positive correlation with cyanobacterial structure, while air temperature (r = -0.57) correlated with cyanobacteria on the negative side of the axis. Water transparency (r = 0.57), total dissolved phosphorus (r = 0.50), pH (r = -0.38), and rotifers (r = -0.59) influenced cyanobacteria in the other four significant axes (Fig. 4).

Samples with the dominance of picocyanobacteria (Ipojuca, Tabocas, and two samples from Cachoeira reservoir) were restricted to the positive side of the first CCA axis, while blooms of colonies tended to be clustered on the negative side. The second axis tended to separate cyanobacterial communities dominated by a single bloom-forming morphotype (Carpina, Cursaí, Tapacurá, and Ipojuca), usually by the colonies, on the negative side. Meanwhile, most of the cases of codominance between the morphotypes were clustered on the positive side of the second axis (Fig. 4a).

Regarding the dominant species, the colonial *Microcystis aeruginosa* (Kützing) Kützing correlated with copepod nauplii and cyclopoid copepods; the colonial *M. flosaquae* (Wittrock) Kirchner, the heterocyted filamentous *Raphidiopsis raciborskii* (Woloszynska) Aguilera et al., and the picocyanobacteria *Aphanocapsa delicatissima* and *M. tenuissima* Lemmermann were influenced by pH, nitrate, and DIN:TDP ratio; the colonial *M. panniformis* Komárek et al. and *M. protocystis* W.B.Crow, and the heterocyted filamentous *Sphaerospermopsis aphanizomenoides* (Forti) Zapomelová et al. correlated with depth, light intensity, mixing zone to depth ratio, air temperature, water transparency, cladocerans, and calanoid copepods; the picocyanobacteria *A. incerta* (Lemmermann) G.Cronberg & Komárek was affected by total dissolved phosphorus, rotifers, and salinity (Fig. 4b).

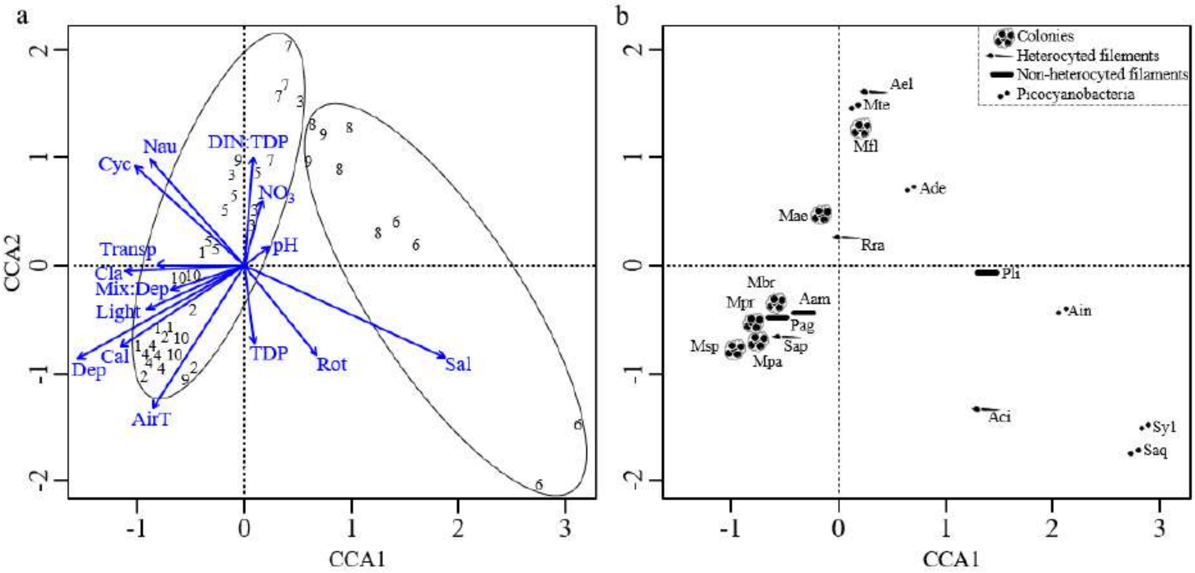


Fig. 4. Canonical correspondence analysis between cyanobacterial biomass and environmental variables in the tropical reservoirs studied, showing the distribution of the samples (a) and species (b). In Figure 4a, left and right ellipses indicate the samples with the dominance of colonies and picocyanobacteria, respectively. Reservoirs: 1 – Carpina, 2 – Cursaí, 3 – Goitá, 4 – Tapacurá, 5 – Cajueiro, 6 – Ipojuca, 7 – Mundaú, 8 – Tabocas, 9 – Cachoeira e 10 – Serrinha. Dep – depth, Transp – water transparency, Light – light intensity, Mix:Dep – mixing zone to depth ratio, AirT – air temperature, pH – pH, Sal – salinity, NO_3 – nitrate, TDP – total dissolved phosphorus, DIN:TDP – DIN:TDP ratio, Cal – calanoids, Cyc – cyclopoids, Cla – cladocerans, Rot – rotifers, Nau – copepod nauplii. Aam – *Anagnostidinema amphibium*, Aci – *Anabaenopsis circularis*, Ael – *A. elenkinii*, Ade – *Aphanocapsa delicatissima*, Ain – *A. incerta*, Mte – *Merismopedia tenuissima*, Mae – *Microcystis aeruginosa*, Mbr – *M. brasiliensis*, Mfl – *M. flosaquae*, Mpa – *M. panniformis*, Mpr – *M. protocystis*, Msp – *Microcystis* sp. (single cells), Pli – *Planktolyngbya limnetica*, Pag – *Planktothrix agardhii*, Rra – *Raphidiopsis raciborskii*, Sap – *Sphaerospermopsis aphanizomenoides*, Saq – *Synechocystis aquatilis*, Sy1 – *Synechococcus* sp.1.

3.3. Structural equation modeling

We created a robust structural equation model applicable to the nature of the tropical environments studied herein ($\chi^2 = 0.009$; d.f. = 1; p = 0.923; CFI = 1; AIC = 4.985; RMSEA = 0) (Fig. 5, Table A.2). Total dissolved phosphorus showed a positive relationship with total cyanobacteria and the colonies. Salinity positively influenced the non-heterocyted filaments and picocyanobacteria, while air temperature positively affected the non-heterocyted filaments.

Solar radiation positively correlated with picocyanobacteria and the colonies, while mixing zone depth negatively impacted picocyanobacteria. The omnivorous and herbivorous crustaceans were positively influenced by total dissolved phosphorus, and negatively by salinity, while air temperature negatively affected omnivores, and mixing zone depth positively correlated with herbivores. Omnivorous crustaceans positively influenced cyanobacterial biomass and the four morphotypes. Meanwhile, herbivores negatively impacted picocyanobacteria, only.

Regarding the variation explained by the model for each cyanobacterial group, the model explained 79.8% of the variation in picocyanobacteria and 62.4% in total cyanobacterial biomass, with higher R^2 values, followed by the heterocyted filaments (59.1%), non-heterocyted filaments (58.2%), and colonies (55.1%). On the other hand, the variation in zooplankton biomass explained by the model was lower than for cyanobacteria, which accounted for 51.9% of the variation in omnivorous crustaceans, and only 29.1% in herbivorous crustaceans. In terms of the correlation between latent variables, the model showed a strong positive association between air temperature and solar radiation, as well as between salinity and total dissolved phosphorus. Positive correlations were also found between total cyanobacteria and all morphotypes, except picocyanobacteria (Fig. 5).

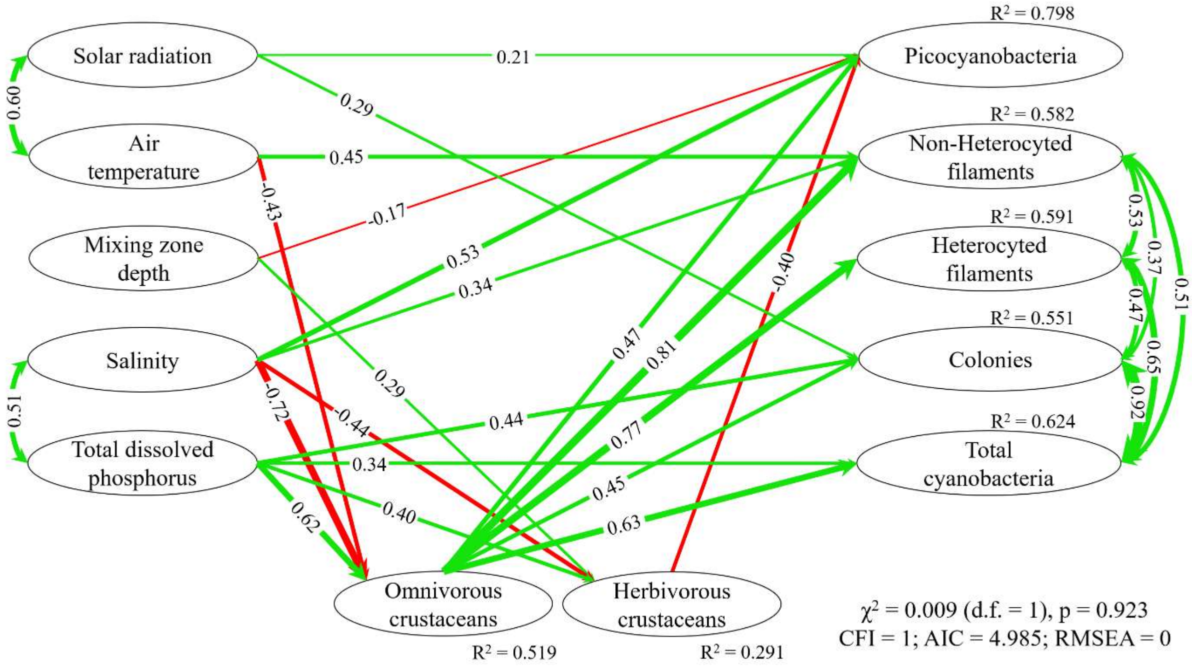


Fig. 5. Structural equation model evaluating the influence of physical (solar radiation, air temperature, and mixing zone depth), chemical (salinity and total dissolved phosphorus), and zooplankton (omnivorous and herbivorous crustaceans) variables on total cyanobacteria, colonies, heterocyted filaments, non-heterocyted filaments, and picocyanobacteria biomass in tropical reservoirs. Arrows between latent variables represent paths with a significant relationship ($p < 0.05$), which can be positive (green arrows) or negative (red arrows). The lines' thickness represents the intensity of the standard coefficients.

4. Discussion

The statistical approach used in our study allowed us to verify the dependence of cyanobacterial biomass and different morphotypes on chemical, physical, and zooplankton variables in tropical reservoirs. A structural equation model proved to be an efficient tool for predicting cyanobacterial biomass, presenting high adjustment, R^2, and standard coefficients, besides no potential error or bias. We verified a strong dependence of cyanobacteria on total dissolved phosphorus and the abundance of omnivorous crustaceans. Besides those variables, cyanobacterial morphotypes were also influenced by solar radiation, air temperature, mixing zone depth, salinity, and herbivorous crustaceans.

4.1. Drivers of cyanobacterial blooms in tropical reservoirs

The partial canonical correspondence analysis revealed that physical and chemical variables were the main drivers of cyanobacterial blooms in tropical reservoirs. Furthermore, greater interactions were observed between chemical variables and zooplankton, and between physical and chemical variables. Accordingly, synergistic interactions between zooplankton and nutrients (e.g. Shurin et al., 2012), and between temperature and nutrients (e.g. Rigosi et al., 2015) have also been demonstrated to affect cyanobacteria and phytoplankton biomass.

With the Spearman correlation and canonical correspondence analyses, we observed that depth negatively correlated to cyanobacterial biomass, suggesting that shallower reservoirs can have higher cyanobacterial biomass, as verified by Kosten et al. (2012). The water transparency showed negative correlations with cyanobacterial biomass and the four morphotypes, representing feedback from the blooms, in which high biomass increases the turbidity. Positive correlations with pH may also be a consequence of the development of cyanobacterial biomass, with a consequent intensification of photosynthetic rates, also increasing the pH. However, pH also determines the concentrations of carbon forms in the water. When pH is higher than 8 (in most eutrophic environments), bicarbonate (HCO_3^-) becomes the main source of available carbon, promoting cyanobacteria (Brito et al., 2018).

The DIN:TDP ratio negatively affected the biomass of total cyanobacteria, indicating that environments potentially limited by phosphorus, are unfavorable for the growth of cyanobacteria. This trend is confirmed by the positive correlation between phosphorus (PO_4, TDP, and TP) and cyanobacterial biomass. Indeed, eutrophication has been recognized as the main regulating factor of cyanobacteria dominance for decades (Shindler et al., 2001; Qin et al., 2019). Among nitrogen forms, only nitrate influenced the cyanobacterial community through the canonical correspondence analysis. In this sense, nitrifying bacteria play an important role in nitrate production in aquatic systems (Sepehri and Sarrafzadeh, 2018). Moreover, heterotrophic bacteria are favored during cyanobacterial blooms, which play an important role in the microbial loop and nutrient cycles (Haraldsson et al., 2018).

The structural equation model showed that omnivorous crustaceans (cyclopoid copepods) were the main factor regulating cyanobacterial biomass and the different morphotypes, followed by total dissolved phosphorus. These two factors also interacted positively in favoring blooms, as also verified by the synergistic relationship between chemical variables and zooplankton in the partial canonical correspondence analysis. Long-term experiments showed that increased eutrophication can increase zooplankton biomass (Shurin et al., 2012). However, there is evidence that cyanobacterial blooms may select smaller zooplankton species that can coexist with cyanobacteria instead of consuming them (Wilson et al., 2006), which justifies the positive relationship between total dissolved phosphorus, omnivorous crustaceans, and cyanobacterial biomass found herein.

Besides cyclopoid copepods, other small zooplankton, such as rotifers and copepod nauplii, also positively correlated with cyanobacteria and the morphotypes in the Spearman correlation analysis, indicating their coexistence with blooms. Smaller zooplankton species are less sensitive to feeding inhibition by blooms composed of large colonial and filamentous cyanobacteria, because they can graze on smaller particles, such as other algae, ciliates, and bacteria (Moustaka-Gouni and Sommer, 2020). Moreover, these alternative food sources are more abundant in eutrophic waters with cyanobacterial blooms, which make blooms “less of a trophic dead end than commonly assumed” (Haraldsson et al., 2018).

Another factor that favors the coexistence of omnivorous crustaceans with cyanobacterial blooms is their ability to select palatable prey through mechanoreceptors (Colina et al., 2016), as shown for *Thermocyclops decipiens*, which consumed palatable prey instead of the filamentous cyanobacterium *Raphidiopsis raciborskii* (Gebrehiwot et al., 2019). Furthermore, herbivorous crustaceans, such as calanoid copepods (the most abundant zooplankton group in our study), can also select their prey, which is a mechanism that favors their coexistence with dense blooms of *Microcystis* (Leitão et al., 2018; Shan et al., 2019). The selective predation exerted by zooplankton can favor the dominance of cyanobacteria by reducing their competitors (Ger et al., 2016). Moreover, blooms of colonial *Microcystis* block the biomass transference to the zooplankton (Filstrup et al., 2014), justifying the absence of effects by herbivorous crustaceans on total cyanobacteria and colonial morphotype. Herbivorous crustaceans negatively affected picocyanobacteria, demonstrating that this group of zooplankton can graze on this morphotype despite their low palatability. Indeed, picocyanobacteria are considered excellent prey for non-selective zooplankton due to their small size (Callieri et al., 2012).

The model showed that picocyanobacteria and colonies were positively influenced by solar radiation, and the non-heterocyted filaments by air temperature, suggesting that the increase in these conditions may favor the development of these cyanobacterial groups. Mixing zone depth, an inverse measure of thermal stratification, negatively correlated with picocyanobacteria, indicating a positive influence of stratification on this morphotype. The influence of photothermal processes in promoting cyanobacterial blooms is well recognized (e.g. Zhang et al., 2018). Air temperature can favor cyanobacterial blooms directly by boosting growth rates, or indirectly through thermal stratification (Paerl and Huisman, 2008). Thermal stratification can further favor the proliferation of buoyant taxa (Wagner and Adrian, 2009) and picocyanobacteria (Callieri et al., 2012). Moreover, air temperature also negatively affected omnivorous crustaceans, whilst mixing zone depth positively impacted the herbivores, demonstrating a negative influence of temperature and thermal stratification on zooplankton, and thus contributing indirectly to promote cyanobacterial blooms.

The model showed a positive influence of salinity on non-heterocyted filaments and picocyanobacteria, while the Spearman correlation revealed its positive effects on total cyanobacteria and heterocyted filaments. As seen in the structural equation model, salinity interacted positively with total dissolved phosphorus to promote cyanobacterial blooms. Indeed, salinity and nutrients have synergistic effects on cyanobacterial biomass (Pearl and Paul, 2012), as many freshwater bloom-forming taxa are salt-tolerant, particularly the genera *Anabaenopsis*, *Dolichospermum*, *Microcystis*, *Oscillatoria*, and *Synechococcus* (Paerl et al., 2016), especially the picocyanobacteria (Callieri et al., 2012). Salinization can further worsen the symptoms of eutrophication in lakes, which tend to be more turbid than freshwater lakes in the same concentration of nutrients (Jeppesen et al., 1994). Another important consequence of salinization is the reduction of zooplankton abundance and grazing rates (Beklioğlu et al., 2011). This fact was verified by the structural equation model in our study, where zooplankton groups were damaged by high salinity levels. Furthermore, herbivorous crustaceans had less influence on cyanobacteria, with negative impacts on picocyanobacteria only.

Total cyanobacteria and all morphotypes, except picocyanobacteria, were positively correlated with each other, suggesting a codominance pattern between them. Indeed, most of cyanobacterial blooms presented higher relative abundances of the colonies, heterocyted, and/or non-heterocyted filaments. Picocyanobacteria usually presented isolated dominance across the reservoirs studied. This morphotype usually forms prolonged blooms, with numerous impacts on aquatic ecosystems (Śliwińska-Wilczewska et al., 2018). Due to their small size and high surface to volume ratio, picocyanobacteria have higher affinity and maximum uptake rates of dissolved phosphorus than other cyanobacteria and eukaryotic algae (Moutin et al., 2002). Moreover, picocyanobacteria can produce potent allelochemicals against other phytoplankton species, constituting one of the major competitive strategies that enhance their dominance in lakes (Bubak et al., 2020; Konarzewska et al., 2020). These factors probably reduced the codominance of other cyanobacterial morphotypes during picocyanobacterial blooms in our study.

4.2. The future of cyanobacterial blooms in tropical reservoirs

Our model can be used for future predictions of cyanobacterial biomass in tropical lakes and reservoirs, especially those used for public supply. Although most generalized models show low performance than single-site models (Rousso et al., 2020), the model proposed herein presented high adjustment metrics (χ^2 <0.01; d.f. = 1; p >0.9), no potential error or bias (RMSEA = 0), and high R^2 values (>55%), which corresponded to the proposed conceptual model. Our structural equation model presented a higher adjustment and proportion of the variation explained by the variables than the models proposed for three subtropical lakes in China (Shan et al., 2019).

Among the modeling studies performed exclusively in tropical lakes and reservoirs, most are site- or taxon-specific, which can be used only locally (Rousso et al., 2020). Only Brasil et al. (2016) modeled the impacts of environmental factors on total cyanobacterial biomass in tropical lakes. However, the authors used water transparency, pH, total nitrogen, and the ratio between zooplankton and phytoplankton biomass as independent variables, which can also act as a consequence of cyanobacterial blooms; furthermore, they did not use any meteorological factors. By comparison, our model included meteorological, physical, chemical, and zooplankton variables, allowing the predictions of the isolated and interactive effects of climate, stratification, eutrophication, salinization, and trophic interactions on cyanobacterial blooms and the biomass of four morphotypes.

The model proposed herein is based on the monitoring of ten tropical reservoirs, with varying environmental conditions and multiple uses. Because of that, it can be used for general managing purposes in a wide geographical range in the tropics (for model output see Table A.2). Likewise, Rousso et al. (2020) highlight that water managers should use generalized models for lakes with similar characteristics. Moreover, to avoid biases in the model, our study was conducted during normal climatic conditions (i.e. without climatic anomalies) during an annual cycle. Therefore, our model may be not precise in testing the effects of environmental variables on cyanobacterial blooms during climatic anomalies, such as during *El Niño* and *La Niña*, when annual amplitudes of temperature are higher. Including these anomalies would introduce bias in our model, as multiple frequency strategies can distort the results (Rousso et al., 2020).

In Northeast Brazil, the temperature rise predicted for the end of the 21st century may reduce the quality and quantity of water, and consequently, increase risks of salinization, anoxia, eutrophication, and cyanobacterial blooms (Brasil et al., 2016). Moura et al. (2018) pointed out that in this region there has been an increase in the frequency and intensity of blooms in the last two decades, accompanied by high concentrations of cyanotoxins; with a possible warning for increased toxic blooms in the coming years. The elevated levels of cyanotoxins in the reservoirs of this region threaten the aquatic biota and the health of the people supplied with these water sources (Lorenzi et al., 2018).

The relationships between environmental variables and cyanobacterial biomass, in addition to proposed strategies for controlling the blooms, are summarized in Fig. 6. Given the predictions of increased eutrophication in aquatic ecosystems globally (Beaulieu et al., 2019), the proposed model suggests that phosphorus concentrations in the water column could lead to an increase in the biomass of omnivorous crustaceans in the future, as these organisms are adapted to coexist with cyanobacteria. This could lead to an increase in the intensity and frequency of cyanobacterial blooms in the tropical region, putting the health of the environment and the people who are supplied by these water sources at risk, in addition to reducing ecosystem services.

Moreover, due to climate change, an increase in meteorological variables, such as solar radiation and air temperature, is expected (Marengo et al., 2017), which can lead to an increase in the biomass of colonies, picocyanobacteria, and non-heterocyted filaments, as these groups are favored under thermal stratification (Wagner and Adrian, 2009; Callieri et al., 2012). Another emerging threat to freshwaters is salinization (Reid et al., 2019), which is further enhanced due to the increased demand for irrigation and the use of fertilizers (rich in nutrients, potassium, sodium chloride, among other salts) in agriculture (Pfister et al., 2011). Reservoirs located in agricultural landscapes, as the case of the present study, are more susceptible to salinization, considering that salts are carried to aquatic systems during precipitation events (Cañedo-Argüelles et al., 2013). Our model also demonstrates that higher salinities can damage zooplankton groups, and consequently reduce their grazing on cyanobacteria, besides the direct positive influence of salinity on heterocyted filaments and picocyanobacteria.

Therefore, we suggest reducing external phosphorus (e.g. from sewage discharge) and salt (e.g. from agriculture) inputs in reservoirs used for public supply to alleviate the effects of eutrophication and salinity on cyanobacterial blooms. Additionally, we recommend bloom mitigation measures be adopted, such as biomanipulation through the addition of piscivorous fish or removal of benthivorous, omnivorous, and zooplanktivorous fish, to allow large herbivorous crustaceans to develop (capable of consuming cyanobacteria) and reduce the resuspension of phosphorus in the sediments (e.g. Jeppesen et al., 2012). These measures might be associated with the introduction of submerged non-invasive macrophytes with allelopathic potential, that can control toxic and non-toxic lineages of cyanobacteria (e.g. Amorim et al., 2019a). This approach proved to be efficient to reduce up to 85% of total cyanobacteria and 99% of filamentous morphotypes, in a tropical reservoir in Northeast Brazil, with bottom-up and allelopathic effects of submerged macrophytes being more expressive (Amorim and Moura, 2020) (Fig. 6).

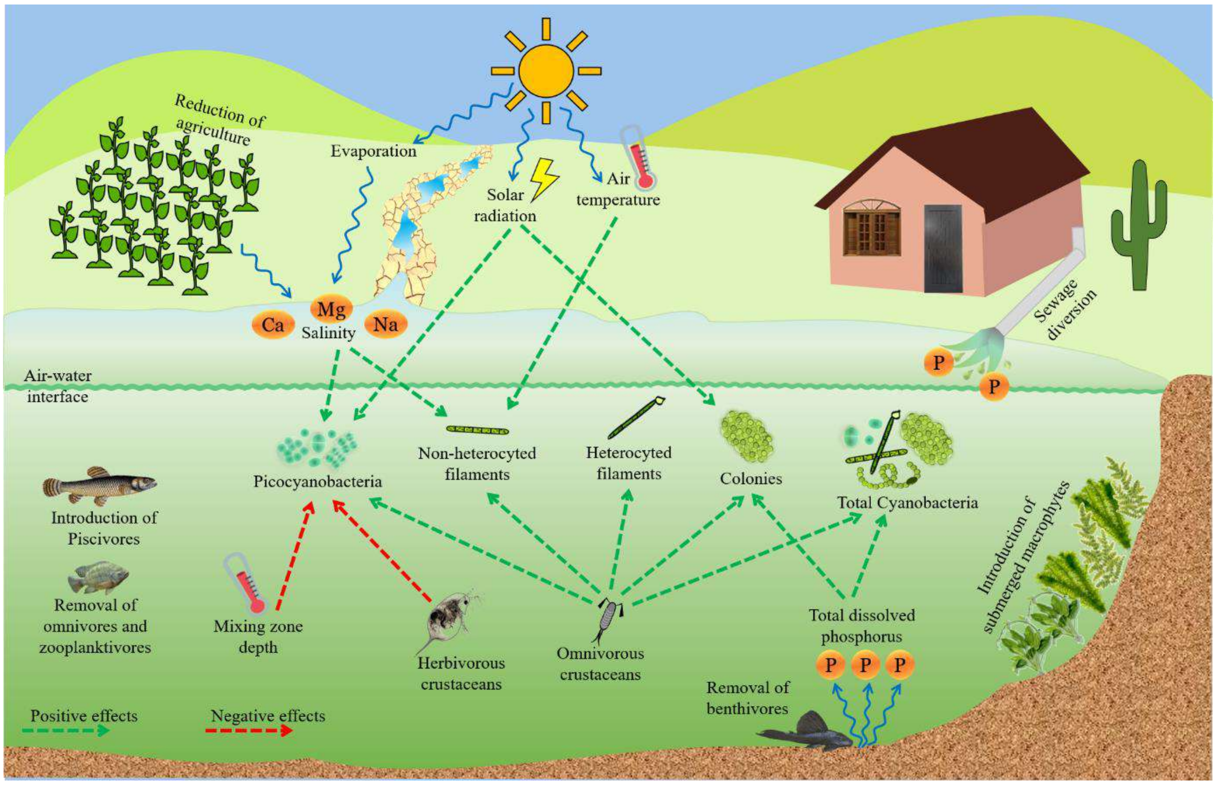


Fig. 6. Conceptual diagram showing the direct effects of physical (solar radiation, air temperature, and mixing zone depth), chemical (salinity and total dissolved phosphorus), and zooplankton (omnivorous and herbivorous crustaceans) variables on total cyanobacteria, colonies, heterocyted filaments, non-heterocyted filaments, and picocyanobacteria assessed through the structural equation model for tropical reservoirs. Green arrows represent positive effects and red arrows represent negative effects. As management strategies, we highlight the reduction of external sources of nutrients and salts, such as agriculture and domestic sewage, in addition to the removal of omnivorous, zooplanktivorous, and benthivorous fish, the introduction of non-invasive piscivorous fish and submerged macrophytes with allelopathic potential.

5. Conclusions

In this study, we evaluated the effects of physical and chemical variables, besides trophic interactions with zooplankton, on cyanobacterial blooms with different intensities using correlation, canonical correspondence analysis (pCCA and CCA), and a structural equation model. These analyses proved to be excellent tools for modeling tropical cyanobacterial blooms in response to changing environmental conditions. The first hypothesis was refuted since air temperature did not significantly affect the total cyanobacterial biomass, instead, total dissolved phosphorus interacted synergistically with the omnivorous crustaceans to promote the blooms. The second hypothesis was confirmed considering that air temperature, mixing water depth, salinity, and total dissolved phosphorus had direct and indirect effects, mediated by zooplankton, on total cyanobacteria and the four morphotypes.

Overall, our results reveal the less efficiency of zooplankton on the control of tropical cyanobacterial blooms. Particularly because the zooplankton community during cyanobacterial blooms is composed mainly of small organisms, such as rotifers, cyclopoids, and copepod nauplii, which are adapted to coexist with blooms instead of consuming cyanobacteria. The proposed model can be used for future predictions of cyanobacterial biomass in tropical lakes and reservoirs, benefiting the management of drinking waters. These results highlight the potential contamination risks of toxic cyanobacteria, considering the projections of increasing temperature, salinity, and eutrophication. Furthermore, we proposed some mitigation strategies to control tropical cyanobacterial blooms, and alleviate the impacts of warming, eutrophication, and salinization on cyanobacteria.

Conflicts of interest

Declarations of interest: none.

Data Availability

Additional data and information may be obtained from the first author (alvescihelio@gmail.com, cihelio.amorim@ufrpe.br).

Author Contributions

Cihelio A. Amorim: Conceptualization, Methodology, Validation, Data curation, Formal analysis, Writing - Original Draft, Writing - Review & Editing. **Ênio W. Dantas:**

Methodology, Formal analysis, Writing - Review & Editing. **Ariadne N. Moura:** Conceptualization, Supervision, Methodology, Writing - Review & Editing.

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Appendix A

Title: Modeling cyanobacterial blooms in tropical reservoirs: the role of physicochemical variables and trophic interactions

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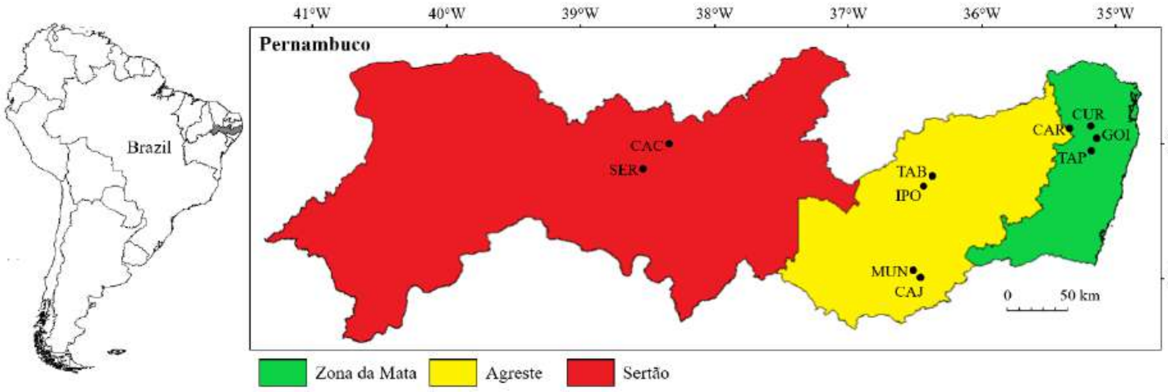

Fig. A.1. Geographic location of the ten tropical reservoirs studied distributed in the phytogeographic regions "Zona da Mata", "Agreste", and "Sertão" of Pernambuco, Brazil. See Table 1 for reservoir abbreviations.

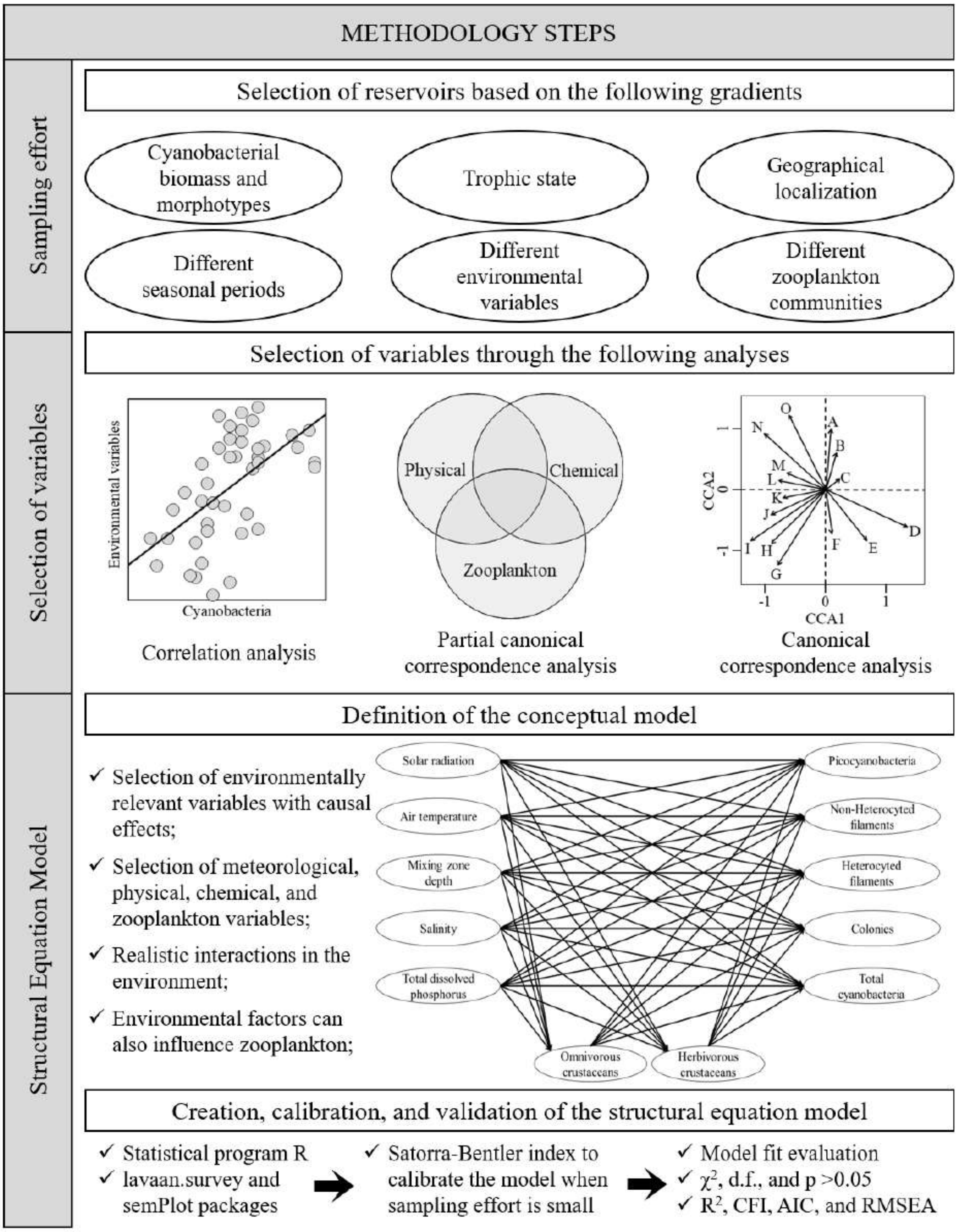

Fig. A.2. Summary of all methodology steps applied to select the reservoirs, environmentally relevant variables, definition of the conceptual model, and the creation, calibration, and validation of the structural equation model. The a priori structural equation model (conceptual model) was used to assess the effects of physical (solar radiation, air temperature, and mixing zone depth), chemical (salinity and total dissolved phosphorus - TDP), and zooplankton (omnivorous and herbivorous crustaceans) variables on total cyanobacteria, colonies, heterocyted filaments, non-heterocyted filaments, and picocyanobacteria in tropical reservoirs.

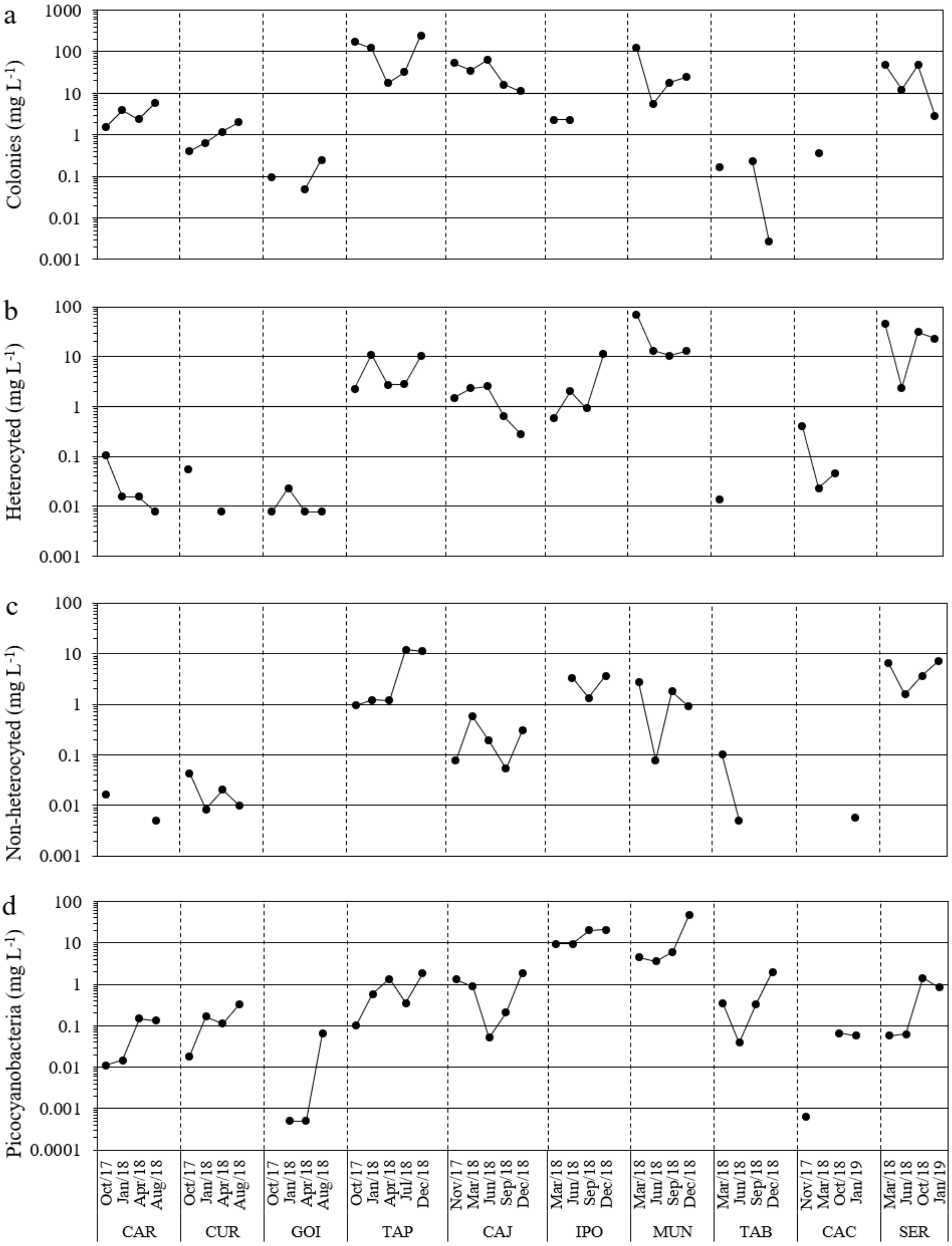

Fig. A.3. Seasonal variation in the biomass of the cyanobacterial morphotypes (a) colonies, (b) heterocyted filaments, (c) non-heterocyted filaments, and (d) picocyanobacteria in the tropical reservoirs studied. See Table 1 for reservoir abbreviations.

Table A.1. Minimum, maximum, mean, and standard deviation (±SD) of the physical, chemical, and zooplankton variables in the tropical reservoirs studied.

	Minimum	**Maximum**	**Mean**	**±SD**
Physical variables				
Depth (m)	2.30	14.70	7.84	3.24
Water transparency (m)	0.05	2.70	1.06	0.71
Light intensity (µmol m^{-2} s^{-1})	114.64	1767.30	653.45	426.55
Water temperature (°C)	24.08	30.50	27.47	1.74
Mixing zone to depth ratio	0.11	1.00	0.77	0.30
Air temperature (°C)	20.04	30.18	25.80	2.72
Solar radiation (kJ m^{-2})	1409.00	2560.00	1955.83	309.61
Chemical variables				
pH	6.54	9.60	8.14	0.79
Salinity (PSU)	0.02	4.93	0.61	1.00
Nitrate (µg L^{-1})	0.11	618.54	63.82	133.32
Nitrite (µg L^{-1})	0.87	294.56	18.48	55.24
Ammonia (µg L^{-1})	4.13	720.34	109.20	156.48
Dissolved inorganic nitrogen (DIN, µg L^{-1})	19.22	1557.84	191.50	318.88
Orthophosphate (µg L^{-1})	3.32	527.88	112.01	166.44
Total dissolved phosphorus (TDP, µg L^{-1})	8.87	573.00	153.87	173.97
Total phosphorus (µg L^{-1})	12.09	1036.89	246.73	299.57
DIN to TDP ratio	0.24	62.27	12.75	16.99
Trophic state index	50.43	67.47	58.77	4.55
Zooplankton biomass				
Rotifers (µg L^{-1})	0.00	151.63	17.09	33.55
Calanoid copepods (µg L^{-1})	0.53	538.59	79.91	139.68
Cyclopoid copepods (µg L^{-1})	0.02	184.38	26.87	42.26
Copepod nauplii (µg L^{-1})	0.11	181.04	22.13	31.36
Cladocerans (µg L^{-1})	0.13	381.55	18.40	59.41
Total zooplankton (µg L^{-1})	14.37	1051.50	164.40	204.40

Table A.2. Statistical summary of the structural equation model (model output) proposed in this study for tropical reservoirs. Bold values represent significant regressions, covariances, and variances.

		Regressions				
		Estimate	Std. Error	z-value	p-value	Std. path coef.
Omnivorous crustaceans ~	Solar radiation	0.293	1.377	0.213	0.832	0.030
	Air temperature	**-6.469**	**2.084**	**-3.104**	**0.002**	**-0.433**
	Mixing zone depth	-0.224	0.368	-0.609	0.542	-0.088
	Salinity	**-2.703**	**0.538**	**-5.026**	**0.000**	**-0.720**
	Total dissolved phosphorus	**0.720**	**0.123**	**5.875**	**0.000**	**0.615**
Herbivorous crustaceans ~	Solar radiation	-0.077	1.515	-0.051	0.959	-0.010
	Air temperature	-2.360	2.155	-1.095	0.273	-0.196
	Mixing zone depth	**0.597**	**0.291**	**2.049**	**0.040**	**0.289**
	Salinity	**-1.339**	**0.459**	**-2.920**	**0.004**	**-0.442**
	Total dissolved phosphorus	**0.378**	**0.133**	**2.838**	**0.005**	**0.400**
Total cyanobacteria ~	Solar radiation	2.582	1.493	1.730	0.084	0.228
	Air temperature	-2.197	2.739	-0.802	0.423	-0.129
	Mixing zone depth	-0.057	0.323	-0.175	0.861	-0.019
	Salinity	0.718	0.649	1.106	0.269	0.167
	Total dissolved phosphorus	**0.452**	**0.187**	**2.423**	**0.015**	**0.338**
	Omnivorous crustaceans	**0.719**	**0.150**	**4.806**	**0.000**	**0.628**
	Herbivorous crustaceans	-0.156	0.185	-0.841	0.400	-0.110
Colonies ~	**Solar radiation**	**3.222**	**1.638**	**1.967**	**0.049**	**0.293**
	Air temperature	-3.451	2.955	-1.168	0.243	-0.208
	Mixing zone depth	0.197	0.374	0.527	0.598	0.069
	Salinity	-0.802	0.692	-1.159	0.247	-0.192
	Total dissolved phosphorus	**0.572**	**0.202**	**2.838**	**0.005**	**0.440**
	Omnivorous crustaceans	**0.498**	**0.184**	**2.706**	**0.007**	**0.448**
	Herbivorous crustaceans	0.064	0.201	0.318	0.751	0.046
Heterocyted filaments ~	Solar radiation	0.960	1.273	0.754	0.451	0.122
	Air temperature	2.136	2.067	1.033	0.301	0.179
	Mixing zone depth	-0.360	0.208	-1.732	0.083	-0.176
	Salinity	0.569	0.507	1.122	0.262	0.190
	Total dissolved phosphorus	0.100	0.128	0.780	0.435	0.107

	Omnivorous crustaceans	**0.613**	**0.096**	**6.381**	**0.000**	**0.769**
	Herbivorous crustaceans	-0.107	0.124	-0.866	0.387	-0.109
Non-heterocyted filaments ~	Solar radiation	-0.438	0.699	-0.626	0.531	-0.092
	Air temperature	**3.228**	**1.278**	**2.526**	**0.012**	**0.449**
	Mixing zone depth	-0.001	0.147	-0.004	0.997	0.000
	Salinity	**0.613**	**0.302**	**2.032**	**0.042**	**0.340**
	Total dissolved phosphorus	0.046	0.088	0.521	0.602	0.081
	Omnivorous crustaceans	**0.389**	**0.061**	**6.329**	**0.000**	**0.809**
	Herbivorous crustaceans	-0.136	0.071	-1.927	0.054	-0.228
Picocyanobacteria ~	**Solar radiation**	**1.316**	**0.542**	**2.430**	**0.015**	**0.212**
	Air temperature	-2.510	1.305	-1.924	0.054	-0.268
	Mixing zone depth	**-0.274**	**0.095**	**-2.868**	**0.004**	**-0.171**
	Salinity	**1.252**	**0.238**	**5.264**	**0.000**	**0.532**
	Total dissolved phosphorus	0.015	0.080	0.182	0.856	0.020
	Omnivorous crustaceans	**0.296**	**0.068**	**4.371**	**0.000**	**0.472**
	Herbivorous crustaceans	**-0.309**	**0.077**	**-4.030**	**0.000**	**-0.397**
			Covariances			
		Estimate	Std. Error	z-value	p-value	Std. path coef.
Solar radiation ~~	**Air temperature**	**0.002**	**0.001**	**3.367**	**0.001**	**0.596**
	Mixing zone depth	-0.001	0.003	-0.451	0.652	-0.072
	Salinity	0.000	0.002	0.084	0.933	0.016
	Total dissolved phosphorus	0.002	0.006	0.297	0.766	0.042
Air temperature ~~	Mixing zone depth	0.003	0.001	1.873	0.061	0.215
	Salinity	-0.001	0.001	-0.633	0.526	-0.075
	Total dissolved phosphorus	0.003	0.004	0.799	0.424	0.109
Mixing zone depth ~~	Salinity	-0.013	0.010	-1.300	0.194	-0.272
	Total dissolved phosphorus	-0.017	0.023	-0.719	0.472	-0.110
Salinity ~~	**Total dissolved phosphorus**	**0.052**	**0.015**	**3.538**	**0.000**	**0.506**
Total cyanobacteria ~~	**Colonies**	**0.218**	**0.048**	**4.573**	**0.000**	**0.920**
	Heterocyted filaments	**0.105**	**0.030**	**3.448**	**0.001**	**0.646**
	Non-heterocyted filaments	**0.050**	**0.016**	**3.147**	**0.002**	**0.507**
	Picocyanobacteria	0.001	0.012	0.091	0.928	0.012
Colonies ~~	**Heterocyted filaments**	**0.081**	**0.028**	**2.892**	**0.004**	**0.470**

	Non-heterocyted filaments	**0.038**	**0.016**	**2.328**	**0.020**	**0.365**
	Picocyanobacteria	-0.013	0.012	-1.161	0.246	-0.142
Heterocyted filaments ~~	**Non-heterocyted filaments**	**0.038**	**0.015**	**2.526**	**0.012**	**0.526**
	Picocyanobacteria	0.008	0.009	0.871	0.384	0.124
Non-heterocyted filaments ~~	Picocyanobacteria	-0.004	0.007	-0.656	0.512	-0.111
		Variances				
		Estimate	Std. Error	z-value	p-value	Std. path coef.
Solar radiation		**0.005**	**0.001**	**5.390**	**0.000**	**1.000**
Air temperature		**0.002**	**0.000**	**5.051**	**0.000**	**1.000**
Salinity		**0.032**	**0.011**	**3.012**	**0.003**	**1.000**
Mixing zone depth		**0.069**	**0.010**	**6.988**	**0.000**	**1.000**
Total dissolved phosphorus		**0.331**	**0.038**	**8.791**	**0.000**	**1.000**
Omnivorous crustaceans		**0.219**	**0.049**	**4.432**	**0.000**	**0.481**
Herbivorous crustaceans		**0.209**	**0.044**	**4.797**	**0.000**	**0.709**
Total cyanobacteria		**0.223**	**0.046**	**4.808**	**0.000**	**0.376**
Colonies		**0.251**	**0.052**	**4.881**	**0.000**	**0.449**
Heterocyted filaments		**0.118**	**0.028**	**4.253**	**0.000**	**0.409**
Non-heterocyted filaments		**0.044**	**0.011**	**4.114**	**0.000**	**0.418**
Picocyanobacteria		**0.036**	**0.011**	**3.379**	**0.001**	**0.202**

ARTIGO 2:

Grupos Funcionais

4 ARTIGO 2

Reynolds' functional groups in tropical drinking water reservoirs: describing, explaining, and predicting habitat templates and assembly rules of phytoplankton

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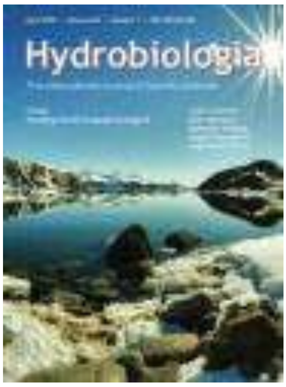

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Abstract: This study aimed to create habitat templates and assembly rules to describe, explain, and predict the occurrence of blooms of Reynolds' phytoplankton functional groups (RFGs) in tropical drinking water reservoirs. We analyzed the structure of RFGs in 10 tropical reservoirs, in humid and semiarid regions of Brazil, and defined their relationships with 10 environmental axes. We designated the habitat template and assembly rules based on niche differentiation, thresholds for the occurrences and bloom formation, Spearman's correlation, and generalized additive models. We identified 136 species, assembled in 20 RFGs. Five habitat templates were recognized based on environmental conditions and dominant RFGs, usually bloom-forming species of cyanobacteria, dinoflagellates, green algae, and diatoms. The RFGs D, X1, and P presented the most restrictive assembly rules, being restricted to some circumstances, while RFGs M and S_N displayed the widest. Positive correlation between RFGs revealed a coexistence pattern. Moreover, salinity was the best predictor of RFGs biomass (higher R^2), followed by trophic state, phosphorus, transparency, zooplankton, and nitrogen. Our approach improves the understanding of how RFGs interacts with environmental gradients in tropical reservoirs, helping water managers to adopt sustainable practices to control algal blooms, based on predictions of future state of dominance.

Keywords: algal blooms; Brazilian Semiarid; cyanobacteria; ecology; predictive models; salinity.

Introduction

Organisms' traits can represent their response to the environmental drivers or their effects on ecosystem functioning, besides increasing the predictability of the community's response to environmental changes (Kruk et al. 2021). Thus, explaining the main processes driving plankton diversity and structure is a central question in community ecology (Hutchinson

1961; Rojo 2021). For phytoplankton assemblages, species coexistence is driven by several biotic and abiotic filters that affect species interactions (Borics et al. 2020). These filters influence more species' traits than in species themselves, generating patterns of dominance when conditions become favorable to some species or not suitable to others (Reynolds et al. 2002).

Considering that phytoplankton is a highly diverse group of photosynthetic organisms (Borics et al. 2021), species-level classifications can often generate chaotic dynamics under certain circumstances, even after the advances in the study of physiology and ecology of the organisms (Benicà et al. 2015). To alleviate this problem, trait-based approaches and functional groups are often used to classify phytoplankton species without losing important features and responses (Reynolds et al. 2002; Salmaso and Pádisak 2007; Kruk et al. 2010). The definition of functional groups can cluster different sets of species with common functional features that respond in a similar way to environmental changes and present the same effects on ecosystem functioning (Kruk et al. 2021).

Based on that, Reynolds (1998, 2002) created the phytoplankton functional groups (hereafter Reynolds' Functional Groups – RFG, Kruk et al. 2017) to cluster species with similar tolerances and preferences and co-occurred in the same environment at the same time. These groups were created based on the habitat template, which included eight environmental axes: mixing zone depth, irradiance, water temperature, acidity and alkalinity, herbivorous zooplankton, soluble reactive phosphorus, dissolved inorganic nitrogen, and soluble reactive silicon (Reynolds 2006). Although this approach has been widely used for many years (Padisák et al. 2009; Kruk et al. 2021) and the environmental axes have been proven to be coherent with the assemblages (Salmaso et al. 2015), some groups are not well defined in terms of habitat template and driving processes (Padisák et al. 2009) and some new environmental axes should be further studied, e.g., salinity and the amount of the inoculum (Kruk et al. 2021).

The diversity and composition of phytoplankton are primarily controlled by local filters and biotic interactions (Borics et al. 2021). Important filters select specific attributes that act stronger in some species or groups, creating different patterns of abundance in response to the environmental gradients (Reynolds 1998). As predicted by the “habitat template” concept, introduced by Southwood (1977), the environment exerts influence on the fitness of the organisms, populations, or groups of species, which will select certain adaptations for better reproduction and survival (Townsend et al. 1997). This approach has been proved to be useful for classifying and predicting phytoplankton community structure without losing important information on the tolerances, and requirements in the natural environment (Reynolds 1998), both for RFGs (Reynolds 2006) and for morphology-based functional groups (Kruk and Segura 2012).

Knowing the spatial-temporal patterns of an assemblage, coupled with the information of its habitat template, can help to define clear assembly rules (i.e., “values and domain of factors that either structure or constrain the properties of ecological assemblages”, according to Keddy and Weiher 1999). Phytoplankton ecologists usually fail in describing assembly rules because of the biased recognition of how environmental factors will be associated with the algal structure and spatial-temporal patterns (Rojo et al. 2021). Moreover, rules must be explicit and quantitative values of key factors, instead of merely describing a pattern (Keddy and Weiher 1999). However, even with clear assembly rules, phytoplankton structure can be different from the expected patterns, either because of the action of other unknown factors (Reynolds 2000) or because species can grow even under unfavorable conditions (Reynolds 2012) and become the inoculum of future dominant assemblages (Rojo and Álvarez-Cobelas 2003). Thus, the habitat template with the environmental thresholds that an assemblage can grow intensely, considering the entire set of species, can represent a precise assembly rule (Rojo 2021).

Phytoplankton functional groups are often used in water quality assessments and algal bloom status (e.g., Padisák et al. 2006; Long et al. 2020; Wang et al. 2020). Indeed, harmful algal blooms are among the main causes of biodiversity loss and the reduction of ecosystem functioning in freshwaters (Reid et al. 2019; Amorim and Moura 2021). Consequently, phytoplankton-dominated shallow lakes support fewer ecosystem services than macrophyte-dominated systems, which can impair the achievement of the United Nations Sustainable Development Goals (Janssen et al. 2021). Therefore, understanding how phytoplankton assemblages interact with environmental gradients will help water managers to improve the sustainable use of drinking water reservoirs, ensuring the provision of food, clean water and sanitation, biodiversity, climate regulation, and sustainable development.

Answering the question "what lives where and why?" (Reynolds 1998) is a big challenge for phytoplankton ecologists over the years. Since the proposal of the RFGs classification, much has been done to its development, especially to describe spatial-temporal patterns in phytoplankton ("what lives where?"), but less often to explain those patterns ("why and how?"), and scarcely to predict ("when and where in new scenarios") (Kruk et al. 2021). To answer the question "what lives where", a descriptive framework aiming to describe the quantitative habitat templates where species are distributed in space and time can be followed (Reynolds 1998). When the focus is to answer "why and how", ecologists must explain the main mechanisms driving community assembly rules, relationships with environmental variables, biological interactions, and ecosystem functioning. Finally, to understand "when and where in new scenarios" the focus should be on predicting future scenarios of community responses to environmental changes and effects on ecosystem functioning and services (Kruk et al. 2021).

Accordingly, in this study, we evaluated the dynamics of RFGs in tropical drinking water reservoirs and created links to the relevant environmental factors driving their biomass, distribution, and bloom development. Our main goal is to create the relevant habitat templates and establish assembly rules capable of describing, explaining, and predicting the occurrence of blooms of each RFG in tropical reservoirs, based on niche differentiation, environmental thresholds for their occurrence and dominance, group coexistence, and generalized additive models. Furthermore, our approach will help water managers to predict the main impacts of algal blooms on biodiversity, water quality, ecosystem functioning, and services, besides supporting the adoption of sustainable practices.

Material and methods

Study sites, sampling, and analyses

We sampled phytoplankton from 10 tropical drinking water reservoirs in Pernambuco state, Brazil. The reservoirs are in the phytogeographic regions "Zona da Mata" (4), "Agreste" (4), and "Sertão" (2), along a longitudinal gradient. The reservoirs presented contrasting environmental conditions, ranging from oligotrophic to hypereutrophic, and are distributed in a climatic gradient, distributed in the humid, dry-subhumid, and semiarid zones of Brazil (Table S1). Moreover, all of them presented algal blooms at least in one sampling period (Amorim et al. 2020; Amorim and Moura 2021). All studied reservoirs provide several ecosystem services for people, supporting the water public supply, irrigation, agriculture, fisheries, livestock, biodiversity, recreation, education, flood control, nutrient retention, primary production, climate regulation, among others.

Samples were collected five (Tapacurá and Cajueiro reservoirs) or four (other reservoirs) times a year from October 2017 to January 2019 (n = 42), comprising an annual cycle in each reservoir, with samples in the rainy and dry seasons. All samples were collected from the deepest location of each reservoir, usually close to the dam and during the morning time (08:00 to 12:00). Water transparency and depth were measured with a Secchi disk and an echo sounder, respectively. Water temperature, salinity, and pH were measured with a HANNA multiparametric probe (HI98194). Water temperature was further verified throughout the water column to establish the mixing zone depth (when there was a difference in the water temperature greater than 0.5 °C at each 0.5 m depth, i.e., the formation of the thermocline in stratified environments). Destratified environments presented a mixing zone depth equal to or almost reaching the sediment, demonstrating a complete mixture of the water column. The concentrations of dissolved inorganic nitrogen (DIN), soluble reactive phosphorus (SRP), and total phosphorus (TP) were determined according to Strickland and Parsons (1965), Golterman et al. (1971), and Valderrama (1981). Chlorophyll-*a* was analyzed spectrophotometrically according to Chorus and Bartram (1999).

Samples collected through surface trawls with a 25 µm mesh-size plankton net, fixed with 4% formaldehyde, were used for phytoplankton species identification following specialized literature (Komárek and Anagnostidis 1986, 2005; Anagnostidis and Komárek 1988; Prescott and Vinyard 1982; Komárek and Fott 1983; Round et al. 1990; Krammer and Lange-Berlalot 1991; John et al. 2002; Wehr et al. 2015; among others). Subsurface samples (20 cm depth) were collected directly from the water column, preserved with 1% acetic Lugol, and analyzed in sedimentation chambers using an inverted microscope for phytoplankton quantification (Utermöhl 1958). Phytoplankton was counted until achieving 400 organisms of the most abundant taxa and until the stabilization of the species curve (i.e., when no species was added) (Lund et al. 1958). The species' biomass (mg L^{-1}, wet weight) was determined according to Hillebrand et al. (1999) and grouped into Reynolds' Functional Groups (RFGs) (Reynolds et al. 2002; Padisak et al. 2009; among others). All species, even the rarest ones, were classified into RFGs, following the recommendations of Rojo (2021).

Zooplankton was collected by filtering 100 L of water from the surface of the reservoirs with a 60 µm mesh-size plankton net, then the samples were fixed with 4% formaldehyde and analyzed in a 1 mL Sedgewick-Rafter chamber (three subsamples per sample) in an optical microscope. Biomass (µg L^{-1}, dry weight) was calculated based on regression formulas relating the bodyweight with length and width of rotifers and microcrustaceans (Ruttner-Kolisko 1977; Dumont et al. 1975).

Calculations and data analyses

All statistical analyzes were run in the statistical program R (version 4.0.5), with a significance level of $p < 0.05$ (R Core Team 2021). Most data are presented as the mean and the standard error. Concentrations of total phosphorus and chlorophyll-*a* were used to estimate the trophic state index (TSI), following the formulas proposed by Cunha et al. (2013). When DIN and SRP concentrations were below approximately 100 and 10 µg L^{-1}, respectively, the reservoirs were considered N or P-limited (Chorus and Spijkerman 2021). Phytoplankton RFGs with relative biomass greater than 50% of the total phytoplankton biomass were classified as dominant, while abundant species presented relative biomass greater than 5% of the total biomass. The relative biomass of herbivorous zooplankton was calculated as the sum of rotifers, calanoid copepods, nauplii, and cladocerans, and then divided by total zooplankton biomass.

To designate the habitat template for each RFG, we selected seven of the eight environmental axes (variables) used by Reynold (2006): mixing zone depth, irradiance, water temperature, pH (instead of CO_2 concentration), herbivorous zooplankton biomass (instead of herbivorous zooplankton filtration rate), SRP, and DIN. We also incorporated three new axes that are important drivers of phytoplankton structure: TSI, water transparency, and salinity. These axes can successfully represent the gradients of resources, energy, and loss processes due to zooplankton grazing. Mean values of each environmental axis were weighted by each RFG biomass to verify niche differentiation among the RFGs and create the habitat template, following the presumptions of Southwood (1977). Weighted means were calculated by multiplying all values of each variable by the biomass of a specific RFG and then the sum of these results was divided by the sum of the biomasses in all samples. This approach allows us to identify the specific range of conditions where each RFG presents higher biomass. To verify the correspondence of the habitat templates with the relative biomass of the RFGs, environmental variables were analyzed through a non-metric multidimensional scaling (NMDS) ordination using the Euclidian distance matrix, in the *vegan* package (Oksanen et al. 2018). Then, the biomass of each RFG was plotted in the ordination and the slopes of trophic state and water transparency, representing the gradients of resources and energy, respectively, were obtained using the function *envfit*. Environmental variables were standardized using the range function, while RFG's biomasses were transformed (log(x+1)).

To establish the assembly rules of each RFG, based on the definitions of Keddy and Weiher (1999) and recommendations of Rojo (2021), we calculated the thresholds of the occurrence of each group as abundant. For that, we selected the minimum and maximum values of each environmental axis in the samples where each RFG reached relative biomass greater than 5%. However, in the case of any group that does not present a relative biomass >5%, or only in one sample, we extracted the minimum and maximum values from all occurrences of this group.

To analyze the influence of the RFGs on each other, we constructed a Spearman correlation matrix to verify the coexistence and inhibition patterns among RFGs. Finally, generalized additive models (GAM) were used to verify the influence of each environmental axis on RFGs. This method account for linear and non-linear relationships between variables. The biomass of each RFG, irradiance, SRP and DIN were log(x+1)-transformed. Model smoothing was evaluated using the estimated degree of freedom (e.d.f.), while R^2-adjusted and p-values ($p < 0.05$) indicated model fit and significance, respectively. All models were fitted using the *gam* function in the *mgcv* package (Wood 2004, 2011).

Results

Environmental variables and Reynolds' Phytoplankton Functional Groups

The 10 studied reservoirs presented contrasting environmental parameters and phytoplankton communities, which can be separated into five main groups (habitat templates). Firstly, Carpina reservoir (hypereutrophic) showed higher mixing zone depth (destratified) and relative biomass of herbivorous zooplankton, besides high water transparency, temperature, pH, SRP, TSI, and salinity. Secondly, Ipojuca reservoir was extremely saline, hypereutrophic, with high pH and herbivorous zooplankton biomass, besides showing the lowest values of mixing depth (stratified), irradiance, and water transparency. The third group was formed by the other hypereutrophic reservoirs (Tapacurá, Mundaú, and Serrinha) and Cajueiro (mesotrophic), which presented, in general, high trophic state and pH, low to moderate irradiance, and herbivorous zooplankton biomass, besides low mixing depth (stratified), water transparency, and salinity. Tapacurá presented the highest concentrations of SRP, and Mundaú the highest concentrations of DIN. The fourth group was represented by Goitá and Cachoeira reservoirs, both eutrophic, which displayed high temperature, water transparency, and herbivorous zooplankton relative biomass, neutral pH, moderate trophic state, in addition to low irradiance and salinity. Finally, the fifth group, formed by Tabocas (mesotrophic) and Cursaí (oligotrophic) exhibited high mixing depth (destratified), irradiance, herbivorous zooplankton biomass, water transparency, neutral pH, in addition to low levels of SRP, DIN, trophic state, and salinity (Fig. S1).

A total of 136 phytoplankton species were identified for the 10 reservoirs studied, distributed mainly in Chlorophyta (51 spp.), Cyanobacteria (40 spp.), and Bacillariophyta (19 spp.). These taxa were classified in 20 RFGs, of which 16 were abundant at least in one sample, usually forming surface blooms. The mean phytoplankton biomass varied greatly in each reservoir, ranging from 1.88 mg L^{-1} (standard error: ±0.57) in Cursaí reservoir to 132.75 mg L^{-1} (standard error: ±44.93) in Tapacurá reservoir (Fig. 1).

Group M was dominant in Tapacurá, Cajueiro, Carpina, Mundaú, Cursaí, and Serrinha reservoirs. Group L_O was dominant in Cachoeira, Goitá, and Mundaú, while L_M was dominant in Cajueiro, Cursaí, Serrinha, and Carpina. Serrinha reservoir also showed a dominance of the group S_N. Finally, Tabocas and Ipojuca reservoirs presented the dominance of the groups F and D, respectively. Groups C, H1, J, K, MP, P, S1, X1, X2, and Y were abundant at least in one sample (>5%), while the groups E, N_A, W1, and W2 always presented low relative biomass (<5%) (Fig. 1, Table 1).

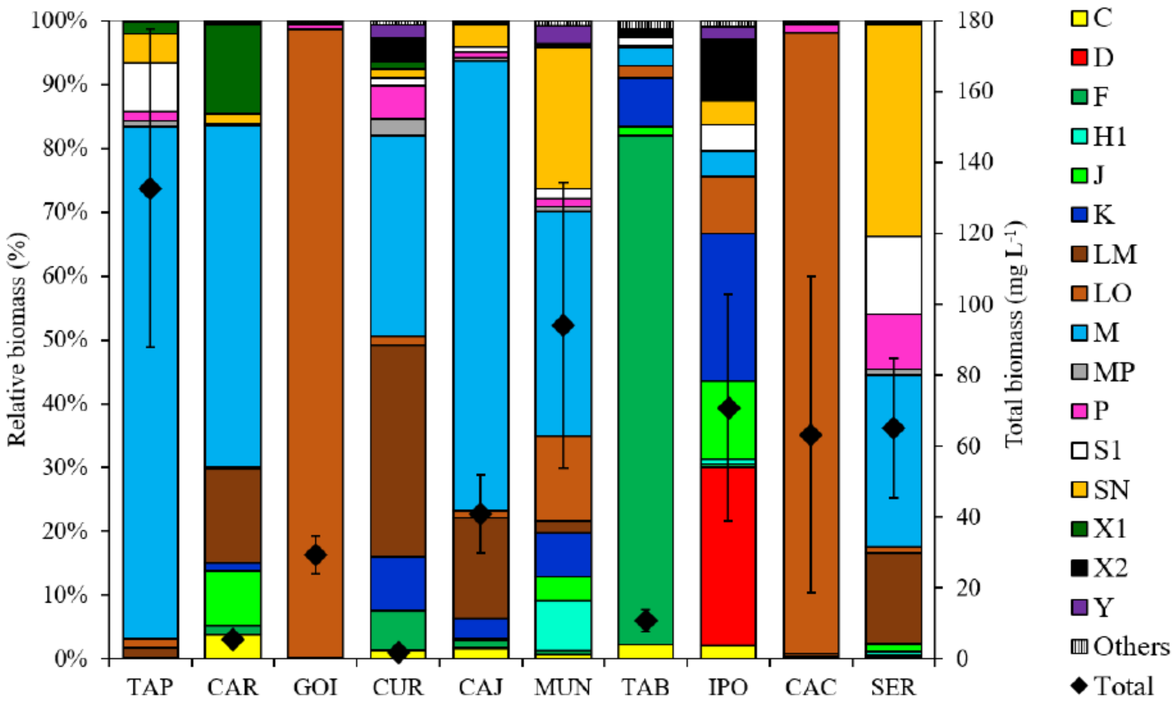

Fig. 1 Total biomass of the phytoplankton assemblages and relative biomass of the Reynolds' functional groups (RFGs) with relative biomass >5% in the tropical reservoirs studied (n = 42). Diamonds are the mean biomass in each reservoir and error bars represent the standard error. Reservoirs: Tapacurá (TAP), Carpina (CAR), Goitá (GOI), Cursaí (CUR), Cajueiro (CAJ), Mundaú (MUN), Tabocas (TAB), Ipojuca (IPO), Cachoeira (CAC), and Serrinha (SER)

Table 1 Reynolds' phytoplankton functional groups (RFGs), their mean relative biomasses (± standard error) in each tropical reservoir studied, and main representative species (n = 42). See Fig. 1 for reservoir abbreviations

Reservoir	**RFG**	**Relative biomass**	**Main representatives**
TAP	M	80.2 (±6.2)	*Microcystis panniformis* Komárek et al.
	S1	6.3 (±4.3)	*Anagnostidinema amphibium* (C.Agardh ex Gomont) Strunecký et al.
	S_N	4.5 (±1.0)	*Raphidiopsis raciborskii* (Woloszynska) Aguilera et al.
	L_O	1.4 (±1.0)	*Snowella lacustris* (Chodat) Komárek & Hindák
CAR	M	53.6 (±19.8)	*Microcystis protocystis* W.B.Crow, *Microcystis aeruginosa* (Kützing) Kützing, *Microcystis botrys* Teling
	L_M	14.7 (±11.9)	*Microcystis protocystis*, *Ceratium furcoides* (Levander) Langhans
	X1	13.9 (±10.5)	*Schroederia setigera* (Schröder) Lemmermann
	J	8.7 (±8.7)	*Scenedesmus ecornis* (Ehrenberg) Chodat
	C	3.8 (±2.6)	*Cyclotella meneghiniana* Kützing
	S_N	1.5 (±1.3)	*Raphidiopsis raciborskii*
GOI	L_O	98.4 (±0.4)	*Ceratium furcoides*
CUR	L_M	33.2 (±19.2)	*Microcystis protocystis*, *Ceratium furcoides*
	M	31.4 (±18.2)	*Microcystis protocystis*
	K	8.3 (±3.2)	*Aphanocapsa delicatissima* West & G.S.West, *Aphanocapsa elachista* West & G.S.West
	F	6.2 (±2.5)	*Botryococcus braunii* Kützing, *Eutetramorus tetrasporus* Komárek
	P	5.1 (±2.4)	*Aulacoseira granulata* (Ehrenberg) Simonsen
	X2	3.7 (±2.1)	*Cryptomonas brasiliensis* A.Castro, C.E.M.Bicudo & D.Bicudo
	MP	2.6 (±1.6)	*Gomphonema argur* Ehrenberg
	Y	2.1 (±1.6)	*Cryptomonas ovata* Ehrenberg
	L_O	1.4 (±1.3)	*Peridinium gatunense* Nygaard
	S_N	1.4 (±1.2)	*Raphidiopsis raciborskii*
CAJ	M	70.6 (±17.7)	*Microcystis aeruginosa*
	L_M	15.8 (±14.7)	*Microcystis aeruginosa*, *Ceratium furcoides*
	S_N	3.5 (±0.7)	*Raphidiopsis raciborskii*
	K	3.1 (±1.8)	*Aphanocapsa delicatissima*
MUN	M	35.1 (±8.7)	*Microcystis flos-aquae* (Wittrock) Kirchner
	S_N	22.1 (±4.7)	*Raphidiopsis raciborskii*
	L_O	13.3 (±12.3)	*Merismopedia tenuissima* Lemmermann
	H1	7.8 (±3.5)	*Anabaenopsis elenkinii* V.V.Miller
	K	6.9 (±3.2)	*Aphanocapsa delicatissima*
	J	3.7 (±3.0)	*Pediastrum duplex* Meyen
	Y	2.7 (±1.6)	*Gymnodinium* sp.
TAB	F	79.6 (±11.8)	*Botryococcus braunii*
	K	23.2 (±3.8)	*Aphanocapsa delicatissima*, *Aphanocapsa holsatica*
	M	2.7 (±2.2)	*Microcystis aeruginosa*
	C	2.3 (±2.1)	*Cyclotella meneghiniana*
	S1	1.5 (±1.4)	*Planktolyngbya limnetica* (Lemmermann) Komárková-Legnerová & Cronberg
IPO	D	28.1 (±16.2)	*Navicula* sp.
	K	23.2 (±7.7)	*Aphanocapsa incerta* (Lemmermann) G.Cronberg & Komárek
	J	12.1 (±8.8)	*Tetradesmus lagerheimii* M.J.Wynne & Guiry
	X2	9.5 (±5.4)	*Plagioselmis lacustris* (Pascher & Ruttner) Javornicky
	L_O	9.0 (±6.5)	*Ceratium furcoides*, *Synechocystis aquatilis* Sauvageau
	S1	4.1 (±2.7)	*Planktolyngbya limnetica*
	M	3.9 (±2.3)	*Microcystis aeruginosa*
	S_N	3.7 (±1.5)	*Raphidiopsis raciborskii*
CAC	L_O	97.3 (±0.5)	*Ceratium furcoides*
SER	S_N	33.2 (±10.6)	*Raphidiopsis raciborskii*
	M	27.0 (±13.6)	*Microcystis protocystis*, *Microcystis panniformis*

L_M	14.2 (±12.3)	*Microcystis panniformis*, *Ceratium furcoides*
S1	8.7 (±3.5)	*Planktolyngbya limnetica*
P	8.6 (±8.3)	*Aulacoseira granulata*
H1	4.1 (±1.4)	*Sphaerospermopsis aphanizomenoides* (Forti) Zapomelová et al.

What lives where? Describing habitat template of RFGs

To test niche differentiation among the RFGs and create the habitat template, mean values of 10 environmental axes (variables) were weighted by each RFG biomass. The mean mixing zone depth weighted by RFGs biomass revealed that groups D, W1, Y, K, and X2 occurred usually in stratified water bodies (mixing depth <3 m), while groups L_M, P, X1, and F preferred destratified environments (mixing depth >6 m). Groups D, W1, L_O, J, and Y occurred in reservoirs with low irradiance (<400 µmol m^{-2} s^{-1}), while groups F, L_M, E, M, and MP presented higher biomass in environments with higher irradiance (>700 µmol m^{-2} s^{-1}). Mean water temperature weighted by RFGs biomass was lower for the RFGs N_A and F (<26 ºC) and higher for the RFGs L_O, X1, M, and MP (>28 ºC). Regarding pH values, groups F, N_A, and E preferred neutral waters (pH 7 – 8), while all other groups were common in alkaline habitats (pH >8). In terms of the percentage of herbivores in the zooplankton community, groups H1 and S_N preferred environments with low biomass of herbivorous zooplankton (<60% of the whole community), on the other hand, groups D, W1, L_O, and C presented higher biomasses in reservoirs with high herbivorous relative biomass (>80% of the whole community) (Table 2).

In terms of the amount of resources, groups F and N_A were found in SRP-limited reservoirs (<15 µg L^{-1}), while groups X1, M, MP, S1, and D presented higher biomasses in reservoirs with high SRP concentrations (>150 µg L^{-1}). For DIN concentration, groups L_O, and D occurred in DIN-limited waters (<100 µg L^{-1}), while N_A, Y, J, H1, MP, and K showed higher biomasses under high DIN concentrations (>300 µg L^{-1}). Group F acted as an indicator of mesotrophic waters (TSI 53.2 – 55.7), groups E and N_A were indicators of eutrophic reservoirs (TSI 55.8 – 58.1), while group L_M was present mainly in supereutrophic habitats (TSI 58.2 – 59.0). All other groups were indicators of hypereutrophic waters, especially groups D, W1, Y, X1, S1, and MP (TSI >59.0). Groups D, W1, H1, Y, K, S1, X2, S_N, and J were usually found in turbid waters (water transparency <0.5 m), while groups E, F, and N_A preferred clear reservoirs (water transparency >1.0 m). Regarding salinity, groups F and E showed low mean values weighted by the biomass (<0.2 PSU), on the other hand, groups Y, X2, K, C, J, and W2 were indicators of saline reservoirs (1 – 3 PSU), while groups D and W1 were usually found in extremely saline waters (>4 PSU) (Table 2).

Table 2 The mean values of the 10 environmental axes weighted by the biomass of each Reynolds' functional group (RFG) to represent the habitat template with the best conditions for their growth in the tropical reservoirs studied (n = 42). Variables: mixing zone depth (ZMix), irradiance, water temperature, pH, the relative biomass of herbivorous zooplankton, soluble reactive phosphorus (SRP), dissolved inorganic nitrogen (DIN), trophic state index (TSI), water transparency, and salinity

RFG	ZMix (m)	Irradiance (µmol m^{-2} s^{-1})	Water temperature (°C)	pH	Herbivorous zooplankton (%)	SRP (µg L^{-1})	DIN (µg L^{-1})	TSI	Water transparency (m)	Salinity (PSU)
C	3.42	482.83	27.40	8.57	0.81	85.19	187.45	61.67	0.60	1.76
D	1.16	198.50	27.93	8.85	0.99	158.34	99.69	67.08	0.20	4.39
E	5.12	833.61	27.17	7.88	0.72	74.62	103.60	56.22	1.16	0.17
F	6.23	1070.68	25.96	7.41	0.64	13.04	124.77	55.17	1.08	0.10
H1	3.05	509.36	27.69	8.72	0.56	87.54	332.57	62.55	0.40	0.83
J	3.08	347.96	27.15	8.34	0.78	53.85	360.59	62.50	0.46	1.64
K	2.47	406.06	26.50	8.56	0.79	88.75	305.61	62.72	0.44	1.92
L_M	7.52	869.02	26.46	8.80	0.75	92.99	166.19	58.53	0.89	0.33
L_O	3.57	254.14	28.70	8.30	0.90	48.61	99.57	61.00	0.95	0.70
M	5.18	776.17	28.32	8.78	0.64	259.17	199.86	62.58	0.61	0.27
MP	5.91	721.85	28.13	8.64	0.64	242.15	326.29	63.05	0.57	0.35
N_A	4.20	545.25	25.31	7.62	0.71	13.13	772.48	58.04	1.04	0.25
P	6.94	612.35	26.48	8.62	0.79	96.95	166.79	59.54	0.79	0.23
S1	5.62	697.23	27.70	8.82	0.63	193.06	156.44	63.21	0.44	0.73
S_N	4.05	566.71	27.73	8.74	0.56	84.88	224.16	62.01	0.45	0.43
W1	1.28	230.96	27.83	8.64	0.95	98.56	229.12	65.82	0.27	4.00
W2	4.32	460.85	27.85	8.69	0.68	75.55	117.48	61.57	0.87	1.31
X1	6.92	609.16	28.58	8.59	0.79	368.97	106.62	63.25	0.77	0.62
X2	2.68	400.83	26.66	8.32	0.77	35.82	131.00	62.25	0.45	1.97
Y	2.18	396.54	26.75	8.39	0.79	80.47	610.25	63.94	0.41	2.35

Why and how? Explaining assembly rules and biological interactions of RFGs

Based on the habitat template, we established the boundaries of environmental variables where RFGs can become abundant (or occur for the RFGs E, MP, N_A, W1, and W2). These thresholds can act as assembly rules of each RFG, creating the limits where these groups can grow and present considerable biomasses. The RFGs D, X1, and P presented the most restrictive assembly rules, while RFGs M and S_N displayed the widest. For example, the assembly rules for the RFG D (*Navicula* sp.) are mixing depth: 1.0-1.5 m, irradiance: 184.4-209.3 µmol m^{-2} s^{-1}, temperature: 27.1-28.2 °C, pH: 8.7-9.2, herbivorous zooplankton relative biomass: 98.4-99.7%, SRP: 132.8-230.7 µg L^{-1}, DIN: 88.4-100.5 µg L^{-1}, TSI: 66.2-67.5, transparency: 0.2 m, and salinity: 3.1-4.9 PSU. For the RFG M (*Microcystis* spp.) the assembly rules are mixing depth: 1.0-13.2 m, irradiance: 114.6-1210.4 µmol m^{-2} s^{-1}, temperature: 26.3-30.5 °C, pH: 6.9-9.6, herbivorous zooplankton relative biomass: 10.9-99.9%, SRP: 6.6-527.9 µg L^{-1}, DIN: 19.2-494.7 µg L^{-1}, TSI: 50.4-67.5, transparency: 0.05-2.7 m, and salinity: 0.1-4.9 PSU. These statements follow the same pattern for the other RFGs (Fig. 2).

The habitat template was well represented by the five groups of reservoirs formed based on similar environmental conditions (Figs. 3a,b). Moreover, RFGs responded to the gradients of resources (trophic state index) and energy (water transparency), as also represented by the mean values of the variables weighted by the biomass of each RFG (Figs. 3c,d).

In general, RFGs displayed significant positive correlations between each other, showing a coexistence pattern, especially between M, MP, S1, and S_N; between H1, J, C, Y, and K; or between D, W1, and W2. Group H1 also positively correlated with S1, S_N, D, and W1; group J with S_N, N_A, and W1; group C with S_N, W1, and W2; group Y with S_N, N_A, and W1; group D with C, Y, and K; group X1 with M, MP, and S1; and group X2 with J, C, Y, and K. Group L_O showed a positive correlation with W2 and a negative with M (Fig. 4).

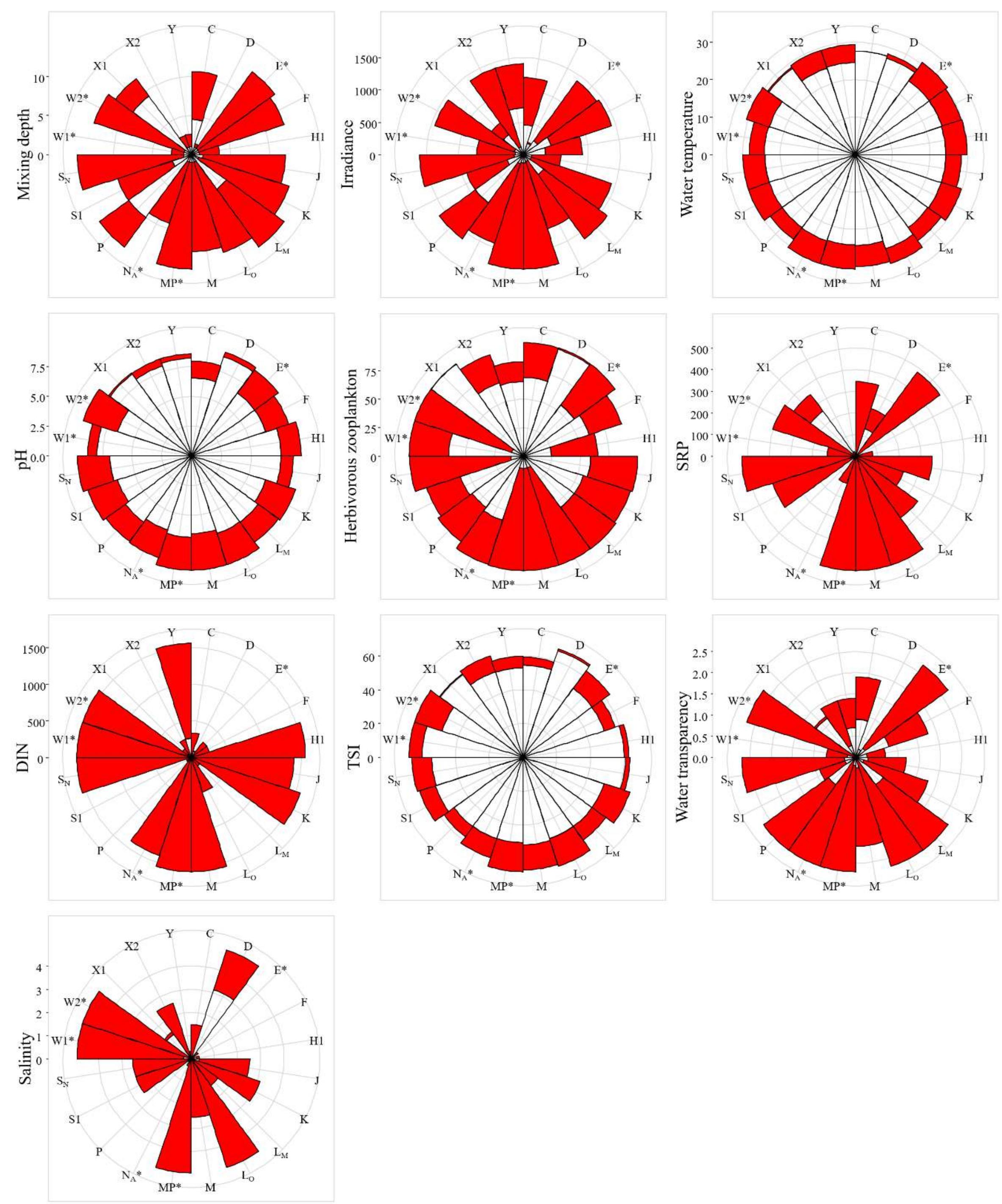

Fig. 2 Polar area charts showing the minimum and maximum values (limits of the red bars) of the 10 environmental axes where each Reynolds' functional group (RFG) can grow intensely and become abundant (relative biomass >5%) to represent the assembly rules (thresholds) of their occurrence as abundant in the tropical reservoirs studied (n = 42). Groups marked with an asterisk (*) represent those that did not reach relative biomass >5%, or only in one sample, so, the limits represent the thresholds of their occurrence. Variables: mixing zone depth, irradiance,

water temperature, pH, the relative biomass of herbivorous zooplankton, soluble reactive phosphorus (SRP), dissolved inorganic nitrogen (DIN), trophic state index (TSI), water transparency, and salinity

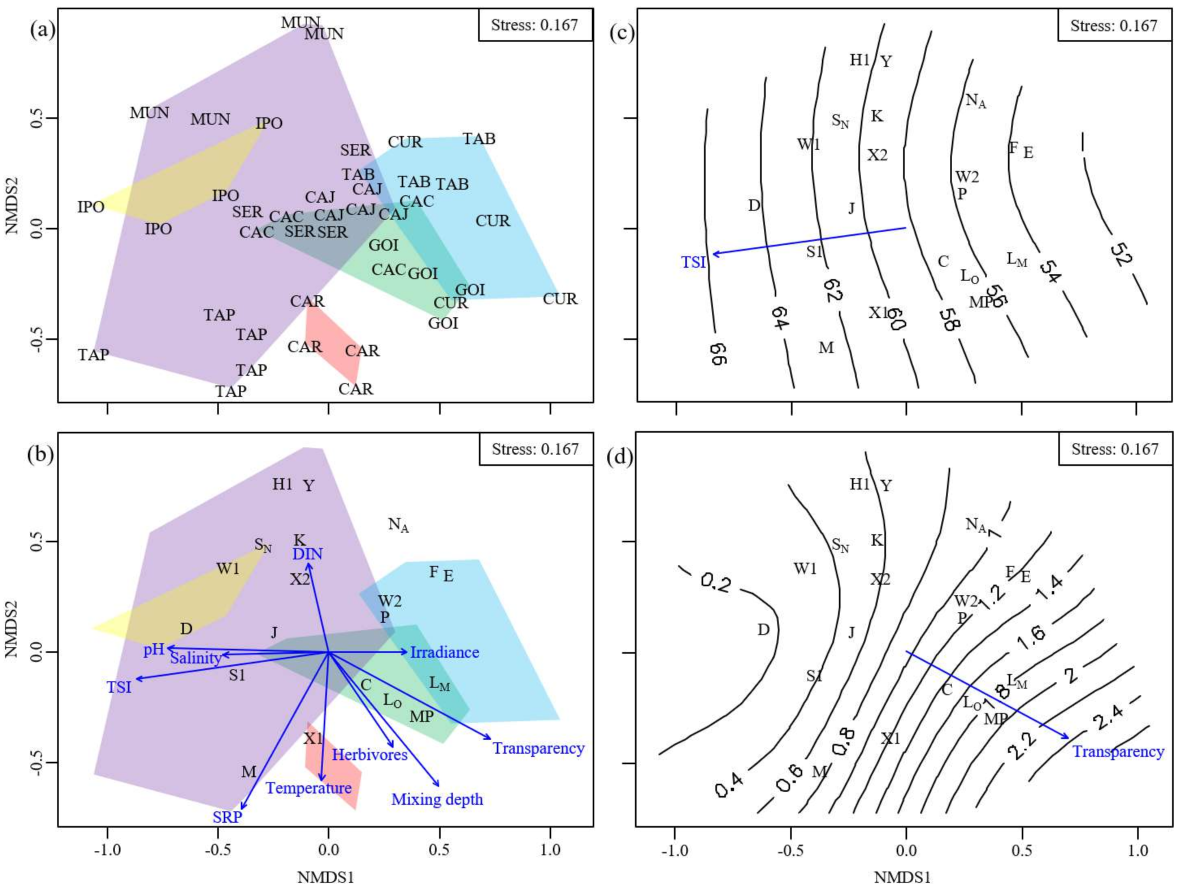

Fig. 3 Non-metric multidimensional scaling (NMDS) ordination showing the clustering reservoirs based on environmental gradients (a) and the distribution of Reynolds' functional groups (RFG) and their relationships with the environmental axes in the tropical reservoirs studied (n = 42). Panels (c) and (d) show the distribution of each RFG along the gradients of resources (trophic state index) and energy (water transparency), respectively. Variables: mixing zone depth, irradiance, water temperature, pH, the relative biomass of herbivorous zooplankton, soluble reactive phosphorus (SRP), dissolved inorganic nitrogen (DIN), trophic state index (TSI), water transparency, and salinity. Red area clusters the Carpina (CAR) reservoir; yellow area represents Ipojuca (IPO) reservoir; purple area indicates Tapacurá (TAP), Mundaú (MUN), Serrinha (SER), and Cajueiro (CAJ) reservoirs; green area groups Goitá (GOI) and Cachoeira (CAC) reservoirs; and the blue area represents Cursaí (CUR) and Tabocas (TAB) reservoirs

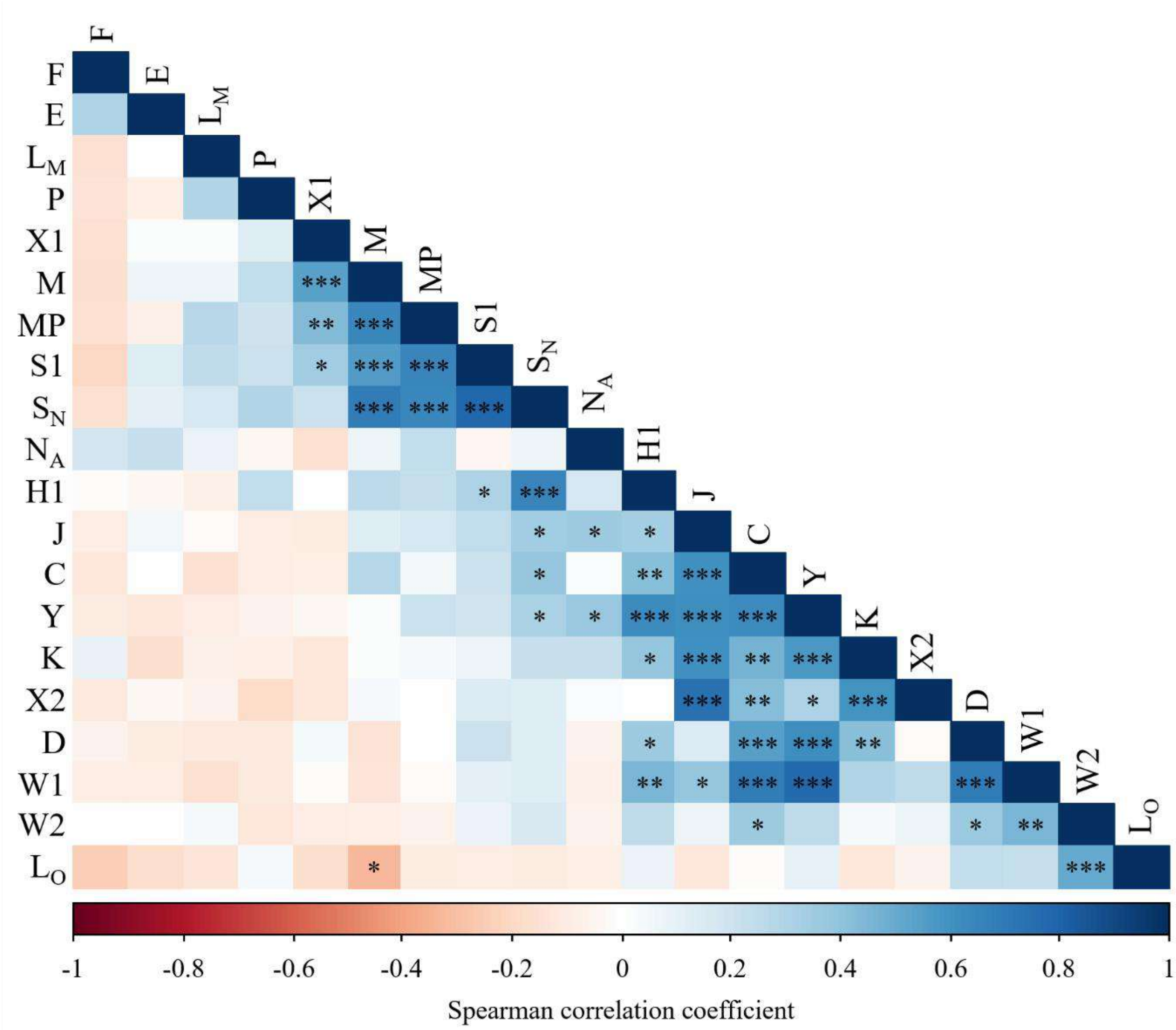

Fig. 4 Spearman rank correlation (rs) matrix to show the coexistence and inhibitory patterns among Reynolds' functional groups (RFGs) in the tropical reservoirs studied (n = 42). Asterisks represent significant correlations (*: $p < 0.05$; **: $p < 0.01$; and ***: $p < 0.001$)

When and where in new scenarios? Predicting the responses of RFGs to environmental changes

Generalized additive models were useful to clearly describe the relationships among RFGs and the 10 environmental axes (variables). Groups H1, M, MP, S1, and S_N were positively influenced by pH and TSI, and negatively by water transparency and herbivorous zooplankton. The latter variable also negatively influenced group W2 until 40% of herbivores relative biomass, while showed a negative influence on group L_O until 70% of relative biomass and positive after this value. Also, higher pH boosted the growth of groups F, L_M, and X1. Irradiance negatively impacted groups D, J, L_O, and W1, while water temperature negatively influenced groups F, K, and N_A (Fig. 5, Figs. S2–S11, Table S2).

Besides that, groups C, J, K, and Y showed a significant correlation with saline, stratified (low mixing depth), hypereutrophic, and turbid conditions. Moreover, groups D, W1, and X2 were also associated with stratified and saline environments. Group X2 was further negatively impacted by higher water transparency. Group F was associated with extremely low values of salinity (<0.05 PSU), group M and S_N showed a positive relationship with salinity until 0.5 PSU and decreased markedly in biomass after this value, however, group S_N showed a positive relationship again after 2 PSU, while group X2 presented higher biomasses at approximately 2.5 PSU (Fig. 5, Figs. S2–S11, Table S2).

Concentrations of DIN positively influenced only groups N_A and Y. Concentrations of SRP positively influenced groups M, MP, S1, and X1, besides showing an opposite relationship with groups E and F. Salinity also increased the biomass of groups D and W2 at concentrations of approximately 100 µg L^{-1} and negatively impacted them at extreme values. Higher values of trophic state index positively influenced group X1, besides D and W1 after a threshold of 65. Only group P did not correlate with any of the environmental axes (Fig. 5, Figs. S2–S11, Table S2).

In general, salinity was the best predictor of the RFGs biomass, accounting for 98.8% of the variance of the RFG D, 92% for X2, 89.2% for W1, 78.3% for K, 72.4% for F, 61.3% for C, and 48.6% for Y. Trophic state was the second-best predictor (75% for W1, 67.7% for D, and 36.7% for S1), followed by SRP (51.9% for E, 39% for X1, and 36.9% for W2), water transparency (37.5% for S1, and 31.4% for S_N), herbivorous zooplankton relative biomass (35.4% for W2, and 31.1% for S_N), and DIN (34.7% for Y). Mixing depth, irradiance, water temperature, and pH always explained less than 30% of the variance in each RFG biomass (Fig. 5, Figs. S2–S11, Table S2).

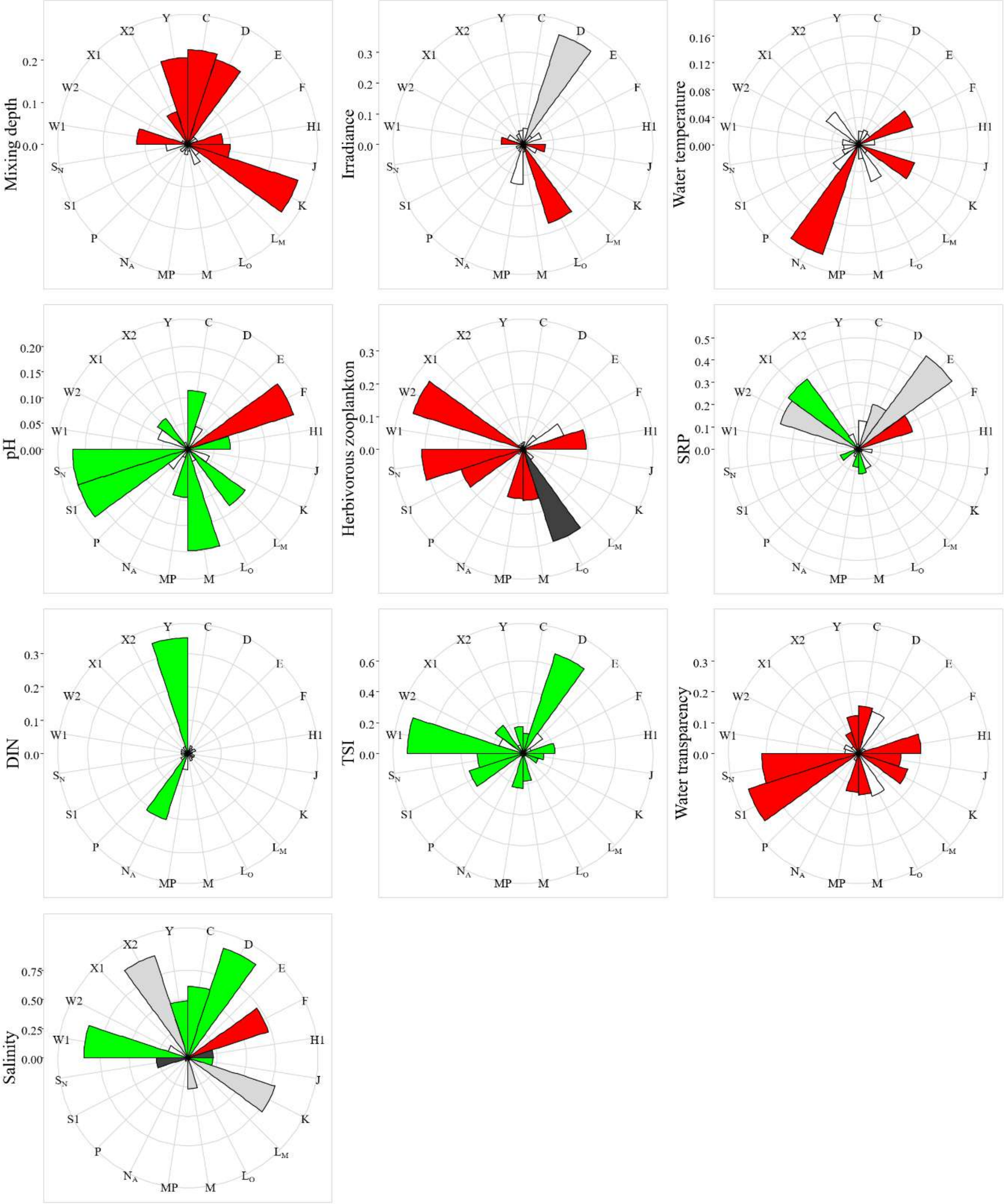

Fig. 5 Polar area charts showing the strength of the influence of the 10 environmental axes on each Reynolds' functional group (RFG) in the tropical reservoirs studied (n = 42), assessed by the R^2-adjusted from generalized additive models - GAM. Variables: mixing zone depth, irradiance, water temperature, pH, the relative biomass of

herbivorous zooplankton, soluble reactive phosphorus (SRP), dissolved inorganic nitrogen (DIN), trophic state index (TSI), water transparency, and salinity. Green bars: positive influence; red bars: negative influence; dark grey bars: humped-type relationship with the main influence negative and then positive; light gray bars: humped-type relationship with the main influence positive and then negative; white bars: non-significant relationships

Discussion

What lives where? Habitat template

There is no doubt about the usefulness and applicability of RFGs in phytoplankton ecology (Kruk et al. 2021). This classification can also be efficiently used to assess the ecological status of lakes (Padisák et al. 2006). Moreover, this approach has been proven to better reflect the anthropogenic impacts in aquatic ecosystems than species-based classifications (Abonyi et al. 2012).

The habitat template of most RFGs was constructed primarily based on samples from the temperate region, during the proposal of this classification much less information was available for tropical regions (e.g., Reynolds 1998, 2006; Reynolds et al. 2002). More recently, Salmaso et al. (2015) graphically represented the habitat template of the RFGs based on data from Reynolds et al. (2002). They statistically confirmed the relationships of the RFGs with the environmental axes. However, more information about tropical habitats is needed. Accordingly, our approach allowed us to explore the specific conditions where RFGs are more successful (as abundant or dominant) and form surface blooms, besides establishing the boundaries of their occurrences at high biomasses in tropical reservoirs.

According to Reynolds (2006), phytoplankton will grow wherever and whenever they can and become abundant if a suitable inoculum and optimum conditions are available. Nevertheless, how the input of a new inoculum affects phytoplankton assembly has not been evaluated yet (Kruk et al. 2021). Considering that, we opted to classify all phytoplankton species into RFGs, even the non-abundant ones (relative biomass <5%), unlike most studies (e.g., Costa et al. 2016; Rodrigues et al. 2018; Kruk et al. 2021). Non-dominant species can act as the inoculum of the future assemblages, because, even at low biomasses, they can compete for resources better than dominant species under certain circumstances (Rojo and Álvarez-Cobelas 2003). Thus, they must not be underestimated (Rojo 2021).

From the 20 RFGs identified in our study, groups E (*Dinobryon* sp. and *Mallomonas caudata*), N_A (*Cosmarium reniforme* and *Staurastrum leptocladum*), W1 (*Euglena viridis*), and W2 (*Trachelomonas volvocina*) did not present enough biomass to be considered as abundant. However, they were present in unique conditions that allowed their growth and tolerance to environmental pressures. If these conditions become more intense, these groups will probably present their best fitness, achieving higher biomasses and possibly becoming dominant in future assemblages. Moreover, what defines the successful development of a phytoplankton species is not its biomass, but how it supports environmental inadequacies when it arrives in a new habitat (Reynolds 2012). Accordingly, evaluating the habitat template and environmental drives of non-dominant groups or species is important to predict the state and structure of future assemblages.

Why and how? Assembly rules, biological interactions, and driving processes

After the establishment of the best environmental conditions for each RFG (habitat template), we defined the assembly rules and delimited the thresholds of their occurrence as abundant. Although some authors ask for a null model to describe assembly patterns (e.g., Götzenberger et al. 2012; Borics et al. 2020), these models do not specify assembly rules properly, as defined by Keddy and Weiher (1999).

The high variability of the environmental conditions in the selected reservoirs accounted for a high diversity of phytoplankton assemblages. The positive correlation between many RFGs revealed a coexistence pattern between them, contradicting the principle of competitive exclusion when the number of species is limited by the number of limiting resources (Hutchinson 1961; Burson et al. 2018). This pattern can be explained by the different ways how species use limiting resources (Amorim and Moura 2021). Based on that, we could separate the reservoirs into five main habitat templates. These clusters better reflected the environmental conditions, besides the RFGs they hosted and their responses to the resource and energy axes. Based on that, we were able to redraw the habitat template of 20 RFGs from Reynolds (2006) with data exclusively from the tropical region and after the inclusion of three new environmental axes (Fig. 6).

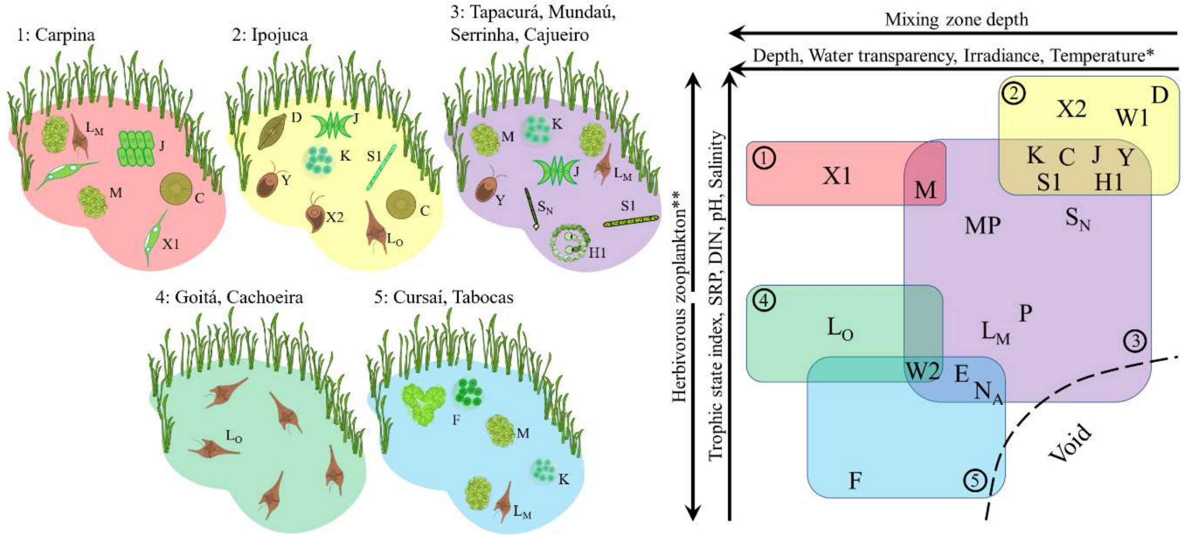

Fig. 6 Representation of the habitat template where the 20 Reynolds' functional groups (RFGs) are more successful in tropical reservoirs. The RFGs were plotted based on the resource (y-axis: trophic state index, soluble reactive phosphorus - SRP, dissolved inorganic nitrogen - DIN, pH, and salinity) and energy (x-axis: mixing zone depth, water transparency, irradiance, and temperature) gradients, besides the gradient of potential loss processes due to herbivores grazing (y-axis: herbivorous zooplankton). See results for a description of the main environmental conditions in each group of reservoirs. *The effects of temperature were minimal due to the small variance across reservoirs; **The herbivorous zooplankton presented higher relative biomasses in both extremes of the loss processes axis

Carpina reservoir presented low phytoplankton biomass but a considerable number of abundant groups (M, *Microcystis* spp.; L_M, *Microcystis* spp. and *Ceratium furcoides*; X1, *Schroederia setigera*; J, *Scenedesmus ecornis*; C, *Cyclotella meneghiniana*, and S_N, *Raphidiopsis raciborskii*). This reservoir was hypereutrophic with higher temperature, pH, SRP, TSI, and salinity, but differed from the other hypereutrophic environments by presenting high mixing zone depth (destratified), water transparency, and relative biomass of herbivorous zooplankton. This discrepancy can be explained by the large stands of the submerged macrophyte *Ceratophyllum demersum* in Carpina reservoirs, which can inhibit phytoplankton growth through allelopathy, especially cyanobacteria (see Amorim and Moura 2020). Moreover, *C. demersum* presents lower or no inhibitory effects on green algae (Amorim et al. 2019a). These conditions allowed higher biomass of RFG X1 in this destratified and clear reservoir. This group was positively influenced by pH, SRP, and TSI, confirming its preference for mixed layers in eutrophic to hypereutrophic habitats (e.g., Reynolds et al. 2002; Padisák et al. 2009). Furthermore, RFG X1 was also found in saline environments (Gómez et al. 2004; Afonina and Tashlykova 2019).

The distinctive environmental characteristics of Ipojuca reservoir restricted the occurrence of the RFG D as abundant to this habitat, although presenting other abundant groups (J, *Tetradesmus lagerheimii*; L_O, *Ceratium furcoides* and *Synechocystis aquatilis*; X2, *Plagioselmis lacustris*; K, *Aphanocapsa incerta*; M, *M. aeruginosa*; S1, *Planktolyngbya limnetica*; and S_N, *R. raciborskii*). Group D was represented by a small-sized (~10 µm), planktonic *Navicula* sp., that was restricted to a shallow, stratified, alkaline, turbid, hypereutrophic, and extremely saline reservoir with lower irradiance and higher biomass of herbivorous zooplankton (mainly rotifers, see Amorim and Moura 2021). Moreover, this group was significantly influenced by mixing depth, irradiance, SRP, TSI, and salinity. Small diatoms are favored under stratification conditions, due to their small surface area to volume ratio

(Winder et al. 2009). Also, some diatom taxa, such as *Navicula* spp. and *Nitzschia* spp., can find optimum conditions and occur strictly in extreme saline inland habitats (Tibby et al. 2007). Besides, freshwater diatoms from turbid environments present higher growth rates and photosynthetic activities under low light intensities (Shi et al. 2015).

In Ipojuca reservoir, different phytoplankton RFGs coexisted, including diatoms (D), green algae (J), dinoflagellates (L_O), cryptophytes (X2), and cyanobacteria (K, L_O, M, S1, and S_N). Among the main resources in Ipojuca, only DIN (<80 µg L^{-1}) and irradiance (<300 µmol m^{-2} s^{-1}) were limited. Considering the principle of competitive exclusion, the number of dominant species is equal to the limiting resources, which favor the most competitive species (Hutchinson 1961). However, different phytoplankton groups present contrasting ecological strategies and use resources more efficiently, allowing coexistence (Amorim and Moura 2021). Besides that, light represents a complex spectrum of different resources, promoting the niche differentiation between species and enabling the coexistence of species specialized in the utilization of different parts of the light spectrum (Stomp et al. 2004). Due to the presence of several photosynthetic pigments, different phytoplankton groups can take advantage of the entire light spectrum and grow even under low irradiance conditions (Burson et al. 2018).

The expressive presence of cyanobacteria in Ipojuca reservoir, especially the RFGs K, L_O, M, S1, and S_N, is explained by the synergistic effects of higher trophic state and extreme salinity levels on cyanobacteria, as already verified by Amorim et al. (2020). Indeed, the RFGs K, M, and S_N were significantly influenced by both TSI and salinity, while S1 was impacted by TSI. Salinization is a common problem in drylands (e.g., Brazilian semiarid region), where the water level reductions and high evaporation rates, associated with agriculture and demand for irrigation, increase the accumulation of salts (Cañedo-Argüelles et al. 2013). These conditions favor cyanobacterial blooms because many freshwater bloom-forming taxa are salt-tolerant (e.g., *Anabaenopsis*, *Dolichospermum*, *Microcystis*, *Oscillatoria*, *Synechococcus*, and other picocyanobacteria) (Paerl et al. 2016).

The group composed of the hypereutrophic reservoirs (Tapacurá, Mundaú, and Serrinha) plus the mesotrophic Cajueiro presented high pH, low to moderate irradiance, and herbivorous zooplankton relative biomass, besides low mixing depth (stratified), water transparency, and salinity. Many cyanobacterial RFGs formed blooms in these reservoirs, mainly M (*Microcystis* spp.) that was dominant in all of them, in addition to Carpina and Cursaí, and abundant in Tabocas and Ipojuca. This group presented the widest range of occurrence as abundant, from oligotrophic to hypereutrophic, clear to turbid, neutral to alkaline, freshwater to saline, and from stratified to destratified habitats. Besides RFG M, *Microcystis* spp. also formed an association with the dinoflagellate *Ceratium furcoides* in the RFG L_M, dominant in Cajueiro, Carpina, Cursaí, and Serrinha. *Microcystis* is one of the most toxigenic and widespread bloom-forming cyanobacteria, occurring in all continents, except Antarctica (Harke et al. 2016).

The RFG S_N (*R. raciborskii*) was dominant in Serrinha and abundant in Tapacurá, Cajueiro, Mundaú, Carpina, Cursaí, and Ipojuca. This group is represented by another potentially toxic and widespread cyanobacterium (Sinha et al. 2012). It also presented a wide habitat template as RFG M, being associated with higher TSI, pH, and salinity, besides low values of transparency and biomass of herbivorous zooplankton. All filamentous cyanobacteria, represented by the RFGs H1 (*Anabaenopsis elenkinii* and *Sphaerospermopsis aphanizomenoides*), S1 (*Anagnostidinema amphibium* and *P. limnetica*), and S_N (*R. raciborskii*) were negatively influenced by the herbivorous zooplankton biomass. This happened because herbivorous zooplankton can cut large filaments, and then ingest the small particles (Amorim et al. 2019b). The other abundant RFGs in the reservoirs with cyanobacterial blooms were L_O (*Snowella lacustris* and *Merismopedia tenuissima*), K (*Aphanocapsa delicatissima*), J (*Pediastrum duplex*), Y (*Gymnodinium* sp.), and P (*Aulacoseira granulata*).

In Goitá and Cachoeira reservoirs (both eutrophic, with high water temperature, transparency, and biomass of herbivorous zooplankton, besides neutral pH, low irradiance, and salinity) only RFG L_O (*C. furcoides*) was dominant, contributing to more than 97% of phytoplankton biomass during blooms. Group L_O was negatively influenced by the irradiance, while showed a humped-type relationship with the biomass of herbivorous zooplankton, being negatively impacted until 70% of relative biomass, and positively after this value. This RFG represents a complex assemblage, composed of small picocyanobacteria (*S. lacustris*, *M. tenuissima*, and *S. aquatilis*) and large dinoflagellates. Both groups were abundant or dominant under low irradiance (Goitá, Cachoeira, Ipojuca, Mundaú, and Tapacurá). *Ceratium furcoides* presents swimming movements and phototaxis that allow it to search for better conditions of light (Clegg et al. 2003). Low insolation and higher temperatures are conditions that favor the growth of *C. furcoides* and the formation of blooms (Lindström 1992). Similarly, the diversity of accessory pigments in cyanobacteria makes them strong competitors for light, which enables them to grow under low light conditions (Carey et al. 2012).

The humped effect of herbivores on RFG L_O, verified in the GAM models, can be explained by the dominance of picocyanobacteria under low herbivorous biomass, and dinoflagellates under high biomass, with a negative effect on picocyanobacteria and positive on dinoflagellates. In previous work, Amorim and Moura (2021) demonstrated that cyanobacterial blooms presented higher biomass of omnivorous cyclopoid copepods, while dinoflagellate blooms increased the biomass of herbivorous calanoid copepods and cladocerans. Herbivorous zooplankton can efficiently consume picocyanobacteria (Amorim et al. 2020). Likewise, calanoid copepods can graze on *C. furcoides* cells, despite their large size (Santer 1996).

The last group of reservoirs, composed of Tabocas (mesotrophic) and Cursaí (oligotrophic), displayed high water transparency, mixing depth (destratified), irradiance, herbivorous zooplankton biomass, neutral pH, low levels of SRP, DIN, and salinity. These reservoirs were characterized by the increased biomass of RFG F (*Botryococcus braunii* and *Eutetramorus tetrasporus*), which was negatively impacted by water temperature, pH, SRP, and salinity, demonstrating its preference for oligo-mesotrophic conditions. *Botryococcus braunii* can form blooms in deep, stratified, clear-water, and oligotrophic lakes (Towsend 2001). The high oil content in its colonies favors its buoyancy under stratification conditions (Wake and Hillen 1980).

When and where in new scenarios? RFGs as predictors of environmental change

Since its proposal, the RFGs approach has been largely used to describe spatiotemporal patterns of phytoplankton and its tolerances and sensitivities to environmental variables (Salmaso et al. 2015). However, much less has been done to explain “why and how” phytoplankton is assembled and more research should focus on explaining “when and where” phytoplankton RFGs will dominate under new scenarios of environmental change (Kruk et al. 2021). As shown above, in our study, GAM models were efficient in predicting the response of 20 RFGs to 10 environmental gradients, providing explanations of the mechanisms involved in their dominance in specific ranges of environmental conditions (habitat template). Based on that, we quantified the relationships between RFGs biomass and each environmental axis for dominant, abundant, and non-abundant groups, which enabled the prediction of the most favorable set of environmental conditions considered optima for their growth in the gradients of resources and energy (Fig. 6). By considering abundant and non-abundant groups, our approach is also useful to predict how environmental changes will affect future phytoplankton assemblages’ structure, and thus can estimate when and where phytoplankton RFGs will be more successful under new scenarios of global change.

Accordingly, the ability of RFGs of predicting environmental change can support the adoption of sustainable practices to avoid eutrophication. In this regard, the knowledge of the key functional traits of dominant phytoplankton groups is essential for the success of management strategies to control algal blooms (Ibelings et al. 2016). Based on that, Mantzouki et al. (2016) developed a schematic framework to inform lake managers of the best practices to control the main RFGs responsible for harmful cyanobacterial blooms. The combination of these practices will maintain the provision of ecosystem services in lakes and reservoirs, allowing the sustainable development and conservation of aquatic resources.

Another valuable applicability of the RFGs approach is the prediction of ecosystem functioning. In this regard, in previous work (see Amorim and Moura 2021), we demonstrated how algal blooms impact water quality, plankton diversity, structure, and ecosystem functioning. In that article, cyanobacterial (RFGs M, SN, and L_O) and mixed blooms (RFGs cyanobacteria: K, diatoms: D, green algae: J, and dinoflagellates: L_O) increased water pH, concentrations of phosphorus, and phytoplankton richness, but reduced water transparency and zooplankton richness. Dinoflagellate blooms (RFG L_O) presented higher water transparency and zooplankton richness, in addition to low phytoplankton richness. Besides that, green algae blooms (RFG F) displayed higher water transparency, but lower pH, phosphorus, and zooplankton richness. All bloom categories boosted phytoplankton resource use efficiency but decreased for zooplankton. These results reinforce the applicability of the RFG approach in predicting water quality, plankton biodiversity, structure, and ecosystem functioning.

Conclusions

To the best of our knowledge, this is the first attempt to recreate the habitat template of RFGs exclusively for ecosystems from the tropical region. In this study, we defined the habitat template for 20 RFGs in tropical drinking water reservoirs, describing the main environmental conditions where they are most successful and can form blooms. Moreover, we designated the assembly rules and the boundaries of 10 environmental axes where the RFGs can become dominant in tropical reservoirs. The positive correlation between many RFGs revealed a coexistence pattern instead of harmful effects. Furthermore, generalized additive models were useful to explain and predict the structure of phytoplankton assemblages, with the most variance in the RFG's biomass explained by salinity, TSI, SRP, transparency, herbivores biomass, and DIN.

Our approach does not substitute the habitat templates proposed by Reynolds et al. (2002) and Pádisak et al. (2009) but complements the understanding of how RFGs interacts with environmental gradients in tropical reservoirs, by considering dominant and non-dominant groups and including three new environmental axes. Based on our results, water managers can predict in which circumstances RFGs will become dominant in tropical drinking water reservoirs. With this knowledge, it will be possible to anticipate the necessity of restoration measures. Although we described the structure of RFGs, their main processes, and mechanisms in five habitat templates, some of them are underrepresented and still need further clarifications. As Reynolds (2006) said: "*the ecology of populations and communities is relevant to many aspects of human existence, from the safety of drinking water to the sustainability of fisheries. The accumulated knowledge is both broad and deep but it is far from complete*". So, more research is needed, especially to explain and predict phytoplankton structure in tropical habitats, including lakes, reservoirs, rivers, estuaries, and oceans.

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Competing interests

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

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Code availability

Not applicable.

Author Contributions

CAA participated in the conceptualization, methodology development, validation, data curation, and formal analysis of all data, wrote the original draft, wrote, reviewed, and edited the final version of the manuscript. ANM participated in the conceptualization, supervision, funding acquisition, methodology of all data, wrote, reviewed, and edited the final version of the manuscript. All authors read and approved the final manuscript.

Ethics approval

Not applicable.

Consent to participate

Not applicable.

Consent for publication

Not applicable.

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Supplementary information

Reynolds' functional groups in tropical drinking water reservoirs: describing, explaining, and predicting habitat templates and assembly rules of phytoplankton

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Table S1 List of the 10 tropical reservoirs selected for this study, their municipalities, geographical coordinates, storage capacity, trophic state, and mean depth

Reservoir - abbreviation	**Municipality**	**Geographical location**	**Storage (10^3 m^3)[a]**	**Trophic state[b]**	**Mean depth[c]**
Carpina-CAR	Lagoa do Carro	7°53'43.3"S; 35°20'26.2"W	270,000	Hypereutrophic	11.05
Cursaí-CUR	Paudalho	7°52'36.6"S; 35°10'45.8"W	13,034	Oligotrophic	12.76
Goitá-GOI	Glória do Goitá	7°58'14.5"S; 35°07'00.8"W	52,536	Eutrophic	9.08
Tapacurá-TAP	São Lourenço da Mata	8°02'39.4"S; 35°11'52.0"W	104,871	Hypereutrophic	8.89
Cajueiro-CAJ	Garanhuns	8°58'09.2"S; 36°28'48.7"W	19,283	Mesotrophic	7.16
Ipojuca-IPO	Belo Jardim	8°20'49.4"S; 36°22'35.2"W	29,336	Hypereutrophic	2.53
Mundaú-MUN	Garanhuns	8°56'33.7"S; 36°29'23.7"W	1,969	Hypereutrophic	3.34
Tabocas-TAB	Belo Jardim	8°14'54.9"S; 36°22'38.4"W	1,168	Mesotrophic	8.68
Cachoeira-CAC	Serra Talhada	7°58'18.9"S; 38°19'25.8"W	21,031	Eutrophic	7.43
Serrinha-SER	Serra Talhada	8°11'41.0"S; 38°31'19.6"W	311,080	Hypereutrophic	7.40

[a]Source: APAC (2020); [b]Data from the present study; [c]Measured at the deepest point and close to the dam.

Fig. S1 Mean values (± standard error) of the 10 environmental axes in the tropical reservoirs studied (n = 42). See Table S1 for reservoir abbreviations

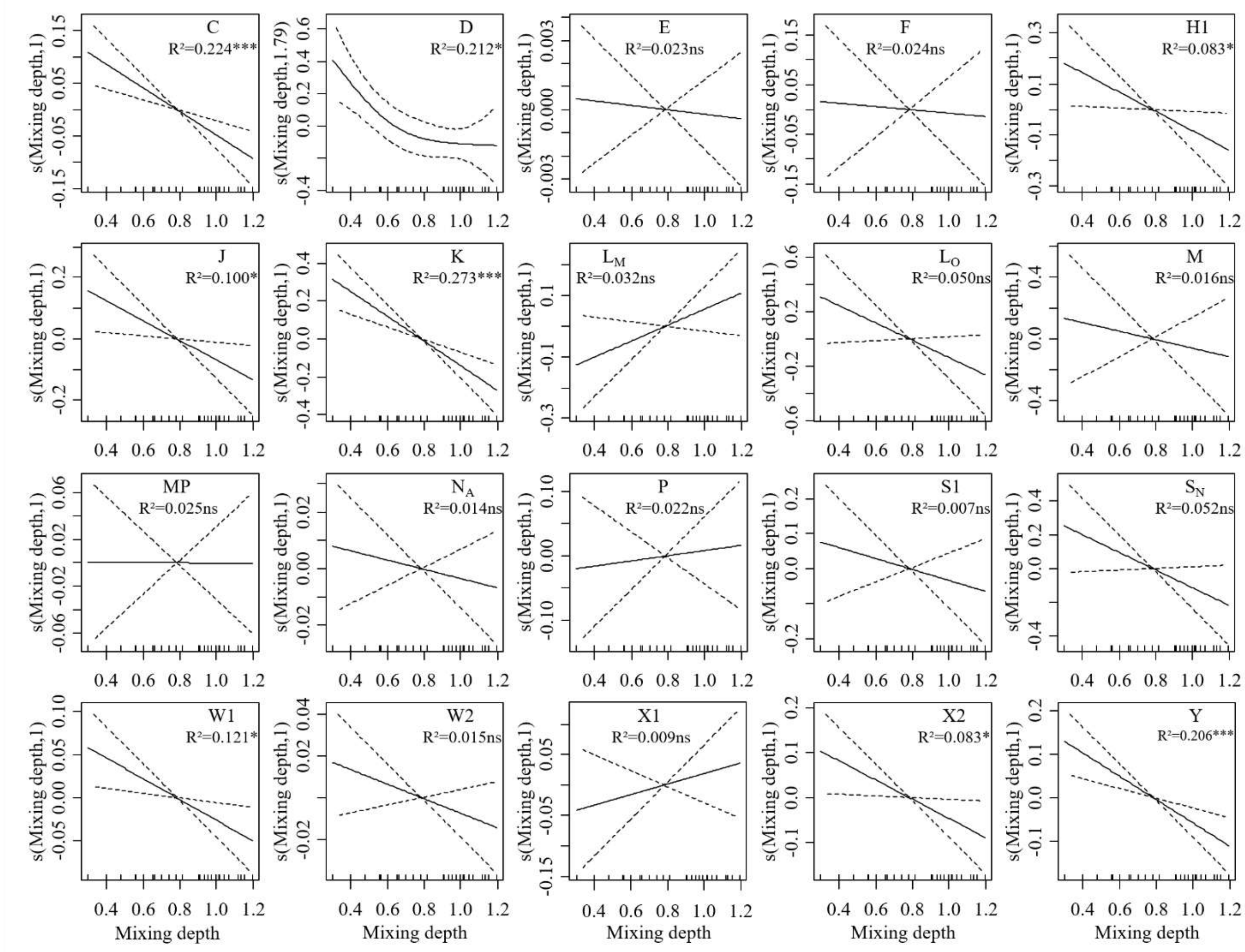

Fig. S2 Generalized additive models - GAM showing the effects of mixing zone depth on each Reynolds' functional group (RFG) in the tropical reservoirs studied (n = 42). Asterisks represent significant correlations (*: p <0.05; **: p <0.01; and ***: p <0.001)

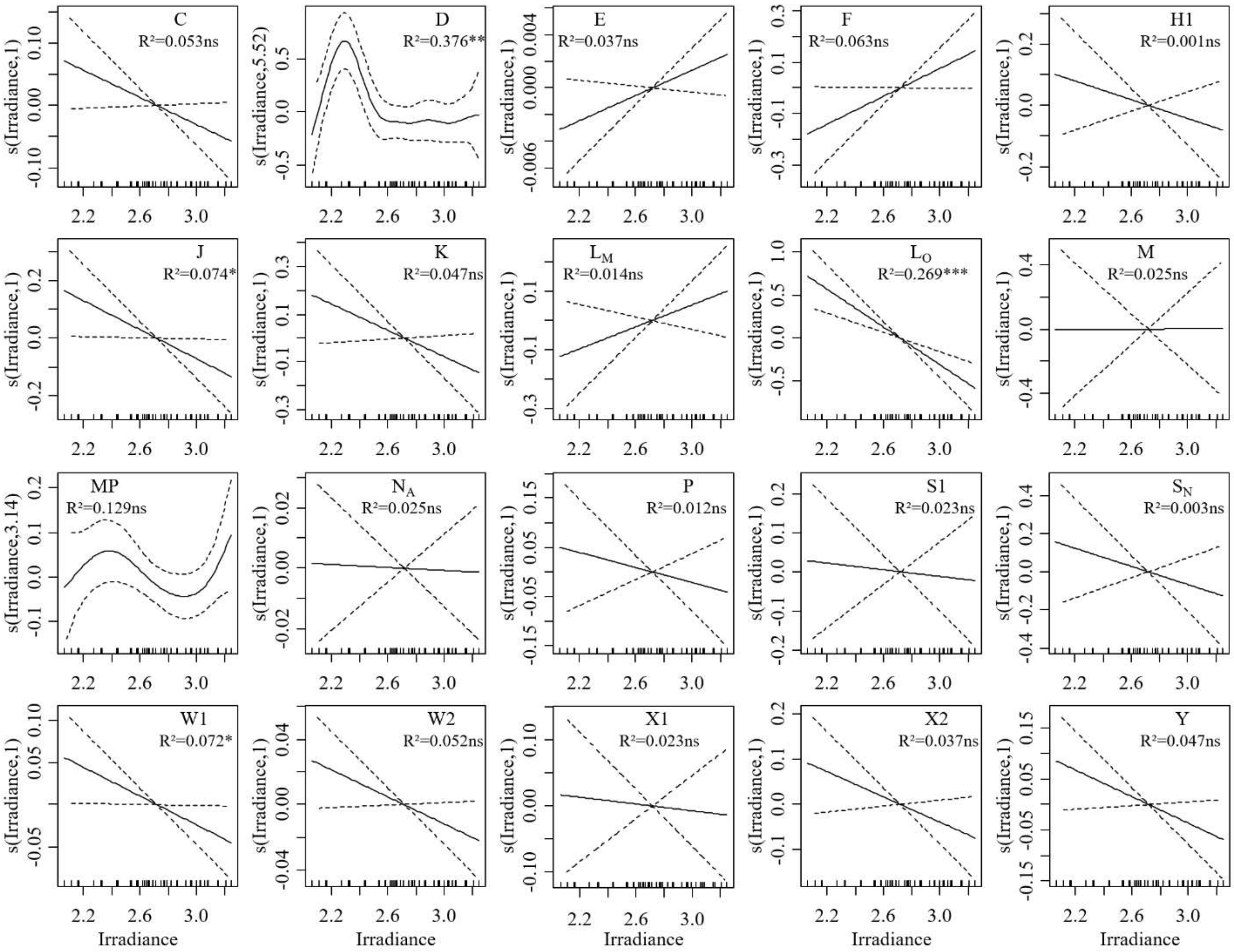

Fig. S3 Generalized additive models - GAM showing the effects of irradiance on each Reynolds' functional group (RFG) in the tropical reservoirs studied (n = 42). Asterisks represent significant correlations (*: p <0.05; **: p <0.01; and ***: p <0.001)

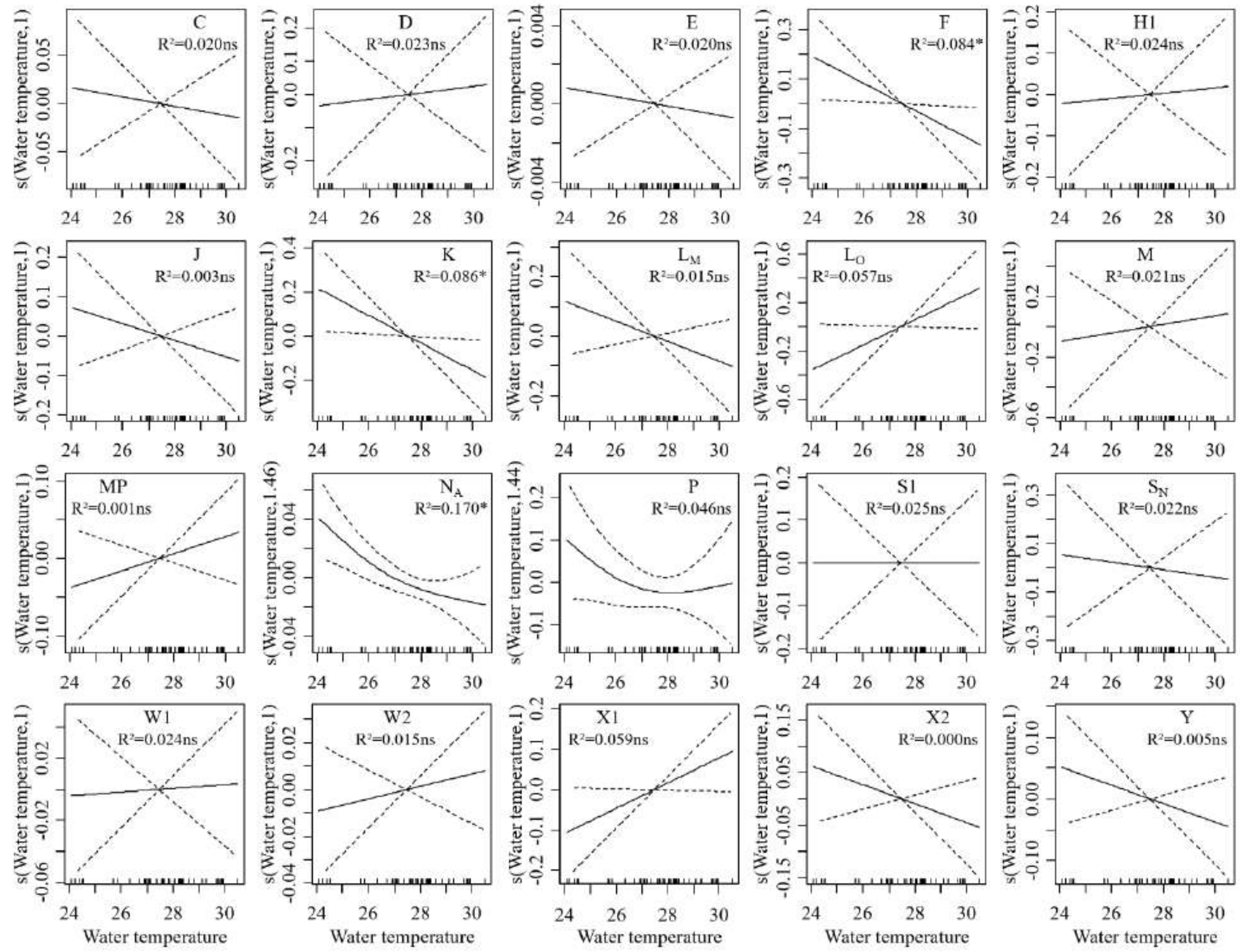

Fig. S4 Generalized additive models - GAM showing the effects of water temperature on each Reynolds' functional group (RFG) in the tropical reservoirs studied (n = 42). Asterisks represent significant correlations (*: p <0.05; **: p <0.01; and ***: p <0.001)

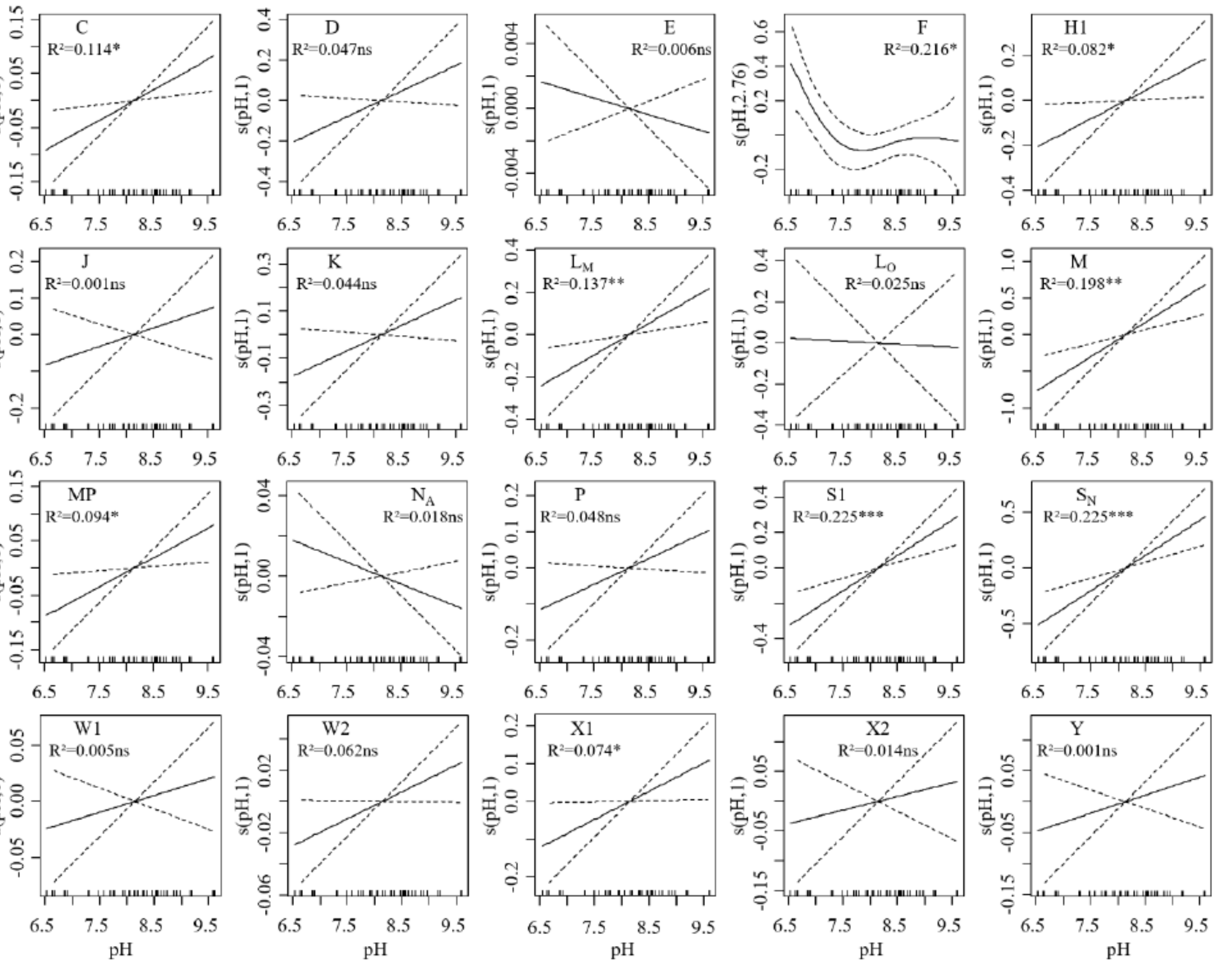

Fig. S5 Generalized additive models - GAM showing the effects of pH on each Reynolds' functional group (RFG) in the tropical reservoirs studied (n = 42). Asterisks represent significant correlations (*: p <0.05; **: p <0.01; and ***: p <0.001)

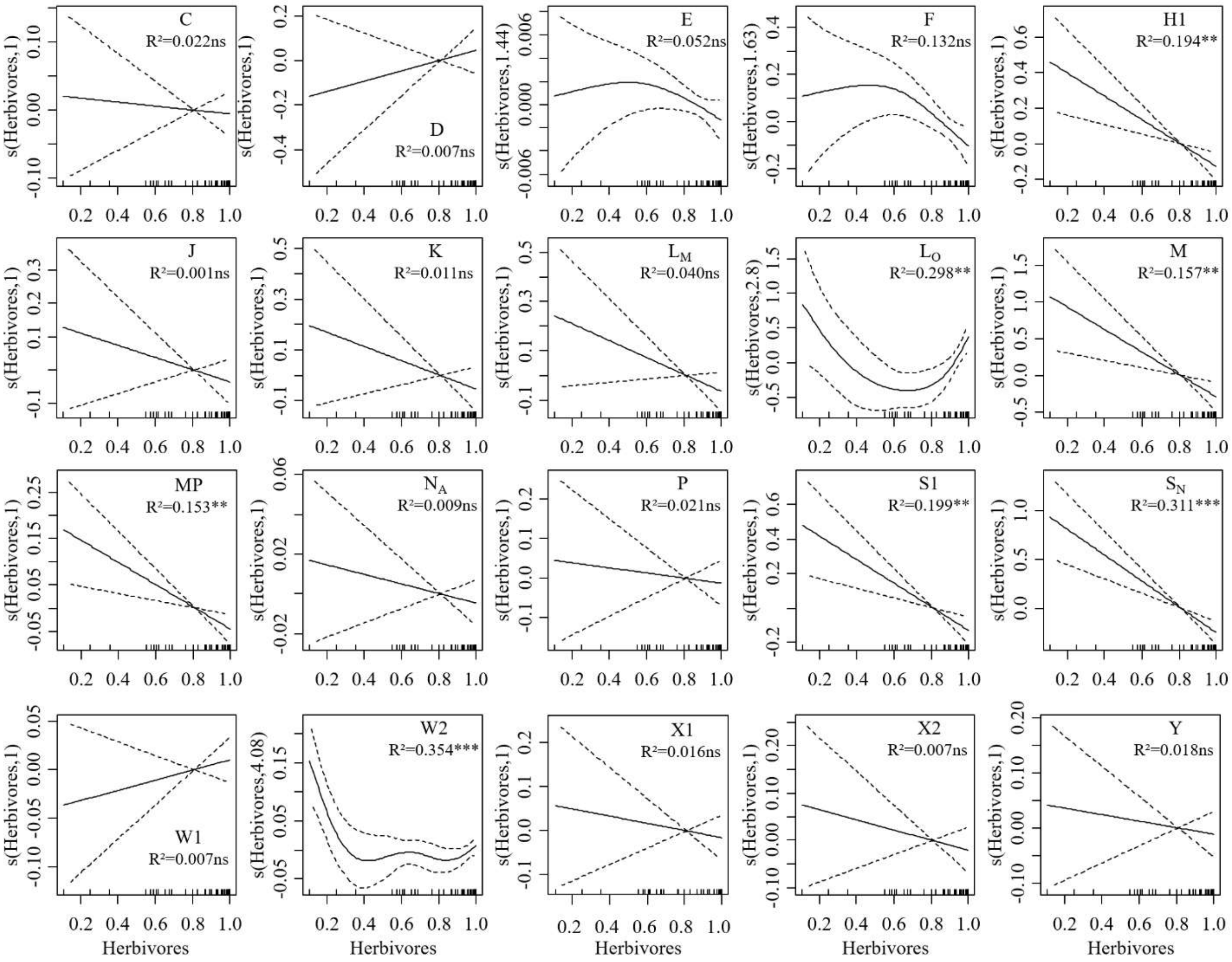

Fig. S6 Generalized additive models - GAM showing the effects of herbivorous zooplankton relative biomass on each Reynolds' functional group (RFG) in the tropical reservoirs studied (n = 42). Asterisks represent significant correlations (*: p <0.05; **: p <0.01; and ***: p <0.001)

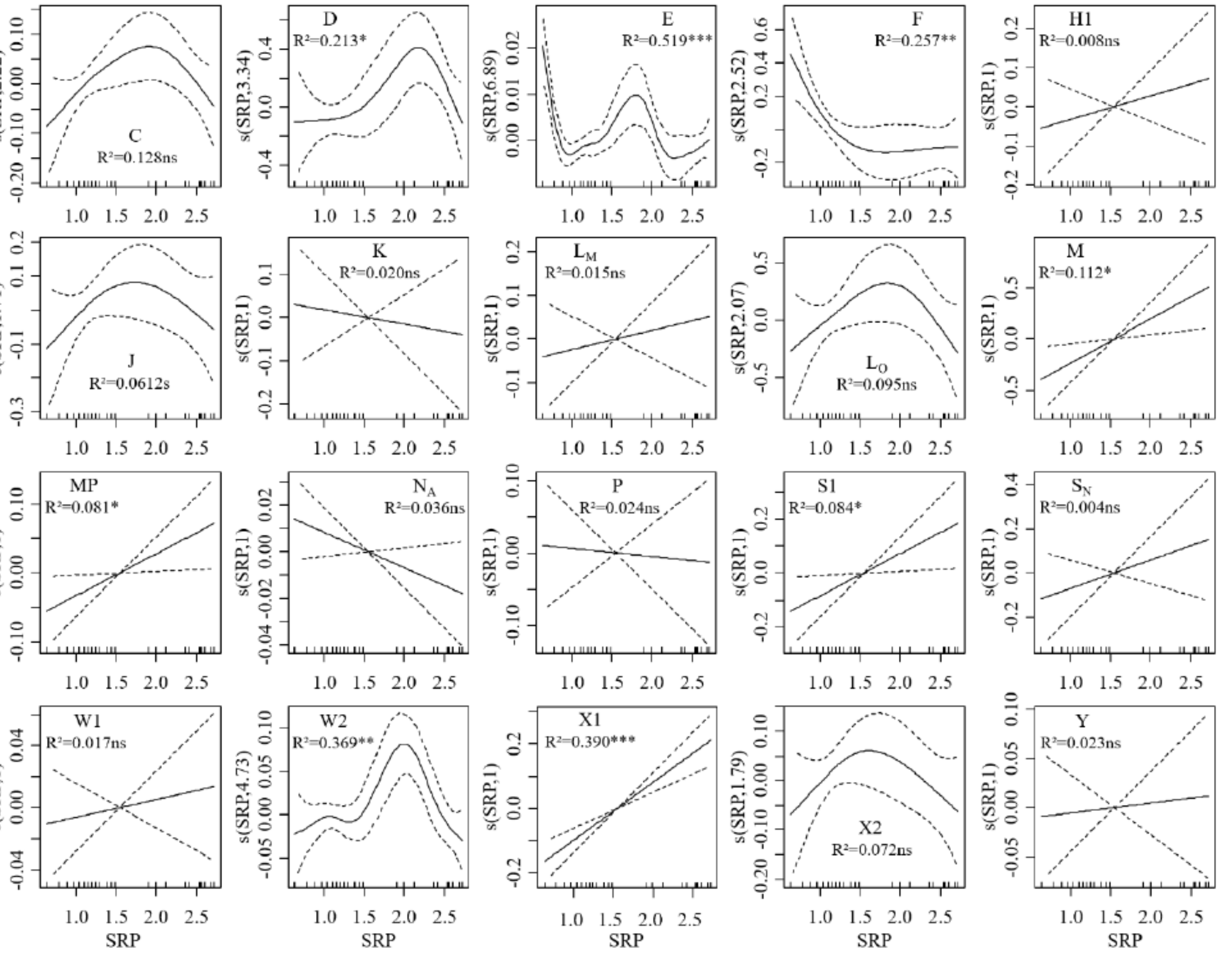

Fig. S7 Generalized additive models - GAM showing the effects of soluble reactive phosphorus (SRP) on each Reynolds' functional group (RFG) in the tropical reservoirs studied (n = 42). Asterisks represent significant correlations (*: $p < 0.05$; **: $p < 0.01$; and ***: $p < 0.001$)

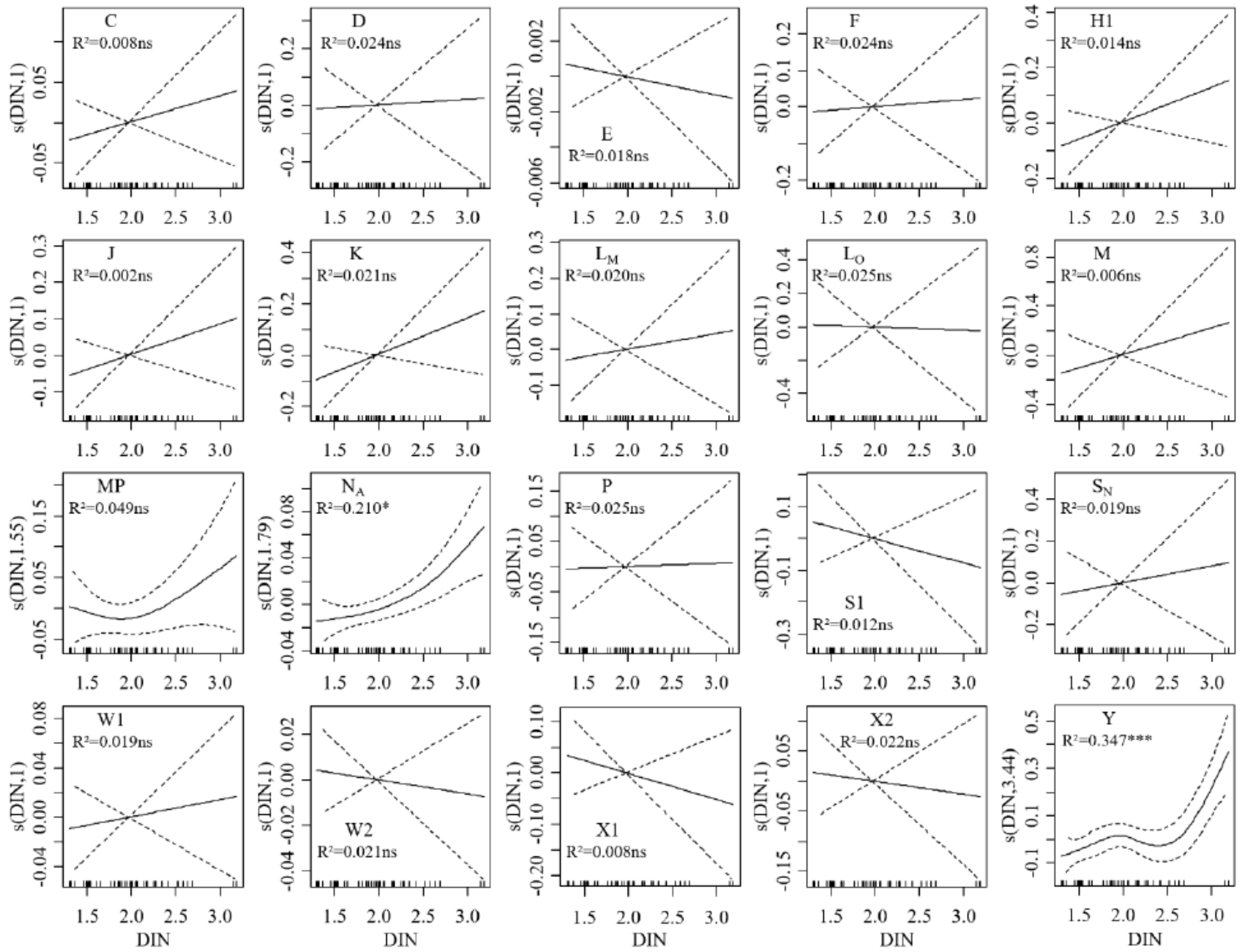

Fig. S8 Generalized additive models - GAM showing the effects of dissolved inorganic nitrogen (DIN) on each Reynolds' functional group (RFG) in the tropical reservoirs studied (n = 42). Asterisks represent significant correlations (*: $p < 0.05$; **: $p < 0.01$; and ***: $p < 0.001$)

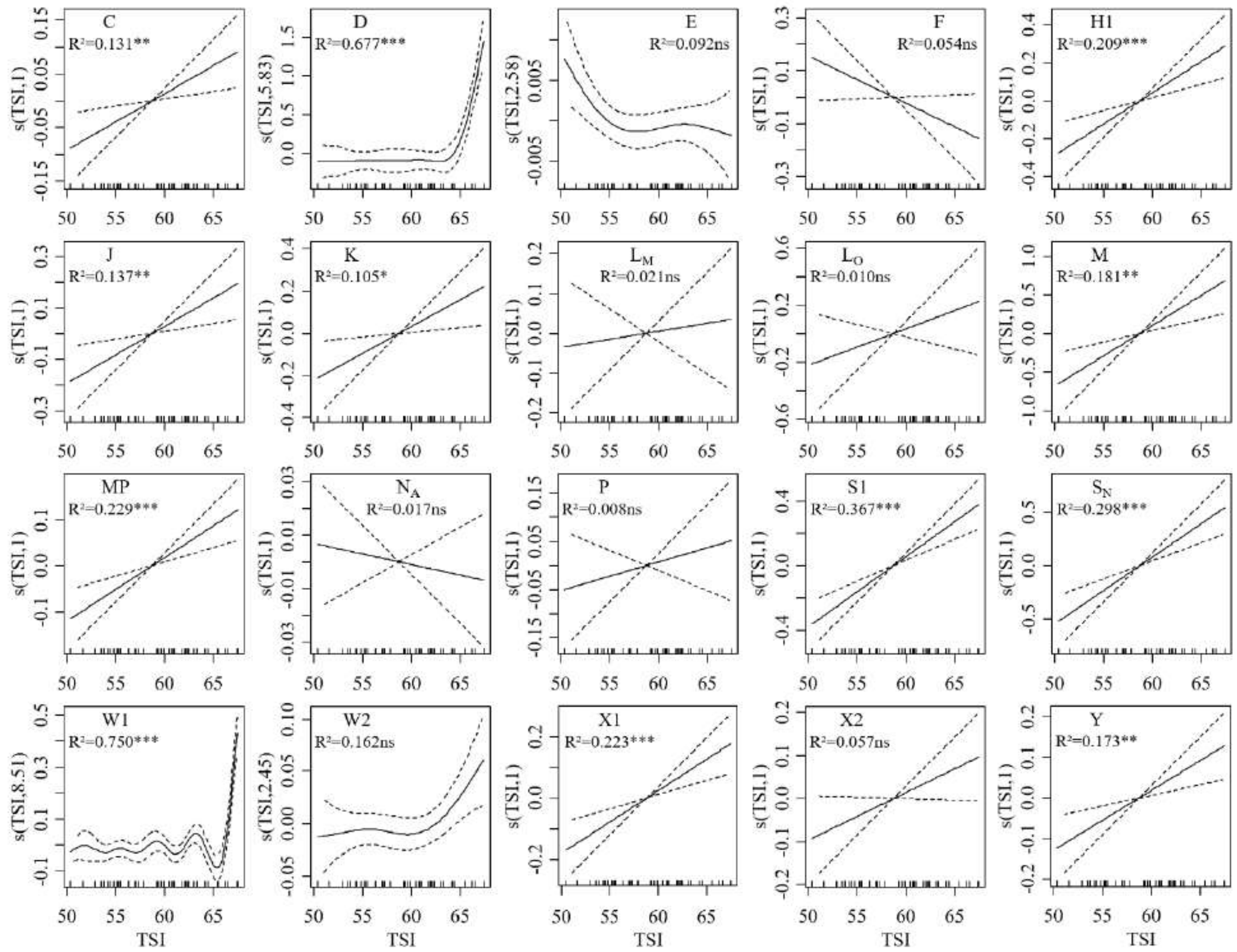

Fig. S9 Generalized additive models - GAM showing the effects of the trophic state index (TSI) on each Reynolds' functional group (RFG) in the tropical reservoirs studied (n = 42). Asterisks represent significant correlations (*: p <0.05; **: p <0.01; and ***: p <0.001)

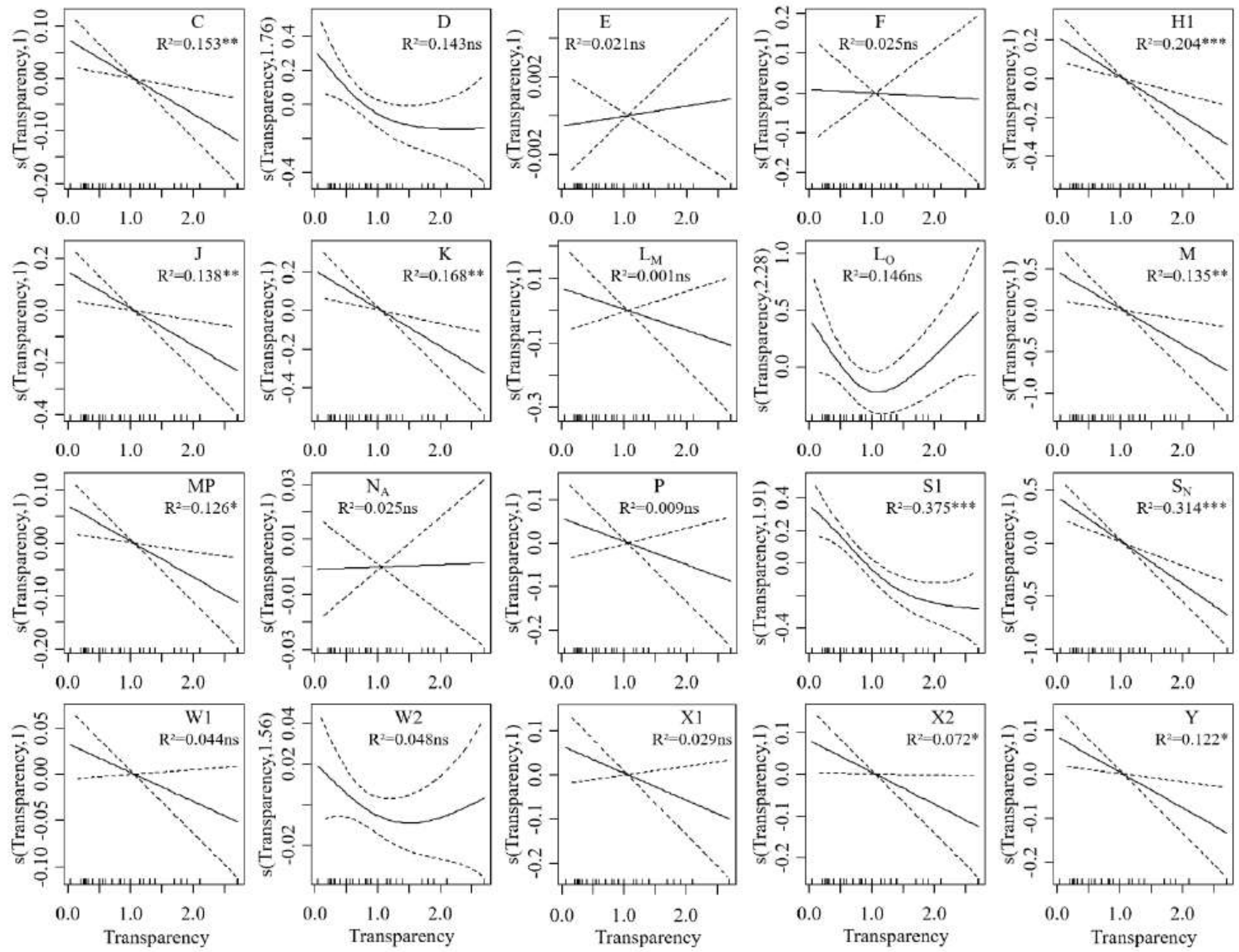

Fig. S10 Generalized additive models - GAM showing the effects of water transparency on each Reynolds' functional group (RFG) in the tropical reservoirs studied (n = 42). Asterisks represent significant correlations (*: p <0.05; **: p <0.01; and ***: p <0.001)

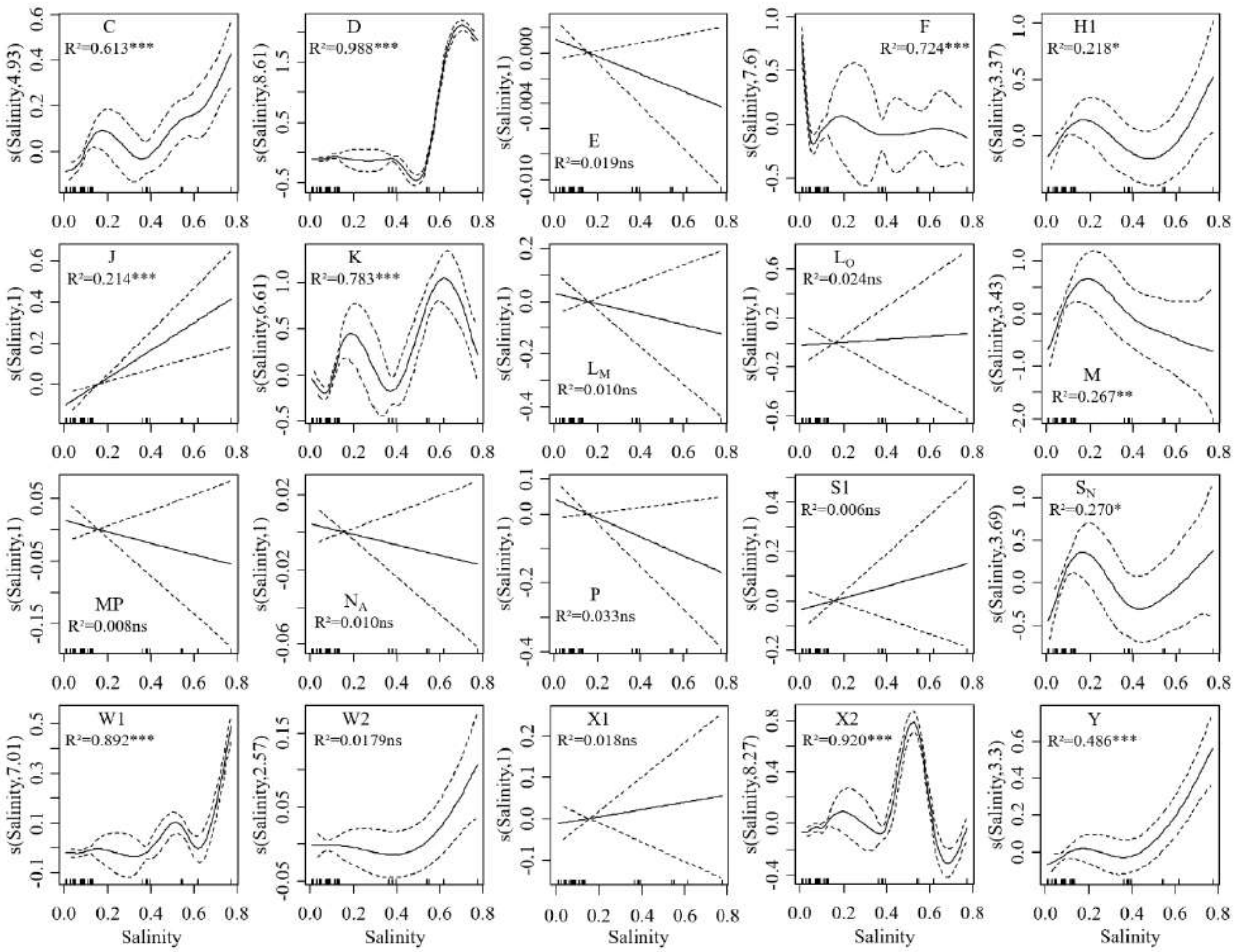

Fig. S11 Generalized additive models - GAM showing the effects of salinity on each Reynolds' functional group (RFG) in the tropical reservoirs studied (n = 42). Asterisks represent significant correlations (*: $p < 0.05$; **: $p < 0.01$; and ***: $p < 0.001$)

Table S2 Generalized Additive Models (GAM) testing the effects of the 10 environmental axes (variables) on each Reynolds' functional group (RFG) in the tropical reservoirs studied (n = 42)

Environmental axis	RFG	Estimate	Std. error	e.d.f.	F	R^2 adj	p-value
Mixing zone depth	C	0.087	0.016	1.00	13.150	0.224	<0.001
Mixing zone depth	D	0.098	0.052	1.79	4.301	0.212	0.014
Mixing zone depth	E	0.002	0.001	1.00	0.078	0.023	0.781
Mixing zone depth	F	0.131	0.045	1.00	0.041	0.024	0.841
Mixing zone depth	H1	0.171	0.045	1.00	4.845	0.083	0.033
Mixing zone depth	J	0.131	0.036	1.00	5.693	0.100	0.022
Mixing zone depth	K	0.207	0.042	1.00	16.800	0.273	<0.001
Mixing zone depth	L_M	0.185	0.045	1.00	2.394	0.032	0.130
Mixing zone depth	L_O	0.452	0.095	1.00	3.234	0.050	0.080
Mixing zone depth	M	0.691	0.122	1.00	0.361	0.016	0.551
Mixing zone depth	MP	0.075	0.019	1.00	0.000	0.025	0.991
Mixing zone depth	N_A	0.014	0.006	1.00	0.452	0.014	0.505
Mixing zone depth	P	0.131	0.325	1.00	0.113	0.022	0.738
Mixing zone depth	S1	0.220	0.049	1.00	0.744	0.007	0.394
Mixing zone depth	S_N	0.402	0.076	1.00	3.343	0.052	0.075
Mixing zone depth	W1	0.021	0.012	1.00	6.789	0.121	0.013
Mixing zone depth	W2	0.021	0.007	1.00	1.672	0.015	0.203
Mixing zone depth	X1	0.093	0.029	1.00	0.631	0.009	0.432
Mixing zone depth	X2	0.076	0.026	1.00	4.828	0.083	0.034
Mixing zone depth	Y	0.064	0.021	1.00	11.910	0.206	<0.001
Irradiance	C	0.087	0.018	1.00	3.396	0.053	0.073
Irradiance	D	0.098	0.046	5.52	3.911	0.376	0.003
Irradiance	E	0.002	0.001	1.00	2.646	0.037	0.112
Irradiance	F	0.131	0.043	1.00	3.830	0.063	0.057
Irradiance	H1	0.171	0.047	1.00	0.996	0.001	0.324
Irradiance	J	0.131	0.037	1.00	4.369	0.074	0.043
Irradiance	K	0.207	0.049	1.00	3.078	0.047	0.087
Irradiance	L_M	0.185	0.045	1.00	1.630	0.014	0.209
Irradiance	L_O	0.452	0.083	1.00	16.480	0.269	<0.001
Irradiance	M	0.691	0.122	1.00	0.000	0.025	0.994
Irradiance	MP	0.075	0.018	3.14	1.822	0.129	0.152
Irradiance	N_A	0.014	0.007	1.00	0.014	0.025	0.907
Irradiance	P	0.131	0.032	1.00	0.533	0.012	0.469
Irradiance	S1	0.220	0.049	1.00	0.071	0.023	0.791
Irradiance	S_N	0.402	0.078	1.00	0.899	0.003	0.349
Irradiance	W1	0.021	0.013	1.00	4.276	0.072	0.045
Irradiance	W2	0.021	0.007	1.00	3.341	0.052	0.075
Irradiance	X1	0.093	0.029	1.00	0.077	0.023	0.783
Irradiance	X2	0.076	0.027	1.00	2.617	0.037	0.113
Irradiance	Y	0.034	0.023	1.00	3.109	0.047	0.085
Water temperature	C	0.087	0.019	1.00	0.193	0.020	0.663
Water temperature	D	0.098	0.058	1.00	0.083	0.023	0.775

Environmental axis	RFG	Estimate	Std. error	e.d.f.	F	R^2 adj	p-value
Water temperature	E	0.002	0.001	1.00	0.191	0.020	0.665
Water temperature	F	0.131	0.042	1.00	4.884	0.084	0.033
Water temperature	H1	0.171	0.048	1.00	0.052	0.024	0.821
Water temperature	J	0.131	0.038	1.00	0.880	0.003	0.354
Water temperature	K	0.207	0.047	1.00	4.997	0.086	0.031
Water temperature	L_M	0.186	0.045	1.00	1.662	0.015	0.205
Water temperature	L_O	0.452	0.094	1.00	3.584	0.057	0.066
Water temperature	M	0.691	0.122	1.00	0.154	0.021	0.697
Water temperature	MP	0.075	0.019	1.00	0.987	0.001	0.326
Water temperature	N_A	0.014	0.006	1.46	7.084	0.170	0.024
Water temperature	P	0.131	0.031	1.44	1.635	0.046	0.367
Water temperature	S1	0.220	0.049	1.00	0.000	0.025	0.999
Water temperature	S_N	0.402	0.078	1.00	0.116	0.022	0.736
Water temperature	W1	0.021	0.013	1.00	0.022	0.024	0.882
Water temperature	W2	0.021	0.007	1.00	0.405	0.015	0.528
Water temperature	X1	0.093	0.028	1.00	3.646	0.059	0.063
Water temperature	X2	0.076	0.027	1.00	1.308	0.007	0.259
Water temperature	Y	0.064	0.023	1.00	1.237	0.005	0.273
pH	C	0.087	0.018	1.00	6.442	0.114	0.015
pH	D	0.098	0.056	1.00	3.112	0.047	0.085
pH	E	0.002	0.001	1.00	0.756	0.006	0.390
pH	F	0.131	0.040	2.76	3.057	0.216	0.026
pH	H1	0.171	0.045	1.00	4.780	0.082	0.035
pH	J	0.131	0.038	1.00	1.084	0.001	0.304
pH	K	0.207	0.049	1.00	1.949	0.044	0.094
pH	L_M	0.185	0.042	1.00	7.673	0.137	0.008
pH	L_O	0.452	0.098	1.00	0.013	0.025	0.911
pH	M	0.691	0.108	1.00	11.430	0.198	0.002
pH	MP	0.075	0.018	1.00	5.375	0.094	0.026
pH	N_A	0.014	0.006	1.00	1.803	0.018	0.187
pH	P	0.131	0.031	1.00	3.161	0.048	0.083
pH	S1	0.220	0.043	1.00	13.210	0.225	<0.001
pH	S_N	0.402	0.068	1.00	13.220	0.225	<0.001
pH	W1	0.021	0.013	1.00	0.814	0.005	0.372
pH	W2	0.021	0.006	1.00	3.800	0.062	0.058
pH	X1	0.093	0.028	1.00	4.385	0.074	0.043
pH	X2	0.076	0.027	1.00	0.435	0.014	0.513
pH	Y	0.064	0.023	1.00	0.969	0.001	0.331
Herbivores relative biomass	C	0.087	0.019	1.00	0.116	0.022	0.735
Herbivores relative biomass	D	0.098	0.058	1.00	0.742	0.007	0.394
Herbivores relative biomass	E	0.002	0.001	1.44	0.643	0.052	0.311
Herbivores relative biomass	F	0.131	0.042	1.63	3.150	0.132	0.075
Herbivores relative biomass	H1	0.171	0.043	1.00	11.110	0.194	0.002
Herbivores relative biomass	J	0.131	0.038	1.00	1.040	0.001	0.314
Herbivores relative biomass	K	0.207	0.049	1.00	1.488	0.011	0.230

Environmental axis	RFG	Estimate	Std. error	e.d.f.	F	R^2 adj	p-value
Herbivores relative biomass	L_M	0.185	0.045	1.00	2.784	0.040	0.103
Herbivores relative biomass	L_O	0.452	0.082	2.80	6.673	0.298	0.003
Herbivores relative biomass	M	0.691	0.111	2.00	8.848	0.157	0.005
Herbivores relative biomass	MP	0.075	0.018	1.00	8.629	0.153	0.005
Herbivores relative biomass	N_A	0.014	0.006	1.00	0.648	0.009	0.426
Herbivores relative biomass	P	0.131	0.032	1.00	0.180	0.021	0.674
Herbivores relative biomass	S1	0.220	0.044	1.00	11.470	0.199	0.002
Herbivores relative biomass	S_N	0.402	0.064	1.00	20.020	0.311	<0.001
Herbivores relative biomass	W1	0.021	0.013	1.00	0.722	0.007	0.400
Herbivores relative biomass	W2	0.021	0.006	4.08	5.615	0.354	<0.001
Herbivores relative biomass	X1	0.093	0.029	1.00	0.373	0.016	0.545
Herbivores relative biomass	X2	0.076	0.027	1.00	0.723	0.007	0.400
Herbivores relative biomass	Y	0.064	0.023	1.00	0.304	0.018	0.584
SRP	C	0.087	0.018	2.22	2.146	0.128	0.089
SRP	D	0.068	0.051	3.34	3.611	0.213	0.038
SRP	E	0.002	0.001	6.89	6.788	0.519	<0.001
SRP	F	0.131	0.039	2.53	6.144	0.257	0.007
SRP	H1	0.171	0.048	1.00	0.709	0.008	0.405
SRP	J	0.131	0.037	1.71	1.192	0.062	0.289
SRP	K	0.207	0.050	1.00	0.188	0.020	0.667
SRP	L_M	0.185	0.046	1.00	0.399	0.015	0.531
SRP	L_O	0.452	0.093	2.07	1.597	0.095	0.170
SRP	M	0.691	0.114	1.00	6.335	0.112	0.016
SRP	MP	0.075	0.018	1.00	4.744	0.081	0.035
SRP	N_A	0.014	0.006	1.00	2.579	0.036	0.116
SRP	P	0.131	0.033	1.00	0.050	0.024	0.824
SRP	S1	0.220	0.047	1.00	4.901	0.084	0.033
SRP	S_N	0.402	0.077	1.00	1.208	0.004	0.278
SRP	W1	0.021	0.013	1.00	0.317	0.017	0.577
SRP	W2	0.021	0.006	4.73	5.227	0.369	0.002
SRP	X1	0.093	0.023	1.00	27.880	0.390	<0.001
SRP	X2	0.076	0.026	1.79	1.742	0.072	0.240
SRP	Y	0.064	0.023	1.00	0.082	0.023	0.776
DIN	C	0.087	0.019	1.00	0.690	0.008	0.411
DIN	D	0.098	0.058	1.00	0.030	0.024	0.864
DIN	E	0.002	0.001	1.00	0.270	0.018	0.606
DIN	F	0.131	0.045	1.00	0.045	0.024	0.883
DIN	H1	0.171	0.047	1.00	1.618	0.014	0.211
DIN	J	0.131	0.038	1.00	1.092	0.002	0.302
DIN	K	0.207	0.049	1.00	1.905	0.021	0.175
DIN	L_M	0.185	0.046	1.00	0.205	0.020	0.653
DIN	L_O	0.452	0.098	1.00	0.009	0.025	0.927
DIN	M	0.691	0.121	1.00	0.759	0.006	0.389
DIN	MP	0.075	0.019	1.55	1.570	0.049	0.364
DIN	N_A	0.014	0.006	1.79	7.323	0.210	0.013

Environmental axis	**RFG**	**Estimate**	**Std. error**	**e.d.f.**	**F**	**R^2 adj**	**p-value**
DIN	P	0.131	0.033	1.00	0.008	0.025	0.931
DIN	S1	0.220	0.049	1.00	0.548	0.012	0.464
DIN	S_N	0.402	0.078	1.00	0.233	0.019	0.632
DIN	W1	0.021	0.013	1.00	0.249	0.019	0.620
DIN	W2	0.021	0.007	1.00	0.163	0.021	0.688
DIN	X1	0.093	0.029	1.00	0.710	0.008	0.405
DIN	X2	0.076	0.027	1.00	0.136	0.022	0.714
DIN	Y	0.064	0.019	3.44	6.850	0.347	<0.001
TSI	C	0.087	0.017	1.00	7.342	0.131	0.010
TSI	D	0.098	0.033	5.83	15.300	0.677	<0.001
TSI	E	0.002	0.001	2.58	1.905	0.160	0.092
TSI	F	0.131	0.043	1.00	3.404	0.054	0.702
TSI	H1	0.171	0.042	1.00	12.160	0.209	<0.001
TSI	J	0.131	0.036	1.00	7.689	0.137	0.008
TSI	K	0.207	0.047	1.00	5.934	0.105	0.019
TSI	L_M	0.185	0.046	1.00	0.157	0.021	0.694
TSI	L_O	0.452	0.097	1.00	1.433	0.010	0.238
TSI	M	0.691	0.109	1.00	10.330	0.181	0.003
TSI	MP	0.075	0.017	1.00	13.510	0.229	<0.001
TSI	N_A	0.014	0.006	1.00	0.305	0.017	0.584
TSI	P	0.131	0.032	1.00	0.680	0.008	0.414
TSI	S1	0.220	0.039	1.00	25.400	0.367	<0.001
TSI	S_N	0.402	0.065	1.00	18.880	0.298	<0.001
TSI	W1	0.021	0.007	8.51	15.870	0.750	<0.001
TSI	W2	0.021	0.007	2.46	3.545	0.162	0.079
TSI	X1	0.093	0.025	1.00	13.110	0.223	<0.001
TSI	X2	0.076	0.026	1.00	3.561	0.057	0.066
TSI	Y	0.064	0.021	1.00	9.837	0.173	0.003
Water transparency	C	0.087	0.017	1.00	8.628	0.153	0.005
Water transparency	D	0.098	0.054	1.76	2.432	0.143	0.062
Water transparency	E	0.002	0.001	1.00	0.162	0.021	0.690
Water transparency	F	0.131	0.045	1.00	0.019	0.025	0.892
Water transparency	H1	0.171	0.042	1.00	11.790	0.204	<0.001
Water transparency	J	0.131	0.036	1.00	7.746	0.138	0.008
Water transparency	K	0.207	0.045	1.00	9.491	0.168	0.004
Water transparency	L_M	0.185	0.046	1.00	1.036	0.001	0.315
Water transparency	L_O	0.452	0.090	2.28	3.617	0.146	0.065
Water transparency	M	0.691	0.112	1.00	7.609	0.135	0.009
Water transparency	MP	0.075	0.018	1.00	7.107	0.126	0.011
Water transparency	N_A	0.014	0.007	1.00	0.008	0.025	0.928
Water transparency	P	0.131	0.032	1.00	1.405	0.009	0.243
Water transparency	S1	0.220	0.039	1.91	10.970	0.375	<0.001
Water transparency	S_N	0.402	0.064	1.00	20.220	0.314	<0.001
Water transparency	W1	0.021	0.013	1.00	2.957	0.044	0.093
Water transparency	W2	0.021	0.007	1.56	0.569	0.048	0.377

Environmental axis	**RFG**	**Estimate**	**Std. error**	**e.d.f.**	**F**	**R^2 adj**	**p-value**
Water transparency	X1	0.093	0.028	1.00	2.277	0.029	0.139
Water transparency	X2	0.076	0.026	1.00	4.297	0.072	0.045
Water transparency	Y	0.064	0.022	1.00	6.884	0.122	0.012
Salinity	C	0.087	0.012	4.93	12.800	0.613	<0.001
Salinity	D	0.098	0.006	8.61	393.500	0.988	<0.001
Salinity	E	0.002	0.001	1.00	1.845	0.019	0.182
Salinity	F	0.131	0.023	7.60	14.520	0.724	<0.001
Salinity	H1	0.171	0.012	3.37	3.657	0.218	0.019
Salinity	J	0.121	0.034	1.00	12.470	0.214	<0.001
Salinity	K	0.207	0.231	6.61	23.060	0.783	<0.001
Salinity	L_M	0.185	0.046	1.00	0.606	0.010	0.441
Salinity	L_O	0.452	0.068	1.00	0.045	0.024	0.883
Salinity	M	0.691	0.104	6.43	3.818	0.267	0.009
Salinity	MP	0.075	0.019	1.00	0.696	0.008	0.409
Salinity	N_A	0.014	0.006	1.00	0.598	0.010	0.444
Salinity	P	0.121	0.132	1.00	2.436	0.033	0.126
Salinity	S1	0.220	0.049	1.00	0.773	0.006	0.384
Salinity	S_N	0.402	0.067	3.69	3.333	0.270	0.013
Salinity	W1	0.021	0.004	7.01	49.720	0.892	<0.001
Salinity	W2	0.021	0.007	2.57	2.405	0.179	0.054
Salinity	X1	0.093	0.029	1.00	0.298	0.018	0.588
Salinity	X2	0.076	0.008	8.27	59.000	0.920	<0.001
Salinity	Y	0.064	0.017	3.30	11.880	0.486	<0.001

ARTIGO 3:

Consequências das Florações

5 ARTIGO 3

Ecological impacts of freshwater algal blooms on water quality, plankton biodiversity, structure, and ecosystem functioning

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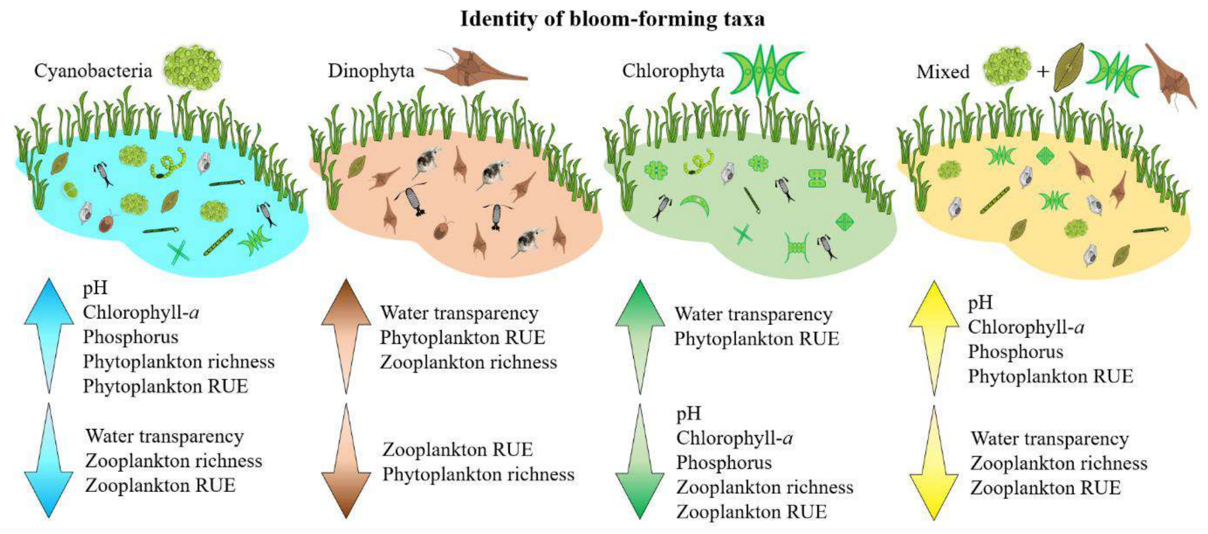
Graphical Abstract



Highlights

- Algal blooms negatively impact water quality, biodiversity, and ecosystem functioning
- Freshwater algal blooms reduce plankton diversity and change dominance patterns
- Blooms increase phytoplankton resource acquisition but decrease for zooplankton
- Cyanobacterial blooms threaten plankton diversity, ecosystem functions, and services
- Species richness loss negatively impact ecosystem functioning during algal blooms

Abstract: Harmful algal blooms are among the emerging threats to freshwater biodiversity that need to be studied further in the Anthropocene. Here, we studied freshwater plankton communities in ten tropical reservoirs to record the impact of algal blooms, comprising different phytoplankton taxa, on water quality, plankton biodiversity, and ecosystem functioning. We compared water quality parameters (water transparency, mixing depth, pH, electrical conductivity, dissolved inorganic nitrogen, total dissolved phosphorus, total phosphorus, chlorophyll-*a*, and trophic state), plankton structure (composition and biomass), biodiversity (species richness, diversity, and evenness), and ecosystem functioning (phytoplankton:phosphorus and zooplankton:phytoplankton ratios as a metric of resource use efficiency) through univariate and multivariate analysis of variance, and generalized additive mixed models in five different bloom categories. Most of the bloom events were composed of Cyanobacteria, followed by Dinophyta and Chlorophyta. Mixed blooms were composed of Cyanobacteria plus Bacillariophyta, Chlorophyta, and/or Dinophyta, while non-bloom communities presented phytoplankton biomass below the threshold for bloom development (10 mg L^{-1}). Higher phytoplankton biomasses were recorded during Cyanobacteria blooms (15.87–273.82 mg L^{-1}) followed by Dinophyta blooms (18.86–196.41 mg L^{-1}). An intense deterioration of water quality, including higher pH, eutrophication, stratification, and lower water transparency, was verified during Cyanobacteria and mixed blooms, while Chlorophyta and Dinophyta blooms presented lower pH, eutrophication, stratification, and higher water transparency. All bloom categories significantly impacted phytoplankton and zooplankton structure, changing the composition and dominance patterns. Bloom intensity positively influenced phytoplankton resource use efficiency (R^2=0.25; p<0.001), while decreased zooplankton resource acquisition (R^2=0.51; p<0.001). Moreover, Cyanobacteria and Chlorophyta blooms negatively impacted zooplankton species richness, while Dinophyta blooms decreased phytoplankton richness. In general, Cyanobacteria blooms presented low water quality and major threats to plankton biodiversity, and ecosystem functioning. Moreover, we demonstrated that biodiversity losses decrease ecosystem functioning, with cascading effects on plankton dynamics.

Keywords: Biodiversity loss; Phytoplankton; Resource use efficiency; Species richness; Tropical reservoirs; Zooplankton.

1. Introduction

Freshwater systems are among the most valuable sources of ecosystem services on Earth (Hilt et al., 2017), although they are intensely threatened (WWF, 2020). However, they are exposed to a variety of chemical, climatic, biological, and anthropogenic stressors, making them sentinels of global and local changes (Adrian et al., 2009; Jenny et al., 2020). Such changes are threatening freshwater biodiversity by increasing extinction rates and population declines (Reid et al., 2019). Accordingly, global estimations demonstrate that freshwater biodiversity is declining faster than the biodiversity in terrestrial and marine environments (84% of population decrease since 1970) (WWF, 2020). Therefore, biodiversity loss is diminishing ecosystem services, including nature’s capacity to provide essential benefits to humanity (Díaz et al., 2019).

The effects of biodiversity in maintaining ecosystem functioning in aquatic habitats are well recognized (Hooper et al., 2012). In general, more diverse communities can sustain higher ecosystem functioning, increasing productivity, temporal stability, and nutrient retention (Tilman et al., 1997; Ptacnik et al., 2008; Allan et al., 2011). However, due to a high degree of biodiversity loss (e.g. Cardinale et al., 2012), freshwater habitats are becoming more vulnerable to global changes, with disastrous consequences to the environment, such as harmful algal blooms (Reid et al., 2019). Among biodiversity-ecosystem functioning metrics, species richness and biomass flow (i.e. resource use efficiency - RUE) play an important role in explaining the relationships in food-webs (Poisot et al., 2013), especially the effects of algal blooms on freshwater ecosystems (e.g. Tian et al., 2017). In this regard, Grey et al. (2000) demonstrated that changes in the dominant phytoplankton taxa to Cyanobacteria hinder carbon assimilation by the zooplankton, which leads to a carbon limited community. Accordingly, food-web dynamics and structure are essential for understanding ecosystem functioning, considering that consumer-resource interactions are usually more important than within trophic level interactions (Filstrup et al., 2014; Schaffner et al., 2019).

It is widely accepted that eutrophication and global climate change have intensified phytoplankton blooms in lakes and reservoirs around the world (Ho et al., 2019; Griffith and Gobler, 2020). Intense algal blooms can trigger serious changes in the aquatic ecosystems (Reid et al., 2019), especially since most of them are composed of potentially toxic cyanobacteria (Huisman et al., 2018; Moura et al., 2018), with significant threats to the aquatic ecosystems, biota, and people who utilize these water sources (Amorim et al., 2017; Olokotum et al., 2020). Additionally, Cyanobacteria blooms can also increase water turbidity and pH (Visser et al., 2016), and can produce taste and odor compounds, reducing ecosystem services, for example, recreational value, and drinking water potability (Jüttner and Watson, 2007). The problems continue during bloom senescence, leading to intense microbial degradation of algal biomass, and consequently causing anoxia and death of aquatic animals (Rabalais et al., 2010). Another problem related to algal bloom senescence is the vicious loop between eutrophication, climate change, and bloom decomposition, which can potentially release more nutrients and greenhouse gases (Yan et al., 2017; Bižić et al., 2020). These factors make algal blooms one of the most severe threats to freshwater biodiversity.

Cyanobacteria is the most harmful bloom-forming taxa for aquatic ecosystems and trophic structure. Although Cyanobacteria blooms can increase the resource use efficiency of phytoplankton, they block the energy transfer from primary producers to zooplankton (Filstrup et al., 2014; Tian et al., 2017). Also, the grazing of zooplankton on phytoplankton is usually interrupted during Cyanobacteria blooms due to cyanotoxin production, less nutritional quality, and the formation of large filaments and colonies (Moustaka-Gouni and Sommer, 2020). This change in energy transfer has been demonstrated through stable isotope analyses, which demonstrated that zooplankton shifted its diet to assimilate carbon from small particles during cyanobacterial blooms (Major et al., 2017), especially from microbial sources (Kluijver et al., 2012).

During Cyanobacteria blooms, an intense modification of plankton structure and composition can be observed, especially during bloom senescence, when bacteria and ciliates can grow intensely (Engström-Öst et al., 2013). These changes also impact zooplankton composition, selecting small forms specialized in the consumption of these alternative food sources (Wilson et al., 2006, Amorim et al., 2020). To date, the effects of Cyanobacteria blooms on phytoplankton and zooplankton structure, and ecosystem functioning are well understood, while the effects of other bloom-forming taxa on aquatic food webs have not yet been evaluated.

Besides Cyanobacteria, the expansion of invasive dinoflagellate *Ceratium* spp. is another emerging threat to freshwater biodiversity (Cavalcante et al., 2016; Accattatis et al., 2020). *Ceratium* blooms can release taste and odor compounds (Ewerts et al., 2018), cause oxygen depletion after bloom collapse, and consequently fish kills, in addition to reducing water treatment efficiency (Meichtry de Zaburlín et al., 2016). Besides influencing environmental conditions, different algal blooms can also severely impact phytoplankton and zooplankton dynamics (Liu et al., 2019; Schaffner et al., 2019), and the effects depend on the bloom-forming identity (Escalas et al., 2019).

To the best of our knowledge, only Escalas et al. (2019) studied the consequences of dominance by different bloom-forming taxa on the structure of phytoplankton in temperate lakes. Accordingly, herein, we describe water quality, plankton dynamics, and ecosystem functioning during algal blooms, composed of different phytoplankton groups, in tropical reservoirs for the first time. For that, we evaluated the changes in environmental conditions, phytoplankton and zooplankton diversity, structure, and resource use efficiency (RUE) in response to different bloom-forming taxa. Our approach is based on three main hypotheses: (1) Cyanobacteria blooms present an intensified water quality deterioration compared to other bloom categories, i.e. lower water transparency, elevated pH values, and higher trophic state; (2) blooms of Cyanobacteria and Dinophyta negatively impact phytoplankton and zooplankton species richness, diversity, and evenness; (3) intense algal blooms, independent of bloom-forming taxa, result in higher RUE for phytoplankton and lower RUE for zooplankton.

2. Material and methods

2.1. Study sites, sampling, and analyses

For this study, we selected ten tropical water reservoirs in Pernambuco state, Northeast, Brazil, with contrasting environmental and climatic conditions. The reservoirs varied from oligotrophic to hypereutrophic and are distributed in the three main phytogeographical regions of Pernambuco: “Zona da Mata” (4), “Agreste” (4), and “Sertão” (Fig. 1). The annual rainfall varies considerably among these regions, with 500-800 mm year^{-1} in “Sertão” and “Agreste”, and up to 2,200 mm year^{-1} in the “Zona da Mata” (APAC, 2019). Water availability is one of the main problems in the Brazilian Northeast, especially in the semiarid region of “Agreste” and “Sertão”, where man-made reservoirs are the main source of drinking water (Barbosa et al., 2012). The selected reservoirs present a storage capacity between 1,168,000 and 270,000,000 m^3 of water, and all of them are inserted in an agricultural landscape (APAC, 2019; Amorim et al., 2020).

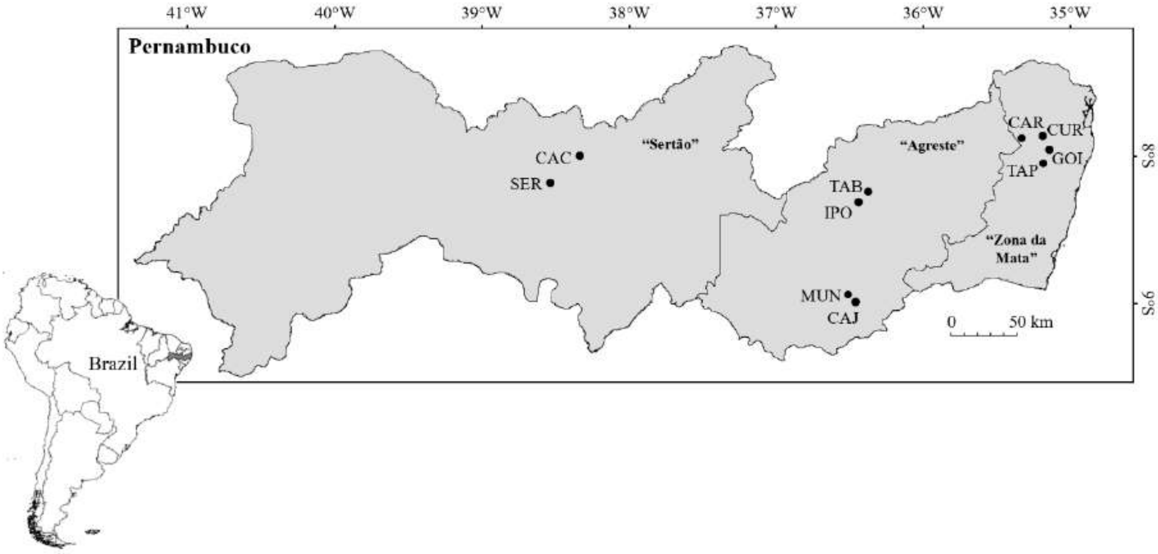


Fig. 1 Geographic location of the ten reservoirs studied distributed in the phytogeographic regions "Zona da Mata", "Agreste", and "Sertão" of Pernambuco, Brazil. Reservoir abbreviations: CAR - Carpina, CUR - Cursaí, GOI – Goitá, TAP - Tapacurá, CAJ - Cajueiro, IPO – Ipojuca, MUN - Mundaú, TAB - Tabocas, CAC - Cachoeira, SER - Serrinha.

Based on environmental monitoring performed during samplings, we selected some ecosystem services that all reservoirs provide for people. The ecosystem services can be classified into the four following categories (Biggs et al., 2017) (1) provisioning services: water availability and quality for drinking, irrigation in agriculture, livestock, and transportation, biodiversity for fish and shrimp production; (2) regulating and maintenance services: riparian vegetation, aquatic macrophytes for nutrient and toxin removal, oxygen production, carbon accumulation, nutrient cycling and retention, control of nuisance species by fish and zooplankton; (3) cultural services: recreation, sense of place, educational value; (4) supporting services: local climate regulation, conservation, primary production, decomposition, among others.

Samples for phytoplankton, zooplankton, and physicochemical analyses were collected quarterly from October 2017 to January 2019, comprising an annual cycle with five (Tapacurá and Cajueiro) or four (other environments) samples in each reservoir (n = 42). Samples were always collected at the same point, in the deepest region of each reservoir, in the morning. Depth was verified with an echo sounder and water transparency with a Secchi disk. Water temperature, electrical conductivity, and pH were analyzed in situ with a HANNA multiparametric probe. Mixing zone depth was established when there was a difference in the water temperature greater than 0.5°C every 0.5 m deep. The concentrations of dissolved inorganic nitrogen (DIN), total dissolved phosphorus (TDP), and total phosphorus (TP) were determined following specific literature (Strickland and Parsons, 1965; Golterman et al., 1971; Valderrama, 1981), using the spectrophotometric method. Before the analysis of dissolved nutrients, samples were filtered in glass fiber filters with 0.45 µm pore-size. Algal biomass retained in the filters was extracted in heated ethanol for chlorophyll-*a* quantification, according to the spectrophotometric method proposed by Chorus and Bartram (1999). Total phosphorus and chlorophyll-*a* concentrations were used to determine the trophic state index (Cunha et al., 2013).

Qualitative samples for phytoplankton identification were collected through surface trawls using a 25 µm mesh-size plankton net and fixed with 4% formaldehyde. Species were identified in a light microscope, following specialized literature, for example, Komárek and Anagnostidis (1986, 2005), Anagnostidis and Komárek (1988), Komárek and Komárková (2002, 2004), Prescott and Vinyard (1982), Komárek and Fott (1983), Round et al. (1990), Krammer and Lange-Berlalot (1991), John et al. (2002), Wehr et al. (2015), among others. Species were grouped into Cyanobacteria, Dinophyta, Chlorophyta (the green algae classes Chlorophyceae, Trebouxiophyceae, and Zygnematophyceae), Bacillariophyta, and other taxa (the sum of the mixotrophic Chrysophyta, Cryptophyta, Euglenophyta, and Xantophyta).

Samples used for phytoplankton quantification were collected directly from the subsurface of reservoirs (20 cm) with a van Dorn bottle, preserved with 1% acetic Lugol, and analyzed with the aid of an inverted microscope in sedimentation chambers (Utermöhl, 1958).

Phytoplankton was counted until achieving 400 organisms of the most abundant taxa and until the stabilization of the species curve (i.e. when no species was added) (Lund et al., 1958). The species' biomass (mg L^{-1}) was determined according to the geometric models proposed by Hillebrand et al. (1999).

Algal blooms were defined based on the World Health Organization (WHO) as alert level 2 (biomass >10 mg L^{-1}) (Chorus and Bartram, 1999), which were grouped into five bloom categories: non-bloom communities (biomass <10 mg L^{-1}), blooms of Cyanobacteria, Dinophyta, Chlorophyta, and mixed blooms (when more than one taxon reached biomass greater than 10 mg L^{-1}). In this regard, each bloom category represents a set of phytoplankton communities dominated by a specific bloom-forming taxon, usually comprising all samples from each reservoir in each category, which were collected quarterly during an annual cycle. Only Tabocas, Cachoeira, and Ipojuca reservoirs presented samples classified in two different bloom categories. The most abundant phytoplankton taxa in each bloom category were the species with a relative abundance greater than 50% of total phytoplankton or with biomass greater than 10 mg L^{-1}.

Samples for zooplankton identification and quantification were collected by filtering 100 L of water from the reservoirs with a 60 µm mesh-size plankton net. Samples were then fixed with 4% formaldehyde and analyzed in a 1 mL Sedgewick-Rafter chamber (three subsamples per sample) with the aid of a light microscope. Biomass (µg L^{-1}) was calculated based on the regression formulas relating the dry weight with the length and width of the organisms, according to Ruttner-Kolisko (1977) for rotifers and Dumont et al. (1975) for microcrustaceans. Species were grouped into rotifers, calanoid copepods, cyclopoid copepods, copepod nauplii, and cladocerans.

2.2. Data analyses

All statistical analyzes were performed in the software R (version 3.5.2), with a significance level set at $p < 0.05$ (R Core Team, 2019). Phytoplankton and zooplankton species richness (S) was defined as the number of species identified per sample. Shannon-Weiner diversity index (H') and Pielou evenness ($J' = H' / \log(S)$) were calculated in the *vegan* package (Oksanen et al., 2018). Resource use efficiency (RUE) was estimated as the community biomass divided by the amount of resources (total dissolved phosphorus for phytoplankton, and algal biomass for zooplankton), as an indicator of resource flow across communities. Phosphorus is the major resource for phytoplankton from freshwater and marine environments, even in eutrophic habitats (Ptacnik et al., 2008). We used total dissolved phosphorus for phytoplankton RUE calculations instead of total phosphorus, because the former is bioavailable as a resource, while a significant part of total phosphorus is attached to algal cells and cannot be instantly consumed by phytoplankton (Shi et al., 2020).

Initially, a PERMANOVA analysis was performed to verify whether phytoplankton and zooplankton composition and structure showed significant multivariate differences among bloom categories, using the Bray-Curtis dissimilarity. The post hoc function *pairwise.adonis* was used for multiple comparisons between bloom categories in the *vegan* package. Environmental conditions, phytoplankton and zooplankton total biomass, the biomass of specific groups, species richness, diversity, evenness, and RUE were compared between bloom categories using a one-way ANOVA for normal and homoscedastic data, while heteroscedastic data were analyzed using the Brown-Forsythe test in the *onewaytests* package (Dag et al., 2018). Pairwise comparisons were performed with Tukey and Bonferroni post hoc tests for ANOVA and Brown-Forsythe test, respectively. Before these analyses, data were tested for normality and homoscedasticity by Kolmogorov-Smirnov and Bartlett tests, respectively. When necessary, data were transformed using the functions *log(x+1)* (plankton biomass, electrical conductivity, DIN, TDP, TP, and chlorophyll-*a*) or *ln* (RUE).

Generalized additive mixed models (GAMM) were used to verify the effects of bloom intensity (phytoplankton biomass) and each bloom-forming taxa on some water quality parameters (water transparency, mixing zone depth, pH, total dissolved phosphorus, total phosphorus, and tropic state index), phytoplankton and zooplankton richness and RUE, in addition to the effects of phytoplankton and zooplankton richness on their respective RUE. This method combines linear mixed models since we have fixed and random variables, and generalized additive models, which account for linear and non-linear relationships between variables. The explanatory variables (phytoplankton biomass and plankton species richness) were treated as fixed parameters, while reservoir identity and time (months of the year) were treated as random variables to remove spatial and temporal autocorrelation effects. Model smoothing was evaluated using the estimated degree of freedom (e.d.f.), while model fit and significance were assessed by the R^2-adjusted and p-values ($p < 0.05$). All models were fitted using *the gamm* function in the *mgcv* package (Wood, 2004, 2011).

3. Results

3.1. Phytoplankton, zooplankton, and environmental variables

Cyanobacteria blooms were more frequent and registered in Tapacurá, Cajueiro, Mundaú, and Serrinha reservoirs, besides one sample in Ipojuca (n = 19); while Dinophyta blooms were present in Goitá and Cachoeira reservoirs (n = 7); and Chlorophyta blooms in Tabocas (n = 3). Mixed blooms, registered only in the Ipojuca reservoir (n = 3), presented higher biomasses of Cyanobacteria plus Chlorophyta, Bacillariophyta, and/or Dinophyta. On the other hand, Carpina and Cursaí, in addition to one community in both Tabocas and Cachoeira (n = 10), did not present algal blooms (Fig. 2A). The specific bloom category, bloom period, and the most abundant phytoplankton taxa are described in Table A.1. Regarding zooplankton, cyclopoid copepods were dominant during Cyanobacteria and Chlorophyta blooms, rotifers during mixed blooms, while calanoid copepods and cladocerans presented higher abundances during Dinophyta blooms (Fig. 2B).

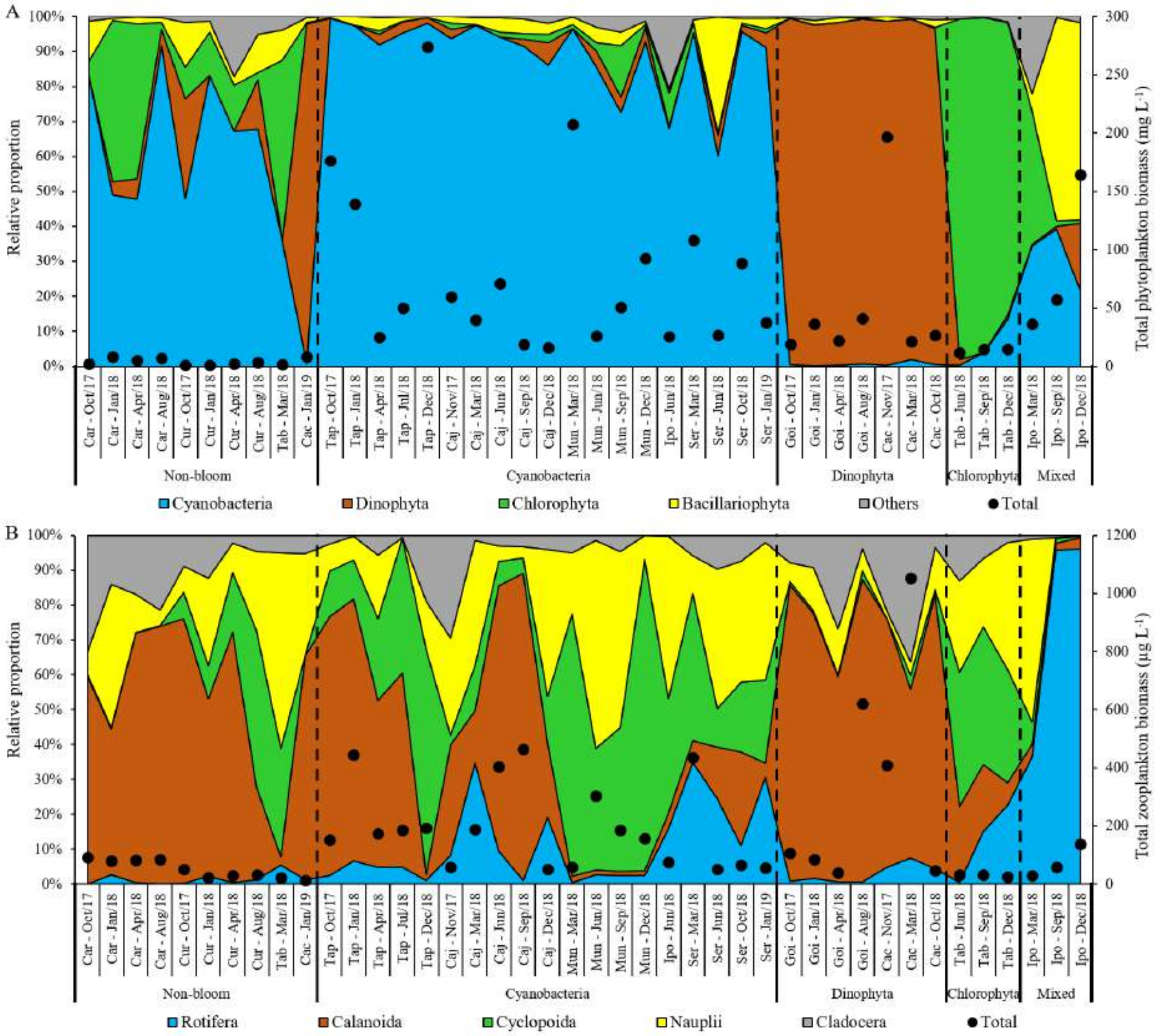


Fig. 2 Temporal variation in phytoplankton (A) and zooplankton (B) total biomass and the relative proportion of different groups among different bloom categories (non-bloom communities, blooms of Cyanobacteria, Dinophyta, Chlorophyta, and mixed blooms) in the tropical reservoirs studied (n = 42). See Fig. 1 for reservoir abbreviations.

Cyanobacteria blooms were dominated by *Microcystis aeruginosa* (Kützing) Kützing (Cajueiro), *M. panniformis* Komárek et al. (Tapacurá and Serrinha), *M. flos-aquae* (Wittrock) Kirchner (Mundaú), *Merismopedia tenuissima* Lemmermann (Mundaú), and *Raphidiopsis raciborskii* (Woloszynska) Aguilera et al. (Mundaú and Serrinha), while Dinophyta blooms were dominated by *Ceratium furcoides* (Levander) Langhans (Goitá and Cachoeira), and Chlorophyta blooms by *Botryococcus braunii* Kützing (Tabocas). Mixed blooms (Ipojuca) showed higher contributions of the cyanobacterium *Aphanocapsa incerta* (Lemmermann) G.Cronberg & Komárek (March to December 2018), plus the diatom *Navicula* sp. (September to December 2018), the green alga *Tetradesmus lagerheimii* M.J.Wynne & Guiry (March 2018), and/or the dinoflagellate *C. furcoides* (December 2018). Furthermore, Carpina and Cursaí reservoirs, although not presenting algal blooms, showed a dominance of *Microcystis protocystis* W.B.Crow (Table A.1).

A PERMANOVA analysis revealed that the phytoplankton structure differed between all bloom categories, except between Chlorophyta and mixed blooms ($F_{model} = 9.15$; $R^2 = 0.4972$; $p < 0.001$) (Table A.2). Similarly, zooplankton structure during Cyanobacteria blooms differed from all other bloom categories. Dinophyta and Chlorophyta blooms did not differ between them or between non-bloom communities, while Chlorophyta blooms were also similar to mixed blooms ($F_{model} = 6.47$; $R^2 = 0.4116$; $p < 0.001$) (Table A.2).

Mixed and Cyanobacteria blooms were registered in shallower reservoirs. Also, mixed blooms were present in reservoirs with lower mixing zone depth and higher electrical conductivities, while Chlorophyta blooms were present in less saline reservoirs (lower electrical conductivity). Total dissolved phosphorus and total phosphorus were higher during mixed and Cyanobacteria blooms, followed by non-bloom communities and Dinophyta blooms. Meanwhile, dissolved inorganic nitrogen did not differ across different bloom-forming taxa. Mixed and Cyanobacteria blooms resulted in lower water transparency, higher pH, and chlorophyll-*a*. Moreover, Cyanobacteria and mixed blooms were generally present in hypereutrophic environments; while Dinophyta blooms in eutrophic reservoirs and Chlorophyta blooms; and non-bloom communities were present in mesotrophic water bodies, according to the mean trophic state of each bloom category (Tables A.3 and A.4).

Generalized additive mixed models showed that bloom intensity, represented by the phytoplankton biomass, decreased water transparency and mixing zone depth, and increased pH, total phosphorus, total dissolved phosphorus, and trophic state index. The positive influence of bloom intensity on total dissolved phosphorus and total phosphorus was more apparent between 10 and 100 mg L^{-1}, stabilizing the curve or even decreasing phosphorus concentrations when algal biomass exceeded 100 mg L^{-1}. The positive influence of bloom intensity on the trophic state index became more evident above 10 mg L^{-1} of algal biomass (Fig. A.1, Table A.6).

3.2. Effects of algal blooms on phytoplankton and zooplankton structure and functioning

A total of 136 phytoplankton taxa (87% at the species level) were identified, composed mainly of chlorophytes (55 spp.), cyanobacteria (40 spp.), and diatoms (19 spp.). Cyanobacteria blooms exhibited higher phytoplankton species richness compared to non-bloom communities and Dinophyta blooms, followed by mixed and Chlorophyta blooms. Non-bloom communities, Cyanobacteria, and mixed blooms presented significantly higher diversity and evenness than Dinophyta and Chlorophyta blooms (Fig. 3A-C, Table A.6).

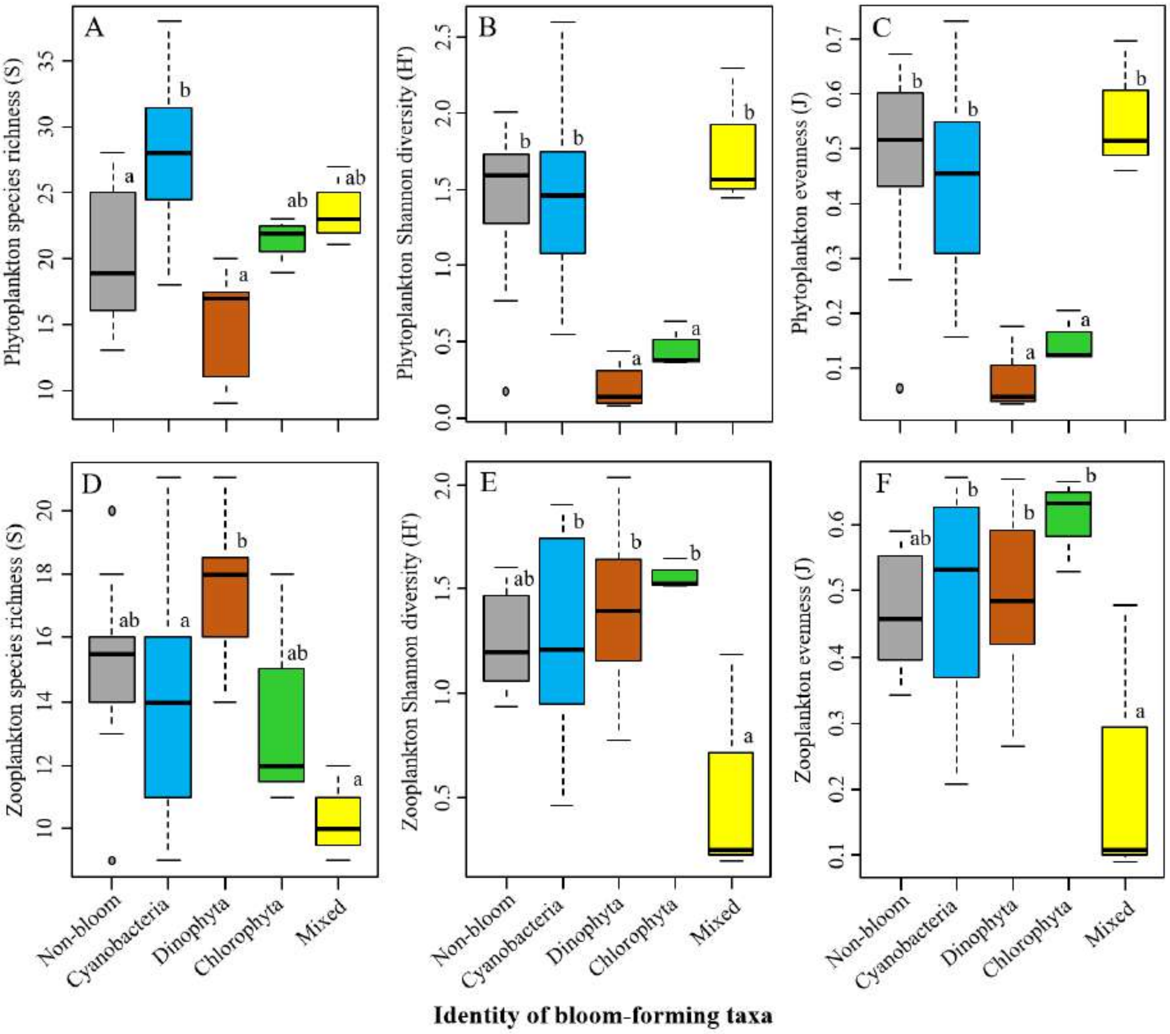


Fig. 3 Species richness, Shannon-Wiener diversity index, and evenness for phytoplankton (A-C) and zooplankton (D-F) during different bloom categories in the tropical reservoirs studied (n = 42). Grey boxes: non-bloom communities; blue boxes: blooms of Cyanobacteria; brown boxes: blooms of Dinophyta; green boxes: blooms of Chlorophyta; and yellow boxes: mixed blooms. Significant differences between bloom categories are represented by different lowercase letters ($p < 0.05$).

Total phytoplankton biomass exhibited significant differences across all bloom-forming taxa, with higher biomasses during Cyanobacteria (15.87 - 273.82 mg L^{-1}), Dinophyta (18.86 - 196.41 mg L^{-1}), and mixed blooms (35.97 - 164.58 mg L^{-1}). On the other hand, total phytoplankton biomass during Chlorophyta blooms (11.95 - 14.88 mg L^{-1}) did not differ from non-bloom communities (0.99 - 8.09 mg L^{-1}). Concerning the biomass of phytoplankton groups, Cyanobacteria, Dinophyta, Chlorophyta, and Bacillariophyta presented higher biomass during their respective bloom events. Regarding non-dominant taxa in each bloom category, Cyanobacteria and Chlorophyta presented lower biomass during Dinophyta blooms. Furthermore, Bacillariophyta exhibited higher biomass during mixed and Cyanobacteria blooms, and lower biomass during Chlorophyta blooms. Additionally, other phytoplankton taxa (mixotrophic organisms) reached higher biomass during Cyanobacteria and mixed blooms (Fig. 4, Table A.6).

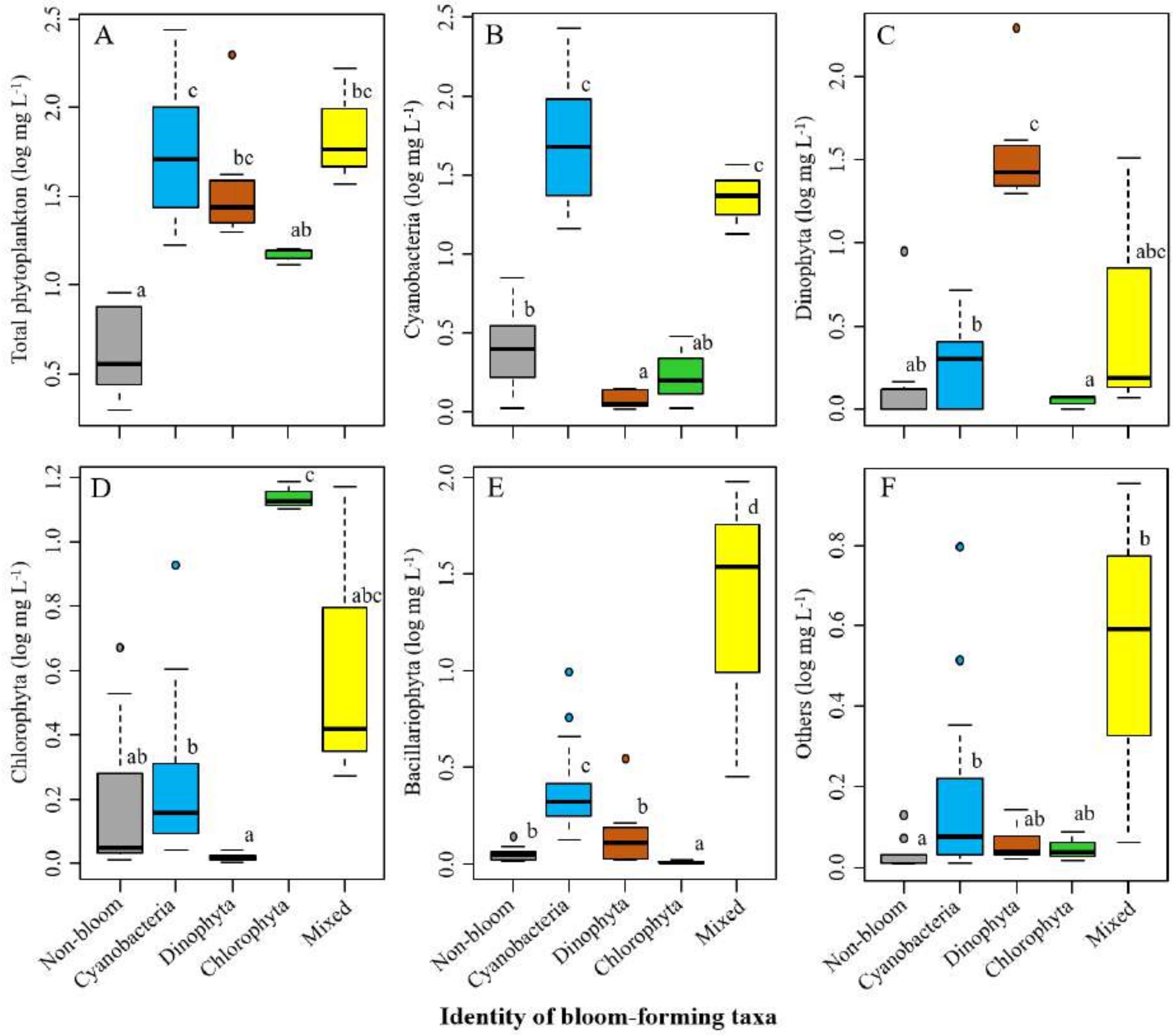


Fig. 4 Total phytoplankton biomass (A), and the biomass of Cyanobacteria (B), Dinophyta (C), Chlorophyta (D), Bacillariophyta (E), and other phytoplankton taxa (F) during different bloom categories in the tropical reservoirs studied (n = 42). Grey boxes: non-bloom communities; blue boxes: blooms of Cyanobacteria; brown boxes:

blooms of Dinophyta; green boxes: blooms of Chlorophyta; and yellow boxes: mixed blooms. Significant differences between bloom categories are represented by different lowercase letters ($p < 0.05$).

A total of 55 zooplankton taxa were identified (89% at the species level), distributed in Rotifera (36 spp.), Cladocera (14 spp.), and Copepoda (5 spp.). In terms of zooplankton species richness, higher values were registered during Dinophyta blooms, and lower during mixed blooms. Cyanobacteria and mixed blooms exhibited lower zooplankton richness than Dinophyta blooms. Concerning diversity and evenness, mixed blooms exhibited lower values than Cyanobacteria, Dinophyta, and Chlorophyta blooms, however, none of them differed from non-bloom communities (Fig. 3D-F, Table A.6).

Cyanobacteria and Dinophyta blooms exhibited higher total zooplankton biomass, differing significantly from Chlorophyta blooms and non-bloom communities. Considering zooplankton groups, all of them presented different patterns across bloom categories. Rotifers presented higher biomass during mixed blooms, followed by Cyanobacteria and Dinophyta blooms, differing significantly from non-bloom communities. On the other hand, calanoid copepods exhibited higher biomass during Dinophyta and Cyanobacteria blooms, differing significantly from mixed blooms, but not from non-bloom communities. Moreover, higher biomass of cyclopoid copepods and copepod nauplii were registered during Cyanobacteria blooms, with a significant difference from non-bloom communities and mixed blooms. Besides, the biomass of cyclopoids during Cyanobacteria blooms was also higher than in Dinophyta blooms. The biomass of cladocerans was higher during Dinophyta blooms, followed by non-bloom communities and Cyanobacteria blooms, which significantly differed from mixed blooms (Fig. 5, Table A.6).

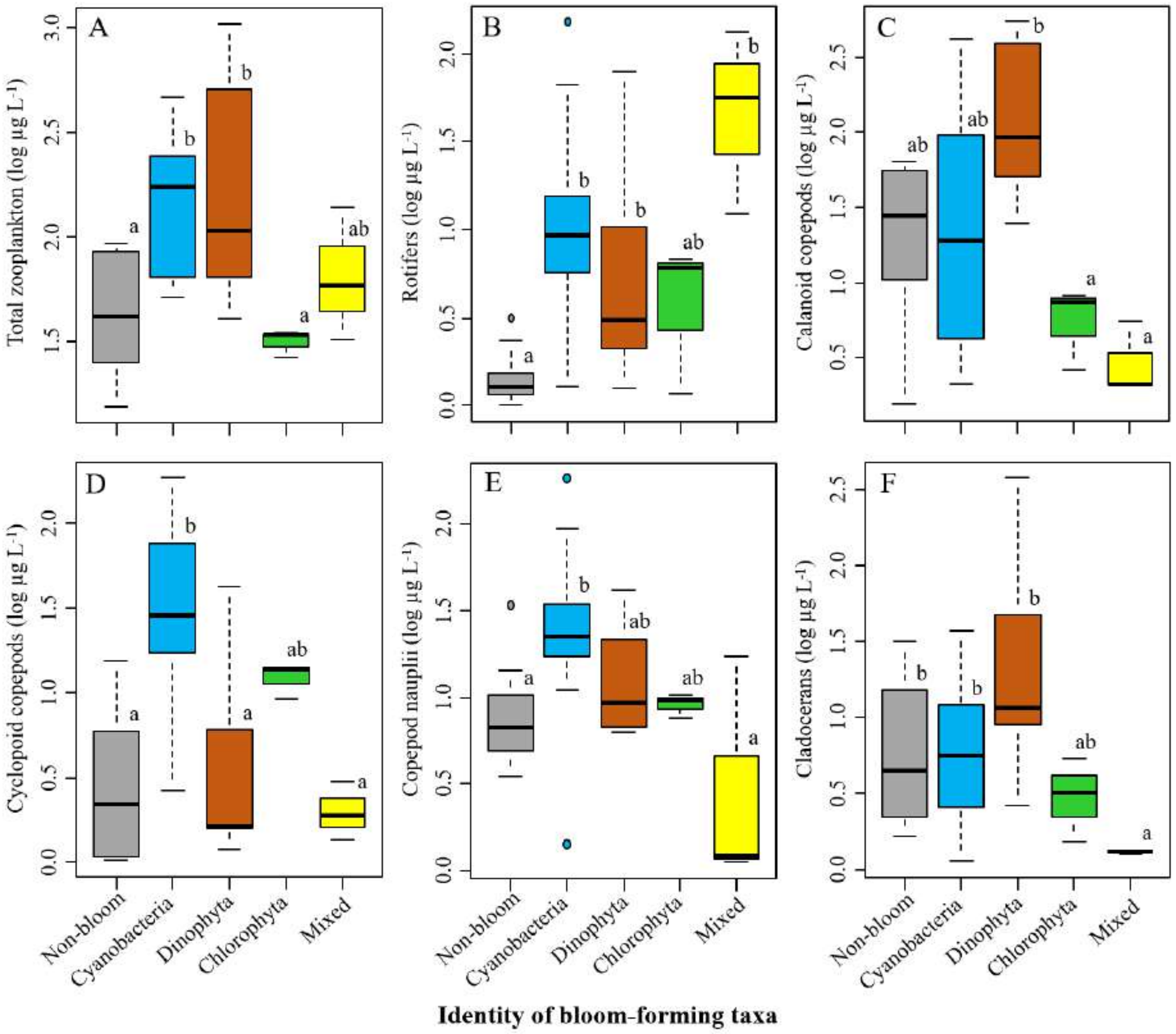


Fig. 5 Total zooplankton biomass (A), and the biomass of rotifers (B), calanoid copepods (C), cyclopoid copepods (D), copepod nauplii (E), and cladocerans (F) during different bloom categories in the tropical reservoirs studied (n = 42). Grey boxes: non-bloom communities; blue boxes: blooms of Cyanobacteria; brown boxes: blooms of Dinophyta; green boxes: blooms of Chlorophyta; and yellow boxes: mixed blooms. Significant differences between bloom categories are represented by different lowercase letters ($p < 0.05$).

Algal blooms composed of Cyanobacteria, Dinophyta, and Chlorophyta exhibited higher phytoplankton RUE, compared to non-bloom communities (Fig. 6A, Table A.6). In contrast, mixed, Cyanobacteria, and Chlorophyta blooms resulted in significantly lower zooplankton RUE, compared to non-bloom communities (Fig. 6B, Table A.6).

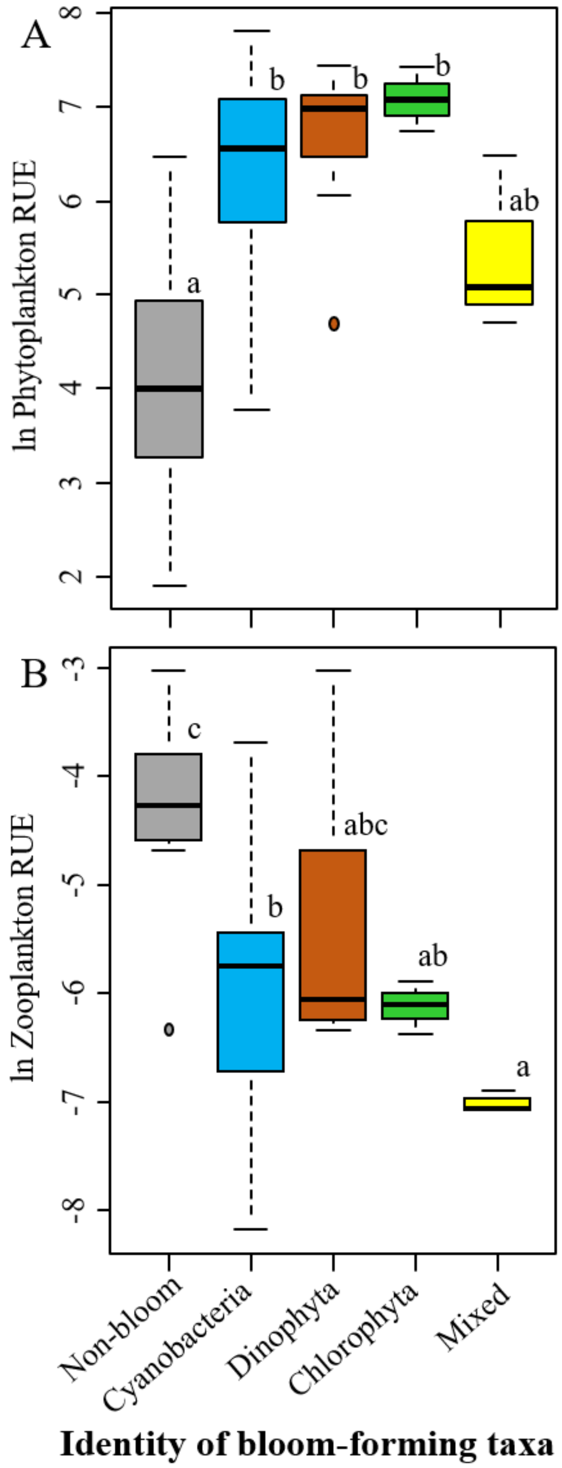


Fig. 6 Resource use efficiency (RUE) for phytoplankton (A) and zooplankton (B) during different bloom categories in the tropical reservoirs studied (n = 42). Grey boxes: non-bloom communities; blue boxes: blooms of Cyanobacteria; brown boxes: blooms of Dinophyta; green boxes: blooms of Chlorophyta; and yellow boxes: mixed blooms. Significant differences between bloom categories are represented by different lowercase letters ($p < 0.05$).

The generalized additive mixed models (GAMM) revealed that the intensity of algal blooms, represented by the phytoplankton biomass, exhibited different relationships with plankton species richness and RUE (Fig. 7). Phytoplankton richness did not show a significant relationship with bloom intensity ($p = 0.430$) (Fig. 7A). In contrast, zooplankton richness displayed a marginally significant negative dependence on algal blooms ($p = 0.067$) (Fig. 7B). Moreover, phytoplankton and zooplankton RUE were oppositely correlated with algal biomass. Phytoplankton RUE significantly increased with rising bloom intensity (Fig. 7C), showing an almost linear relationship ($p < 0.001$), while zooplankton RUE was significantly and negatively impacted by bloom intensity ($p < 0.001$) (Fig. 7D). Furthermore, zooplankton species richness significantly increased its RUE ($p = 0.014$), while phytoplankton richness did not significantly alter its RUE ($p = 0.333$) (Fig. 7E-F, Table A.5).

Cyanobacteria biomass showed contrasting significant effects on plankton richness, increasing for phytoplankton ($p = 0.019$) and decreasing for zooplankton ($p = 0.004$). Dinophyta biomass negatively influenced phytoplankton richness when biomass exceeded 1 mg L^{-1} ($p = 0.016$). On the other hand, lower biomasses of Chlorophyta significantly increased phytoplankton richness ($p < 0.001$), however, this relationship became stabilized or almost negative above 1 mg L^{-1}. Regarding RUE, Cyanobacteria biomass presented divergent significant unimodal effects, with peaks at approximately 1 mg L^{-1}. Phytoplankton RUE decreased at low Cyanobacteria biomass (<1 mg L^{-1}) and strongly increased above 1 mg L^{-1} ($p = 0.009$). The contrary was observed for zooplankton RUE, which increased until 1 mg L^{-1} and intensely decreased after this threshold ($p < 0.001$). Dinophyta biomass presented an almost significant and positive effect on phytoplankton RUE ($p = 0.075$). Zooplankton RUE was also negatively associated with Bacillariophyta biomass, especially above 0.1 mg L^{-1} ($p = 0.009$) (Fig. A.2, Table A.5).

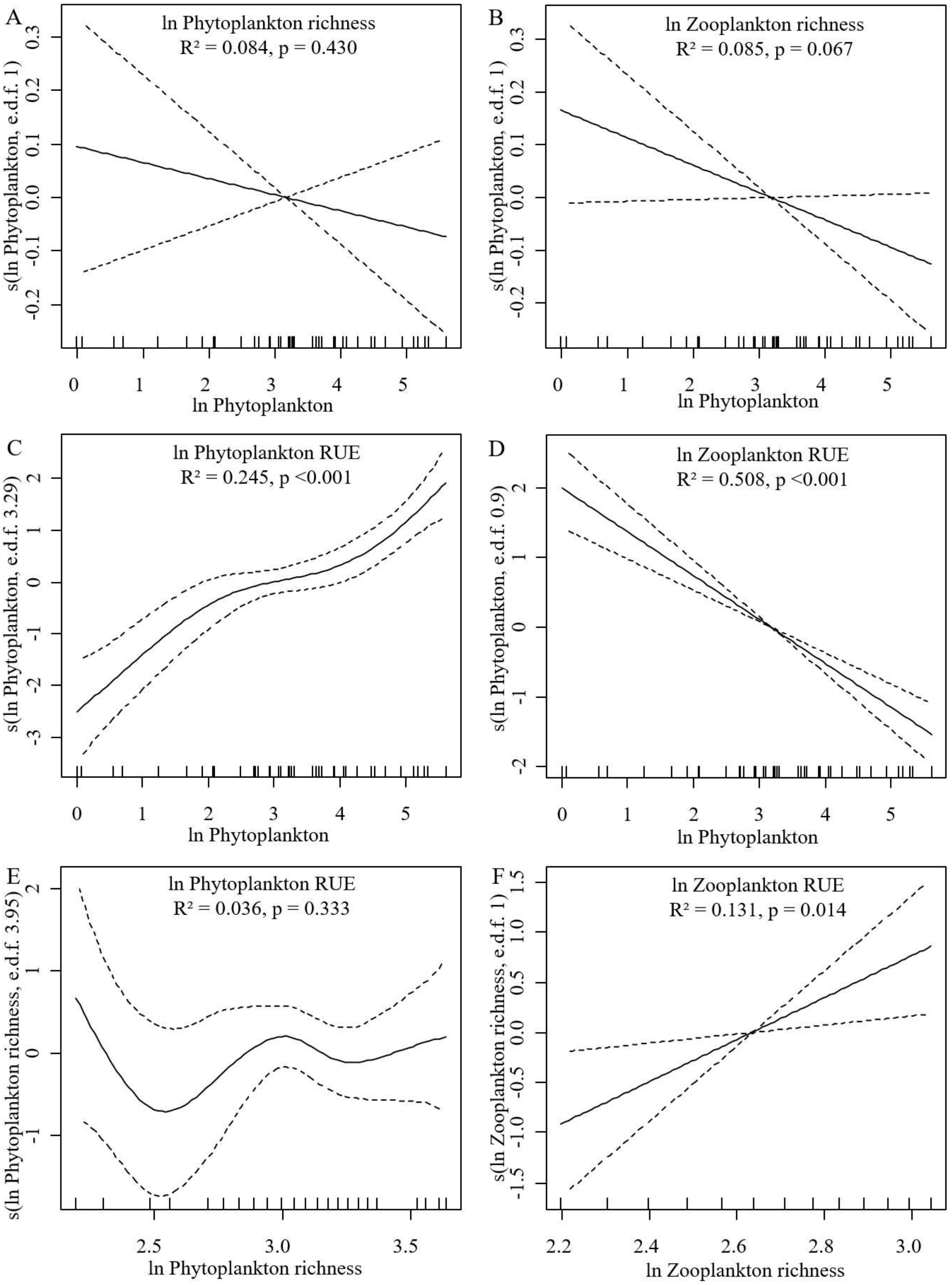


Fig. 7 Fitted Generalized Additive Mixed Models (GAMM) to estimate the impacts of bloom intensity (phytoplankton biomass) on phytoplankton (A) and zooplankton (B) species richness, phytoplankton (C) and zooplankton (D) resource use efficiency (RUE), besides the effects of phytoplankton (E) and zooplankton (F) species richness on their respective resource use efficiency, during different bloom categories in the tropical

reservoirs studied (n = 42). Explanatory variables were fixed, while reservoir location and sampling periods were randomized to account for spatial and temporal autocorrelation. The Y-axis corresponds to GAMM modeled results. All models were significant ($p < 0.0001$). Dashed lines represent the 95% confidence interval.

4. Discussion

In this study, we showed how algal blooms intensify water quality deterioration, impact plankton structure, biodiversity, and ecosystem functioning in tropical reservoirs. More intense algal blooms changed phytoplankton and zooplankton communities, leading to a decrease in zooplankton species richness and resource use efficiency, while phytoplankton resource use efficiency increased. Cyanobacteria and mixed blooms (also composed of Cyanobacteria plus other taxa) presented an intense deterioration of water quality, which became more turbid, stratified, saline, alkaline, and hypereutrophic. These results are important to predict the effects of algal blooms on biodiversity and ecosystem service losses in freshwater environments.

The impacts of Cyanobacteria blooms on aquatic ecosystems are widely known (Sukenik et al., 2015). The most harmful consequence is related to cyanotoxin production, with serious impacts on aquatic communities (Amorim et al., 2017) and people who are supplied by the contaminated waters (Moura et al., 2018; Olokotum et al., 2020). These effects also decrease freshwater ecosystem services, including water quality for drinking and irrigation purposes, recreation, livestock, and fish production.

In our study, Cyanobacteria and mixed blooms created conditions that could further increase their dominance, such as higher eutrophication, pH, and lower water transparency (Fig. A.1, Table A.4). These factors can favor Cyanobacteria bloom development (Carey et al., 2012, Moura et al., 2018). The increased turbidity caused by Cyanobacteria blooms has serious consequences on pelagic and benthic communities, reducing prey visual capture and forcing animals to migrate to clearer areas (Sukenik et al., 2015). Furthermore, Cyanobacteria blooms can release more nutrients (Yan et al., 2017) and increase photosynthetic rates (Verspagen et al., 2014), and consequently boost eutrophication and pH, respectively. Therefore, these conditions can hamper the development of plant and animal communities, especially those sensitive to high turbidity, nutrients, and pH, which will find unfavorable conditions for their growth.

Also, Cyanobacteria and mixed blooms were registered in stratified water bodies, i.e. lower mixing depth than other bloom categories. Despite being stimulated by higher temperatures and thermal stratification (Paerl and Huisman, 2008), cyanobacteria can modify their environment to increase light attenuation and surface temperatures, and thus, intensify stratification (Rinke et al., 2010). Moreover, large cyanobacterial colonies and filaments absorb more energy and consequently warm faster at high biomass, also increasing the stability of the water column (Kumagai et al., 2000).

Besides influencing environmental conditions, all bloom-forming taxa significantly altered phytoplankton and zooplankton composition and structure (Table A.2). These relationships are well documented in our study by the influence of different bloom-forming taxa on plankton diversity, biomass, and RUE. Cyanobacteria and mixed blooms increased phytoplankton species richness, diversity, and evenness (Fig. 3), which is contradictory with some literature reports (e.g. Escalas et al., 2019), but similar to others (e.g. Carey et al., 2014; Yang et al., 2017; Liu et al., 2019). In this sense, Cyanobacteria can benefit phytoplankton diversity through nutrient releasing (Carey et al., 2014). Furthermore, phytoplankton species richness can be stable during Cyanobacteria blooms due to species resilience, which can recover their biomass even after long periods of blooms (Yang et al., 2017). Accordingly, more diverse communities present higher ecosystem functioning, which favors higher temporal stability (Allan et al., 2011), and consequently the maintenance of greater species richness for extended periods. Besides, generalized additive mixed models revealed that phytoplankton species richness positively correlated with Cyanobacteria and Chlorophyta biomass, and negatively with Dinophyta. Dinoflagellate blooms created a cascading effect on zooplankton, increasing the biomass of large herbivorous crustaceans (calanoid copepods and cladocerans) (Fig. 5), and consequently decreasing phytoplankton richness. Moreover, significantly lower Shannon-Weiner diversity and evenness were registered during Dinophyta and Chlorophyta blooms (Fig. 3).

The biomass of Cyanobacteria and Chlorophyta was lower during Dinophyta blooms (Fig. 4). As discussed above, this pattern could be related to the increased biomass of calanoid copepods and cladocerans. These zooplankton groups can select their prey and avoid consuming large and inedible particles (Colina et al., 2016). On the other hand, *Ceratium* spp., due to its large size and the presence of horns, is not grazed by the most zooplankton species (Hart and Wragg, 2009). Also, the higher mixing depth during dinoflagellate blooms impaired the development of buoyant cyanobacteria and colonial green algae, as previously reported (Winder and Hunter, 2008; Wagner and Adrian, 2009). Accordingly, thermal stability is an important factor that regulates the fluctuation between Cyanobacteria species, during stratification periods, and *Ceratium* spp., during mixing periods, in reservoirs (Naselli-Flores and Barone, 2003; Bordet et al., 2017).

Additionally, diatoms presented higher biomass during mixed and Cyanobacteria blooms in hypereutrophic and stratified reservoirs, demonstrating a coexistence with Cyanobacteria (Fig. 4). This result can also be associated with thermal stratification, that not only selects Cyanobacteria species, but also small diatoms, due to their small surface area to volume ratio (Winder et al., 2009). Besides that, resource acquisition and utilization are similar between Cyanobacteria and diatoms, which justify their coexistence pattern (Tilman et al., 1982). So, diatoms can act as a good indicator of cyanobacterial blooms, especially in man-made lakes (Liu et al., 2020), as in the present study. The same coexistence pattern was observed for other phytoplankton taxa (mixotrophic organisms) during Cyanobacteria and mixed blooms (Fig. 4). In this regard, mixotrophic algae are benefited in (hyper)eutrophic environments due to high bacterial development, an alternative food source for mixotrophic organisms (Flynn et al., 2018).

Unlike the coexistence pattern seen in the phytoplankton community during mixed blooms, zooplankton presented lower species richness, diversity, and evenness during these blooms (Fig. 3), which were also negatively related to the biomass of Cyanobacteria (Fig. A.2). Cyanobacteria and mixed blooms had contrasting effects on zooplankton groups, decreasing the biomass of large species (calanoids and cladocerans) and increasing that of the small forms (rotifers, cyclopoids, and nauplii) (Fig. 5). Accordingly, cyanobacteria can limit herbivorous crustaceans' growth through several morphological, physiological, and nutritional factors (Ger et al., 2019; Amorim and Moura, 2020), reducing reproduction and body size (Ghadouani et al., 2003). On the other hand, Cyanobacteria can select small zooplankton species to coexist with blooms (Wilson et al., 2006), especially rotifers, copepod nauplii, and cyclopoid copepods (Amorim et al., 2020). As a result, these small organisms can be benefited by consuming heterotrophic bacteria and small protozoans during Cyanobacteria blooms (Engström-Öst et al., 2013).

Dinoflagellate blooms increased zooplankton richness (Fig. 3) because of the increased development of calanoids and cladocerans (Fig. 5). Some studies have demonstrated that calanoid copepods and some cladocerans are not affected during *Ceratium* blooms (Hart and Wragg, 2009). Moreover, despite its large size, *C. furcoides* can be efficiently grazed by large species of copepods, including calanoids (Santer, 1996). This fact can explain the higher biomass of large calanoid copepods and cladocerans during Dinophyta blooms, which could graze on *C. furcoides* cells, especially when other phytoplankton groups presented lower abundances.

As also demonstrated in other studies (e.g. Filstrup et al., 2014; Tian et al., 2017), herein, algal blooms had contrasting effects on plankton RUE, increasing for phytoplankton and decreasing for zooplankton (Fig. 6). These results reinforce the importance of studying both intra- and inter-community relationships. All bloom categories presented higher phytoplankton RUE compared to non-bloom communities, demonstrating a higher acquisition of resources, as previously observed (Filstrup et al., 2014; Escalas et al., 2019). Interestingly, blooms of Chlorophyta and Dinophyta presented high phytoplankton RUE but lower total phosphorus concentrations (mesotrophic to eutrophic), while Cyanobacteria and mixed blooms exhibited high phytoplankton RUE and higher total phosphorus (hypereutrophic) (Table A.4), demonstrating that blooms increase resource acquisition by phytoplankton despite the trophic state. This result also indicates that bloom-forming species can use limiting resources more efficiently than other phytoplankton species.

Cyanobacteria blooms were more frequent, although other bloom categories also presented high phytoplankton RUE. This result can be explained by the greater number of bloom-forming taxa and higher phytoplankton richness during Cyanobacteria blooms, which tend to present different ecological strategies and use limited resources more efficiently. Furthermore, zooplankton RUE was significantly and positively related to its species richness, indicating that higher biodiversity also increases resource acquisition (Fig. 7). In this regard, Tilman et al. (1997) showed that a high number of coexisting species, with contrasting nutrient requirements, boost community productivity and nutrient retention, and consequently influence ecosystem functioning. Furthermore, zooplankton grazing is benefited under high phytoplankton diversity, with increased top-down control even during algal blooms in tropical (Amorim et al., 2019), subtropical (Sarnelle, 2005), and temperate lakes (McCauley and Briand, 1979). This characteristic reinforces the negative impact of biodiversity loss, caused by algal blooms, on community productivity and ecosystem dynamics in freshwater habitats.

Zooplankton RUE was severely impacted during all blooms, especially in mixed blooms, followed by Chlorophyta and Cyanobacteria blooms (Fig. 6). Cyanobacteria can form large colonies and filaments, besides being unpalatable to grazers due to its low nutritional quality (Moustaka-Gouni and Sommer, 2020). Similarly, blooms of the green alga *Botryococcus* are not efficiently grazed by zooplankton due to large particle size (Ramírez and Corbacho, 2005). Moreover, Chlorophyta and Cyanobacteria blooms presented higher abundances of omnivorous cyclopoid copepods, while mixed blooms presented higher biomass of rotifers, which are primarily small organisms and not efficient grazers of phytoplankton (e.g., Colina et al., 2016; Amorim and Moura, 2020). Additionally, Cyanobacteria and Bacillariophyta exhibited a negative influence on zooplankton RUE (Fig. A.2). Altogether, these factors contributed to lower zooplankton resource acquisition during such blooms.

Once established, algal blooms created conditions that can further enhance their biomass, including an intensification of the trophic state, pH, water stratification, and decrease in water transparence, as previously demonstrated (e.g. Filstrup et al., 2014; Escalas et al., 2019). All bloom categories altered abundance patterns in phytoplankton and zooplankton, with consequences on biodiversity, ecosystem functioning, biomass flow, and trophic interactions. Cyanobacteria blooms, expected to increase in both frequency and intensity in tropical lakes and reservoirs in the coming years (Moura et al., 2018; Amorim et al., 2020), presented major threats to water quality, plankton biodiversity, and ecosystem functioning (Fig. 8).

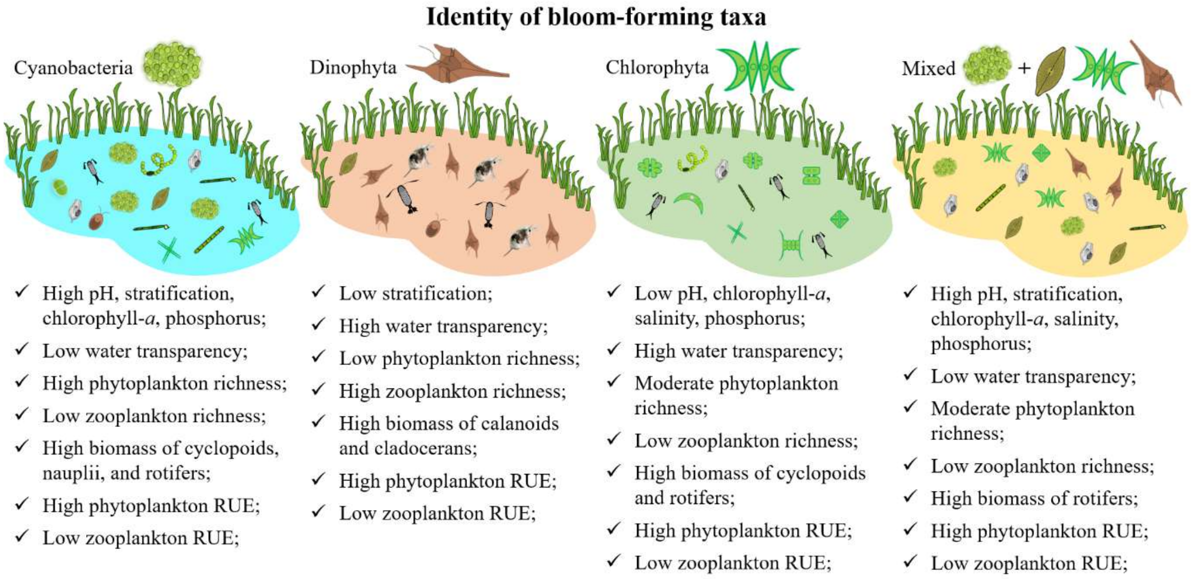


Fig. 8 Schematic representation of the main ecological consequences of algal blooms, composed of different bloom-forming phytoplankton taxa, on water quality, biodiversity (plankton structure and diversity), and ecosystem functioning (plankton biomass and resource use efficiency (RUE)), in freshwater reservoirs.

Our findings support the idea that biodiversity loss decreases ecosystem functioning, with cascading effects on plankton dynamics. Harmful algal blooms are listed as one of the most serious threats to freshwater biodiversity, leading to intense biodiversity loss (Cardinale et al., 2012; Reid et al., 2019). For example, in our study, blooms of Cyanobacteria reduced zooplankton richness, while Dinophyta blooms decreased phytoplankton richness (Fig. A.2). Besides species richness, Dinophyta and Chlorophyta blooms negatively impacted phytoplankton diversity and evenness, while mixed blooms diminished zooplankton diversity and evenness (Fig. 3). Moreover, Cyanobacteria and Dinophyta intense blooms are known to cause fish kills, due to biomass decomposition and consequently decrease in oxygen concentrations (Rabalais et al., 2010; Meichtry de Zaburlín et al., 2016), indicating severe changes in the whole aquatic food webs, as well as affecting ecosystem functioning and the provision of ecosystem services (Sukenik et al., 2015).

5. Conclusions

Our first hypothesis was confirmed, as Cyanobacteria and mixed blooms presented lower water quality (i.e. higher pH, chlorophyll-*a*, trophic state, stratification, and lower water transparency). The second hypothesis was partially confirmed since Dinophyta blooms decreased phytoplankton richness, diversity, and evenness, while the contrary was observed during Cyanobacteria blooms. Also, Cyanobacteria blooms decreased zooplankton richness, while dinoflagellates positively influenced this richness. Our results also supported the third hypothesis since all bloom categories presented a positive impact on phytoplankton RUE and a negative influence on zooplankton RUE.

To the best of our knowledge, this article shows how freshwater algal blooms, composed of different bloom-forming taxa, intensify water quality deterioration, impact plankton trophic interactions, biodiversity, and ecosystem functioning for the first time. Blooms composed of Cyanobacteria presented major threats to plankton biodiversity and resource acquisition, besides showing an intense water quality deterioration in tropical man-made reservoirs. However, all bloom categories impacted plankton dynamics and should be studied further, as their impacts on aquatic biodiversity and ecosystem services are likely to increase under anthropogenic and climatic pressures. Our research focused on artificial man-made reservoirs, especially due to their relevance to people as an important source of ecosystem services. Therefore, more research is needed to clarify how people will be impacted by the biodiversity and ecosystem services losses during algal blooms. We also highlight the importance of biodiversity conservation measures in aquatic ecosystems to sustain high ecosystem functioning and maintain trophic interactions.

Conflicts of interest

Declarations of interest: none.

Data Availability

Additional data and information may be obtained from the first author (alvescihelio@gmail.com, cihelio.amorim@ufrpe.br).

Author Contributions

Cihelio A. Amorim: Conceptualization, Methodology, Validation, Data curation, Formal analysis, Writing - Original Draft, Writing - Review & Editing. **Ariadne N. Moura:** Conceptualization, Supervision, Methodology, Writing - Review & Editing.

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APPENDIX A

Title: Ecological impacts of freshwater algal blooms on water quality, plankton biodiversity, structure, and ecosystem functioning
Journal: Science of the Total Environment

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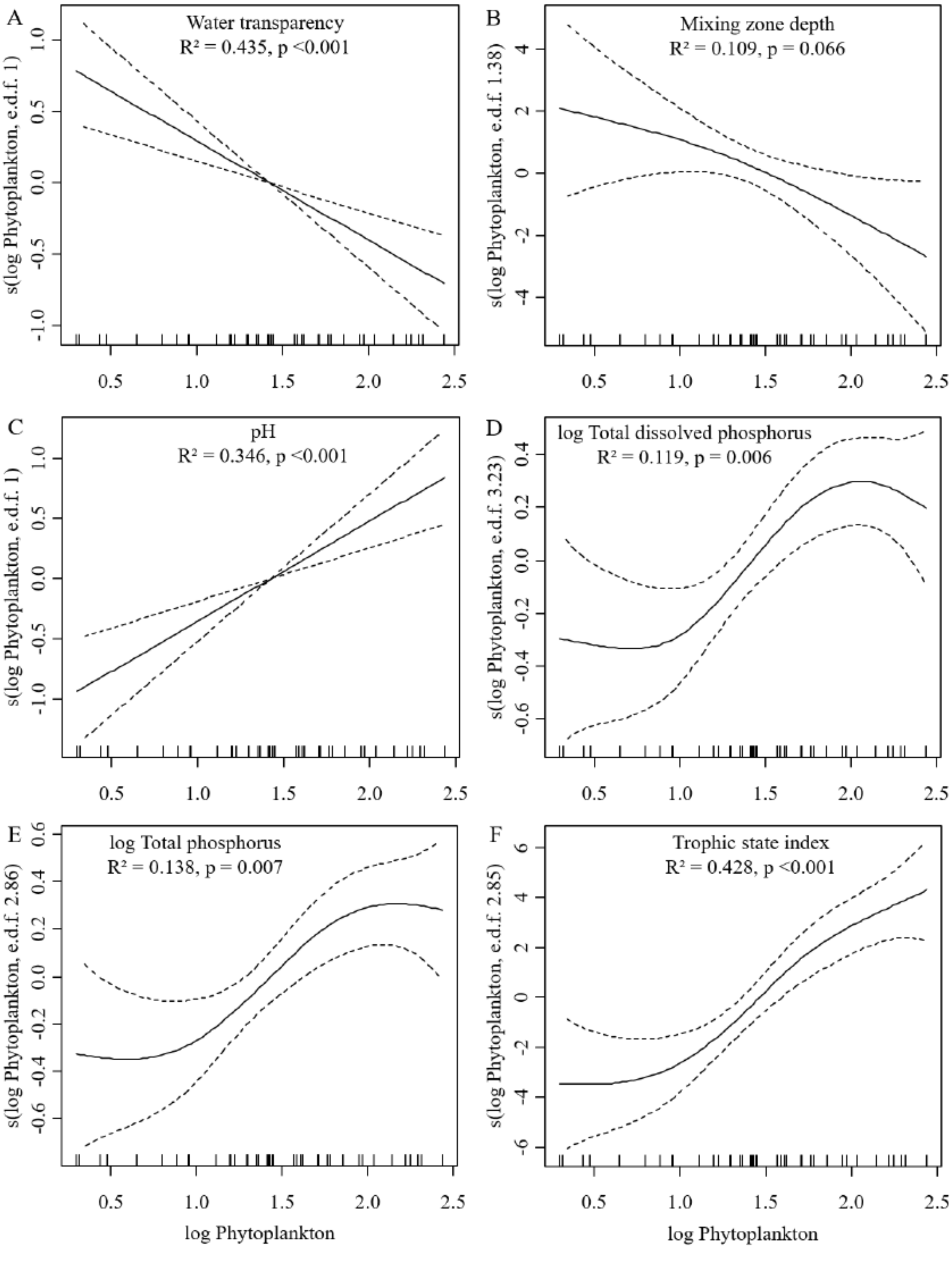

Fig. A.1 Fitted Generalized Adittive Mixed Models (GAMM) showing the relationships between bloom intensity (log-transformed phytoplankton biomass) and some water quality parameters: water transparency (A), mixing zone depth (B), pH (C), total dissolved phosphorus (D), total phosphorus (E), and trophic state index (according to Cunha et al., 2013) (F) during different bloom categories in the tropical reservoirs studied (n = 42). Explanatory variables were fixed, while reservoir location and sampling periods were randomized to account for spatial and temporal autocorrelation. The Y-axis corresponds to GAMM modeled results. All models were significant ($p < 0.0001$). Dashed lines represent the 95% confidence interval.

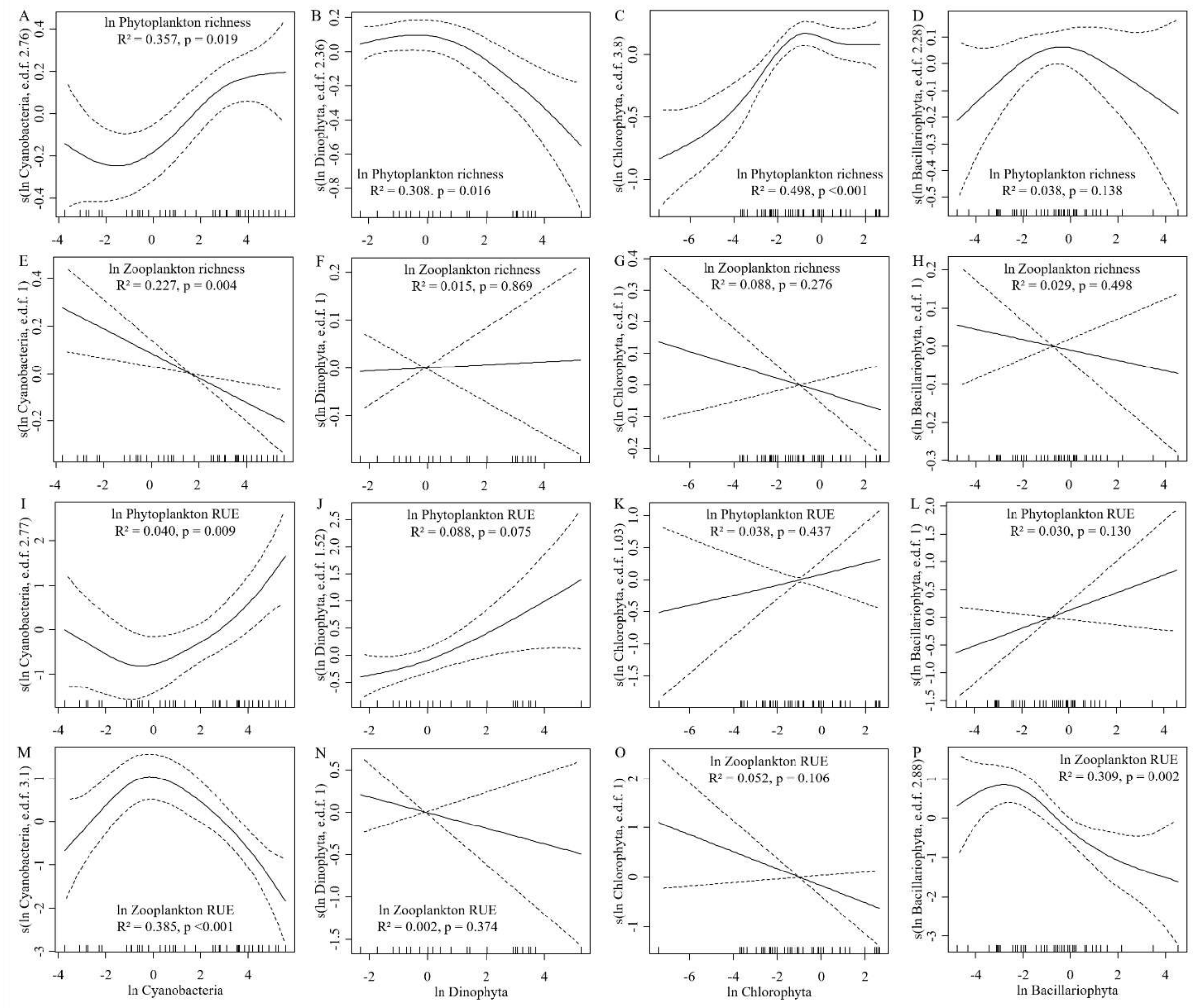

Fig. A.2 Fitted Generalized Adittive Mixed Models (GAMM) to estimate the impacts of each bloom-forming taxa, i.e. the biomass of Cyanobacteria (A, E, I, M), Dinophyta (B, F, J, N), Chlorophyta (C, G, K, O), and Bacillariophyta (D, H, L, P), on phytoplankton (A-D) and zooplankton (E-H) species richness, and phytoplankton (I-L) and zooplankton (M-P) resource use efficiency (RUE) during different bloom categories in the tropical reservoirs studied (n = 42). Explanatory variables were fixed, while reservoir location and sampling periods were randomized to account for spatial and temporal autocorrelation. The Y-axis corresponds to GAMM modeled results. All models were significant (p <0.0001). Dashed lines represent the 95% confidence interval.

Table A.1 Classification of the studied reservoirs based on the presence, the main bloom-forming taxa, duration of algal blooms, and most abundant phytoplankton species (biomass >10 mg L^{-1} or relative contribution >50%).

Reservoir	**Bloom type**	**Bloom period**	**Most abundant phytoplankton species**
Carpina	Non-bloom	Absence of blooms	*Microcystis protocystis* W.B.Crow, *Schroederia setigera* (Schröder) Lemmermann
Cursaí	Non-bloom	Absence of blooms	*Microcystis protocystis*
Goitá	Dinophyta	Oct/17 – Aug/18	*Ceratium furcoides* (Levander) Langhans
Tapacurá	Cyanobacteria	Oct/17 – Dec/18	*Anagnostidinema amphibium* (C.Agardh) Strunecký et al., *Microcystis brasiliensis* (De Azevedo & C.L.Sant'Anna) Rigonato et al., *Microcystis panniformis* Komárek et al., *Microcystis protocystis*
Cajueiro	Cyanobacteria	Nov/17 – Dec/18	*Microcystis aeruginosa* (Kützing) Kützing
Ipojuca	Cyanobacteria, mixed	Mar/18 – Dec/18	*Aphanocapsa incerta* (Lemmermann) G.Cronberg & Komárek, *Synechocystis aquatilis* Sauvageau, *Ceratium furcoides*, *Navicula* sp., *Tetradesmus lagerheimii* M.J.Wynne & Guiry
Mundaú	Cyanobacteria	Mar/18 – Dec/18	*Anabaenopsis elenkinii* V.V.Miller, *Merismopedia tenuissima* Lemmermann, *Microcystis aeruginosa*, *Microcystis flos-aquae* (Wittrock) Kirchner, *Raphidiopsis raciborskii* (Woloszynska) Aguilera et al.
Tabocas	Non-bloom, Chlorophyta	Jun/18 – Dec/18	*Botryococcus braunii* Kützing
Cachoeira	Non-bloom, Dinophyta	Nov/17 – Oct/18	*Ceratium furcoides*
Serrinha	Cyanobacteria	Mar/18 – Jan/19	*Microcystis panniformis*, *Microcystis protocystis*, *Raphidiopsis raciborskii*

Table A.2 Pairwise multiple comparisons of phytoplankton and zooplankton composition and structure for each bloom category (n = 42). Bold values represent significant differences verified using the function *pairwise.adonis* after the PERNANOVA analysis (p <0.05).

Variable	Pairwise comparisons	p-adjusted
Phytoplankton	**Non-bloom vs. Cyanobacteria**	**0.01**
	Non-bloom vs. Dinophyta	**0.01**
	Non-bloom vs. Chlorophyta	**0.04**
	Non-bloom vs. Mixed	**0.04**
	Cyanobacteria vs. Dinophyta	**0.01**
	Cyanobacteria vs. Chlorophyta	**0.02**
	Cyanobacteria vs. Mixed	**0.02**
	Dinophyta vs. Chlorophyta	**0.04**
	Dinophyta vs. Mixed	**0.05**
	Chlorophyta vs. Mixed	1.00
Zooplankton	**Non-bloom vs. Cyanobacteria**	**0.01**
	Non-bloom vs. Dinophyta	0.10
	Non-bloom vs. Chlorophyta	0.30
	Non-bloom vs. Mixed	**0.04**
	Cyanobacteria vs. Dinophyta	**0.01**
	Cyanobacteria vs. Chlorophyta	**0.02**
	Cyanobacteria vs. Mixed	**0.02**
	Dinophyta vs. Chlorophyta	0.23
	Dinophyta vs. Mixed	**0.04**
	Chlorophyta vs. Mixed	1.00

Table A.3 Mean and standard error (±SE) of environmental conditions registered in each reservoir studied (n = 42). Reservoir abbreviations: CAR - Carpina, CUR - Cursaí, GOI – Goitá, TAP - Tapacurá, CAJ - Cajueiro, IPO – Ipojuca, MUN - Mundaú, TAB - Tabocas, CAC - Cachoeira, SER - Serrinha. Variables abbreviations: EC - electrical conductivity, DIN - dissolved inorganic nitrogen, TDP - total dissolved phosphorus, TP - total phosphorus. Trophic state abbreviations (according to Cunha et al., 2013): Oligo. - oligotrophic, Meso. - mesotrophic, Eutro. - eutrophic, Hyper. - hypereutrophic.

	Reservoirs																			
	CAR		CUR		GOI		TAP		CAJ		IPO		MUN		TAB		CAC		SER	
	Mean	±SE	Mean	±SE	Mean	±SE	Mean	±SE	Mean	±SE	Mean	±SE	Mean	±SE	Mean	±SE	Mean	±SE	Mean	±SE
Depth (m)	11.1	0.7	12.8	0.8	9.1	0.4	8.9	1.1	7.2	0.3	2.5	0.1	3.3	0.5	8.7	1.0	7.4	0.9	7.4	1.0
Water transparency (m)	1.6	0.2	2.2	0.3	1.8	0.3	0.6	0.1	1.1	0.1	0.3	0.0	0.5	0.1	1.1	0.2	0.9	0.4	0.6	0.1
Mixing depth (m)	11.1	0.7	7.0	2.0	9.8	2.6	7.6	1.0	4.9	1.1	1.7	0.3	2.5	0.5	5.9	1.4	3.7	1.6	6.9	1.5
pH	8.3	0.1	7.3	0.3	7.7	0.3	8.6	0.3	8.5	0.2	8.6	0.3	8.6	0.5	7.1	0.5	7.9	0.3	8.6	0.1
EC (µS cm^{-1})	2724.3	75.2	246.0	9.9	474.8	7.4	438.0	16.4	661.6	17.7	6008.0	987.9	705.8	13.0	58.8	8.0	252.5	62.3	434.3	29.2
DIN (µg L^{-1})	256.4	95.8	70.7	14.8	81.2	60.8	181.5	93.0	129.7	27.8	76.1	14.7	831.7	375.7	95.0	36.9	170.9	108.7	39.6	11.0
TDP (µg L^{-1})	303.8	24.6	18.2	3.6	40.4	18.6	479.7	66.4	25.5	4.6	289.6	76.9	146.8	29.8	12.0	1.1	86.9	42.7	86.5	39.2
TP (µg L^{-1})	475.4	42.6	22.7	7.7	71.8	25.2	850.1	91.3	36.3	11.2	470.1	144.4	205.6	50.6	24.2	7.3	97.5	47.0	115.6	54.6
Chlorophyll-*a* (µg L^{-1})	7.7	2.5	3.0	0.6	15.3	8.0	63.2	39.3	11.0	1.7	140.2	63.4	96.5	46.9	12.1	3.8	25.3	18.6	49.8	11.1
Trophic state index	60.0	0.4	52.2	0.7	56.8	1.4	64.1	0.7	55.4	0.6	64.8	1.2	62.4	1.2	54.4	1.4	57.0	1.8	60.2	0.9
Trophic state	Hyper.		Oligo.		Eutro.		Hyper.		Meso.		Hyper.		Hyper.		Meso.		Eutro.		Hyper.	

Table A.4 Statistics, mean, and standard error (±SE) of environmental conditions registered during different bloom categories in the tropical reservoirs studied (n = 42). The trophic state index was calculated according to Cunha et al. (2013). Significant differences between bloom categories were verified by the one-way ANOVA or Brown-Forsythe (when F values are marked with an asterisk) tests and are represented by different superscript letters after the mean value (Tukey or Bonferroni tests p <0.05).

	ANOVA/Brown-Forsythe			Identity of bloom-forming taxa									
				Non-bloom (n=10)		Cyanobacteria (n=19)		Dinophyta (n=7)		Chlorophyta (n=3)		Mixed (n=3)	
	d.f.	F	p-value	Mean	±SE	Mean	±SE	Mean	±SE	Mean	±SE	Mean	±SE
Depth (m)	4	11.36	<0.001	11.31^{c}	0.62	6.62^{b}	0.62	8.10^{bc}	0.64	8.73^{bc}	1.37	2.50^{a}	0.15
Water transparency (m)	4	8.07*	0.001	1.76^{c}	0.20	0.69^{b}	0.08	1.35^{abc}	0.33	1.14^{abc}	0.28	0.23^{a}	0.03
Mixing depth (m)	4	3.35	0.019	7.82^{b}	1.30	5.41^{b}	0.66	7.51^{b}	1.89	6.33^{b}	1.86	1.33^{a}	0.17
pH	4	5.77	0.001	7.63^{ab}	0.23	8.55^{c}	0.14	7.87^{abc}	0.22	7.28^{a}	0.63	8.82^{bc}	0.18
Electrical conductivity (µS cm^{-1})	4	17.8*	<0.001	1217.20^{b}	411.41	777.95^{b}	221.15	385.00^{b}	53.55	52.67^{a}	7.31	6436.33^{c}	1258.91
Dissolved inorganic nitrogen (µg L^{-1})	4	0.35	0.839	144.77^{a}	46.83	267.13^{a}	101.90	130.41^{a}	69.24	112.09^{a}	46.28	90.20^{a}	5.50
Total dissolved phosphorus (µg L^{-1})	4	3.45	0.017	131.28^{ab}	47.83	190.94^{ab}	45.99	70.96^{ab}	25.55	11.83^{a}	1.56	329.96^{b}	92.61
Total phosphorus (µg L^{-1})	4	2.71	0.045	202.84^{ab}	75.84	312.32^{ab}	81.51	94.14^{ab}	26.91	26.20^{a}	9.92	554.22^{b}	165.96
Chlorophyll-*a* (µg L^{-1})	4	9.53	<0.001	6.05^{a}	1.31	53.40^{bc}	14.90	22.32^{ab}	10.81	12.40^{ab}	5.30	167.40^{c}	81.06
Trophic state index	4	6.95	<0.001	55.68^{a}	1.24	60.53^{bc}	0.87	57.36^{ab}	1.08	54.39^{ab}	2.04	65.58^{c}	1.32

Table A.5 Generalized Additive Mixed Models (GAMM) testing the effects of phytoplankton biomass (log-transformed) on water quality parameters, and the effects of phytoplankton biomass and the biomass of different bloom-forming taxa (ln-transformed) on phytoplankton and zooplankton species richness and resource use efficiency (RUE) during different bloom categories in the tropical reservoirs studied (n = 42). Explanatory variables were fixed, while reservoir location and sampling periods were randomized to account for spatial and temporal autocorrelation. Bold values correspond to significant effects (p <0.05).

Response variable	Explanatory variable	Estimate	Std. error	t-value	e.d.f.	F	R^2_{adj}	p-value
Water transparency	log Phytoplankton	1.05	0.12	9.03	1.00	17.61	0.435	**<0.001**
Mixing zone depth	log Phytoplankton	6.07	0.82	7.38	1.38	2.65	0.109	0.066
pH	log Phytoplankton	8.14	0.12	66.79	1.00	18.46	0.346	**<0.001**
log Total dissolved phosphorus	log Phytoplankton	1.86	0.15	12.08	3.23	4.64	0.119	**0.006**
log Total phosphorus	log Phytoplankton	2.02	0.16	12.69	2.86	4.70	0.138	**0.007**
Trophic state index	log Phytoplankton	58.79	0.95	61.81	2.85	12.15	0.428	**<0.001**
ln Phytoplankton richness	ln Phytoplankton	3.08	0.09	34.05	1.00	0.64	0.084	0.430
ln Zooplankton richness	ln Phytoplankton	2.63	0.06	45.13	1.00	3.53	0.085	0.067
ln Phytoplankton RUE	ln Phytoplankton	5.83	0.36	16.14	3.29	15.08	0.245	**<0.001**
ln Zooplankton RUE	ln Phytoplankton	-5.54	0.14	-40.61	0.90	54.22	0.508	**<0.001**
ln Phytoplankton RUE	ln Phytoplankton richness	5.81	0.39	14.90	3.95	1.19	0.036	0.333
ln Zooplankton RUE	ln Zooplankton richness	-5.53	0.25	-22.14	1.00	6.51	0.131	**0.014**
ln Phytoplankton richness	ln Cyanobacteria	3.09	0.06	54.52	2.76	3.59	0.357	**0.019**
ln Phytoplankton richness	ln Dinophyta	3.08	0.06	48.91	2.36	3.34	0.308	**0.016**
ln Phytoplankton richness	ln Chlorophyta	3.08	0.05	58.99	3.80	7.67	0.498	**<0.001**
ln Phytoplankton richness	ln Bacillariophyta	3.08	0.08	36.55	2.28	1.89	0.038	0.138
ln Zooplankton richness	ln Cyanobacteria	2.63	0.05	51.09	1.00	9.16	0.227	**0.004**
ln Zooplankton richness	ln Dinophyta	2.64	0.06	42.57	1.00	0.03	0.015	0.869
ln Zooplankton richness	ln Chlorophyta	2.64	0.06	47.26	1.00	1.22	0.088	0.276
ln Zooplankton richness	ln Bacillariophyta	2.64	0.06	44.30	1.00	0.47	0.029	0.498
ln Phytoplankton RUE	ln Cyanobacteria	5.84	0.40	14.52	2.77	4.32	0.040	**0.009**
ln Phytoplankton RUE	ln Dinophyta	5.80	0.38	15.21	1.52	2.34	0.088	0.075
ln Phytoplankton RUE	ln Chlorophyta	5.81	0.41	14.02	1.03	0.59	0.038	0.437
ln Phytoplankton RUE	ln Bacillariophyta	5.81	0.40	14.61	1.00	2.39	0.030	0.130
ln Zooplankton RUE	ln Cyanobacteria	-5.55	0.20	-28.32	3.10	7.72	0.385	**<0.001**
ln Zooplankton RUE	ln Dinophyta	-5.53	0.27	-20.41	1.00	0.81	0.002	0.374
ln Zooplankton RUE	ln Chlorophyta	-5.53	0.26	-21.19	1.00	2.74	0.052	0.106
ln Zooplankton RUE	ln Bacillariophyta	-5.54	0.17	-32.74	2.88	5.91	0.309	**0.002**

Table A.6 Statistical summary of one-way ANOVA or Brown-Forsythe tests comparing phytoplankton and zooplankton species richness, Shannon-Weiner diversity, evenness, total biomass, the biomass of each taxonomic group, and resource use efficiency between the five bloom categories (n = 42).

Variable	**d.f.**	**F**	**p-value**	**Test**
Phytoplankton species richness	4	10.31	<0.001	One-way ANOVA
Phytoplankton Shannon-Weiner diversity	4	18.85	<0.001	One-way ANOVA
Phytoplankton evenness	4	1.91	<0.001	One-way ANOVA
Zooplankton species richness	4	3.68	0.013	One-way ANOVA
Zooplankton Shannon-Weiner diversity	4	3.28	0.021	One-way ANOVA
Zooplankton evenness	4	3.64	0.013	One-way ANOVA
Phytoplankton total biomass	4	21.92	<0.001	One-way ANOVA
Cyanobacteria biomass	4	82.77	<0.001	One-way ANOVA
Dinophyta biomass	4	3.04	0.024	Brown-Forsythe
Chlorophyta biomass	4	3.77	0.025	Brown-Forsythe
Bacillariophyta biomass	4	28.33	<0.001	One-way ANOVA
Other groups biomass (mixotrophic organisms)	4	4.43	0.005	One-way ANOVA
Zooplankton total biomass	4	5.74	0.001	One-way ANOVA
Rotifers biomass	4	11.93	<0.001	One-way ANOVA
Calanoid copepods biomass	4	4.65	0.004	One-way ANOVA
Cyclopoid copepods biomass	4	12.01	<0.001	One-way ANOVA
Copepod nauplii biomass	4	5.219	0.002	One-way ANOVA
Cladocerans biomass	4	16.09	0.009	Brown-Forsythe
Phytoplankton resource use efficiency	4	9.27	<0.001	One-way ANOVA
Zooplankton resource use efficiency	4	10.20	<0.001	Brown-Forsythe

ARTIGO 4:

Controle das Florações

6 ARTIGO 4

Effects of the manipulation of submerged macrophytes, large zooplankton, and nutrients on a cyanobacterial bloom: A mesocosm study in a tropical shallow reservoir

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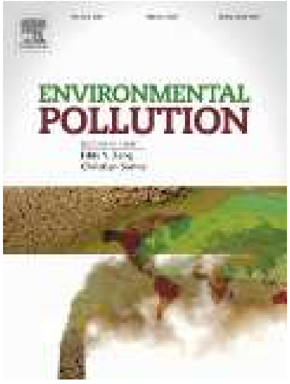
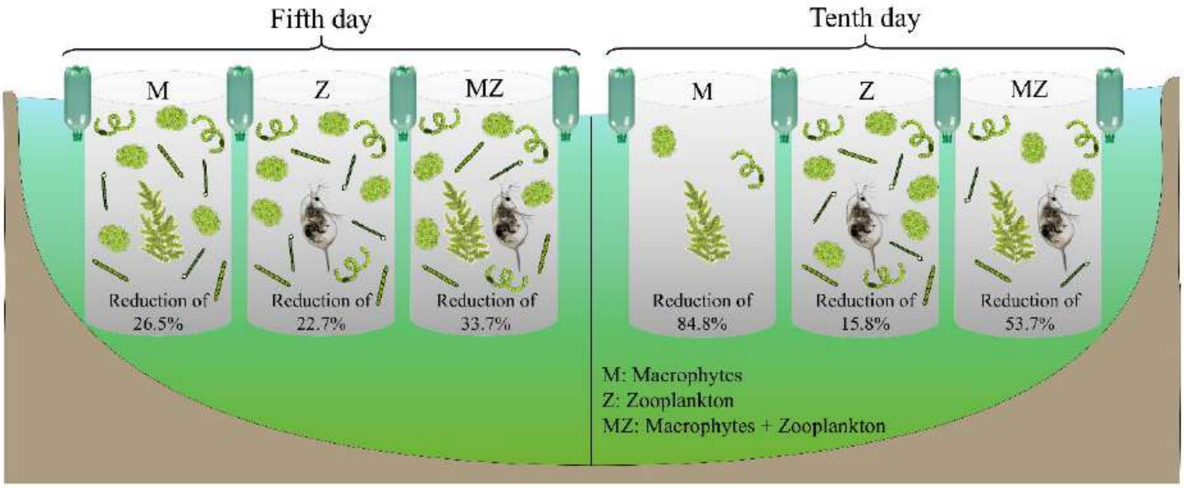


Graphical Abstract



Highlights

- Biomanipulation of submerged plants could control a tropical cyanobacterial bloom
- Submerged macrophytes could control a cyanobacterial bloom through allelopathy
- Macrophyte additions are more efficient at controlling blooms than large zooplankton
- Top-down effects of large zooplankton were significant only on the fifth day
- This article helps to understand biomanipulation as a tool to control tropical blooms

Abstract: Biomanipulation is an efficient tool to control eutrophication and cyanobacterial blooms in temperate lakes. However, the effects of this technique are still unclear for tropical ecosystems. Herein, we evaluated the effects of the biomanipulation on cyanobacterial biomass in a tropical shallow reservoir in Northeast Brazil. A mesocosm experiment was conducted in Tapacurá reservoir (Pernambuco) with eight treatments, in which we factorially manipulated the presence of submerged macrophytes (*Ceratophyllum demersum)*, large herbivorous zooplankton (*Sarsilatona serricauda*), and nutrients (0.4 mg L^{-1} of nitrogen and 0.5 mg L^{-1} of phosphorus). On the first, fifth, and tenth days, we analyzed the total biomass of cyanobacteria, and the morphotypes coccoid, heterocyted filamentous, and non-heterocyted filamentous cyanobacteria; these components were compared through a three-way ANOVA. The bloom was composed mainly of five *Microcystis* morphospecies (coccoids) and *Raphidiopsis raciborskii* (heterocyted filaments). On the fifth day of the experiment, the combined addition of macrophytes and zooplankton was more efficient at controlling cyanobacterial biomass. On the tenth day, all macrophyte treatments showed significant cyanobacterial biomass reduction, decreasing up to 84.8%. On the other hand, nutrients and zooplankton, both isolated and combined, had no significant effect. Macrophytes also reduced the biomass of coccoids, heterocyted filaments, and non-heterocyted filaments when analyzed separately on the tenth day. ***Ceratophyllum** demersum* was more efficient at controlling the bloom than the addition of large herbivorous zooplankton, which could be related to allelopathy since cyanobacterial biomass was also reduced when nutrients were added. The addition of submerged macrophytes with allelopathic potential, associated with the increase of large herbivorous zooplankton, proved to be an efficient technique for controlling tropical cyanobacterial blooms.

Keywords: Allelopathy; Biomanipulation; Blooms of *Microcystis*; Control of cyanobacteria; Tropical shallow lakes.

Summary of Results

We showed how submerged macrophytes and large zooplankton can reduce up to 85% of cyanobacterial biomass during a bloom, benefiting in the restoration of tropical shallow lakes.

Introduction

In the last few decades, eutrophication has become a major problem for aquatic environments, decreasing their multiple uses and ecosystem services (Hilt et al., 2017). Moreover, there is a need for controlling eutrophication to promote access to potable water and reduce harmful algal blooms (Paerl, 2009). Amongst the consequences of eutrophication, blooms of potentially toxic cyanobacteria are most problematic, as they affect the use of aquatic environments, and pose health risks to aquatic and human communities due to the release of cyanotoxins (Paerl and Otten, 2013; Carmichael and Boyer, 2016; Moura et al., 2018).

The excessive inputs of nitrogen and phosphorus into the water bodies favor the increase of cyanobacterial blooms, composed by both nitrogen-fixing (e.g. *Aphanizomenon*, *Dolichospermum* (=*Anabaena*) and *Raphidiopsis* (=*Cylindrospermopsis*)) and non-nitrogen-fixing (e.g. *Microcystis* and *Planktothrix*) genera, therefore, for effective control, a significant reduction in concentrations of both nutrients is required (Paerl, 2009). However, large water bodies remain eutrophic, and still contain cyanobacterial blooms, even considering the many efforts to reduce nutrient loading, such as in Taihu Lake in China (e.g. Qin et al., 2019). Biomanipulation may be an alternative solution in mitigating bloom development; this consists of the alteration of important components in biological communities and presents improved cost-benefit over large-scale nutrient reductions (Triest et al., 2016).

In temperate regions, fish biomanipulation (through the removal of planktivores and benthivores, or by the addition of piscivores) has generated satisfactory results (Jeppesen et al., 2012). This manipulation causes a trophic cascade in aquatic communities, favoring the development of zooplankton, the main phytoplankton consumer (Triest et al., 2016). However, in tropical regions, the effects of the trophic cascade are less evident, mainly because of the dominance of fast-growing omnivorous fish, higher densities of juveniles, and lower abundance of piscivores (Jeppesen et al., 2007, 2012). In this sense, only massive fish removals would be enough to alter the other trophic levels and control phytoplankton biomass (Okun et al., 2008).

Another problem in most tropical eutrophic lakes is the predominance of small zooplankton, such as rotifers, cyclopoid copepods, and small cladocerans (Jeppesen et al., 2007). This is dissimilar to temperate regions, where the zooplankton community is generally represented by large cladocerans, such as *Daphnia* (Jeppesen et al., 2012). Additionally, cyanobacteria present adaptations to avoid predation by zooplankton, such as nutritional deficiency, gathering in large colonies and filaments, and the ability to produce cyanotoxins, which further weaken the trophic cascade in tropical regions (Ger et al., 2016). Although some laboratory experiments show that tropical zooplankton can control cyanobacterial growth (Kâ et al., 2012; Amorim et al., 2019b), in natural environments zooplankton presents a negligible effect on cyanobacteria (e.g. Lacerot et al., 2013; Hong et al., 2015; Severiano et al., 2018). Alternatively, the addition of submerged macrophytes efficiently reduces the phytoplankton biomass, especially cyanobacteria (Vanderstukken et al., 2014). The principal action mode of the submerged macrophytes is the release of allelochemicals (Hilt and Gross, 2008), competition for nutrients and light, reduction of sediment resuspension, and providing refuge for zooplankton (Scheffer et al., 1993).

Despite recent advances in the field of biomanipulation, little is known about how the manipulation of the aquatic communities can control eutrophication and algal blooms in tropical regions (Jeppesen et al., 2007, 2012). Firstly, it is known that in (sub)tropical regions, macrophytes are considered a poor refuge for zooplankton from predation by fish, as young fish can aggregate densely inside all biological forms of macrophytes (Meerhoff et al., 2006). Therefore, for higher biomanipulation efficiency in tropical regions, multiple strategies must be adopted, combining different changes in aquatic communities. In this regard, some recent studies have shown the effectiveness of aquatic macrophytes in reducing phytoplankton biomass after omnivorous fish removal in shallow lakes in China, with the bottom-up effects provided by macrophytes being more prominent (Yu et al., 2016; Liu et al., 2018).

Accordingly, in this study, we factorially manipulated the presence of submerged macrophytes, large herbivorous zooplankton, and nutrients to evaluate the potential control of cyanobacterial biomass and the biomass of different morphotypes in a tropical shallow system in Northeast Brazil. We tested three main hypotheses: (i) The isolated addition of submerged macrophytes or large herbivorous zooplankton is less efficient at controlling cyanobacterial blooms than the combined addition in a tropical shallow reservoir; (ii) Allelopathy, rather than the competition for nutrients, can be the main action mode of submerged macrophytes to control cyanobacterial blooms; (iii) Submerged macrophytes, even in a nutrient-enrichment scenario, are more efficient at controlling cyanobacterial blooms from a tropical shallow reservoir than the addition of large herbivorous zooplankton.

Materials and Methods

Study site

The experiment was conducted in the pelagic zone of Tapacurá reservoir, in the municipality of São Lourenço da Mata, in the state of Pernambuco, Northeast Brazil (8º2'36"S, 35º11'52"W). The reservoir is located in a tropical climate zone, characterized by dry summers (Alvares et al., 2013) and a rainy period from March to August. This reservoir is inserted in Capibaribe river basin, has a storage capacity of 94,200,000 m^3 of water, and is used for multiple purposes, like supply nearby towns, fishing, agriculture, and livestock (APAC, 2019).

Although Tapacurá reservoir is typically a large system, during the experiment it had a storage capacity of 24,524,000 m^3 of water (26% of the total volume), and a mean depth of 2 m due to an intense drought (APAC, 2019). Although located in a protected area, the Ecological Station of Tapacurá, this reservoir is subject to multiple anthropic pressures, such as sewage discharge, deforestation, and extensive sugarcane plantations. Tapacurá reservoir is considered a hypereutrophic environment with large stands of the floating macrophyte *Eichhornia crassipes* (Mart.) Solms, and annual blooms of *Microcystis* spp. The zooplankton community is composed mainly of rotifers and cyclopoid copepods, while fish assemblage is dominated by small zooplanktivores, such as the characid *Astyanax bimaculatus (Linnaeus, 1758)* and the omnivorous *Oreochromis niloticus* (Linnaeus, 1758).

Submerged macrophytes and large herbivorous zooplankton, used in the experiment, were collected from Carpina reservoir, which has extensive stands of the submerged macrophyte *Ceratophyllum demersum* L., and a zooplankton community dominated by large cladocerans, such as *Sarsilatona serricauda* (Sars, 1901). Carpina reservoir is another large (storage capacity of 270,000,000 m^3 of water) and hypereutrophic environment, also located in Capibaribe river basin, Lagoa do Carro municipality (22 km from Tapacurá reservoir). This water system is used for the same purposes as Tapacurá (i.e. water supply, fishing, agriculture, and livestock) (APAC, 2019).

Experimental design

The experiment was carried out between February 08th and 18th, 2019. We used 32 mesocosms with a capacity of 100 L, divided into two blocks of 16, which were made from PVC tube supports and transparent polyethylene bags (1.25 m depth and 0.32 m width). The mesocosms were open to the atmosphere and closed at the bottom, not reaching the sediment, ensuring the absence of fish into the mesocosms. PET bottles were used as floats to maintain the opening of mesocosms placed 25 cm above the water surface. The mesocosm blocks were attached with ropes and then fixed to two trees in the limnetic region, at a point with a depth of 4.2 m. Mesocosms were filled with unprocessed water at the same time to ensure that all treatments were in the same initial conditions. Mesocosms have been proved to be relevant to evaluate the effects of large-scale processes and can be applied to larger natural environments, as the mesocosm volume does not affect algal abundances (Spivak et al., 2011).

The experiment had a factorial design (3×3), which allowed for an evaluation of the effects of the addition of submerged macrophytes, large herbivorous zooplankton, and nutrients (isolated and combined). Eight treatments, each one with four replicates, were designated, which were randomly arranged between the two blocks of mesocosms. The treatments were assigned as follows: C - control; M - macrophytes; Z - zooplankton; MZ - macrophytes + zooplankton; N - nutrients; MN - macrophytes + nutrients; ZN - zooplankton + nutrients; and MZN - macrophytes + zooplankton + nutrients.

We selected the macrophyte *C. demersum* based on its allelopathic potential against toxic and non-toxic strains of *Microcystis* spp. (Amorim et al., 2019a). Young branches were collected and transported to Tapacurá reservoir in plastic bags filled with reservoir water. They were then washed several times with tap and distilled water to remove epiphytic organisms and sediment. The macrophyte biomass used in the mesocosms was 3.5 gWW L^{-1} (g of wet weight per liter), this biomass was selected based on the mean biomass found in Carpina reservoir in 0.1 m^3 (100 L, the same volume of the mesocosms). Zooplankton was collected by filtering 200 L of water from Carpina reservoir with a 320 µm mesh-opening plankton net to gather only large organisms. For each mesocosm with zooplankton, we added 85 µg L^{-1} of large herbivorous zooplankton. Zooplankton samples were transported to Tapacurá reservoir in polyethylene bottles and maintained without food until the inoculation into the mesocosms (approximately three hours). Additional samples were collected in Carpina reservoir and fixed with 4% formalin for further analysis. The added nutrients were ammonium (NH_4^+) and phosphate (PO_4^{3-}) at a concentration of 0.4 and 0.5 mg L^{-1}, respectively. Concentrations were estimated based on the annual mean of these nutrients in the reservoir, which were weighed to the desired molar concentration from ammonium chloride (NH_4Cl) and potassium phosphate (KH_2PO_4) for NH_4^+ and PO_4^{3-}, respectively; they were then diluted in 200 mL of autoclaved distilled water. The biomass of macrophytes and zooplankton, besides nutrient concentration, was equal to all their respective treatments. After the inoculation, the mesocosms were covered with a net of 5 mm mesh-opening to prevent the entrance of birds, insects, and debris. At the end of the experiment, macrophytes were removed and discarded far from the reservoir, and the water from the mesocosms was filtered with a 60 µm mesh-opening plankton net to collect zooplankton samples.

Sampling and analyses

Samples for physicochemical variables and chlorophyll-*a* analysis were collected after the addition of macrophytes, zooplankton, and nutrients, and again after ten days, always between 8 and 10 am (n = 4). Longer periods could allow periphyton settling into the mesocosm. In each mesocosm, the values of water temperature, pH, electrical conductivity, total dissolved solids, and salinity were measured with a HANNA multiparameter probe, and light intensity was measured with an LI-COR photometer. Meteorological data (air temperature, wind speed, and solar radiation) were obtained from the nearest meteorological station on the first and tenth days of the experiment (INMET, 2019). Water samples were collected with a 1 m long PVC tube at different points of the mesocosms to obtain an integrated sample from all depths. Samples (n = 4) were collected to quantify nitrate, nitrite, ammonium, dissolved inorganic nitrogen (DIN), orthophosphate, total dissolved phosphorus (TDP), and total phosphorus concentrations, following specific methodologies (Strickland and Parsons, 1965; Golterman et al., 1971; Valderrama, 1981), and chlorophyll-*a*, by the spectrophotometric method after extraction of pigments in 90% ethanol (Chorus and Bartram, 1999), which was considered an estimate of phytoplankton biomass in the mesocosms. Nutrient limitation was assessed through chlorophyll-*a* to DIN or TDP ratios, as described by Kosten et al. (2009). In this case, lower values indicate that phytoplankton is limited by factors other than nitrogen or phosphorus.

Samples of 200 mL were collected for cyanobacteria quantification at each mesocosm on the first, fifth, and tenth days (n = 4); they were then preserved with 1% acetic Lugol and analyzed under an inverted microscope in sedimentation chambers to determine the density, and further converted to biomass (mg L^{-1}), using the geometric models proposed by Hillebrand et al. (1999). Species were identified based on specific literature. The biomass was grouped into total biomass, and the biomass of coccoids (non-nitrogen-fixing), heterocyted filaments (nitrogen-fixing), and non-heterocyted filaments (non-nitrogen-fixing). Samples for zooplankton quantification (100 L) were collected on the initial day (n = 4) before mesocosms were filled, and after ten days (n = 4), by filtering the water of the mesocosms in a 60 µm mesh-opening plankton net and fixed with 4% formalin. Species were quantified in a 1 mL Sedgewick-Rafter chamber, with the analysis of three subsamples per sample under a light microscope. Biomass was calculated using regressions between dry weight of organisms (µg L^{-1}), length and width of rotifer (Ruttner-Kolisko, 1977) and microcrustacean (Dumont et al., 1975) specimens. Zooplankton biomass was separated into rotifers, copepod nauplii, calanoid copepod, cyclopoid copepod, and cladocerans.

Statistical analyses

Initially, a PERMANOVA analysis was performed to verify whether the physicochemical variables (except nutrient concentrations) on the first day, as well as in all treatments on the tenth day, showed significant multivariate differences. Nutrient concentrations in the treatments C and N on the first day were compared by the T-test, and normality was checked by the Shapiro-Wilk test. Concentrations of nutrients, chlorophyll-*a* to DIN or TDP ratios, total zooplankton biomass, chlorophyll-*a*, total cyanobacterial biomass, and the biomass of coccoid, heterocyted and non-heterocyted filamentous cyanobacteria on the fifth and/or tenth days were compared using a three-way ANOVA to verify significant differences between the factors macrophytes, zooplankton, and nutrients, and possible interactions between them. For example, the factor macrophytes compares all macrophyte treatments to all treatments without macrophytes. The total cyanobacterial biomass and the biomass of the three morphotypes were compared between the sampling days in the treatment control by a one-way ANOVA. Before ANOVA analyses, data were tested for normality and homoscedasticity by Kolmogorov-Smirnov and Bartlett tests, respectively. When necessary, data were transformed ($\log(x + 1)$) to meet ANOVA premises. All analyses were performed using the R 3.5.2 statistical program, with a significance level of 5% ($p < 0.05$) (R Core Team, 2019).

Results

Physicochemical variables, nutrients, and zooplankton

The water of the mesocosms showed greater electrical conductivity, total dissolved solids, salinity, and temperature on the tenth day. The water presented alkaline pH values (>9.9) on both sampling days and in all treatments, while light intensity was higher on the first day. Air temperature and wind speed were higher on the tenth day, while solar radiation was higher on the first. However, no significant multivariate differences were found for the physicochemical parameters (except nutrients) between the treatments and sampling days (PERMANOVA: $F_{model} = 1.18$; $R^2 = 0.2669$; $p = 0.278$) (Supplementary Table S1).

After ten days, there was an increase of 108.5 µg L^{-1} in DIN concentrations in the treatment N, with significant differences based on factor nutrients. For TDP, the concentration increase was 175.6 µg L^{-1} in the treatment N on the tenth day, with significant differences based on factors macrophytes, zooplankton, nutrients, and the interaction between macrophytes + zooplankton, and macrophytes + zooplankton + nutrients (Figure 1, Table 1). Regarding other forms of nutrients, concentrations of nitrate did not differ significantly between treatments, while ammonium showed higher values in all treatments with nutrient additions, and nitrite in the treatments N, MN, and ZN. Higher values of orthophosphate were observed in all treatments with nutrient addition, besides the treatment M, while total phosphorus was higher in the treatment N (Supplementary Table S1). Low values of chlorophyll-*a* to DIN or TDP ratios indicated that phytoplankton was limited by factors other than nutrients, especially in the treatments M and MN (Table 1, Supplementary Figure S1).

The biomass of large herbivorous zooplankton collected in Carpina reservoir, which was added into the mesocosms, was 85 µg L^{-1} (± 10.4), consisting of 92% of *S. serricauda* and 8% of *Notodiaptomus cearensis* (Wright, 1936); both are herbivorous crustaceans with an average body size greater than 1 mm. The initial zooplankton biomass of Tapacurá reservoir was 178.6 µg L^{-1} (± 9.8), consisting of 54.5% of cyclopoid copepods (*Thermocyclops decipiens* (Kiefer, 1929)), 24.8% of cladocerans (mainly *Diaphanosoma spinulosum* (Herbst, 1975)), 15.2% of copepod nauplii, 4.5% of calanoid copepods, and 1% of rotifers, representing the initial biomass in all treatments of the experiment. At the end of the experiment, all treatments with macrophyte additions showed higher biomasses of rotifers, followed by cyclopoid copepods. In the treatments with zooplankton additions, cladocerans maintained biomass between 15.4 µg L^{-1} (Z) and 69.5 µg L^{-1} (MZN), with relative contributions from 16.5 to 39.9%. Significant differences in the total zooplankton biomass were verified based on factors macrophytes, nutrients, and the interaction between zooplankton + nutrients (Figure 2, Table 1).

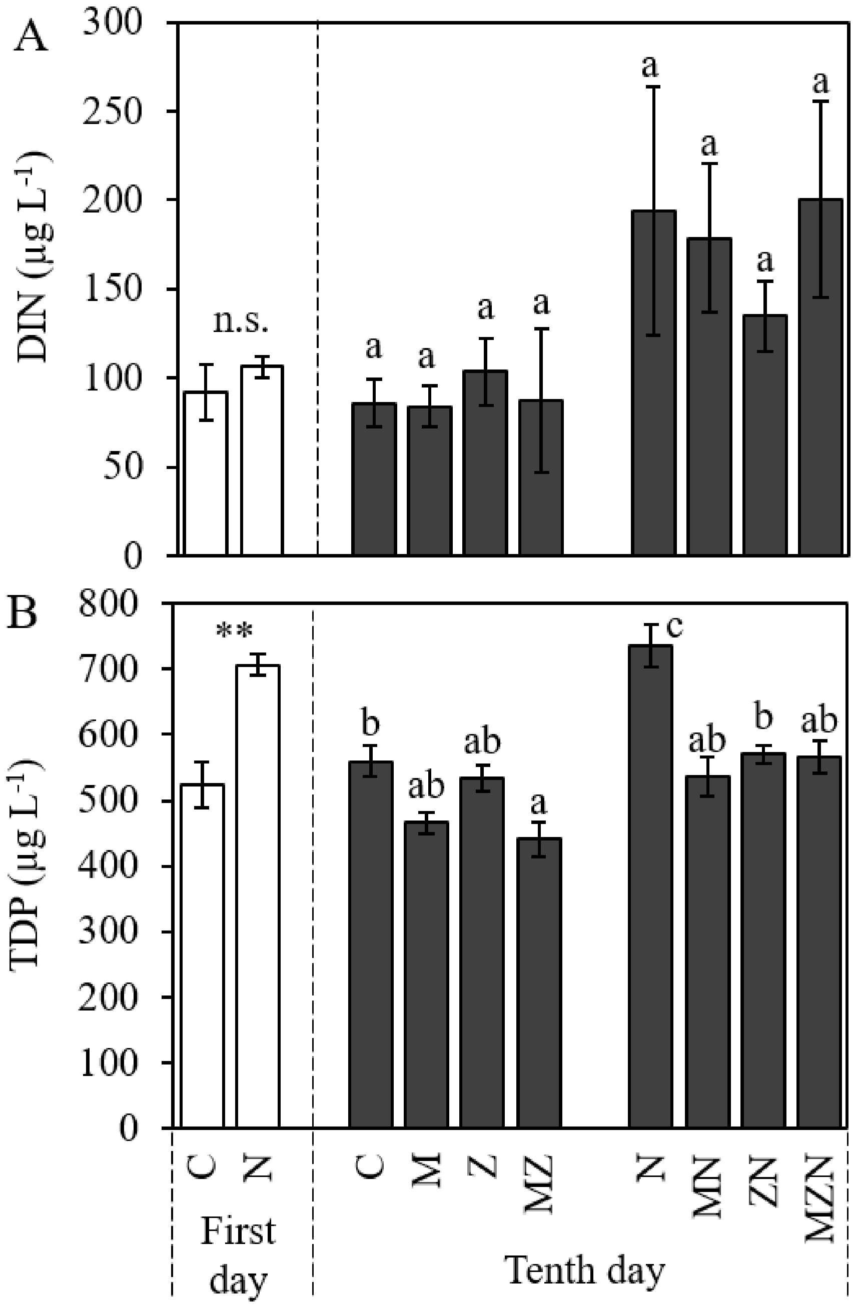


Figure 1. Mean values (± standard error) of the concentrations of (A) dissolved inorganic nitrogen (DIN) and (B) total dissolved phosphorus (TDP) in the treatments control (C), macrophytes (M), zooplankton (Z), nutrients (N), and their possible interactions, on the first and tenth days of the biomanipulation experiment. Significant differences between initial concentrations in treatments C and N are represented by n.s.: not significant; or **: p <0.01. Different letters represent significant differences between the treatments on the tenth day (p <0.05).

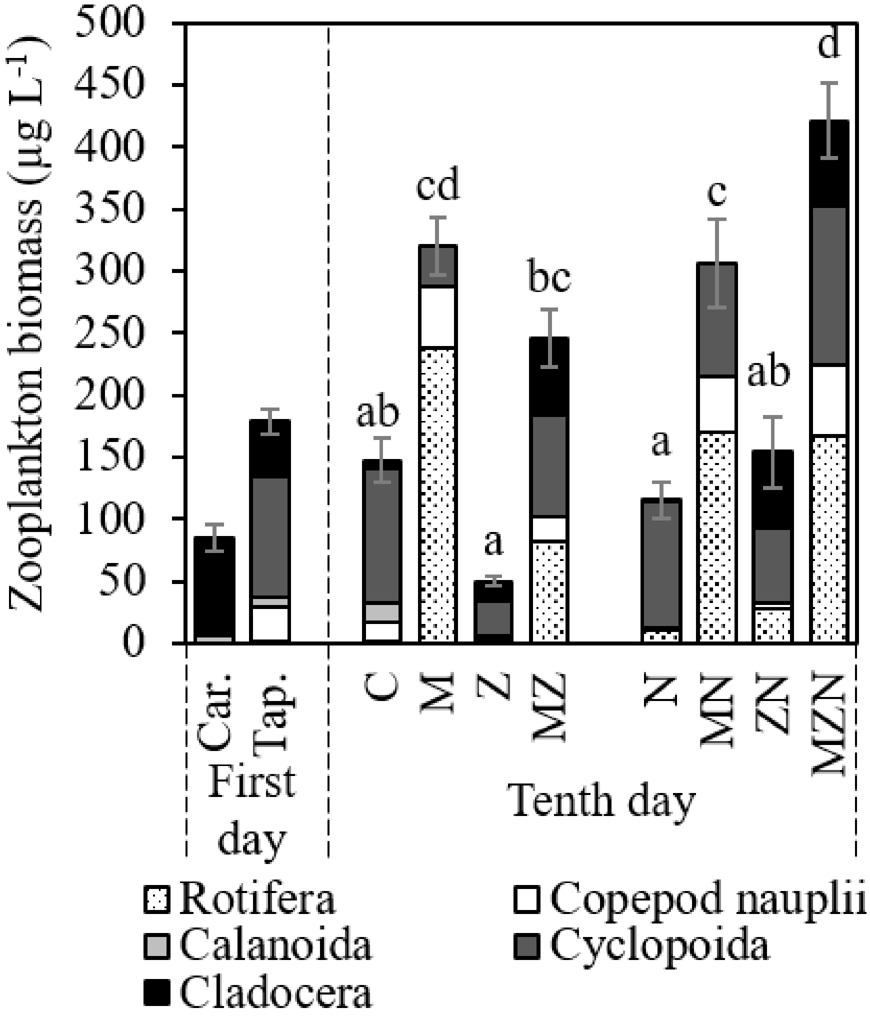


Figure 2. Mean values (± standard error for total biomass) of zooplankton biomass distributed in rotifers, copepod nauplii, calanoid copepods, cyclopoid copepods, and cladocerans in the treatments control (C), macrophytes (M), zooplankton (Z), nutrients (N), and their possible interactions, on the first and tenth days of the biomanipulation experiment. The initial biomass of Tapacurá reservoir (Tap.) represents the initial biomass into the mesocosm in all treatments, while the biomass of Carpina reservoir (Car.) represents the biomass of herbivorous crustaceans introduced in the treatments with zooplankton additions. Different letters represent significant differences between the treatments on the tenth day for the total zooplankton biomass ($p < 0.05$).

Table 1. Results of the three-way ANOVA analysis comparing dissolved inorganic nitrogen (DIN), total dissolved phosphorus (TDP), chlorophyll-*a*:DIN ratio, chlorophyll-*a*:TDP ratio, and total biomass of zooplankton, based on factors macrophytes (M), zooplankton (Z), nutrient (N), and their possible interactions on the tenth day of the biomanipulation experiment. Bold values represent significant effects ($p < 0.05$).

Variables	**Factors**	**F**	**p**
Inorganic dissolved nitrogen (DIN)	M	0.085	0.775
	Z	0.020	0.889
	N	**9.697**	**0.007**
	M:Z	0.357	0.558
	M:N	0.375	0.549
	Z:N	0.275	0.607
	M:Z:N	0.731	0.405
Total dissolved phosphorus (TDP)	M	**32.860**	**< 0.001**
	Z	**7.460**	**0.015**
	N	**35.710**	**<0.001**
	M:Z	**8.232**	**0.011**
	M:N	0.064	0.804
	Z:N	1.541	0.232
	M:Z:N	**8.031**	**0.012**
Chlorophyll-*a*:DIN	M	**14.765**	**0.001**
	Z	0.012	0.915
	N	**11.543**	**0.004**
	M:Z	**9.712**	**0.007**
	M:N	3.869	0.067
	Z:N	0.119	0.735
	M:Z:N	2.115	0.165
Chlorophyll-*a*:TDP	M	**32.239**	**< 0.001**
	Z	1.023	0.327
	N	2.233	0.155
	M:Z	**16.787**	**<0.001**
	M:N	**17.140**	**<0.001**
	Z:N	0.201	0.660
	M:Z:N	0.198	0.662
Total zooplankton	M	**144.721**	**< 0.001**
	Z	0.018	0.894
	N	**12.996**	**0.001**
	M:Z	1.560	0.224
	M:N	1.298	0.266
	Z:N	**22.286**	**< 0.001**
	M:Z:N	0.579	0.454

Chlorophyll-a and cyanobacterial biomass

High phytoplankton biomasses were recorded in Tapacurá reservoir at the beginning of the experiment, expressed by chlorophyll-*a* concentrations (276.8 µg L^{-1}, ± 15.2). Strong negative effects of macrophytes on chlorophyll-*a* were observed, besides the interaction between macrophytes + zooplankton and macrophytes + nutrients (Table 2). Macrophytes were able to significantly reduce phytoplankton biomass by 82.1% compared to the control, when associated with nutrients or zooplankton, the reduction was 60.0 and 57.5%, respectively. Although zooplankton additions did not significantly inhibit phytoplankton biomass alone, they reduced chlorophyll-*a* in ZN treatment by 45.7% (Figure 3, Table 3).

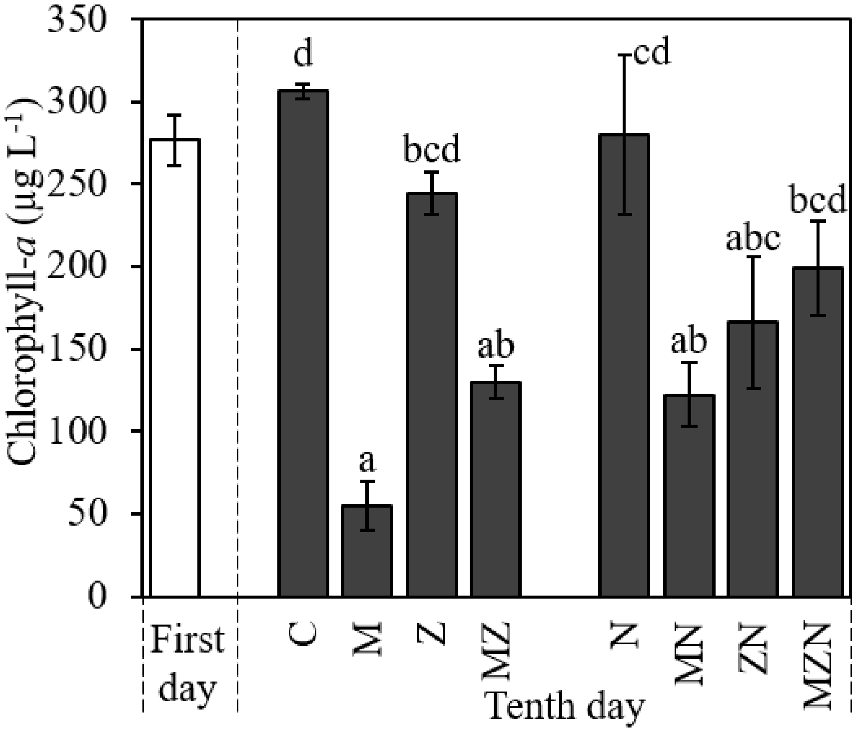


Figure 3. Mean values (± standard error) of chlorophyll-*a* concentrations in the treatments control (C), macrophytes (M), zooplankton (Z), nutrients (N), and their possible interactions, on the first and tenth days of the biomanipulation experiment. Different letters represent significant differences between the treatments on the tenth day (p <0.05).

The cyanobacterial bloom was composed mainly of coccoids, with higher biomass of five *Microcystis* morphospecies: *M. aeruginosa* (Kützing) Kützing, *M. brasiliensis* (De Azevedo & C.L.Sant'Anna) Rigonato et al., *M. panniformis* Komárek et al., *M. protocystis* W.B.Crow, and *Microcystis* sp. (isolated cells), as well as *Raphidiopsis raciborskii (Woloszynska) Aguilera* et al. (heterocyted filamentous). The initial cyanobacterial biomass in the control treatment was 204.6 mg L^{-1} (± 7.9), which increased significantly to 285.3 mg L^{-1} (± 13.8) on the fifth day, and to 294.8 mg L^{-1} (± 12.7) on the tenth. Higher biomasses were registered in the treatment N, with 440.7 mg L^{-1} (± 9.6) on the fifth day, and 330.3 mg L^{-1} (± 8.9) on the tenth (Figure 4).

The cyanobacterial biomass exhibited different responses to the treatments at different times. On the fifth day, cyanobacterial biomass was negatively influenced by macrophytes and zooplankton, and positively by nutrients, as well as by the interactions between macrophytes + zooplankton, macrophytes + nutrients, zooplankton + nutrients, and macrophytes + zooplankton + nutrients (Table 2). On the fifth day, greater reductions in biomass (compared to control) were observed in the treatment MZ (33.7%), followed by MZN (27.7%), M (26.5%), Z (22.7%), and MN (19.9%). Nutrient additions significantly increased cyanobacterial biomass to 54.5% (Figure 4, Table 3). On the tenth day, cyanobacterial biomass was significantly influenced by macrophytes, and the interactions between macrophytes + zooplankton, and zooplankton + nutrients (Table 2). Macrophytes significantly reduced cyanobacterial biomass (compared to the control) in all treatments, especially in the treatments M (84.8%) and MN (81.3%), followed by MZN (66.7%) and MZ (53.7%). Cyanobacterial biomass was not significantly altered by zooplankton or nutrients on the tenth day, neither isolated nor combined (Figure 4, Table 3).

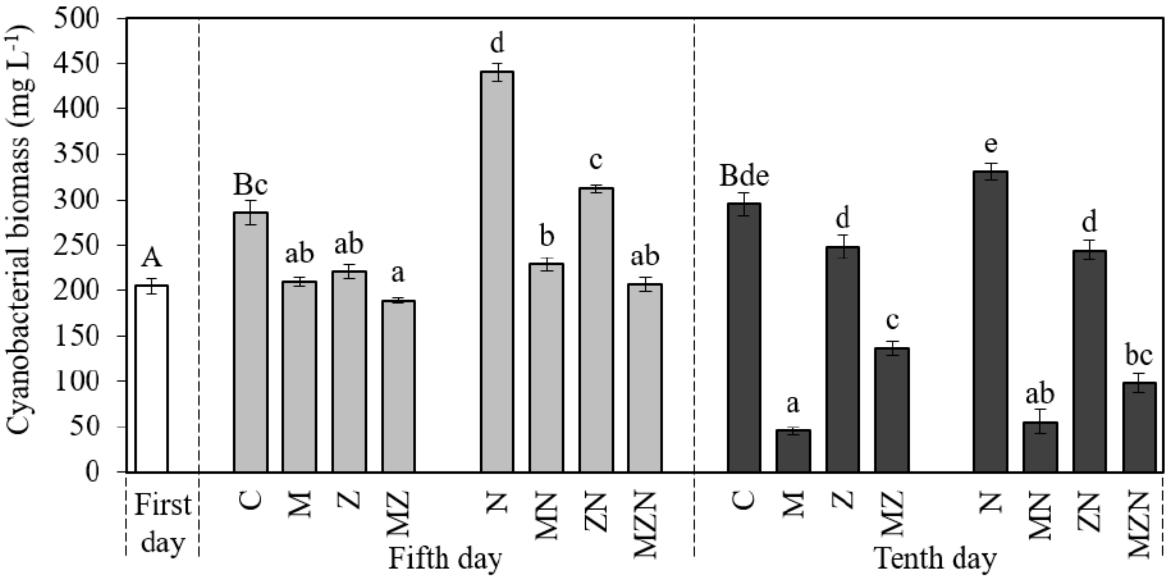


Figure 4. Mean values (± standard error) of total cyanobacterial biomass in the treatments control (C), macrophytes (M), zooplankton (Z), nutrients (N), and their possible interactions, on the first, fifth, and tenth days of the biomanipulation experiment. Different uppercase letters represent significant differences between sampling days for the control treatment ($p < 0.05$). Different lowercase letters represent significant differences between treatments for each sampling day ($p < 0.05$).

Regarding the control treatment variation in biomass of the cyanobacterial morphotypes during the sampling days, the coccoids showed a significant increase in their biomass on the fifth and tenth days. The heterocyted filaments exhibited a decrease in biomass on the fifth day, while no temporal variation was verified for non-heterocyted filaments (Figure 5). Coccoid cyanobacteria were influenced by all factors and their interactions on the fifth day. On the tenth day, significant effects were observed for the factors macrophytes, the interactions between macrophytes + zooplankton, macrophytes + nutrients, and zooplankton + nutrients (Table 2). On the fifth day, the coccoids were inhibited in the treatments M, Z, MZ, MN, and MZN, with an opposite response in treatments N and ZN, in which they were stimulated. On the tenth day, coccoids were inhibited in all treatments with macrophytes and were stimulated with nutrient additions (Figure 5, Table 3). Heterocyted filamentous cyanobacteria were affected by zooplankton on the fifth day, and by macrophytes on the tenth, as well as by the interactions between macrophytes + zooplankton and macrophytes + nutrients on both sampling days (Table 2). On the fifth day, no treatment significantly influenced heterocyted filaments, while on the tenth, all treatments with macrophyte additions (besides the ZN treatment) showed a negative effect (Figure 5, Table 3). Similarly, non-heterocyted filaments were influenced only by zooplankton on the fifth day, and by macrophytes on the tenth (Table 2). Furthermore, this morphotype was not affected by any treatment on the fifth day but was significantly inhibited in all macrophyte treatments on the tenth (Figure 5, Table 3).

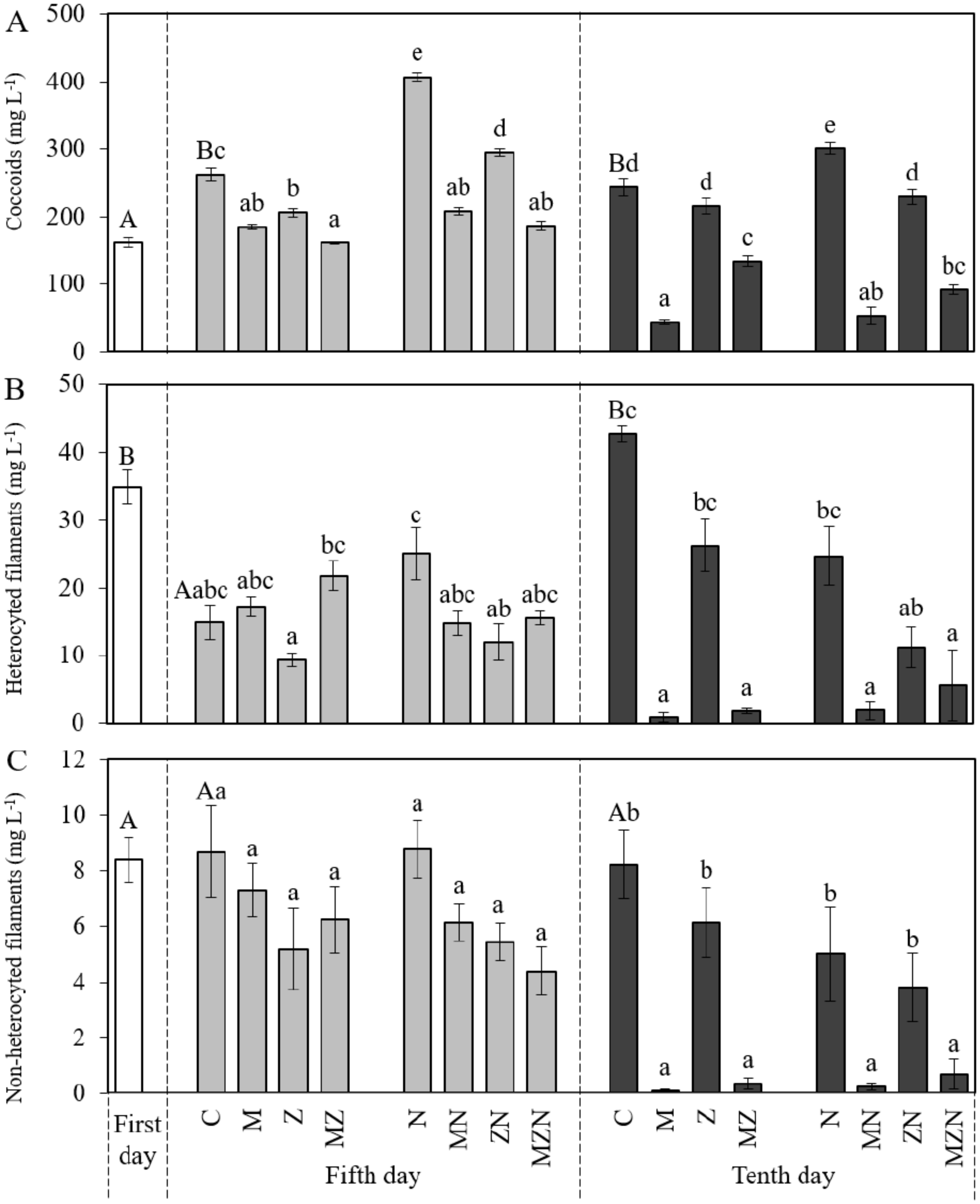


Figure 5. Mean values (± standard error) of (A) coccoids, (B) heterocyted filamentous, and (C) non-heterocyted filamentous cyanobacterial biomass in the treatments control (C), macrophytes (M), zooplankton (Z), nutrients (N), and their possible interactions, on the first, fifth, and tenth days of the biomanipulation experiment. Different uppercase letters represent significant differences between sampling days for the control treatment ($p < 0.05$). Different lowercase letters represent significant differences between treatments for each sampling day ($p < 0.05$).

Table 2. Results of the three-way ANOVA analysis comparing the values of chlorophyll-*a*, total cyanobacterial biomass, and the biomass of the morphotypes coccoids, heterocyted, and non-heterocyted filaments, based on factors macrophytes (M), zooplankton (Z), nutrient (N), and their possible interactions on the fifth and tenth day of the biomanipulation experiment. Bold values represent significant effects.

Variables		**Fifth day**		**Tenth day**	
	Factors	**F**	**p**	**F**	**p**
Chlorophyll-*a*	M	-	-	**42.828**	**< 0.001**
	Z	-	-	0.098	0.758
	N	-	-	0.187	0.671
	M:Z	-	-	**19.205**	**< 0.001**
	M:N	-	-	**10.378**	**0.005**
	Z:N	-	-	0.452	0.511
	M:Z:N	-	-	0.510	0.486
Total biomass of cyanobacteria	M	**413.813**	**< 0.001**	**679.692**	**< 0.001**
	Z	**140.363**	**< 0.001**	0.042	0.839
	N	**168.566**	**< 0.001**	0.072	0.791
	M:Z	**58.127**	**< 0.001**	**82.757**	**< 0.001**
	M:N	**93.715**	**< 0.001**	3.554	0.072
	Z:N	**8.562**	**0.008**	**8.710**	**0.007**
	M:Z:N	**8.334**	**0.008**	0.091	0.766
Coccoids	M	**674.480**	**< 0.001**	**594.692**	**< 0.001**
	Z	**184.740**	**< 0.001**	1.175	0.290
	N	**267.150**	**< 0.001**	1.921	0.179
	M:Z	**62.390**	**< 0.001**	**73.841**	**< 0.001**
	M:N	**116.620**	**< 0.001**	**13.288**	**0.001**
	Z:N	**9.030**	**0.006**	**12.022**	**0.002**
	M:Z:N	**12.090**	**0.002**	0.087	0.771
Heterocyted filaments	M	1.548	0.226	**109.075**	**< 0.001**
	Z	**4.726**	**0.040**	1.608	0.217
	N	0.348	0.561	2.617	0.119
	M:Z	**15.789**	**< 0.001**	**7.105**	**0.014**
	M:N	**10.822**	**0.003**	**8.937**	**0.007**
	Z:N	3.078	0.093	0.011	0.918
	M:Z:N	0.345	0.563	0.194	0.664
Non-heterocyted filaments	M	1.344	0.258	**79.015**	**< 0.001**
	Z	**9.785**	**0.005**	0.411	0.528
	N	0.739	0.399	0.618	0.440
	M:Z	1.824	0.190	0.796	0.382
	M:N	1.227	0.280	3.028	0.095
	Z:N	0.035	0.852	0.036	0.852
	M:Z:N	0.074	0.788	0.185	0.671

Table 3. Percentages of inhibition or stimulus of chlorophyll-*a*, total cyanobacterial biomass, and the biomass of the morphotypes coccoids, heterocyted, and non-heterocyted filaments, in the treatments macrophytes (M), zooplankton (Z), nutrient (N), and their possible interactions, compared to the control (C), after five and ten days of the biomanipulation experiment. Negative (-) and positive (+) symbols represent inhibition and stimulus, respectively. Bold values represent significant inhibitions and stimuli ($p < 0.05$).

	Treatments						
	M	Z	MZ	N	MN	ZN	MZN
Chlorophyll-*a* (tenth day)	**-82.1**	-20.2	**-57.5**	-8.6	**-60.0**	**-45.7**	**-34.9**
Total cyanobacterial biomass (fifth day)	**-26.5**	**-22.7**	**-33.7**	**+54.5**	**-19.9**	+9.5	**-27.7**
Total cyanobacterial biomass (tenth day)	**-84.8**	-15.8	**-53.7**	+12.0	**-81.3**	-17.2	**-66.7**
Coccoids (fifth day)	**-29.2**	**-21.4**	**-38.5**	**+55.5**	**-20.7**	**+12.7**	**-28.8**
Coccoids (tenth day)	**-82.0**	-11.5	**-45.0**	**+23.2**	**-78.3**	-6.0	**-62.3**
Heterocyted filaments (fifth day)	+15.7	-36.9	+46.2	+68.2	-0.6	-19.1	+4.6
Heterocyted filaments (tenth day)	**-98.0**	-38.5	**-95.7**	-42.1	**-95.5**	**-73.6**	**-86.8**
Non-heterocyted filaments (fifth day)	-15.7	-40.2	-28.0	+1.2	-29.1	-37.3	-49.4
Non-heterocyted filaments (tenth day)	**-98.8**	-25.5	**-95.6**	-39.1	**-97.1**	-53.8	**-91.6**

Discussion

During the biomanipulation experiment, we recorded significant top-down and bottom-up effects of macrophytes, zooplankton, and nutrients on cyanobacterial biomass in the studied tropical shallow reservoir. The response of cyanobacteria to the treatments was time-dependent, considering that all tested factors had significant effects on the fifth day, while on the tenth day only macrophytes treatments displayed significant reductions on cyanobacterial biomass. The submerged macrophyte *Ceratophyllum demersum* was more efficient in the control of phytoplankton (chlorophyll-*a*) and cyanobacterial biomass than the addition of large herbivorous zooplankton; this could be attributed to allelopathy since the control was efficient even with nutrient additions.

Although Tapacurá reservoir is typically a large reservoir, during the experiment, it was shallow (mean depth of 2 m) and with a lower storage capacity due to an intense drought. Hence, our study describes the process of biomanipulation in shallow systems, using mesocosms as a model. The use of macrophytes and zooplankton from another reservoir (Carpina) did not affect the results, considering that both reservoirs are hypereutrophic, located in the same watershed, and used for the same purposes. So, the organisms collected in Carpina reservoir were already acclimated to the trophic conditions found in Tapacurá reservoir. Furthermore, the chosen submerged macrophyte, *C. demersum*, can grow intensely even in hypereutrophic environments. This macrophyte can inhibit the phytoplankton and maintain the clear state in shallow lakes, through competition for nutrients, light, and allelopathy, even at high concentrations of phosphorus (e.g. Jasser, 1995; Mjelde and Faafeng, 1997; Dong et al., 2014). However, mechanisms other than nutrient limitation can cause a long-term inhibition of phytoplankton by *C. demersum* (Lombardo and Cooke, 2003).

The increased cyanobacterial biomass in the control treatment throughout the experimental period could be related to the increased water and air temperatures when compared to the initial day. Under natural conditions, the rising temperature can increase cyanobacterial biomass directly by boosting growth rates, or indirectly through thermal stratification and increased phosphorus release from sediments (Paerl and Huisman, 2008). Higher temperatures also have implications for the effectiveness of biomanipulation strategies, intensifying the effects of eutrophication (Rigosi et al., 2015), and enhancing the reproduction of omnivorous fish, which favor phytoplankton through trophic cascade effects (Jeppesen et al., 2012). Because of this, biomanipulation in tropical regions is more complex than in temperate zones, as higher temperatures hamper the control of eutrophication and cyanobacterial blooms.

Nutrient treatments were important to elucidate the effects of submerged macrophytes and large herbivorous zooplankton, isolated and combined, even in a scenario of higher nutrient concentration, and to exclude the possibility of nutrient limitation in macrophyte treatments. Nutrients (treatment N) stimulated cyanobacterial biomass only on the fifth day, which can be a response to the temperature increase that occurred in the final days of the experiment since the warming of surface water can decrease the influence of nutrients on phytoplankton biomass (e.g. Shurin et al., 2012). Another possibility may be the increased rates of nitrogen and phosphorus uptake by heterotrophic bacteria in eutrophic environments, making them less available for phytoplankton (Kirchman, 1994); considering that on the tenth day, nutrient concentrations also did not differ between treatments N and C.

During the experiment, the decrease in cladoceran biomass in treatment Z, compared to the initial biomass, could be related to a reduction in the reproductive capacity of zooplankton due to high cyanobacteria biomass, as demonstrated by Guo and Xie (2006). This reduced biomass made the top-down control of zooplankton difficult on the tenth day, allowing the growth of cyanobacteria. Besides that, the combined addition of herbivorous zooplankton and nutrients caused a significant reduction in chlorophyll-*a*, but not in the total cyanobacterial biomass. In this sense, experimental studies show that eutrophication may increase zooplankton biomass (Shurin et al., 2012). Indeed, in our experiment, cladoceran biomass in the ZN treatment was higher than the treatments C and Z; so, they may have reduced chlorophyll-*a* by top-down control. On the other hand, the absence of a reduction in cyanobacterial biomass can be justified by the selective zooplankton grazing, consuming only the palatable algae. Furthermore, some zooplankton species have food selectivity, being able to identify their prey through mechanoreceptors and select only palatable ones (Heuschele and Selander, 2014). This selectivity was demonstrated for the calanoid copepod *Notodiaptomus iheringi* (Wright, 1935), which favored the dominance of *Microcystis aeruginosa* (Leitão et al., 2018), and for the cyclopoid copepod *Thermocyclops decipiens,* which preferred to graze on a diatom rather than the cyanobacteria *R. raciborskii* (Gebrehiwot et al., 2019). Consequently, selective grazing may favor the dominance of cyanobacteria, which will have a competitive advantage through the decline of palatable organisms.

During the experiment, zooplankton significantly consumed cyanobacteria only on the fifth day. The absence of top-down control of zooplankton on cyanobacteria on the final days of the experiment may be associated with possible prevention of consumption by the large herbivorous zooplankton, as they could have acclimated to the coexistence with cyanobacterial blooms and selected only palatable prey. In a laboratory study, Ger et al. (2011) have also shown that only five days were enough for the calanoid copepod *Eudiaptomus gracilis* (Sars, 1863) to acclimate and avoid the consumption of toxic *Microcystis aeruginosa*, shifting its diet to palatable chlorophytes. The mentioned authors also demonstrated that the exposure of zooplankton to sublethal abundances of *Microcystis* may increase tolerance to blooms. Furthermore, the pre-exposure of cladocerans to high cyanobacterial biomass (first five days of the experiment), can induce tolerance against cyanobacteria (Gustafsson and Hansson, 2004), allowing zooplankton to coexist rather than feed on cyanobacteria.

In contrast to the less efficient top-down control exerted by zooplankton, the introduction of submerged macrophytes was able to reduce chlorophyll-*a* and cyanobacterial biomass in all treatments. Macrophytes can interfere in numerous ways with cyanobacterial growth, either through the release of allelochemicals or competition for light and nutrients (Hilt and Gross, 2008). In our experiment, the possibility of competition for light and nutrients was excluded since there were no differences in nutrient concentrations or light intensity between the treatment with macrophytes and the control. Besides that, on the tenth day, the addition of submerged macrophytes, even in a nutrient enhancement scenario, was more efficient at reducing cyanobacterial biomass. In those treatments, the chlorophyll-*a* to nutrients ratio showed the lowest values, suggesting that nutrients were not the limiting factor for phytoplankton. Therefore, the main action mode of the submerged macrophyte could be allelopathy.

Most of the rooted submerged macrophytes are sensitive to high nutrient concentrations, being suppressed in eutrophic environments, because of turbid waters and poor growth conditions (Søndergaard et al., 2010), or under intense cyanobacterial biomass (Amorim et al., 2017). However, the use of *C. demersum* in our study could prevent the damage caused by eutrophication for three reasons: firstly, *C. demersum* is well adapted to high nutrient conditions (Mjelde and Faafeng, 1997); secondly, as a free-living macrophyte (without a root system), it can develop in large stands near the surface of lakes, without being shaded by floating plants or phytoplankton; additionally, *C. demersum* has potent antioxidant and biotransformation mechanisms to alleviate the effects of cyanotoxins on its physiology (Pflugmacher, 2004); and finally, *C. demersum* is one of the most allelopathic active macrophytes (Hilt and Gross, 2008), preventing cyanobacterial bloom development.

On the fifth day, the introduction of submerged macrophytes combined with the large herbivorous zooplankton was more efficient at controlling cyanobacteria than the isolated additions of macrophytes or zooplankton. This pattern was not seen on the tenth day when the isolated addition of macrophytes was more effective. The reason why the combined addition of macrophytes and zooplankton did not present greater reductions on cyanobacterial biomass on the tenth day could be the death of large cladocerans, which potentially released nutrients into the water, and consequently, increased cyanobacterial growth. Also, nutrient recycling by zooplankton can be an important source of nutrients for phytoplankton growth (Attayde and Hansson, 1999). Another important point is that, in our experiment, *C. demersum* favored the growth of rotifers in macrophyte treatments. However, considering that most rotifers are small and unable to consume large colonies and filaments, their effects on cyanobacteria were negligible.

In sub-tropical lakes, submerged macrophytes have also been shown to be responsible for the reduction of phytoplankton biomass, especially cyanobacteria, with allelopathy being considered the main mechanism of action responsible for algal control (e.g. Vanderstukken et al., 2014; Dong et al., 2014). Macrophytes possess the potential to control allelopathically toxic and non-toxic cyanobacteria but have no adverse effects on green algae species, moreover, toxic cyanobacterial strains are more sensitive to allelochemicals when coexisting with submerged macrophytes (Amorim et al., 2019a). Hence, the use of submerged macrophytes can be an efficient tool for controlling blooms, as they can reduce cyanobacterial biomass (including those potentially toxic) without affecting other organisms.

Most biomanipulation studies in tropical lakes were performed applying the removal of omnivorous or zooplanktivorous fish to establish a trophic cascade on zooplankton and consequently reducing phytoplankton. In Brazil, the effects of these fish on phytoplankton biomass are controversial and may show negative effects (e.g. Menezes et al., 2010; Torres et al., 2016), no effect, or stimulus (e.g. Okun et al., 2008; Dantas et al., 2019). Accordingly, the use of only this type of biomanipulation is not recommended in tropical lakes, as fish may return to their previous biomass due to high reproduction rates (Okun et al., 2008; Jeppesen et al., 2012). Particularly in Northeast Brazil, including Tapacurá reservoir, the fish assemblage of eutrophic water bodies is dominated by zooplanktivores (*Astyanax* spp.) and omnivores (*Oreochromis niloticus*) (Lazzaro et al., 2003), making it difficult to control their biomasses because of rapid reproduction.

In a shallow lake in China, Liu et al. (2018) showed that the introduction of submerged macrophytes, associated with the introduction of piscivorous fish and the removal of zooplanktivores, helped to improve water quality, reduce nutrient and chlorophyll-*a* concentrations, and increase water transparency. As in our study, the results of Liu et al. (2018) revealed that bottom-up effects, mediated by submerged macrophyte introduction, were more expressive than top-down effects, considering that the biomass of crustacean zooplankton remained unchanged and zooplanktivorous fish returned to their previous abundance in the environment. However, the authors did not attribute the effects of macrophytes to allelopathy, considering that chlorophyll-*a* to phosphorus ratio remained unchanged. Similar results were verified by Yu et al. (2016) in sub-tropical lakes in China. Different from those studies, we verified a markedly decrease in chlorophyll-*a* to nutrients ratio in macrophyte treatment, suggesting allelopathy as the main mechanism for cyanobacterial bloom control.

To the best of our knowledge, we showed for the first time how biomanipulation of submerged macrophytes and large herbivorous zooplankton can control tropical cyanobacterial blooms in shallow lakes. Nevertheless, for a long-term successful restoration in eutrophic lakes, and the maintenance of high biomass of large herbivorous zooplankton, repeated fish removal is required (Okun et al., 2008; Søndergaard et al., 2008). Notwithstanding, zooplankton may not be adapted to coexisting with cyanobacterial blooms and submerged macrophytes may be damaged in eutrophic and hypereutrophic environments. To solve this problem, we created a conceptual diagram with some steps that should be followed for a successful biomanipulation in tropical shallow lakes: (1) stop or reduce external loading of phosphorus (e.g. sewage and agriculture); (2) removal of zooplanktivorous, omnivorous, and benthivorous fish; (3) introduction of piscivores; (4) transplantation of submerged macrophytes; (5) introduction of large-bodied herbivorous crustaceans (Figure 6).

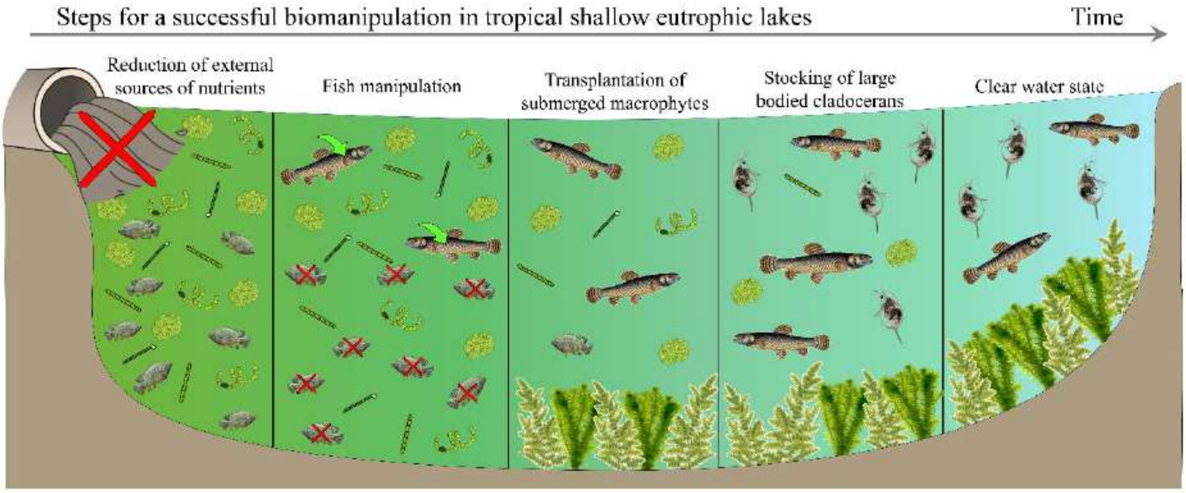


Figure 6. Conceptual diagram summarizing the steps that should be followed to achieve a successful biomanipulation in tropical shallow eutrophic lakes for cyanobacterial bloom control.

Conclusions

In this study, the first hypothesis was partially confirmed, since the combined addition of macrophytes and zooplankton to control cyanobacteria was more efficient than the isolated additions only on the fifth day of the experiment, both with and without nutrient additions. The second hypothesis was confirmed, considering that the reductions in chlorophyll-*a* and cyanobacterial biomass were verified in all treatments with the submerged macrophytes, without a significant reduction in nutrient concentrations; this revealed that allelopathy may have been the main factor responsible for the inhibition of cyanobacteria. The results of the present study also supported the third hypothesis, since macrophytes reduced chlorophyll-*a* concentrations and cyanobacterial biomass in all treatments on both sampling days, while zooplankton negatively affected cyanobacteria only on the fifth day, but in a less remarkable way when compared to macrophytes. The submerged macrophyte also reduced the biomass of coccoids, heterocyted, and non-heterocyted filamentous cyanobacteria, including potentially toxic ones. Thus, the addition of submerged macrophytes with allelopathic potential, associated with the increase of large herbivorous zooplankton, proved to be an efficient method to control tropical cyanobacterial blooms in shallow lakes.

Conflicts of interest

Declarations of interest: none.

Data Availability

Additional data may be obtained from the first author (alvescihelio@gmail.com, cihelio.amorim@ufrpe.br).

Author Contributions

Cihelio A. Amorim: Conceptualization, Methodology, Validation, Data curation, Formal analysis, Writing - Original Draft, Writing - Review & Editing. **Ariadne N. Moura:** Conceptualization, Supervision, Methodology, Writing - Review & Editing.

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Supplementary Material

Title: Effects of the manipulation of submerged macrophytes, large zooplankton, and nutrients on a cyanobacterial bloom: A mesocosm study in a tropical shallow reservoir

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Table S1. Mean values (± standard error: ±SE) of the physical and chemical (electrical conductivity, total dissolved solids, salinity, water temperature, pH, and light intensity), nutrients (nitrate, nitrite, ammonium, orthophosphate, and total phosphorus), and meteorological variables (air temperature, wind speed, and solar radiation) in the treatments control (C), macrophytes (M), zooplankton (Z), nutrient (N), and their possible interactions, on the first and tenth days of the biomanipulation experiment. Significant differences between the treatments for nutrient concentrations on the tenth day are represented by different lowercase letters (Tukey: $p < 0.05$).

	First day		Tenth day															
			C		M		Z		MZ		N		MN		ZN		MZN	
	Mean	±SE	Mean	±SE	Mean	±SE	Mean	±SE	Mean	±SE	Mean	±SE	Mean	±SE	Mean	±SE	Mean	±SE
Electrical conductivity (µS cm^{-1})	515.25	4.82	543.67	0.88	535.75	0.85	557.75	4.33	539.00	1.08	557.75	2.72	543.75	2.29	563.50	4.35	548.75	0.75
Total dissolved solids (mg L^{-1})	257.75	2.50	272.67	0.33	268.00	0.41	277.50	2.99	269.75	0.48	278.75	1.31	272.00	1.00	281.00	2.35	274.50	0.29
Salinity (PSU)	0.25	0.00	0.26	0.00	0.26	0.00	0.27	0.00	0.26	0.00	0.27	0.00	0.26	0.00	0.27	0.00	0.26	0.00
Water temperature (°C)	30.27	0.14	30.89	0.01	30.93	0.07	30.85	0.03	30.90	0.08	31.22	0.11	31.05	0.03	31.06	0.04	31.09	0.03
pH	10.12	0.06	10.83	0.09	9.95	0.09	10.74	0.13	10.43	0.11	10.12	0.09	10.16	0.06	9.87	0.11	10.10	0.03
Light intensity (µmol m^{-2} s^{-1})	1067.8	77.7	851.4	38.4	961.3	31.7	680.4	158.6	841.8	99.0	944.8	37.2	820.2	240.2	872.9	52.9	904.2	25.6
Nitrate (µg L^{-1})	8.96	1.20	17.95^{a}	7.00	11.97^{a}	2.95	8.30^{a}	2.55	44.25^{a}	35.40	11.52^{a}	1.32	16.90^{a}	4.06	16.96^{a}	2.10	15.78^{a}	1.15
Nitrite (µg L^{-1})	1.92	0.19	1.54^{ab}	0.10	1.44^{ab}	0.17	1.63^{ab}	0.19	1.06^{ab}	0.35	2.79c	0.10	1.83^{ab}	0.25	2.02^{bc}	0.29	0.67^{a}	0.25
Ammonium (µg L^{-1})	81.16	16.21	66.39^{ab}	6.09	70.71^{ab}	13.41	93.76^{ab}	16.31	42.31^{a}	5.96	179.63^{b}	69.27	159.89^{b}	46.11	115.93^{b}	18.71	184.04^{b}	56.28
Orthophosphate (µg L^{-1})	328.13	17.18	224.65^{a}	8.70	328.13^{b}	15.49	210.82^{a}	6.28	249.00^{a}	7.84	442.67^{d}	22.87	365.75^{bc}	16.33	436.58^{d}	12.89	409.47^{cd}	9.11
Total phosphorus (µg L^{-1})	900.85	21.45	899.85^{ab}	24.46	765.83^{a}	34.31	946.2^{ab}	56.26	813.19^{a}	38.04	1080.2^{b}	39.27	927.05^{ab}	57.53	939.15^{ab}	8.25	868.61^{a}	13.33
Air temperature (°C)	25.26	1.69	27.48	0.96	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Wind speed (m s^{-1})	0.94	0.35	1.52	0.42	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Solar radiation (kJ m^{-2})	1948.4	818.9	1550.9	556.3	-	-	-	-	-	-	-	-	-	-	-	-	-	-

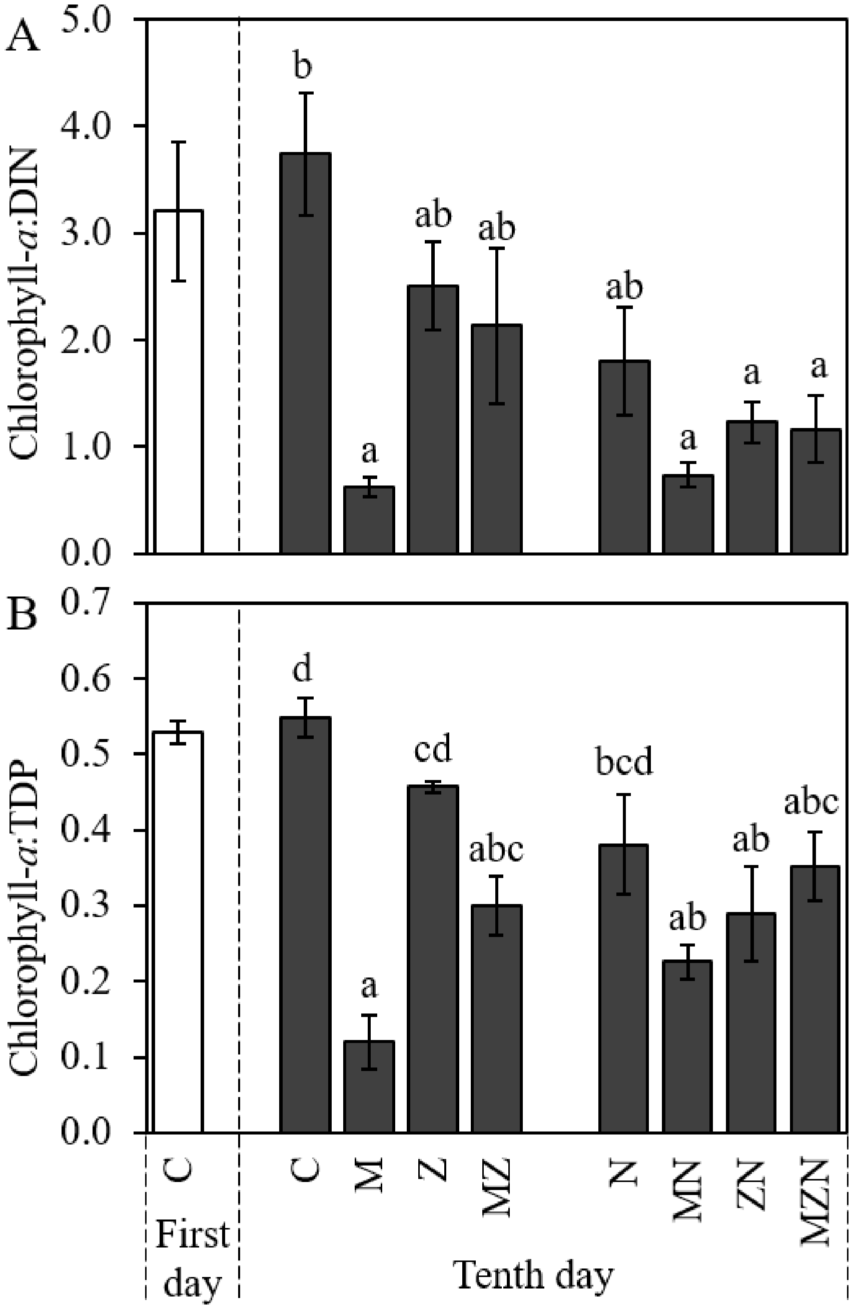

Figure S1. Mean values (± standard error) of (A) chlorophyll-*a* to dissolved inorganic nitrogen (DIN) ratio and (B) chlorophyll-*a* to total dissolved phosphorus (TDP) ratio, as possible indicators of nutrient limitation, in the treatments control (C), macrophytes (M), zooplankton (Z), nutrients (N), and their possible interactions, on the first and tenth days of the biomanipulation experiment. Different letters represent significant differences between the treatments on the tenth day ($p < 0.05$).

ARTIGO 5:

Efeitos da Biomanipulação no Plâncton

7 ARTIGO 5

Biomanipulation of submerged macrophytes, large cladocerans, and nutrients to control cyanobacterial blooms: effects on phytoplankton and zooplankton

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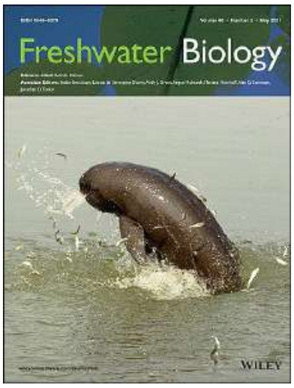

Abstract: Eutrophication and cyanobacterial blooms are among the main threats to water management and freshwater biodiversity. Accordingly, we experimentally tested the effects of the manipulation of submerged macrophytes and large-bodied zooplankton on native phytoplankton and zooplankton assemblages, with and without nutrient additions in a tropical shallow reservoir. A mesocosm experiment was conducted in Tapacurá reservoir, Brazil, in which we designated eight treatments, with the factorial (3×3) manipulation of the submerged macrophyte *Ceratophyllum demersum*, the large zooplankton *Sarsilatona serricauda*, and the concentration of nutrients (0.4 mg L^{-1} of nitrogen and 0.5 mg L^{-1} of phosphorus), for ten days. We compared the effects of the treatments on phytoplankton and zooplankton composition and structure through analyses of variance. The phytoplankton community was composed mainly of cyanobacteria, with relative biomasses greater than 98% in all treatments. At the end of the experiment, the submerged macrophytes significantly inhibited the biomass of cyanobacteria (84.8%), diatoms (79.6%), and green algae (77.9%), while the large cladoceran efficiently consumed the diatoms (95.5%) in the treatment with zooplankton and nutrients. Moreover, both submerged macrophytes and the large cladocerans negatively influenced the native zooplankton community. The submerged macrophytes presented stronger effects on phytoplankton and zooplankton diversity indexes, composition, and structure. However, the introduction of the large cladoceran also reduced the species richness, diversity, and evenness of native zooplankton. Furthermore, nutrient additions did not alter the main effects of macrophytes and large cladocerans. Therefore, our results demonstrate that the introduction of submerged macrophytes could control an intense potentially toxic cyanobacterial bloom, even without the influence of zooplankton grazing, or with nutrient additions in a tropical shallow lake.

Keywords: Eutrophication; Biomanipulation Experiment; *Ceratophyllum demersum*; *Microcystis*; Shallow Lake; Water Management.

Introduction

Harmful algal blooms have arisen as an emergent threat to freshwater biodiversity, reducing water quality and ecosystem services in the aquatic ecosystems (Reid et al., 2019). Most of the freshwater blooms are composed of cyanobacteria species, which can release cyanotoxins into the water and jeopardize freshwater communities and people who are supplied with these water sources (Huisman et al., 2018; Olokotum et al., 2020). Furthermore, nutrient input into the water systems is the most important factor triggering algal blooms, which are favored under conditions of higher eutrophication (Qin et al., 2019; Griffith and Gobler, 2020). Consequently, many countries have adopted restoration measures to reduce eutrophication and algal blooms (Jeppesen et al., 2017).

One important restoration technique is biomanipulation, which consists of the modification of important components of aquatic communities (Jeppesen et al., 2012; Triest et al., 2016). Most successful cases of biomanipulation came from the temperate region, showing an efficient reduction of nutrient concentrations, turbidity, and phytoplankton biomass (e.g., Søndergaard et al., 2008; Ha et al., 2013; Ekvall et al., 2014). The success of this technique usually depends on the recovery of submerged vegetation, which stabilizes the clear state of the lakes (Søndergaard et al., 2017; Hilt et al., 2018).

For the tropical regions, the efficiency of biomanipulation is not fully understood yet. Some characteristics of tropical lakes can impair the success of the biomanipulation through fish removals, such as the dominance of fast-growing omnivorous fish, lower densities of piscivores (Jeppesen et al., 2012), and the predominance of small zooplankton (Jeppesen et al., 2007). On the other hand, the removal of both benthivorous and planktivorous fish can decrease phytoplankton biomass in tropical eutrophic shallow lakes, due to the reduced sediment resuspension and feeding on zooplankton after fish removals (Dantas et al., 2019). However, a 10-fold increase of the zooplankton community, or even the addition of the large cladoceran

Daphnia, was not enough to reduce phytoplankton biomass dominated by cyanobacteria or green algae in a subtropical lake (Lacerot et al., 2013). Therefore, only massive fish removals, associated with the increase of large zooplankton, would be enough to control phytoplankton growth in warmer lakes (Okun et al., 2008; Lacerot et al., 2013).

Despite the low efficiency of zooplankton, some studies have demonstrated that the introduction of submerged macrophytes can decrease phytoplankton biomass in subtropical (Dong et al., 2014; Vanderstukken et al., 2011) and tropical lakes (Amorim and Moura, 2020). Submerged macrophytes can inhibit phytoplankton biomass through several mechanisms, including the reduction of light and nutrient availability, secretion of allelopathic substances, provision of refuges for zooplankton against fish predation, and reduction of sediment resuspension (Scheffer et al., 1993; Søndergaard and Moss, 1998). Among these mechanisms, allelopathy has a strong influence on phytoplankton structure, because of the different sensibility of the main algal groups; for example, cyanobacteria and diatoms are more sensitive to the allelochemicals, while green algae are tolerant (Hilt and Gross, 2008; Amorim et al., 2019). Therefore, the introduction of submerged macrophytes in a shallow lake can shift the dominance of cyanobacteria to green algae (Dong et al., 2014).

Although the combined addition of submerged macrophytes and large zooplankton can efficiently suppress phytoplankton biomass (e.g., Ha et al., 2013), the introduction of a different species can cause substantial effects on the native plankton community (Lee et al., 2016). Submerged macrophytes can significantly alter the diversity and dominance patterns of phytoplankton and zooplankton, reducing the biomass and changing the dominant groups depending on the macrophyte coverage (Ferreira et al., 2018). Similarly, the introduction of a non-native zooplankton species can reduce the abundance of native organisms through competition for similar food items (Lee et al., 2016). Therefore, besides evaluating the impact of the introduction of non-native species on eutrophication and cyanobacterial bloom control, it is also important to estimate the impacts of the introduction on the diversity, composition, and structure of native planktonic communities.

As demonstrated, the structure of planktonic communities is strongly controlled from the top-down through predation, and from the bottom-up by resources availability and competition (George, 2021). Nevertheless, the efficiency of these forces to control phytoplankton growth is dependent on eutrophication, as there are many critical thresholds of nutrients that control the shift from turbid to clear stable states, which are influenced by lake size, depth, and climate (Scheffer and van Nes, 2007). For instance, top-down control of phytoplankton biomass is unlikely in eutrophic and hypereutrophic deep lakes even after biomanipulation, as phytoplankton can be represented by inedible species and phosphorus reduction might be insufficient to start bottom-up control through nutrient limitation (Bennforf et al., 2002). Furthermore, higher eutrophication might impair submerged macrophytes growth and thus reduce bottom-up control of phytoplankton (Søndergaard and Moss, 1998; Scheffer and van Nes, 2007). Nutrient content can also affect the sensitivity of phytoplankton to macrophyte allelochemicals (Hilt and Gross, 2008), as allelopathic interactions are stronger under nutrient limitation conditions (Reigosa et al., 1999).

Thus, in this study, we experimentally evaluated the isolated and combined effects of the introduction of a submerged macrophyte, a large cladoceran, and nutrients on phytoplankton and zooplankton composition and structure, in a tropical shallow reservoir with cyanobacterial blooms. We tested three main hypotheses: (i) Submerged macrophytes are more efficient in controlling cyanobacteria biomass, while large cladocerans can efficiently reduce the biomass of eukaryotic algae; (ii) The input of nutrients reduces the efficiency of top-down and bottom-up effects of large cladocerans and submerged macrophytes on phytoplankton; (iii) The environmental heterogeneity created by the submerged macrophyte drives the diversity, composition, and structure of phytoplankton and zooplankton native communities.

Materials and Methods

Study site and experimental design

We performed a mesocosm experiment in the Tapacurá reservoir, inserted in the Capibaribe watershed, Pernambuco, Brazil (8º2'36"S, 35º11'52"W), from February 08th to February 18th, 2019. The reservoir is hypereutrophic, located in the Ecological Station of Tapacurá, an Atlantic Rain Forest fragment, and has a storage capacity of 94,200,000 m^3 of water (APAC, 2020). During the experiment, Tapacurá presented only 26% of its maximum storage capacity, and a mean depth of 2 m. Although this reservoir is used for multiple purposes, including water supply, fishing, irrigation in agriculture, livestock, and transportation, it is hypereutrophic and exposed to multiple stressors, such as pollution, habitat degradation, and agriculture. Tapacurá reservoir has large stands of the floating macrophyte *Eichhornia crassipes* (Mart.) Solms, and annual blooms of *Microcystis* spp. Rotifers and cyclopoid copepods are dominant in the zooplankton community, while fish assemblage is composed of small zooplanktivorous and omnivorous species (Amorim and Moura, 2020).

A total of 32 mesocosms, constructed with PVC tubes and transparent polyethylene bags (1.25 m depth and 0.32 m width), were kept open to the atmosphere and closed at the bottom. The mesocosms were fixed to two trees in the limnetic region of the reservoir and maintained floating with the support of PET bottles. Each mesocosm was filled with 100 L of non-processed water from the reservoir, ensuring the same initial conditions for all mesocosms. We designated eight treatments (each one with four replicates): C - control; M - macrophytes; Z - zooplankton; MZ - macrophytes + zooplankton; N - nutrients; MN - macrophytes + nutrients; ZN - zooplankton + nutrients; and MZN - macrophytes + zooplankton + nutrients. The submerged macrophyte *Ceratophyllum demersum* L. and the large cladoceran *Sarsilatona serricauda* (Sars, 1901) (species with higher biomass and a mean body size greater than 1 mm) were added to the treatments with macrophytes and zooplankton, respectively. These organisms were collected from the Carpina reservoir, another hypereutrophic system located in the same watershed (22 km from Tapacurá reservoir) (Amorim et al. 2020).

The submerged macrophytes were washed several times with tap and distilled water and then introduced into the mesocosms with biomass of 3.5 g L^{-1} (wet weight), based on the mean biomass in the Carpina reservoir. For the sampling of the large cladocerans, 200 L of water from the Carpina reservoir was filtered with a 320 µm mesh-opening plankton net, which allowed the selection of only large organisms. The mean zooplankton biomass added in each mesocosm was 85 mg L^{-1} (± 10.4), composed of 92% of *S. serricauda* and 8% of *Notodiaptomus cearensis* (Wright, 1936), a large calanoid copepod also larger than 1 mm and already present in Tapacurá reservoir. In the treatments with nutrient additions, we added 0.4 and 0.5 mg L^{-1} (molar concentration) of ammonium (NH_4^+) and phosphate (PO_4^{3-}), respectively, weighted from ammonium chloride (NH_4Cl) and potassium phosphate (KH_2PO_4), diluted in 200 mL of autoclaved distilled water.

Sampling and analyses

Phytoplankton analyzes were performed based on integrated samples collected with a 1 m long PVC tube at different points of the mesocosms on days zero, five, and ten (n = 4), and then preserved with 1% acetic Lugol. Species identification was performed in a light microscope (400× and 1000× magnifications) and based on specialized literature. The density of each species was analyzed in sedimentation chambers in an inverted microscope, following the recommendations of Utërmohl (1958). Phytoplankton biomass (mg L^{-1}) was determined using the geometric formulas from Hillebrand et al. (1999). The biomass was grouped into total biomass, and the biomass of cyanobacteria, green algae, diatoms, and phytoflagellates (Cryptophyta, Dinophyta, and Euglenophyta).

Zooplankton was analyzed in samples collected on days 0 and 10 (n = 4), by filtering the water of the mesocosms (100 L) in a 60 µm mesh-opening plankton net and fixed with 4% formalin. Three subsamples per sample were analyzed in a 1 mL Sedgewick-Rafter chamber in a light microscope for species identification and quantification. Species were identified following specialized literature. Regressions between dry weight (µg L^{-1}), length, and width of the organisms were used to estimate the biomass of the rotifers (Ruttner-Kolisko, 1977), cladocerans, and copepods (Dumont et al., 1975).

Data analyses

Initially, we calculated the proportion of decrease or increase in the phytoplankton and zooplankton biomass in all treatments, compared with the control, where zero represents the mean biomass in the control, while positive and negative values represent the percentage of increase or decrease in the biomass of each treatment, respectively. Species with relative biomasses greater than 50% were considered as dominant, while abundant species contributed to more than 5% of the total biomass. Phytoplankton and zooplankton species richness (S) indexes were defined as the number of species identified per sample. Shannon diversity index (H') and Pielou evenness (J' = H'/log(S)) were calculated in the *vegan* package of the R software (Oksanen et al., 2018).

A three-way ANOVA was used to verify significant differences between the proportion of decrease or increase for phytoplankton and zooplankton biomass, species richness, diversity, and evenness, based on the factors macrophytes, zooplankton, and nutrients, and possible interactions between them on the fifth and/or tenth days. The normality and homoscedasticity of the data before the ANOVA analyses were tested by the Kolmogorov-Smirnov and Bartlett tests, respectively. PERMANOVA analyses were used to test significant differences in the phytoplankton and zooplankton composition (presence and absence matrix) and structure (biomass matrix) between all treatments on the tenth day. Then, data were graphically represented in non-parametric multidimensional scaling plots (NMDS) in the *vegan* package of the R software. All statistical analyses were performed in the R software (version 4.0.5), with a significance level set at $p < 0.05$ (R Core Team, 2021).

Results

Phytoplankton

A total of 57 phytoplankton taxa were identified, composed of 24 species of cyanobacteria and 33 eukaryotic algae (21 green algae, six diatoms, and six phytoflagellates) (Table S1). The initial phytoplankton biomass in the control treatment was 206.5 mg L^{-1} (standard error ± 7.8), which increased to 286.5 mg L^{-1} (standard error ± 14.0) on the fifth day, and to 296.9 mg L^{-1} (standard error ± 12.5) on the tenth. Cyanobacteria always contributed to more than 98% of the phytoplankton biomass, with the dominance of *Microcystis panniformis* Komárek et al. in most of the samples (>50%), while *M. aeruginosa* (Kützing) Kützing, *M. brasiliensis* (De Azevedo & C.L.Sant'Anna) Rigonato et al., *M. protocystis* W.B.Crow, *Microcystis* sp. (isolated cells), *Raphidiopsis raciborskii (Woloszynska) Aguilera* et al., *Sphaerospermopsis aphanizomenoides* (Forti) Zapomelová et al., and *Woronichinia karelica* Komárek & Komárková-Legnerová were abundant (>5%). After cyanobacteria, the second most abundant group was the diatoms, followed by green algae, and phytoflagellates, but always with relative biomasses lower than 2% (Figure 1).

Due to the higher biomass of cyanobacteria in all treatments, the effects of the treatments on total phytoplankton and cyanobacteria biomass were the same, which were significantly influenced by all the factors on the fifth day, besides the factors macrophytes, macrophytes + zooplankton, and zooplankton + nutrients on the tenth. On the fifth day, higher inhibitions of the total phytoplankton and cyanobacteria biomass were observed in the treatment MZ (33.7%), followed by MZN (27.7%), M (26.5%), Z (22.9%), and MN (19.9%). The isolated addition of nutrients (treatment N) significantly stimulated total phytoplankton and cyanobacteria by 54.4% on the fifth day. For the tenth day, higher inhibitions in the total phytoplankton and cyanobacteria biomass were observed in the treatments M (84.7%), MN (81.2%), MZN (66.7%), and MZ (53.8%). Looking at the treatments with nutrient additions, there was a significant reduction in the phytoplankton and cyanobacteria biomass in the treatment ZN when compared to N (Table S2; Figure 2a, b).

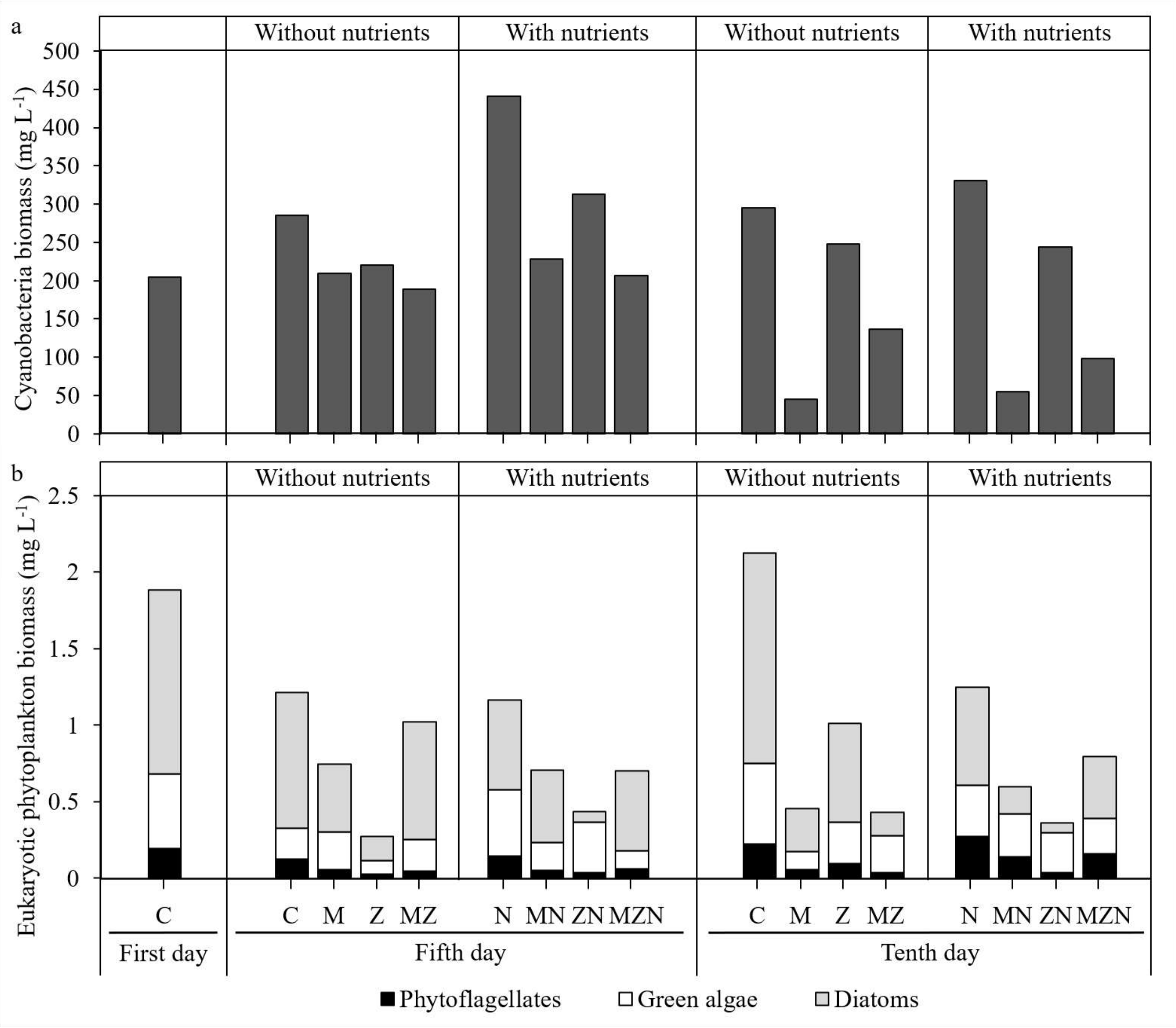

Figure 1. Mean biomass of cyanobacteria (a) and eukaryotic phytoplankton (b), separated into phytoflagellates, green algae, and diatoms, in the treatments control (C), macrophytes (M), zooplankton (Z), nutrients (N), and their possible interactions, on the first, fifth, and tenth days of the biomanipulation experiment.

On the fifth day, only the factor macrophytes + nutrients influenced the biomass of green algae but none of the treatments presented significant differences from the control. On that day, green algae biomass was higher in treatment N compared to Z and MZN. On the tenth day, only the factor macrophytes presented significant effects, with a reduction of 77.9% of the green algae biomass in the treatment M (Table S2; Figure 2c). The biomass of diatoms on the fifth day was influenced by the factor macrophytes + zooplankton and significantly inhibited in the treatments ZN and Z (92.2% and 82.6%, respectively). For the tenth day, the factors macrophytes, zooplankton, nutrients, the interaction between macrophytes + zooplankton, and macrophytes + nutrients presented significant effects. On that day, diatom biomass was reduced by 95.5% in the treatment ZN, followed by MZ (89.0%), MN (87.1%), and M (79.6) (Table S2; Figure 2d). The phytoflagellates biomass was influenced by the factors macrophytes, zooplankton, and macrophytes + zooplankton, where it was significantly reduced in the treatments Z (77.2%) and ZN (71.0%). On the tenth day, only the factors zooplankton and the interaction between macrophytes + zooplankton significantly influenced phytoflagellates biomass, but without significant differences from the control. However, in the set of mesocosms with higher eutrophication, there was a significant reduction in the phytoflagellates biomass in the treatment ZN when compared to N (Table S2; Figure 2e).

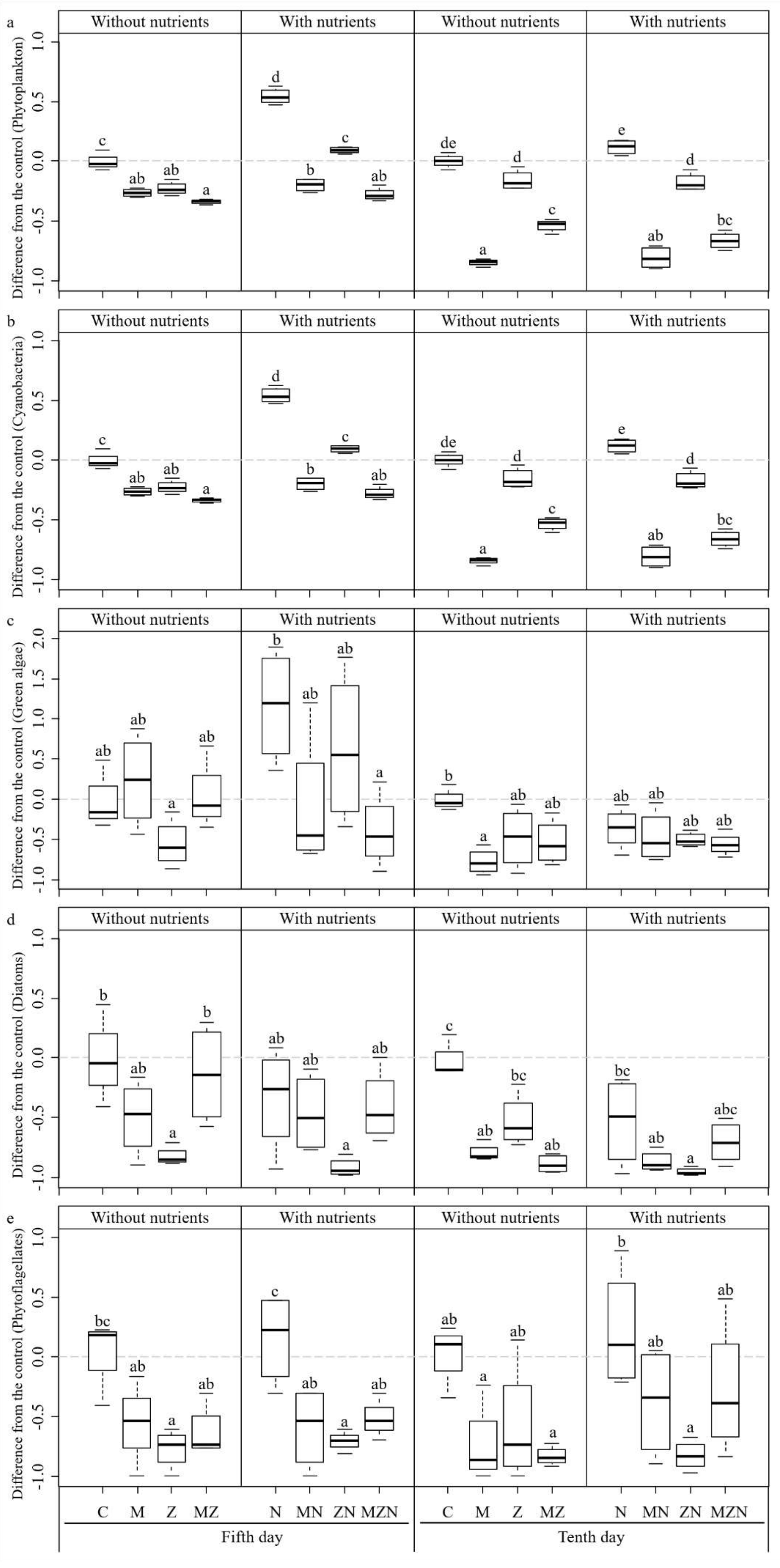
a
Without nutrients
With nutrients
Without nutrients
With nutrients
Difference from the control (Phytoplankton)
1.0
0.5
0.0
-0.5
-1.0
c
ab
ab
a
d
b
c
ab
de
a
d
c
e
ab
d
bc
b
Without nutrients
With nutrients
Without nutrients
With nutrients
Difference from the control (Cyanobacteria)
1.0
0.5
0.0
-0.5
-1.0
c
ab
ab
a
d
b
c
ab
de
a
d
c
e
ab
d
bc
c
Without nutrients
With nutrients
Without nutrients
With nutrients
Difference from the control (Green algae)
2.0
1.5
1.0
0.5
0.0
-0.5
-1.0
ab
ab
a
ab
b
ab
ab
a
b
a
ab
ab
ab
ab
ab
ab
d
Without nutrients
With nutrients
Without nutrients
With nutrients
Difference from the control (Diatoms)
1.0
0.5
0.0
-0.5
-1.0
b
ab
a
b
ab
ab
a
ab
c
ab
bc
ab
bc
ab
a
abc
e
Without nutrients
With nutrients
Without nutrients
With nutrients
Difference from the control (Phytoflagellates)
1.0
0.5
0.0
-0.5
-1.0
bc
ab
a
ab
c
ab
a
ab
ab
a
ab
a
b
ab
a
ab
C
M
Z
MZ
N
MN
ZN
MZN
C
M
Z
MZ
N
MN
ZN
MZN
Fifth day
Tenth day

Figure 2. The proportion of decrease or increase (compared to the mean biomass of the control) of the total phytoplankton (a), cyanobacteria b), green algae (c), diatoms (d), and phytoflagellates (e) in the treatments control (C), macrophytes (M), zooplankton (Z), nutrients (N), and their possible interactions, on the fifth, and tenth days of the biomanipulation experiment. Dashed grey lines indicate the mean difference in the control treatment (zero). Different lowercase letters represent significant differences between treatments for each sampling day ($p < 0.05$).

Zooplankton

A total of 19 zooplankton taxa were identified, composed mainly of rotifers (13 spp.), copepods (3 spp.), and cladocerans (3 spp.) (Table S3). The initial zooplankton biomass in the control treatment was 178.6 µg L^{-1} (standard error ± 9.8), which decreased to 147.3 µg L^{-1} (standard error ± 18.1) on the tenth day. The copepods presented higher biomasses in most of the treatments, represented by the cyclopoid *Thermocyclops decipiens* (Kiefer, 1929) as the dominant species, followed by copepod nauplii and *N. cearensis* as abundant, especially in Z (96.0%), C (93.8%), N (90.2%), ZN (69.0%), MZ (55.2%), and MZN (52.3%). All treatments with macrophytes presented high biomass of rotifers, which became dominant in the treatments M (74.4%), MN (55.8%), MZN (47.3%), and MZ (44.8%), represented mainly by *Lepadella patella* (Müller, 1773) as dominant, and *Lecane bulla* (Gosse, 1851), and *Brachionus calyciflorus* Pallas, 1766 as abundant species. The cladocerans started with a mean relative biomass of 24.8% but declined to less than 5% in all treatments, becoming completely absent in the treatments M, MZ, and MN. Among cladoceran species, only *Diaphanosoma spinulosum* Herbst, 1975 became abundant in the treatments C and ZN (Figure 3).

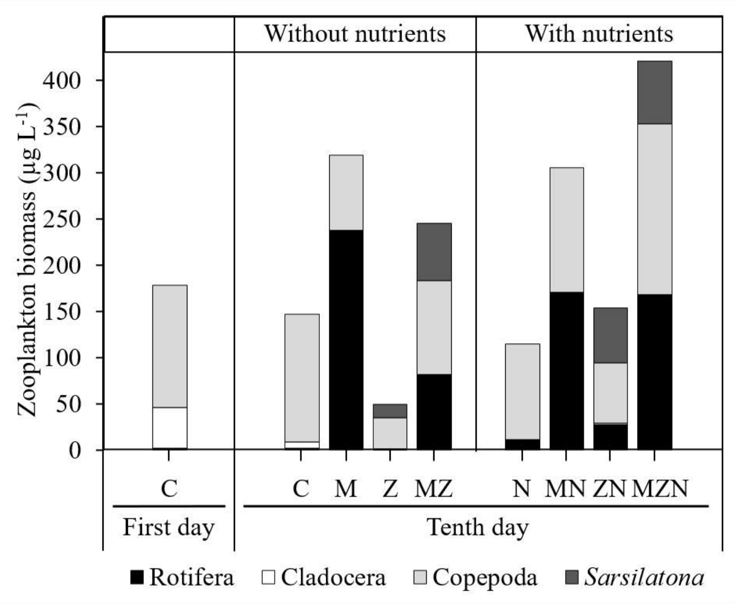

Figure 3. Mean biomass of the zooplankton groups Rotifera, Cladocera, and Copepoda, besides the introduced *Sarsilatona serricauda*, in the treatments control (C), macrophytes (M), zooplankton (Z), nutrients (N), and their possible interactions, on the first and tenth days of the biomanipulation experiment.

On the tenth day, the biomass of the total zooplankton native from the reservoir was significantly influenced by the factors macrophytes, zooplankton, nutrients, and the interactions between zooplankton + nutrients. On that day, the natural zooplankton community from Tapacurá reservoir was significantly enhanced by the submerged macrophyte in the treatments MZN (139.6%), M (117.0%), and MN (107.5%), with higher biomasses than the control and the treatments Z, MZ, N, and ZN (Table S4; Figure 4a). The biomass of rotifers was significantly influenced by the factors macrophytes, zooplankton, and zooplankton + nutrients. Higher biomass of this groups compared to the control was observed in the treatments M (12,147%), MN (8,692%), MZN (8,510%), and MZ (4,131%), but did not significantly differ from the control due to the higher heterogeneity of the data (Table S4; Figure 4b). Cladocerans were affected by the interaction between zooplankton + nutrients. Although there were complete inhibitions (100%) in the treatments M, MZ, and MN, followed by Z (89.9%), N (85.1%), MZN (84.3%), and ZN (76.2%), the increased heterogeneity of the data did not allow the identification of significant differences (Table S4; Figure 4c). For the biomass of copepods, there were significant effects of the factors macrophytes, nutrients, and macrophytes + zooplankton. However, only the treatment ZN differed from MZN in the set of mesocosms with higher eutrophication (Table S4; Figure 4d). The introduced *S. serricauda* presented a decline of 81.1% on its biomass in the treatment Z on the tenth day when compared to the first day; in the other treatments, *S. serricauda* showed a reduction of only 12-23% (Figure 4e).

Phytoplankton and zooplankton diversity, composition, and structure

On the fifth day, phytoplankton species richness was affected by the factors macrophytes + zooplankton and macrophytes + nutrients, Shannon diversity by macrophytes + zooplankton, and Pielou evenness by the factor macrophytes. However, these indexes did not differ from the control in any treatment. For the tenth day, only macrophytes influenced phytoplankton species richness. On the other hand, macrophytes, the interaction between macrophytes + zooplankton, and macrophytes + nutrients impacted phytoplankton diversity and evenness, besides the significant effect of nutrients on Pielou evenness. None of the treatments presented a significant difference from the control for species richness, while Shannon diversity and Pielou evenness were significantly lower than the control in the treatments M, MZ, MN, ZN, and MZN (Table S2; Figure 5).

Zooplankton species richness was significantly influenced by the factors macrophytes + nutrients, and diversity by the factor macrophytes, without significant differences from the control in all treatments. Moreover, Pielou evenness was affected by the factors macrophytes and macrophytes + zooplankton + nutrients. Although none of the treatments differed from the control, there were significant differences based on the factor nutrients, where the treatment MZ presented higher evenness than Z, and MZN higher than N (Table S4; Figure 6).

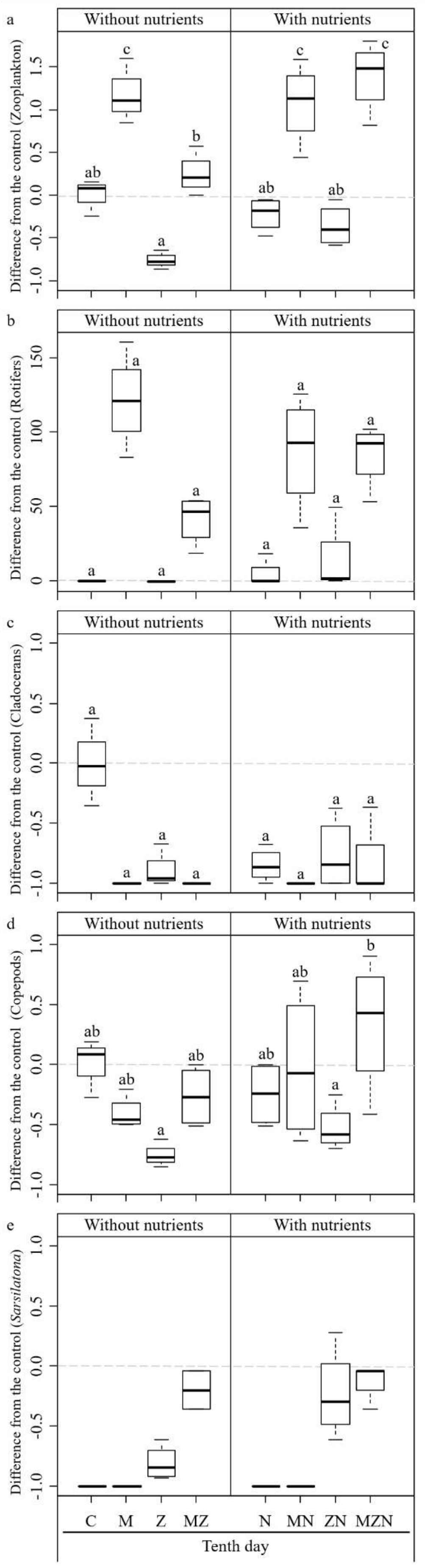
a
Without nutrients
With nutrients
Difference from the control (Zooplankton)
1.5
1.0
0.5
0.0
-0.5
-1.0
ab
c
a
b
ab
c
ab
c
b
Without nutrients
With nutrients
Difference from the control (Rotifers)
150
100
50
0
a
a
a
a
a
a
a
a
c
Without nutrients
With nutrients
Difference from the control (Cladocerans)
1.0
0.5
0.0
-0.5
-1.0
a
a
a
a
a
a
a
a
d
Without nutrients
With nutrients
Difference from the control (Copepods)
1.0
0.5
0.0
-0.5
-1.0
ab
ab
a
ab
ab
ab
a
b
e
Without nutrients
With nutrients
Difference from the control (Sarsilatona)
1.0
0.5
0.0
-0.5
-1.0
C
M
Z
MZ
N
MN
ZN
MZN
Tenth day

Figure 4. The proportion of decrease or increase (compared to the mean biomass of the control for native groups and the initial biomass for *Sarsilatona serricauda*) of the total zooplankton (a), rotifers (b), cladocerans (c), copepods (d), and the introduced *Sarsilatona serricauda* (e) in the treatments control (C), macrophytes (M), zooplankton (Z), nutrients (N), and their possible interactions, on the fifth, and tenth days of the biomanipulation experiment. Dashed grey lines indicate the mean difference in the control treatment for native groups and the initial biomass for *Sarsilatona serricauda* (zero). Different lowercase letters represent significant differences between treatments (p <0.05).

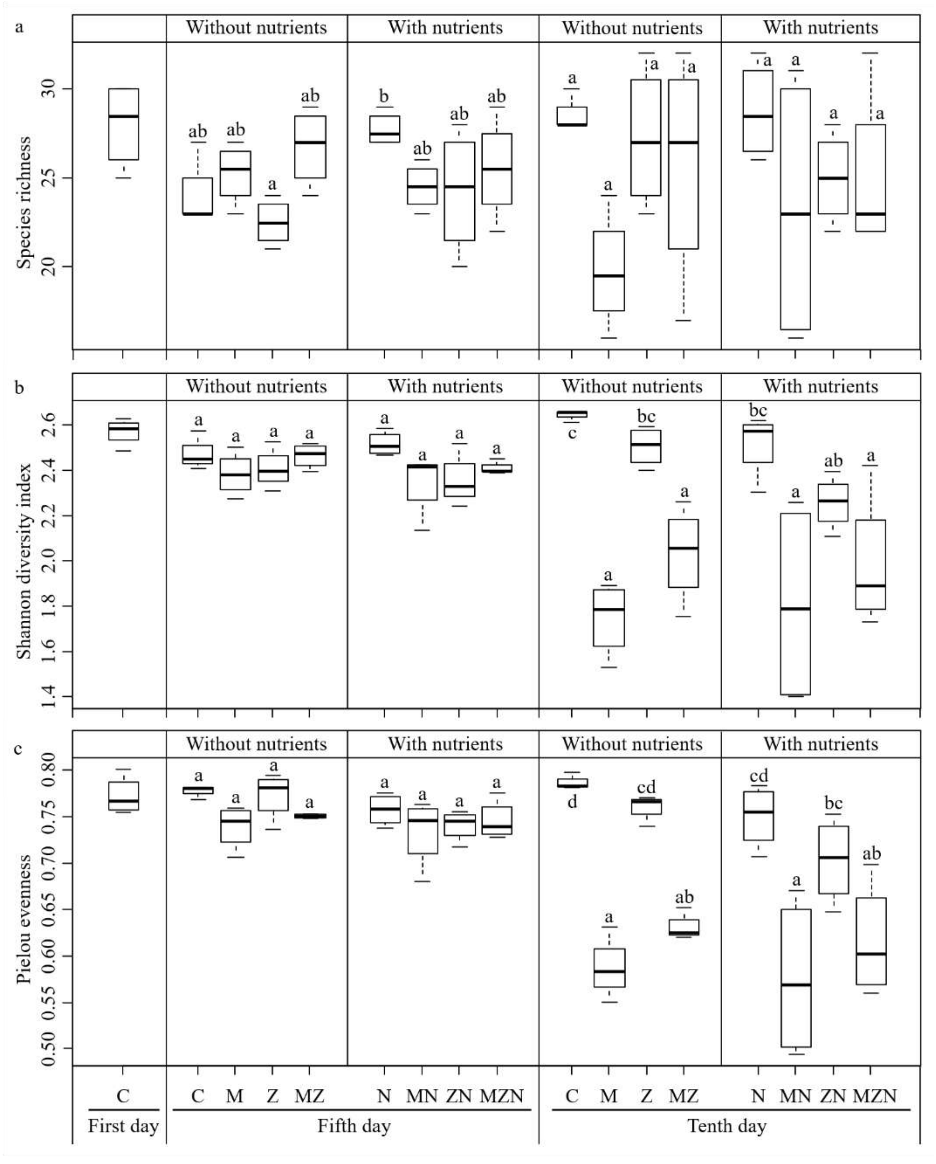

Figure 5. Species richness (a), Shannon diversity index (b), and Pielou evenness (c) of phytoplankton in the treatments control (C), macrophytes (M), zooplankton (Z), nutrients (N), and their possible interactions, on the

first, fifth, and tenth days of the biomanipulation experiment. Different lowercase letters represent significant differences between treatments for each sampling day (p <0.05).

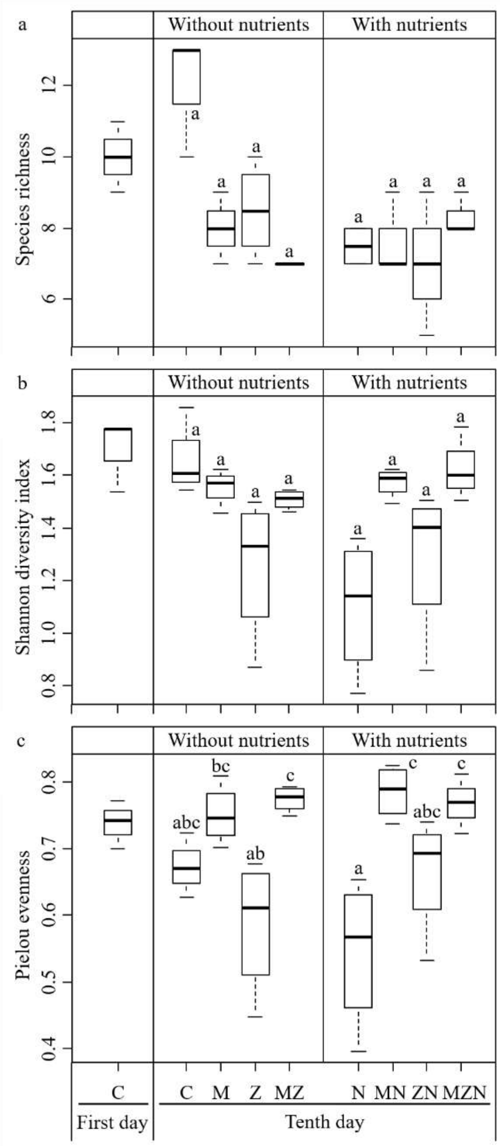

Figure 6. Species richness (a), Shannon diversity index (b), and Pielou evenness (c) of zooplankton in the treatments control (C), macrophytes (M), zooplankton (Z), nutrients (N), and their possible interactions, on the first and tenth days of the biomanipulation experiment. Different lowercase letters represent significant differences between treatments (p <0.05).

The presence of the submerged macrophytes was the most important factor driving the composition and structure of phytoplankton and zooplankton, always presenting highly significant differences between treatments with or without macrophytes (PERMANOVA: p <0.001). Phytoplankton composition and structure were also influenced by the factors macrophytes + nutrients and macrophytes + zooplankton, respectively. Besides macrophytes, zooplankton structure also showed significant differences based on the factors nutrients, macrophytes + zooplankton, and zooplankton + nutrients (Table S5, Figure 7).

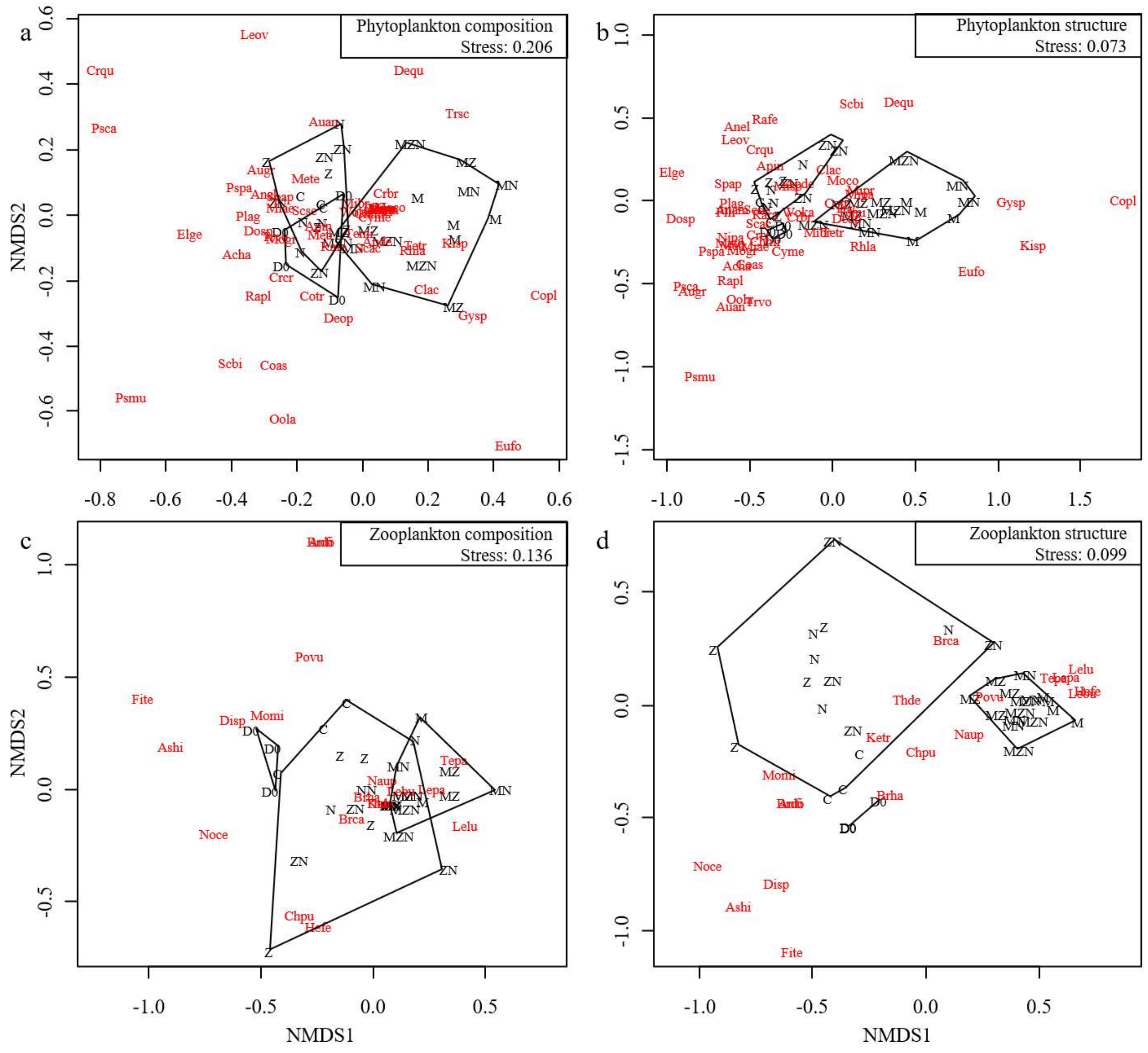

Figure 7. Non-parametric multidimensional scaling plots (NMDS) showing the composition (a) and structure (b) of phytoplankton, and the composition (c) and structure (d) of zooplankton in the treatments control (C), macrophytes (M), zooplankton (Z), nutrients (N), and their possible interactions, on the first (D0) and tenth days of the biomanipulation experiment. The shapes cluster the samples from the first day and the treatments with or without macrophytes. See Tables S1 and S3 for the species abbreviations of phytoplankton and zooplankton, respectively.

Discussion

The introduction of a submerged macrophyte and a large-bodied cladoceran caused significant changes in phytoplankton and zooplankton composition and structure. The submerged macrophyte *Ceratophyllum demersum* was responsible for major shifts in phytoplankton, with inhibition of all phytoplankton groups, depending on the time. For the zooplankton, the introduction of *C. demersum* negatively affected the biomass of cladocerans, while it stimulated the biomass of rotifers. The macrophytes also reduced phytoplankton species diversity and evenness. Indeed, submerged macrophytes have a key role in structuring shallow lakes, mainly because of their positive feedback between abundance and water clarity (Hilt et al., 2017). Consequently, submerged macrophytes help to maintain the clear state of shallow lakes, supporting the provision of several ecosystem services (Janssen et al., 2021).

The cyanobacteria bloom from the Tapacurá reservoir was efficiently reduced up to 85% in all treatments with submerged macrophytes. Considering the possible explanations for this efficient bottom-up control, the most reasonable can be the release of allelopathic substances. The reasons for the allelopathy as the main mechanism are: (1) there were no significant reductions in the dissolved inorganic nitrogen, total dissolved phosphorus, and other physicochemical parameters, including light intensity (see Amorim and Moura, 2020); (2) the treatments with submerged macrophytes reduced phytoplankton biomass without changing nutrient concentration, i.e., lower chlorophyll-*a* to nutrients ratio, indicating no nutrient limitation, even in the treatments without nutrient additions (see Amorim and Moura, 2020); (3) the treatments with submerged macrophytes completely inhibited the potential grazers of cyanobacteria, the herbivorous crustaceans *Notodiaptomus cearensis* and *Diaphanosoma spinulosum*; and (4) *C. demersum* has a high allelopathic potential among submerged macrophytes, and controlled cyanobacteria biomass in other studies (e.g., Mjelde and Faafeng, 1997; Hilt and Gross, 2008; Dong et al., 2014).

Although the submerged macrophytes boosted the rotifer biomass up to 12,147%, this increase was not responsible for the outstanding reduction of cyanobacterial biomass. In this regard, the dominant rotifers in the treatments with macrophytes were the small species *Lecane bulla* (~124 µm) and *Lepadella patella* (~272 µm), which are supposed to be unable to graze on large *Microcystis* colonies (>50 µm) (Kâ et al., 2012). Moreover, rotifers can present higher abundances inside stands of submerged macrophyte in shallow lakes (Ferreira et al., 2018). Accordingly, rotifer species can aggregate inside submerged macrophyte stands, due to higher habitat complexity and food availability, presenting higher densities than in emergent macrophytes and open water sites (Kuczyńska-Kippen, 2005, 2007).

Besides cyanobacteria, the additions of submerged macrophytes also negatively impacted the biomass of green algae and diatoms on the tenth day. The inhibition of cyanobacteria and diatoms is expected due to their higher sensitivities to the macrophyte allelochemicals (Hilt and Gross, 2008). On the other hand, green algae are known to be less affected by the allelopathic effects of macrophytes (e.g., Zhu et al., 2010). In this case, these groups of small eukaryotic phytoplankton could be grazed by the rotifers, which dominated the zooplankton community in all treatments with macrophytes. These groups are considered nutritious food for zooplankton (Wilson et al., 2006). Due to the inhibition of most phytoplankton groups, the addition of submerged macrophytes also reduced species diversity and evenness. Similar results were verified by Ferreira et al. (2018), who reported lower species richness and Shannon diversity inside submerged macrophyte stands.

Contrasting to the effects of submerged macrophytes, the introduction of the large cladoceran *S. serricauda* was less efficient in the top-down control of total phytoplankton and cyanobacteria, reducing their biomass only on the fifth day. Besides being unpalatable to zooplankton, cyanobacteria also present some adaptative strategies to avoid zooplankton grazing, such as large size, toxicity, and poor nutritional quality (Moustaka-Gouni and Sommer, 2020). On the other hand, the introduction of *S. serricauda* decreased the biomass o diatoms on the fifth and tenth days in the treatments Z and ZN, besides the biomass of phytoflagellates in all treatments with zooplankton on the fifth day. As discussed above, these groups are more susceptible to zooplankton grazing. The selectivity of zooplankton can also stimulate cyanobacterial growth through the exclusion of the main competitors (Ger et al., 2019). Moreover, the biomass of the introduced *S. serricauda* showed a decline in its biomass in the treatment Z on the tenth day, explaining the absence of top-down control on phytoplankton on that day. This decreased biomass of *S. serricauda* can be a result of the absence of adaptative strategies to coexist with dense cyanobacterial blooms. Despite the less efficiency of zooplankton in controlling the phytoplankton growth in the treatment Z, it reduced the biomass of total phytoplankton, cyanobacteria, diatoms, and phytoflagellates in the treatment ZN, when compared to the treatment N. This result agrees with Shurin et al. (2012), where the eutrophication can increase zooplankton grazing on phytoplankton.

The combined addition of macrophytes and zooplankton, or macrophytes and nutrients, on phytoplankton usually presented the same effects of the isolated addition of macrophytes, reinforcing the crucial role of submerged plants in structuring phytoplankton communities in shallow lakes. Similarly, Liu et al. (2018) confirmed that the bottom-up effects of submerged macrophytes are more important than the top-down effects of fish manipulation, considering that the grazing potential of zooplankton was maintained low. Moreover, submerged macrophytes can control phytoplankton biomass even without the intervention of zooplankton grazing (Vanderstukken et al., 2011). In our study, macrophytes reduced all phytoplankton groups but did not change the dominant group due to the short duration of the experiment. Probably, longer periods of coexistence between submerged macrophytes and the natural phytoplankton would change the cyanobacterial dominance to green algae, as expected for some subtropical lakes (e.g., Dong et al., 2014; Vanderstukken et al., 2011).

Besides the impacts on phytoplankton structure, the introduction of *S. serricauda* had strong consequences for the native zooplankton in the treatment Z, reducing the total biomass and some abundant species. Accordingly, non-native species can cause serious impacts on the native organisms, changing the interactions between the different levels of the food web and competing for resources with the native community (Lee et al., 2016). The most notorious changes caused by the introduction of *S. serricauda* were the reduction of the abundant copepods and cladocerans (although non-significant). Considering that *S. serricauda* is a large species, it requires more edible food, making the resources less available for native species and, thus, reducing their growth.

It was clear that the presence of submerged macrophytes was the most important driving factor of phytoplankton and zooplankton composition and structure, as demonstrated by the PERMANOVA analyses, always with highly significant differences ($p < 0.001$) and higher values of R^2. Submerged macrophytes play an important role in the structuration of plankton communities and stabilization of the clear water phase (van Donk and van de Bund, 2002). The degree of influence usually depends on fish abundance and macrophyte coverage, besides the interactive effects of the several mechanisms of action of the submerged plants (Søndergaard and Moss, 1998). The reduced effects of zooplankton on plankton community structure are because in eutrophic and hypereutrophic deep lakes, the bottom-up forces can be more evident than the top-down effects on phytoplankton (Bennforf et al., 2002). Under these conditions, changes in the trophic cascade can strongly reduce edible phytoplankton but have no effect, or even stimulate, the biomass of unpalatable organisms, such as cyanobacteria, and thus increasing total biomass (Bennforf, 1995).

Different from what we expected, higher eutrophication did not reduce the main bottom-up and top-down effects of submerged macrophytes and large zooplankton, respectively. The isolated additions of nutrients into the mesocosms influenced phytoplankton biomass only on the fifth day, returning to values close to the control on the tenth day. The reduced influence of nutrients on phytoplankton on the tenth day can indicate that cyanobacteria had used the exceeding amount of dissolved inorganic forms for their growth, making them less available for future generations. However, the high efficiency of submerged macrophytes on the cyanobacterial bloom control was observed even in the treatment MN for both sampling days, suggesting that this approach can be useful in conditions of higher eutrophication. Nevertheless, for a more efficient long-term effect on algal bloom control, additional measures should be implemented; for example, external nutrient control, and successive removals of planktivorous and benthivorous fish, to increase the abundance of native large-bodied crustaceans.

Conclusions

The most pronounced effect of the biomanipulation experiment was the strong inhibition of the cyanobacterial bloom (up to 85%), with biomass higher than 296 mg L^{-1}, on the tenth day in the treatments with macrophyte additions, which confirmed the first hypothesis. Moreover, the introduction of the large cladoceran strongly inhibited the diatoms and phytoflagellates in the treatments Z or ZN, on the fifth or tenth days. The second hypothesis was not confirmed, since the additions of nutrients did not alter the main effects of the submerged macrophytes and large zooplankton on phytoplankton biomass. Finally, the results also corroborated the third hypothesis, since the introduction of submerged macrophytes acted as the main driver of phytoplankton and zooplankton, diversity, composition, and structure.

The submerged macrophyte could control the cyanobacterial bloom without the influence of higher zooplankton grazing in a tropical shallow reservoir. Although we tested the effects of a submerged macrophyte and a large-bodied cladoceran, common in the same watershed as the Tapacurá reservoir, the introduction of the large cladocerans into the mesocosms had negative effects on the diversity and biomass of native zooplankton species. However, we tested only the short-term effects of the biomanipulation (ten days). So, more studies are needed to verify if the effects of the introduction of submerged macrophytes will persist in the long-term and achieve the clear-water state, or if these measures will increase the zooplankton grazing potential on phytoplankton.

Conflicts of interest

Declarations of interest: none.

Data Availability

Additional data may be obtained from the first author (alvescihelio@gmail.com, cihelio.amorim@ufrpe.br).

Author Contributions

Cihelio A. Amorim: Conceptualization, Methodology, Validation, Data curation, Formal analysis, Writing - Original Draft, Writing - Review & Editing. **Ariadne N. Moura:** Conceptualization, Supervision, Methodology, Writing - Review & Editing.

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SUPPLEMENTARY MATERIAL

Title: Biomanipulation of submerged macrophytes, large cladocerans, and nutrients to control cyanobacterial blooms: effects on phytoplankton and zooplankton

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Table S1. List and abbreviation of phytoplankton species in the treatments control (C), macrophytes (M), zooplankton (Z), nutrients (N), and their possible interactions, on the first, fifth, and tenth days of the biomanipulation experiment.

	Abbreviation
Cyanobacteria	
Anabaenopsis elenkinii	Anel
Anagnostidinema amphibium	Anam
Aphanocapsa delicatissima	Apde
Aphanocapsa incerta	Apin
Chroococcus minutus	Chmi
Coelomoron tropicale	Cotr
Dolichospermum sp.	Dosp
Merismopedia tenuissima	Mete
Merismopedia tranquilla	Metr
Microcystis aeruginosa	Miae
Microcystis brasiliense	Mibr
Microcystis panniformis	Mipa
Microcystis protocystis	Mipr
Microcystis sp. (isolated cells)	Misp
Planktolyngbya limnetica	Plli
Planktothrix agardhii	Plag
Pseudanabaena mucicola	Psmu
Pseudanabaena papillaterminata	Pspa
Pseudoanabaena catenata	Psca
Radiocystis fernandoii	Rafe
Raphidiopsis raciborskii	Rara
Sphaerospermopsis aphanizomenoides	Spap
Sphaerospermopsis torques-reginae	Spto
Woronichinia karellica	Woka
Green algae	
Actinastrum hantzschii	Acha
Closteriopsis aciculare	Clac
Coelastrum astroideum	Coas
Crucigenia quadrata	Crqu
Crucigeniella crucifera	Crcr
Desmodesmus opoliensis	Deop
Desmodesmus quadricauda	Dequ
Elakatothrix gelatinosa	Elge
Eutetramorus fottii	Eufo
Kirchneriella sp.	Kisp
Monoraphidium contortum	Moco
Monoraphidium griffithii	Mogr
Oocystis lacustris	Oola
Radiococcus planktonicus	Rapl
Scenedesmus acuminatus	Scac
Scenedesmus bijugus	Scbi
Schroederia setigera	Scse
Tetraedron gracile	Tegr
Tetraedron minimum	Temi
Tetraedron trigonum	Tetr
Treubaria schimidlei	Trsc
Diatoms	
Aulacoseira granulata	Augr
Aulacoseira granulata var. *angustissima*	Auan

Cocconeis placentula	Copl
Cyclotella meneghiniana	Cyme
Nitzschia palea	Nipa
Ulnaria ulna	Ulul
Phytoflagellates	
Cryptomonas brasiliensis	Crbr
Cryptomonas ovata	Crov
Gymnodinium sp.	Gysp
Lepocynclis ovum	Leov
Plagioselmis lacustris	Plla
Trachelomonas volvocina	Trvo

Table S2. Results of the three-way ANOVA analyses comparing the proportion of decrease or increase in the biomass, compared to the control, for the total phytoplankton, cyanobacteria, green algae, diatoms, and phytoflagellates, based on factors macrophytes (M), zooplankton (Z), nutrient (N), and their possible interactions on the tenth day of the biomanipulation experiment. Bold values represent significant effects.

Variables	**Factors**	**Fifth day**		**Tenth day**	
		F	**p**	**F**	**p**
Total phytoplankton	M	**403.204**	**< 0.001**	**678.293**	**< 0.001**
	Z	**138.342**	**< 0.001**	0.022	0.882
	N	**164.201**	**< 0.001**	0.057	0.814
	M:Z	**58.054**	**< 0.001**	**83.380**	**< 0.001**
	M:N	**91.836**	**< 0.001**	3.279	0.083
	Z:N	**8.374**	**0.008**	**8.556**	**0.008**
	M:Z:N	**8.001**	**0.010**	0.090	0.767
Cyanobacteria	M	**413.813**	**< 0.001**	**679.692**	**< 0.001**
	Z	**140.363**	**< 0.001**	0.042	0.839
	N	**168.566**	**< 0.001**	0.072	0.791
	M:Z	**58.127**	**< 0.001**	**82.757**	**< 0.001**
	M:N	**93.715**	**< 0.001**	3.554	0.072
	Z:N	**8.562**	**0.008**	**8.710**	**0.007**
	M:Z:N	**8.334**	**0.008**	0.091	0.766
Green algae	M	2.782	0.109	**6.528**	**0.018**
	Z	3.486	0.075	1.233	0.278
	N	2.615	0.120	0.010	0.922
	M:Z	0.563	0.460	4.115	0.054
	M:N	**11.364**	**0.003**	3.427	0.077
	Z:N	0.009	0.926	0.002	0.965
	M:Z:N	0.024	0.879	3.572	0.071
Diatoms (5th day: log(x+1))	M	3.533	0.073	**6.891**	**0.015**
	Z	4.208	0.052	**5.696**	**0.026**
	N	1.534	0.228	**7.979**	**0.010**
	M:Z	**15.945**	**0.001**	**7.922**	**0.010**
	M:N	0.245	0.625	**14.960**	**0.001**
	Z:N	0.057	0.814	0.635	0.434
	M:Z:N	1.017	0.324	3.662	0.068
Phytoflagellates	M	**5.133**	**0.033**	2.775	0.109
	Z	**16.367**	**0.001**	**7.562**	**0.011**
	N	0.754	0.394	2.639	0.118
	M:Z	**17.299**	**< 0.001**	**7.908**	**0.010**
	M:N	0.109	0.745	2.836	0.106
	Z:N	0.029	0.867	0.189	0.668
	M:Z:N	0.381	0.543	1.259	0.273
Species richness	M	0.963	0.337	**5.485**	**0.028**
	Z	0.942	0.342	0.164	0.689
	N	0.964	0.336	0.013	0.911
	M:Z	**6.899**	**0.015**	3.793	0.064
	M:N	**5.049**	**0.035**	0.634	0.434
	Z:N	0.457	0.506	0.872	0.360
	M:Z:N	0.138	0.713	0.110	0.744
Shannon diversity index	M	1.162	0.292	**77.309**	**< 0.001**
	Z	0.418	0.524	1.831	0.189
	N	0.594	0.449	2.723	0.113
	M:Z	**8.676**	**0.007**	**10.985**	**0.003**
	M:N	0.339	0.566	**6.932**	**0.015**
	Z:N	0.678	0.419	0.794	0.382
	M:Z:N	0.303	0.588	0.005	0.942

Pielou evenness index	M	**5.757**	**0.025**	**115.859**	**< 0.001**
	Z	0.048	0.828	0.795	0.382
	N	3.615	0.070	**5.230**	**0.032**
	M:Z	1.837	0.189	**7.532**	**0.012**
	M:N	1.807	0.192	**6.072**	**0.022**
	Z:N	0.172	0.683	0.152	0.700
	M:Z:N	0.172	0.683	0.021	0.886

Table S3. List and abbreviation of zooplankton species in the treatments control (C), macrophytes (M), zooplankton (Z), nutrients (N), and their possible interactions, on the first and tenth days of the biomanipulation experiment.

	Abbreviation
Rotifers	
Anureopsis fissa	Anfi
Asplanchnopus hialinus	Ashi
Brachionus calyciflorus	Brca
Brachionus dolabratus	Brdo
Brachionus havanaensis	Brha
Filinia terminalis	Fite
Hexarthra fennica	Hefe
Keratella tropica	Ketr
Lecane bulla	Lebu
Lecane lunaris	Lelu
Lepadella patella	Lepa
Polyarthra vulgaris	Povu
Testudinella patina	Tepa
Copepods	
Nauplii	Naup
Notodiaptomus cearensis	Noce
Thermocyclops decipiens	Thde
Cladocerans	
Chydorus pubescens	Chpu
Diaphanosoma spinulosum	Disp
Moina micrura	Momi

Table S4. Results of the three-way ANOVA analyses comparing the proportion of decrease or increase in the biomass, compared to the control, for the total zooplankton, rotifers, copepods, and cladocerans, based on factors macrophytes (M), zooplankton (Z), nutrient (N), and their possible interactions on the tenth day of the biomanipulation experiment. Bold values represent significant effects.

Variables	**Factors**	**Tenth day**	
		F	**p**
Total zooplankton	M	**149.660**	**< 0.001**
	Z	**11.371**	**0.003**
	N	**9.289**	**0.006**
	M:Z	0.258	0.616
	M:N	3.576	0.071
	Z:N	**18.854**	**< 0.001**
	M:Z:N	2.036	0.167
Rotifers (ART)	M	**5.256**	**0.022**
	Z	**4.008**	**0.047**
	N	0.000	0.984
	M:Z	3.462	0.064
	M:N	0.037	0.848
	Z:N	**8.744**	**0.006**
	M:Z:N	2.389	0.125
Cladocerans (ART)	M	0.855	0.355
	Z	2.540	0.114
	N	2.090	0.151
	M:Z	3.768	0.053
	M:N	3.809	0.052
	Z:N	**4.101**	**0.043**
	M:Z:N	3.580	0.060
Copepods	M	**6.465**	**0.018**
	Z	0.801	0.380
	N	**4.708**	**0.041**
	M:Z	**8.438**	**0.008**
	M:N	3.710	0.067
	Z:N	1.851	0.187
	M:Z:N	0.261	0.615
Species richness (ART)	M	2.534	0.115
	Z	3.006	0.086
	N	3.501	0.063
	M:Z	2.437	0.123
	M:N	**4.347**	**0.038**
	Z:N	3.371	0.068
	M:Z:N	0.467	0.499
Shannon diversity index (ART)	M	**4.614**	**0.032**
	Z	0.087	0.770
	N	1.097	0.300
	M:Z	0.036	0.850
	M:N	1.194	0.275
	Z:N	1.451	0.229
	M:Z:N	2.097	0.151
Pielou evenness index	M	**37.551**	**< 0.001**
	Z	0.305	0.586
	N	0.002	0.967
	M:Z	0.167	0.687
	M:N	0.373	0.547
	Z:N	2.289	0.144
	M:Z:N	**5.849**	**0.024**

Table S5. Results of the PERMANOVA analyses comparing the phytoplankton and zooplankton composition and structure, based on factors macrophytes (M), zooplankton (Z), nutrient (N), and their possible interactions on the tenth day of the biomanipulation experiment. Bold values represent significant effects.

Variables		**Tenth day**		
	Factors	$\mathbf{F_{model}}$	$\mathbf{R^2}$	**p**
Phytoplankton composition	M	**9.395**	**0.249**	**< 0.001**
	Z	1.460	0.039	0.215
	N	-0.053	-0.001	0.996
	M:Z	0.829	0.022	0.549
	M:N	**2.198**	**0.058**	**0.050**
	Z:N	0.306	0.008	0.916
	M:Z:N	0.524	0.014	0.797
Phytoplankton structure	M	**40.710**	**0.525**	**< 0.001**
	Z	2.131	0.027	0.120
	N	2.231	0.029	0.114
	M:Z	**5.566**	**0.072**	**0.009**
	M:N	1.744	0.022	0.145
	Z:N	1.161	0.015	0.249
	M:Z:N	1.051	0.014	0.315
Zooplankton composition	M	**5.656**	**0.164**	**< 0.001**
	Z	1.259	0.036	0.318
	N	1.888	0.055	0.146
	M:Z	0.999	0.029	0.456
	M:N	1.985	0.057	0.108
	Z:N	-0.165	-0.005	0.981
	M:Z:N	-0.047	-0.001	0.943
Zooplankton structure	M	**47.730**	**0.539**	**< 0.001**
	Z	0.999	0.011	0.336
	N	**4.194**	**0.047**	**0.016**
	M:Z	**4.298**	**0.049**	**0.021**
	M:N	2.514	0.028	0.068
	Z:N	**4.037**	**0.046**	**0.021**
	M:Z:N	1.714	0.019	0.156

Considerações Finais

8 CONSIDERAÇÕES FINAIS

Neste trabalho de tese, foram evidenciadas as principais causas das florações de cianobactérias em reservatórios tropicais (artigo 1), como as espécies e grupos funcionais se relacionam com os gradientes ambientais e demais grupos fitoplanctônicos (artigo 2), as principais consequências para a qualidade da água, biodiversidade e funcionamento dos ecossistemas aquáticos (artigo 3), além de ferramentas de biomanipulação eficientes no controle das florações (artigos 4 e 5). Muitas destas informações eram restritas para ambientes de regiões temperadas e subtropicais (América do Norte, Europa, Ásia e Austrália), com pouca aplicação nos ecossistemas aquáticos de clima mais quente. Portanto, houve um avanço no entendimento das causas, consequências e controle das florações de cianobactérias tropicais.

As florações de cianobactérias em reservatórios tropicais foram direcionadas principalmente pela eutrofização (fósforo total dissolvido) e interações com os crustáceos onívoros, com uma significativa participação da temperatura do ar, radiação solar, zona de mistura, salinidade e crustáceos herbívoros. A eutrofização interagiu sinergicamente com os crustáceos onívoros e salinidade no favorecimento das florações, indicando que cenários futuros de salinização dos reservatórios do semiárido podem intensificar as florações em ambientes eutróficos, através de mudanças nas interações tróficas com o zooplâncton. Com estes resultados, foi possível construir um modelo de equações estruturais capaz de prever cenários futuros das florações de cianobactérias em lagos e reservatórios tropicais, considerando as previsões de incremento da eutrofização, temperatura e salinização para as próximas décadas.

Levando em consideração as respostas do fitoplâncton, incluindo cianobactérias formadoras de florações, aos gradientes ambientais, foram estabelecidos os modelos de habitat e regras de assembleia de 21 grupos funcionais de Reynolds para reservatórios tropicais. Foram definidas as principais variáveis ambientais e seus limites mais favoráveis ao desenvolvimento dos grupos, nos quais eles podem se tornar abundantes. A maioria dos grupos mostrou um padrão de coexistência, demonstrando um efeito positivo na biodiversidade. Modelos generalizados aditivos foram úteis para explicar e prever a abundância dos grupos funcionais em 10 gradientes ambientais, com efeitos principalmente da salinidade, estado trófico, nutrientes, transparência e zooplâncton herbívoro. Com isso, houve um avanço no entendimento das relações dos grupos funcionais com o ambiente e as demais espécies, através de ferramentas capazes de descrever a dinâmica fitoplanctônica, explicar a variabilidade e prever cenários futuros de dominância de espécies potencialmente prejudiciais ao ambiente.

Também foram avaliadas as consequências ecológicas das florações algais sobre a qualidade da água, diversidade e estrutura do plâncton e funcionamento do ecossistema em reservatórios tropicais. Florações de cianobactérias e mistas apresentaram menor qualidade da água, reduzida diversidade e eficiência do uso dos recursos pelo zooplâncton e maiores valores dessas métricas para o fitoplâncton. No entanto, todas as categorias de florações precisam ser monitoradas, devido seus efeitos adversos na diversidade do plâncton. A diminuição da biodiversidade também reduziu o funcionamento do ecossistema, limitando os serviços ecossistêmicos. Estes resultados são importantes para prever os efeitos das florações algais na qualidade da água, biodiversidade e na provisão de serviços ecossistêmicos em reservatórios de abastecimento público. Nesse sentido, faz-se necessário um maior investimento em pesquisas e na conservação dos recursos hídricos, especialmente os reservatórios de abastecimento público.

Ferramentas de biomanipulação das comunidades aquáticas, um método natural, foram aplicadas para controle de uma densa floração de cianobactérias em um experimento de mesocosmos em um reservatório raso tropical. A introdução de macrófitas submersas foi eficiente na redução de 85% da biomassa total e 99% das cianobactérias filamentosas, mesmo com o incremento da eutrofização, indicando a alelopatia como principal mecanismo de ação. Além disso, as macrófitas inibiram as algas verdes e o zooplâncton consumiu as diatomáceas e fitoflagelados. A adição de zooplâncton foi menos eficiente ao final do experimento, mas potencializou a ação das macrófitas nos primeiros dias da manipulação. As macrófitas foram as principais direcionadoras da diversidade, composição e estrutura do fitoplâncton e zooplâncton. Com isso, a adição de macrófitas submersas nativas com potencial alelopático, associada com o incremento do zooplâncton herbívoro de grande tamanho, mostrou ser um método eficiente para controlar as florações de cianobactérias em reservatórios hipereutróficos rasos tropicais.

Apesar dos avanços, mais pesquisas são necessárias em regiões tropicais, especialmente utilizando dados de monitoramento a longo prazo, para previsões mais precisas do estado das florações algais no futuro. Também é importante relacionar como os impactos das ações antrópicas, alterações no uso e ocupação do solo, eutrofização e secas extremas podem alterar a dinâmica das florações algais no semiárido brasileiro. Além disso, pesquisas relacionando o impacto das florações nos serviços ecossistêmicos, saúde humana e potenciais perdas econômicas são necessárias. Outro ponto relevante, é o aumento da disseminação do conhecimento sobre os impactos das florações algais para comunidades com pouco ou nenhum acesso às informações produzidas nas universidades e centros de pesquisa, como escolas de ensino fundamental e médio, organizações não governamentais e comunidades do entorno dos reservatórios de abastecimento público.

9 NORMAS DE SUBMISSÃO DAS REVISTAS CIENTÍFICAS

Science of the Total Environment

https://www.elsevier.com/journals/science-of-the-total-environment/0048-9697/guide-for-authors

Hydrobiologia

https://www.springer.com/journal/10750/submission-guidelines

Environmental Pollution

https://www.elsevier.com/journals/environmental-pollution/0269-7491/guide-for-authors

Freshwater Biology

https://onlinelibrary.wiley.com/page/journal/13652427/homepage/forauthors.html

Divulgação Científica

10 DIVULGAÇÃO CIENTÍFICA

1. International Network on Limnology of Drylands – INLD. Comitê Executivo: Jovens Limnólogos. Função: atividades de pesquisa, metanálises, estudos cienciométricos, manutenção das redes sociais e atividades de divulgação científica. Coordenação da Profa. Luciana Barbosa. Disponível em: https://www.inld.pro.br/c%C3%B3pia-executive-committee; https://www.instagram.com/inld.limnology/ (Instagram @inld.limnology).
2. Associação Brasileira de Limnologia – ABLimno. Núcleo de Popularização da Limnologia e Núcleo Regional do Nordeste. Função: organização de eventos, manutenção das redes sociais e atividades de divulgação científica. Coordenação da Profa. Luciana Barbosa. Disponível em: https://www.ablimno.org.br/arquivos/news_1593097990_PLANO_ESTRATEGICO_ABLIMNO_2019.pdf; http://novo.ablimno.org.br/nucleos-regionais/; https://www.youtube.com/channel/UCwDJebxw487W6PeKS9mVCzw (Canal no YouTube Popularização ABLimno); https://www.instagram.com/ab.limno/ (Instagram @ab.limno).
3. LimnoEcos – Revista de Popularização da Ciência da Associação Brasileira de Limnologia. Função: Editor Associado, edição de vídeos e textos de divulgação científica. Coordenação da Profa. Luciana Barbosa. Disponível em: https://www.ablimno.org.br/arquivos/news_1593097990_PLANO_ESTRATEGICO_ABLIMNO_2019.pdf; http://novo.ablimno.org.br/limnoecos/; https://www.youtube.com/channel/UCwDJebxw487W6PeKS9mVCzw (Canal no YouTube Popularização ABLimno); https://www.instagram.com/ab.limno/ (Instagram @ab.limno).
4. Grupo de Estudos Aquatic Ecology/Ecologia Aquática. Função: coordenação, organização das palestras, convite dos palestrantes, divulgação e transmissão das palestras para o canal do YouTube. Coordenação principal de Tayná Delgado. Disponível em: https://www.youtube.com/channel/UCdRG0ObmbVfHY1gbeIc1_pw/videos (Canal no YouTube Aquatic Ecology); https://www.instagram.com/aquatic_ecology/ (Instagram @aquatic_ecology).

5. Organização e participação da **Semana C da Ciência do Programa de Pós-Graduação em Botânica - PPGB**. Universidade Federal Rural de Pernambuco – UFRPE. 17 a 25 de outubro de 2018. Atividades de Divulgação Científica em espaços públicos da cidade do Recife: Parque da Jaqueira, Praia de Boa Viagem e Espaço Ciência – Museu Interativo de Ciência de Pernambuco. Público-alvo: sociedade em geral, estudantes e professores do ensino fundamental e médio. Disponível em: https://www.instagram.com/semanadaciencia/ (Instagram @semanadaciencia).
6. Organização e participação da **I Jornada de Botânica: Biodiversidade e Interdisciplinaridade na Botânica**. Programa de Pós-Graduação em Botânica – PPGB, Universidade Federal Rural de Pernambuco – UFRPE. 10 a 12 de novembro de 2020. Evento online. Público-alvo: sociedade em geral, estudantes e professores do ensino fundamental, médio, graduação e pós-graduação. Disponível em: https://www.youtube.com/channel/UCAzhs-kX7nv8fs8ndcCHoDg (Canal no YouTube PPGBio UFRPE); https://www.instagram.com/jobot.ufrpe/ (Instagram @jobot.ufrpe).
7. Organização e participação do **III Ciclo de Palestras da Biologia: Agenda da Biodiversidade Neotropical: Desafios para o Século XXI, Circulando Informação, Pensamento Crítico e Ações Propositivas**. Programa de Pós-Graduação em Biodiversidade – PPGBio, Universidade Federal Rural de Pernambuco – UFRPE, Universidade Federal do Estado do Rio de Janeiro – UNIRIO, Universidade Federal de São Carlos – UFSCar, Associação Brasileira de Limnologia – ABLimno. 23 a 25 de março de 2021. Evento online. Público-alvo: sociedade em geral, estudantes e professores do ensino fundamental, médio, graduação e pós-graduação. Disponível em: https://www.youtube.com/channel/UCwDJebxw487W6PeKS9mVCzw (Canal no YouTube Popularização ABLimno); https://www.instagram.com/cp_bio/ (Instagram @cp_bio).
8. Minicurso ministrado na **XIII Semana de Biologia: Ciência e Tecnologia na Formação dos Profissionais das Ciências Biológicas**. Departamento de Biologia, Universidade Federal Rural de Pernambuco. Título: **Introdução à Limnologia**. 16 a 18 de outubro de 2018. Carga horária: 12h.
9. Palestra ministrada no **Encontro de Jovens Limnólogos da ABLimno**. Associação Brasileira de Limnologia – ABLimno. Título: **Florações de Cianobactérias no Semiárido Brasileiro**. 31 de julho de 2020. Disponível em:

https://www.youtube.com/watch?v=cqyI-qRSDxU (Canal no YouTube Popularização ABLimno).

10. Palestra ministrada no **Webinário do Grupo de Estudos em Ecologia Aquática**. Sem vínculo Institucional. Título: **Florações de Cianobactérias: Causas, Consequências e Controle**. 08 de agosto de 2020. Disponível em: https://www.youtube.com/watch?v=5NxO5PF3VrQ (Canal no YouTube Aquatic Ecology).
11. Minicurso ministrado no **Simpósio Virtual de Limnologia e Saneamento do Semiárido**. Instituto Nacional do Semiárido – INSA. Título: **Monitoramento e Controle de Florações de Cianobactérias no Semiárido Brasileiro**. 21 a 25 de setembro de 2020. Disponível em: https://www.even3.com.br/semiarido2020/; https://www.youtube.com/channel/UCatcJ0-UqtrJTYsdR-sCeCA (Canal no YouTube INSA MCTI).
12. Trabalho apresentado no **Simpósio Virtual de Limnologia e Saneamento do Semiárido**. Instituto Nacional do Semiárido – INSA. Título: **Consequências ecológicas das florações algais sobre o fitoplâncton e zooplâncton em reservatórios do Nordeste do Brasil**. 21 a 25 de setembro de 2020. Disponível em: https://www.even3.com.br/semiarido2020/; https://www.youtube.com/watch?v=RFiqMEENowE (Canal no YouTube INSA MCTI).
13. Oficina ministrada na **31ª Semana do Instituto de Ciências Biológicas**. Universidade Federal de Goiás – UFG. Título: **Impacto das florações de cianobactérias em reservatórios de abastecimento**. 05 de novembro de 2020. Carga horária: 2h. Disponível em: https://www.even3.com.br/31semanadoicb/.
14. Palestra ministrada no **I Symposium on Aquatic Resources**. Programa de Pós-Graduação em Recursos Aquáticos e Pesca, Universidade Estadual do Maranhão – UEMA. Título: **Cyanobacteria and Toxic Algae**. 15 a 18 de dezembro de 2020. Disponível em: https://www.youtube.com/watch?v=JBNVULcGfnw (Canal no YouTube UEMANET Oficial); https://eventos.uemanet.net/.
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16. Cartaz de divulgação científica: AMORIM, C.A.; FALCÃO, R.H.M.; MOURA, A.N. **Macrófitas Aquáticas do Açude de Apipucos, Recife, Pernambuco.** Universidade Federal Rural de Pernambuco, 2017.
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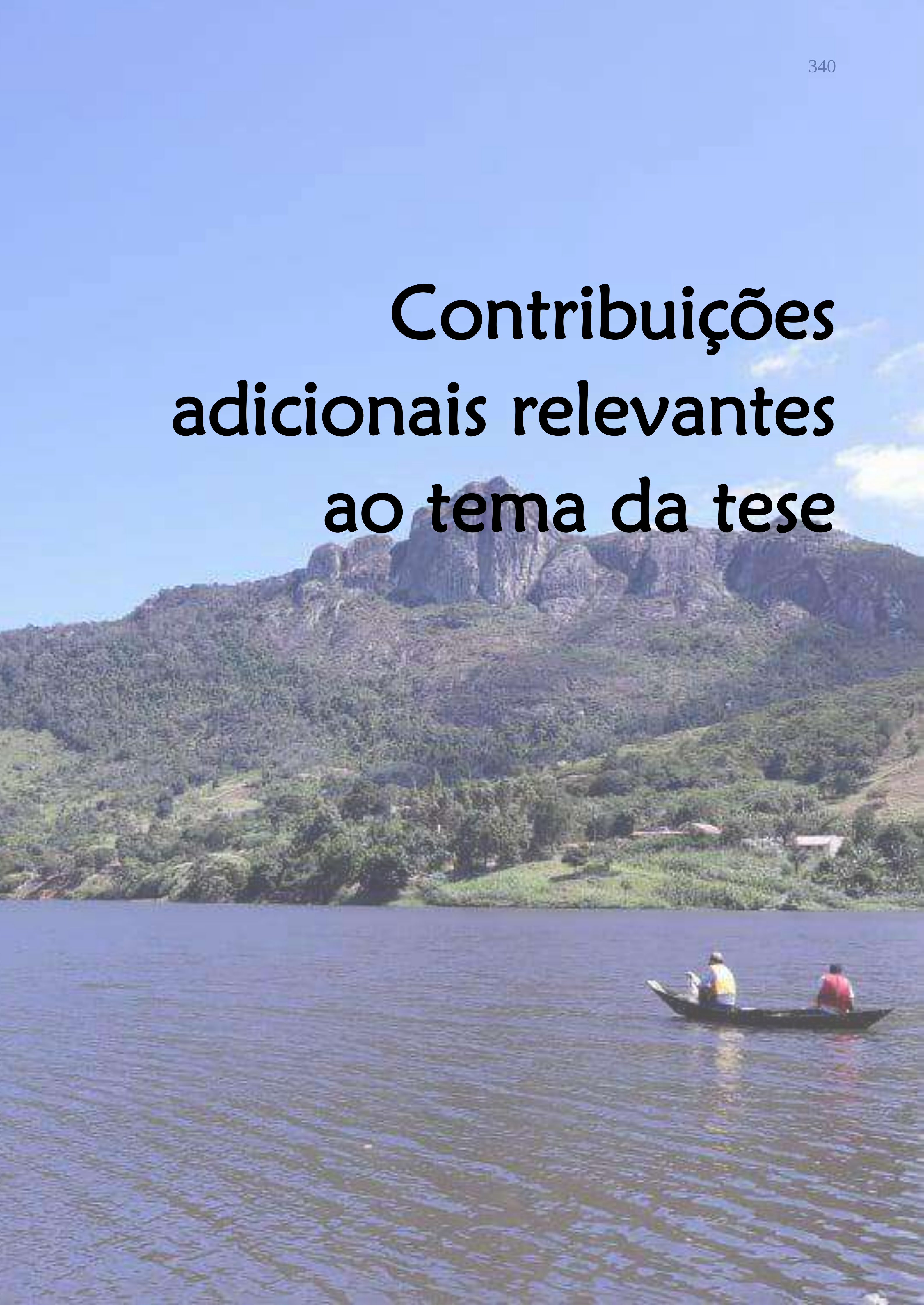
20. Cartaz de divulgação científica: DINIZ, A.S.; AMORIM, C.A.; NASCIMENTO FILHO, S.L.; MOURA, A.N. **Macrófitas Aquáticas: o que sabemos sobre elas?** Universidade Federal Rural de Pernambuco, 2021.
21. Cartaz de divulgação científica: NASCIMENTO FILHO, S.L.; DINIZ, A.S.; AMORIM, C.A.; MOURA, A.N. **Comunidades Aquáticas do Reservatório Jazigo.** Universidade Federal Rural de Pernambuco, 2021.
22. Cinco palestras ministradas para outras instituições, incluindo Complexo Educacional Ferreira e Silva – FESN, Universidade Federal de Goiás, Universidade Estadual Vale do Acaraú, Universidade Federal do Rio Grande do Norte e Universidade Regional do Cariri.

Contribuições adicionais relevantes ao tema da tese



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REVIEW

Cyanobacterial blooms in freshwater bodies from a semiarid region, Northeast Brazil: A review

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ABSTRACT

Harmful cyanobacterial blooms have caused several problems in freshwater environments due to their prolific growth and the harmful cyanotoxins produced by some species. The occurrence of these organisms has increased in recent decades due to climate change and eutrophication, although most studies are from temperate regions in the Northern hemisphere. This review presents data about cyanobacteria occurrence, dominance, and toxicity events in freshwater bodies in a semiarid region of Northeast Brazil, in the tropical Southern hemisphere. We performed a literature survey of cyanobacteria publications from 1930 to 2016. We made a list of all the dominant species registered in each state, noted their distribution and occurrence of dominance events involving one or more species, and the registered records of toxic blooms, including information about the toxins involved and the range of values. We selected 102 publications that described cyanobacteria occurrence from states in Northeast Brazil; these publications included relevant contributions regarding cyanobacteria distribution, richness, density, and biomass. Forty-nine dominant species were recorded, with the most representation found in the state of Pernambuco (30 spp.). The genera with the highest occurrences were *Microcystis*, *Cylindrospermopsis*, *Planktothrix*, *Dolichospermum* (=*Anabaena*), and *Geitlerinema*, especially the species *Cylindrospermopsis raciborskii*, *Microcystis aeruginosa*, and *Planktothrix agardhii*. Episodes of toxic blooms were observed in four states. Microcystins, cylindrospermopsin, saxitoxins, and anatoxin-a(S) were found to be associated with these blooms. In Northeast Brazil, harmful cyanobacterial blooms are common in urban and public reservoirs. However, in recent years, cyanobacterial blooms in this region have been more intense and perennial, with high biomass occurring throughout the year.

Key words: Continental waters; cyanotoxins; *Cylindrospermopsis raciborskii*; harmful algal blooms; tropical blooms.

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INTRODUCTION

The accelerated growth of toxic cyanobacteria has generated serious problems in the management of inland waters that are used for irrigation, fishing, and human supply. Anthropic interferences through the input of nutrients in aquatic environments (in addition to climatic changes) can increase cyanobacterial harmful algal blooms (cyanoHABs) (Paerl and Huisman, 2009; Paerl and Otten, 2013; Paerl *et al.*, 2016). The conditions that cause these blooms have become increasingly more frequent in inland waters worldwide, especially during dry seasons (Carmichael and Boyer, 2016). Global warming favours cyanobacterial dominance because the changes in precipitation patterns promote an elevated water residence time in reservoirs during the dry season or more time for loading nutrients in the rainy season (Paerl and Huisman, 2008).

In Northeast Brazil, which is characterized by a semiarid climate, the cyanoHABs are more pronounced (*e.g.*, Bittencourt-Oliveira *et al.*, 2014; Lopes *et al.*, 2015; Brasil *et al.*, 2016; Lins *et al.*, 2016), which are favored by water scarcity, high temperatures, elevated water residence time, and artificial eutrophication. The recurrence of cyanoHABs became a public health problem due to toxins produced by these organisms. These secondary metabolites can cause serious damage to humans and animals, including irritation and acute, chronic, or even lethal poisoning (Carmichael and Boyer, 2016). Several studies focusing on the presence and detection of cyanotoxins in public water supply reservoirs were carried out in some states in Northeast Brazil. These studies have described the presence of microcystins (Chellappa *et al.*, 2008; Bittencourt-Oliveira *et al.*, 2010; Piccin-Santos and Bittencourt-Oliveira, 2012; Vasconcelos *et al.*, 2013; Mendes *et al.*, 2016), cylindrospermopsin (Bittencourt-Oliveira *et al.*, 2011, 2014), saxitoxins (Molica *et al.*, 2002; Fonseca *et al.*, 2015; Lopes *et al.*, 2015), and anatoxin-a(S) (Molica *et al.*, 2005).

The first studies focused mainly on certain taxonomical aspects (Drouet, 1937, 1938; Carvalho-de-La-Mora, 1989). Studies focusing on the dominance and toxicity of these organisms emerged only after the

"Caruaru tragedy" in 1996, when dozens of renal patients in a haemodialysis clinic died after intravenous exposure to microcystins (Carmichael *et al.*, 2001; Azevedo *et al.*, 2002). After this incident, research efforts were directed to monitor various supply reservoirs, including the important report by Bouvy *et al.* (2000), which monitored water quality and the occurrence of bloom-forming cyanobacteria *Cylindrospermopsis* Seenayya & Subba Raju in 39 reservoirs in Pernambuco state. Afterwards, many other studies have taken place, and the number of cyanoHABs associated with frequent episodes of toxicity has increased over time. However, more knowledge about the dynamics of these blooms and the species responsible for these phenomena is needed to further understand these processes in aquatic environments.

After the deaths of the "Caruaru tragedy", as well as a higher incidence of cyanoHABs, Brazil became the first country to adopt specific legislation that includes the analysis of cyanobacteria and cyanotoxins as parameters when monitoring water supply. For example, the regulation of the Brazilian Ministry of Health, Number 2.914/2011, which makes analysis of microcystins and saxitoxins in reservoirs destined for human supply mandatory. Additionally, this regulation establishes maximum limits of 1.0 µg L^{-1} of microcystins and cylindrospermopsin, and 3.0 µg L^{-1} of saxitoxins in these water sources (Brazil, 2011). However, further efforts are still needed to monitor these toxins, as well as public policies focused on populations with risk of contamination, since reports of toxin-producing species and the registration of newly affected regions have increased in recent years (Grattan *et al.*, 2016).

To analyse the various problems that are associated with cyanoHABs in waterbodies used for human supply, we reviewed studies to describe and characterize these organisms. Based on published material between 1930 and 2016, we show the distribution, dominance, and toxic events associated with the presence of cyanobacteria in lentic water bodies in Northeast Brazil.

METHODS

Data was obtained from articles published in indexed journals accessed through the Scielo (for articles in Brazilian journals), Scopus, and the Web of Science (for articles in international journals) databases. To obtain further information on the occurrence of cyanobacteria in freshwater bodies in Northeast Brazil, we also used Google Scholar to search for articles published between 1930 and 2016.

This bibliographical search was carried out from April to July 2016. We used different combinations of keywords to find appropriate articles, including cyanobacteria, cyanotoxins, toxic blooms, reservoirs, and Northeast Brazil (in English and Portuguese). We used journals that specialize in various disciplines, such as taxonomy, ecology, toxicology, molecular biology, and floristic. Only papers reporting the occurrence of planktonic cyanobacteria in lentic freshwater bodies in Northeast Brazil were selected. These papers also needed to include information about dominance events or present an elevated contribution in richness during at least one sample period. We established the following criteria to include articles: i) if more than one study presented data from the same environment and sample period, only one paper was used; ii) the studies about a phytoplankton community where cyanobacteria had no contributions in richness, density, biomass, or relative abundance were excluded; and iii) in studies that evaluated different depths in the same environment (with or without the formation of scums), only surface data of water bodies was considered. Furthermore, we selected studies that used quantitative density or biomass data to report the presence of cyanoHABs or establish dominance. Then, all species classified as dominant were listed to analyse the distribution and frequency of cyanobacteria representatives. Additionally, we investigated whether these events consisted of a single dominant species or involved two or more alternating or codominant species. The frequency of the dominant species in all dominance events was assessed in all states (n=149).

A multivariate analysis of variance (PERMANOVA) was carried out in order to verify if the species composition of cyanoHABs differed among the states in Northeast Brazil. Afterwards, using the R package IndicSpecies, we performed an indicator species analysis to identify what bloom-forming species were most representative of the states of Northeast Brazil. These analyses were performed in software R 3.4.0 with a significance level of $P<0.05$ (R Core Team, 2017).

We analysed the occurrence of cyanotoxins in different states in the Northeast region. We identified the various types of cyanotoxins and their detected values. Finally, we described the main species present in every cyanoHAB.

RESULTS

We surveyed 102 papers that recorded cyanobacteria through different approaches. These papers contained information about cyanobacteria found in six states as follows: Pernambuco was the most recorded state (55), followed by Rio Grande do Norte (25), Ceará (8), Paraíba (8), Bahia (3), and Maranhão (3) (Fig. 1). We found no records of cyanobacteria in the states of Alagoas, Sergipe, and Piauí. Among the different disciplines, ecological studies (72) presented the most published studies (in all states analysed, more than 60% of the articles were ecological studies), followed by toxicology (22), floristic surveys (4), taxonomy (2), and molecular techniques (2) (Fig. 1).

The history of cyanobacteria research in Northeast Brazil

We found an increasing trend in the number of papers published over the years. Pioneering studies about cyanobacteria were published in the 1930s in Ceará state. However, over the next 60 years, only three studies were published, all focusing on floristic and/or taxonomic surveys. For instance, there was only one paper published in the 1980s that included descriptions of cyanobacteria in Pernambuco state (Drouet, 1937, 1938; Carvalho-de-La-Mora, 1989). These studies represented only the initial step towards recording cyanobacterial species in this region (Fig. 2).

After the Caruaru tragedy in 1996, there was an increase in the number of publications involving the Northeast region of Brazil, primarily in Pernambuco state. Between 2007 and 2011 most articles involved ecological descriptions in the states of Pernambuco and Rio Grande do Norte (19 and 11 papers, respectively) (Fig. 2). In the past 10 years, the interest in these types of studies has increased in this region, and the number of studies in other states, such as Paraíba, Ceará, and Bahia, has also increased during this period (Fig. 2).

Among the 102 papers that we selected, 67 studies presented dominance of one or more cyanobacteria species in at least one sample period (Supplementary Tab. 1), which amounted to 79 environments (33 in Pernambuco, 25 in Paraíba, 14 in Rio Grande do Norte, 5 in Ceará and 2 in Bahia). Most of these studies were carried out in reservoirs used for public supply (97.5%), with only a small percentage carried out in lakes (2.5%).

Occurrence and distribution of dominant cyanobacteria in Northeast Brazil

We recorded 49 dominant cyanobacterial species in lentic ecosystems in Northeast Brazil. These species belong to 19 genera and are distributed in the Chroococcales (20 spp.), Nostocales (16 spp.) and Oscillatoriales (13 spp.) orders. Of all the registered species, 30 were from Pernambuco state, 27 from Rio Grande do Norte, 7 from Paraíba, 4 from Ceará and 3 from Bahia. No dominant species were found in Maranhão state (Tab. 1).

Regarding the dominant genera recorded in most studies, we found that *Microcystis* Lemmermann and *Planktothrix* Anagnostidis & Komárek were present in all states, *Cylindrospermopsis* Seenayya & Subba Raju was present in all states except Bahia, *Dolichospermum* (Bornet & Flahault) P. Wacklin *et al.* (=*Anabaena* Bornet & Flahault) was distributed in the states of Rio Grande do Norte, Pernambuco, and Bahia, and *Geitlerinema* (Anagnostidis & Komárek) Anagnostidis was only present in Rio Grande do Norte and Pernambuco (Fig. 3). Among the dominant species, *Cylindrospermopsis raciborskii* (Woloszynska) Seenayya & Subba Raju was the species most frequently found in aquatic environments in Northeast Brazil (55%), followed by *Microcystis aeruginosa* (Kützing) Kützing (26%) and *Planktothrix agardhii* (Gomont) Anagnostidis & Komárek (25%) (Tab. 1).

The species composition of bloom-forming cyanobacteria presented significant differences among the states, with a greater number of species in the states of Pernambuco and Rio Grande do Norte (PERMANOVA: F=3.5196, P<0.001). Among the indicator species, *M. protocystis* was considered an indicator of blooms in the state of Rio Grande do Norte, *M. aeruginosa* of blooms in Paraíba and Rio Grande do Norte, and *P. agardhii* an indicator of blooms in the states of Bahia and Ceará. A greater number of dominance events (blooms) occurred in Pernambuco state, and usually involved a single

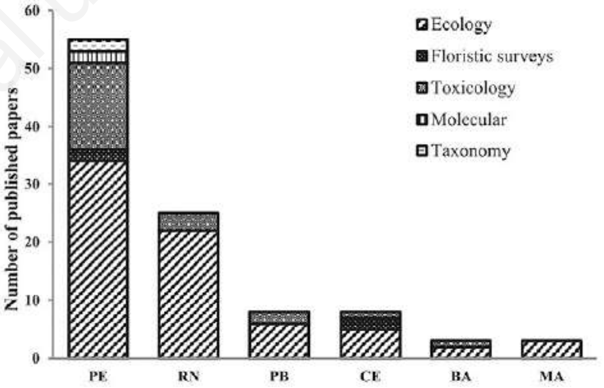

Fig. 1. Distribution of the number of papers about cyanobacteria using varied approaches in different states in Northeast Brazil. PE, Pernambuco; RN, Rio Grande do Norte; PB, Paraíba; CE, Ceará; BA, Bahia; MA, Maranhão.

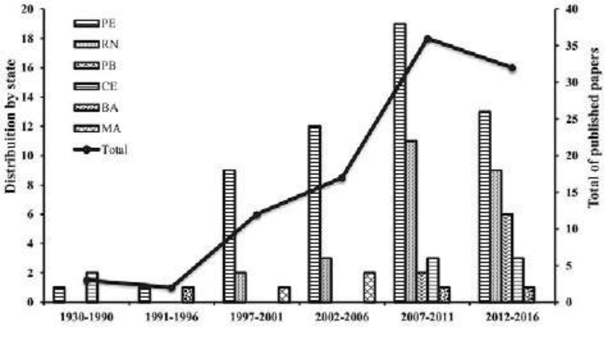

Fig. 2. Distribution and chronological evolution of the number of published works about cyanobacteria by state in Northeast Brazil.

Tab. 1. Number of cyanobacterial dominance events, occurrence frequency (OF%) (n=149), and species distribution in different states of Northeast Brazil.

Species	PE	RN	PB	CE	BA	OF(%)
Chroococcales						
Aphanocapsa cumulus Komárek & Cronberg	2					1
Aphanocapsa delicatissima West & G.S.West		1				1
Aphanocapsa incerta (Lemmermann) Cronberg & Komárek		1				1
Aphanocapsa nubila Komárek & Kling			1			1
Aphanocapsa spp.		1				1
Coelosphaerium naegelianum Unger		1				1
Merismopedia glauca (Ehrenberg) Kützing		1				1
Merismopedia punctata Meyen	1					1
Merismopedia tenuissima Lemmermann	1					1
Microcystis aeruginosa (Kützing) Kützing	10	14	15			26
Microcystis botrys Teiling	1					1
Microcystis flosaquae (Wittrock) Kirchner	1					1
Microcystis novacekii (Komárek) Compère	3	1				3
Microcystis panniformis Komárek *et al.*	11	12				15
Microcystis protocystis W.B.Crow	2	11	3			11
Microcystis spp.	5	6		1	1	9
Sphaerocavum brasiliense Azevedo & Sant' Anna		2				1
Sphaerocavum spp.		1				1
Synechocystis aquatilis Sauvageau	1					1
Woronichinia botrys (Skuja) Komárek & Hindák	1					1
Nostocales						
Anabaena circinalis Bornet & Flahault (=*Dolichospermum circinale* (Bornet & Flahault) Wacklin *et al.*)		6				4
Anabaena constricta (Szafer) Geitler (=*Johanseninema constrictum* (Szafer) Hasler *et al.*)	1					1
Anabaena planctonica Brunnthaler (=*Dolichospermum planctonicum* (Brunnthaler) Wacklin *et al.*)		2				1
Anabaena spiroides Klebahn (=*Dolichospermum spiroides* (Klebhan) Wacklin *et al.*)	1					1
Anabaena spp.	2	2			1	3
Aphanizomenon gracile Lemmermann		5				3
Aphanizomenon cf. *issatschenkoi* (Usacev) Proshkina-Lavrenko		1				1
Aphanizomenon cf. *manguinii* Bourrelly		1				1
Cylindrospermopsis acuminatocrispa Couté & Bouvy	1					1
Cylindrospermopsis catemaco Komárková-Legnerová & Tavera	3					2
Cylindrospermopsis philippinensis (W.R.Taylor) Komárek	3					2
Cylindrospermopsis raciborskii (Woloszynska) Seenayya & Subba Raju	48	16	14	4		55
Cylindrospermopsis spp.	2	1				2
Dolichospermum circinale (Bornet & Flahault) Wacklin *et al.*			1			1
Raphidiopsis curvata Fritsch & M.F.Rich	1	1				1
Sphaerospermopsis aphanizomenoides (Forti) Zapomelová *et al.*	1					1
Oscillatoriales						
Geitlerinema amphibium (Gomont) Anagnostidis	10					7
Geitlerinema splendidum (Gomont) Anagnostidis		1				1
Geitlerinema unigranulatum (R.N.Singh) Komárek & M.T.P.Azevedo	1					1
Oscillatoria splendida Gomont (=*G. splendidum*)	1					1
Oscillatoria tenuissima Gomont			1			1
Oscillatoria spp.	1	1				1
Phormidium fragile Gomont (=*Leptolyngbya fragilis* (Gomont) Anagnostidis & Komárek)		1				1
Planktolyngbya limnetica (Lemmermann) Komárková-Legnerová & Cronberg		3				2
Planktothrix agardhii (Gomont) Anagnostidis & Komárek	15	8	10	3	2	26
Planktothrix isothrix (Skuja) Komárek & Komárková	1	1				1
Planktothrix spp.				1		1
Pseudanabaena catenata Lauterborn	2					1
Pseudanabaena spp.	1					1
Total of species	30	27	7	4	3	

cyanobacterium species (monospecific dominance) per environment. The same pattern was observed for Paraíba state (Fig. 4). However, we observed a distinct pattern in Rio Grande do Norte state, with a high number of dominance events involving the coexistence or alternation of four or more cyanobacterial species (Fig. 4). Although we had very few records of dominance events for the states of Ceará and Bahia, they were constituted by one, two, or three species (Fig. 4).

Toxicity events associated with cyanobacterial dominance

Several cyanoHABs with proven toxicity were registered in Northeast Brazil. Toxin analysis was usually performed using biochemical/immunochemical methods or bioassays in mice or fish. Harmful algal blooms were identified in the states of Pernambuco, Rio Grande do Norte, Ceará, and Paraíba. The highest number of toxic bloom events was registered in Pernambuco state, and included toxins, such as microcystins (among them microcystin-LR), cylindrospermopsin, five variants of saxitoxins and anatoxin-a(S). In Rio Grande do Norte state, microcystins and three variants of saxitoxins were recorded, while for Ceará only three types of saxitoxins were registered, and in Paraíba only microcystins (including microcystin-LR) were recorded (Tab. 2).

DISCUSSION

The increasing occurrence of cyanobacteria has been recorded in freshwater ecosystems all over the world. However, this scenario is becoming more pronounced in Northeast Brazil due to water scarcity in reservoirs (associated with cultural eutrophication), which has been shown to promote recurring cyanoHABs events. According to Saad and Atia (2014), these events have harmful consequences on aquatic environments around the world, mainly due to the increase of nutrient inputs, such as nitrogen and phosphorus.

Reservoirs have been the focus of these studies not only because they are often located in semi-arid regions that are favourable for the development of cyanobacteria, but also because most of these organisms are potential producers of toxins that affect aquatic biota and, consequently, have harmful effects on human health. In this review, we assessed the contribution of over half a century (1930s to 2016) of cyanobacteria studies and found that in recent decades, more attention has been focused on these studies. The number of papers has increased particularly since the 1990s in the Northeast region, especially after the occurrence of the tragedy involving cyanobacteria poisonings in Pernambuco state (Carmichael *et al.*, 2001; Azevedo *et al.*, 2002).

The occurrence of cyanoHABs is usually associated with an interaction of various factors, such as eutrophication, low turbulence, and high temperatures (Dokulil and Teubner, 2000; Bittencourt-Oliveira *et al.*, 2014; Soares *et al.*, 2013; Paerl and Otten, 2013). These factors enable the dominance of colonial and filamentous species that can cause numerous negative effects on domestic, industrial, and recreational uses, especially in lakes and reservoirs (Dokulil and Teubner, 2000). The incidence and intensity of these blooms in recent years support that an increase in water temperature and changes in rainfall patterns (both consequences of climate change) are playing an important role in cyanobacterial

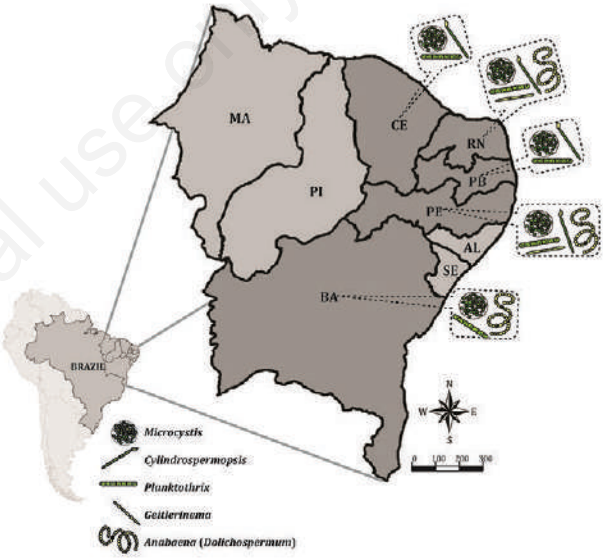

Fig. 3. Distribution of the main dominant cyanobacteria genera in different states in Northeast Brazil.

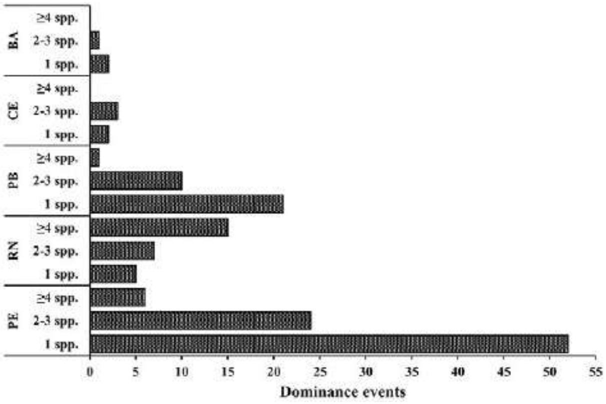

Fig. 4. Number of dominance events and cyanobacteria species per event (n=79) in different states in Northeast Brazil.

proliferation (Elliott, 2012; Paerl and Paul, 2012; Paerl and Otten, 2013). Given that global temperatures are expected to increase in the 21st century (Corlett, 2012) and that rainfall reductions and drought increases are predicted for the next 30 years (Dai, 2013), cyanoHABs issues become even more problematic as these environmental variables may increase the occurrence and biomass of these organisms.

Extreme climatic events are already being observed in Northeast Brazil, causing changes in the phytoplankton community, especially in the dominance of cyanobacteria (Lira *et al.*, 2014; Câmara *et al.*, 2015; Brasil *et al.*, 2016; Costa *et al.*, 2016). Bouvy *et al.* (2000) made one of the most important reports on cyanoHABs (in almost 40 reservoirs in the Northeast) associated with climate change, when the severe drought of 1998 (a consequence of the El Niño in 1997) made the environment favourable for cyanobacteria proliferation, specially *Cylindrospermopsis*

Tab. 2. Occurrence and distribution of dominant cyanobacteria species present in toxic blooms, detected toxins, amount of toxins, and papers that cited the occurrence of these cyanotoxins.

Species	Detected cyanotoxins	Amount	Reference
Pernambuco			
*Anabaena spiroides**	MCs	10.3 to 836,280 ng g^{-1}	
*Aphanocapsa cumulus**	MC-LR	0.08 to 3.7 ng mg^{-1}	
Cylindrospermopsis raciborskii	CYL	33.3 to 2.718 ng g^{-1}	Bouvy *et al.* (1999)[a],
Geitlerinema amphibium	STX	52 ng L^{-1}	Domingos *et al.* (1999),
Merismopedia tenuissima	neoSTX	51 ng L^{-1}	Molica *et al.* (2002; 2005),
Microcystis aeruginosa	GTX	+	Bittencourt-Oliveira *et al.* (2010; 2011; 2014),
M. botrys	dcSTX	+	Piccin-Santos and Bittencourt-Oliveira (2012),
M. novacekii	Analogue of STX	+	and Lorenzi *et al.* (2015)
M. panniformis	ATX-a(s)	+	
Planktothrix agardhii			
P. isothrix			
Sphaerospermopsis aphanizomenoides			
Rio Grande do Norte			
Aphanizomenon gracile			
Ap. cf. *issastschenkoi*	MCs	0.0023 to 24.195 µg L^{-1}	
Ap. cf. *manguinii*			
Anabaena circinalis			
An. planctonica			
Anabaena spp.	STXs	0.003 to 3.14 µg L^{-1}	
C. raciborskii			Costa *et al.* (2006)[a],
M. aeruginosa	STX	+	Chellappa *et al.* (2008),
M. novacekii			and Fonseca *et al.* (2015)
M. panniformis	C-toxins	+	
M. protocystis			
Microcystis spp.	GTX	+	
P. agardhii			
Ceará			
*C. raciborskii**	STX	+	Lopes *et al.* (2015)
*P. agardhii **	dcSTX	+	
	dcGTX	+	
Paraíba			
C. raciborskii	MCs	0.1 to 41.16 µg L^{-1}	Vasconcelos *et al.* (2013),
Dolichospermum circinale			Lins *et al.* (2016), and
M. aeruginosa	MC-LR	27.3 µg L^{-1}	Mendes *et al.* (2016)
P. agardhii			

**Species isolated for cyanotoxin detection; [a]toxicity confirmed through bioassays with mice and/or fish; MC, microcystin; CYL, cylindrospermopsin; STX, saxitoxin; neoSTX, neosaxitoxin; GTX, gonyautoxin; dcSTX, decarbamoylsaxitoxin; ATX-a(s), anatoxin-a(s); dcGTX, decarbamoylgonyautoxin; +, cyanotoxin detected but not quantified.*

raciborskii. Thus, global warming is expected to promote an increasing number of cyanoHABs episodes (Paerl, 2009; Whitehead *et al.*, 2009; Wilk-Woźniak *et al.*, 2016).

In temperate and subtropical regions an increase in water temperature and total phosphorus are expected to promote highly hazardous cyanoHABs (Davis *et al.*, 2009; Rigosi *et al.*, 2015). However, the response to global warming and eutrophication depends on the trophic state of the water bodies and the bloom-forming species, with nutrients being the main factor influencing blooms in oligotrophic lakes, and water temperature becoming more influential in mesotrophic lakes. In eutrophic and hypereutrophic lakes, published evidence suggests nutrients and temperature interact synergistically to promote cyanobacteria (Rigosi *et al.*, 2014). Extreme climate events, like changes in precipitation patterns, can also favour cyanoHABs in subtropical lakes (Haakonsson *et al.*, 2017). In tropical regions, blooms are more frequent and persistent throughout the year, with higher biomass and toxic records (Bouvy *et al.*, 2000; Bittencourt-Oliveira *et al.*, 2014), in contrast with temperate regions where cyanoHABs are restricted to shorter periods of the year, mainly in the summer (Ger *et al.*, 2016).

In our analysis, we observed that *Cylindrospermopsis raciborskii*, *Microcystis aeruginosa*, and *Planktothrix agardhii* were the most frequent species occurring in aquatic environments in Northeast Brazil. These species are often responsible for the formation of blooms in tropical ecosystems (Mowe *et al.*, 2015). They share important adaptive traits, such as the presence of aerotopes and the ability to produce toxins (Komárek and Johansen, 2015a, 2015b), that favour the dominance of this group in reservoirs (Soares *et al.*, 2013; Moura *et al.*, 2015). Previously, the emergence of these species in freshwater ecosystems, under various hydrologic, physical, and chemical conditions, has been found to be related to a set of adaptive strategies developed to reflect the changes caused by global warming, such as wide phenotypic plasticity and a greater ecological tolerance (Kling, 2009), resulting in the successful proliferation of these organisms (Paerl and Huisman, 2009). The species *C. raciborskii*, *M. aeruginosa*, and *P. agardhii*, which were present in most bloom events, were found to be involved in perennial events of cyanobacterial dominance in the Northeast eutrophic reservoirs (Bouvy *et al.*, 1999; Huszar *et al.*, 2000). As noted in most studies on cyanobacteria in Pernambuco and Paraiba states, most dominance events consisted of only one species (monospecific dominance), with rare cases of codominance between two or more species. In addition, in Rio Grande do Norte, most cases of dominance involved codominance of several species. In a recent review about Brazilian water bodies, Soares *et al.* (2013) showed that the most cases of cyanobacterial dominance were constituted by several representatives of the *Microcystis* genus. Currently, the species *C. raciborskii* has a wide distribution on all continents, especially in temperate regions, where it is considered an invasive taxon, due to its buoyancy and nitrogen fixation capacity, tolerance to low light, and resistance to predation by zooplankton (Padisák, 1997; Briand *et al.*, 2004). On the other hand, blooms of *Microcystis* species are becoming more prolific around the world, with records on all continents, especially in temperate European, North American and Australasian countries. In these cases, many blooms are accompanied by microcystin records (Harke *et al.*, 2016). In the tropics, the most prevalent bloom-forming cyanobacteria are *Microcystis* spp. in Africa and Asia, and *C. raciborskii* in South America and Australia (Mowe *et al.*, 2015).

Compared to other semiarid regions around the world, the waters of Northeast Brazil have similar bloom-forming species as those found in semiarid Mediterranean reservoirs, represented by the genera *Microcystis* and *Planktothrix* (Naselli-Flores *et al.*, 2007). In semiarid regions, reservoirs are the main water source for people, and a reduction in water volume during the summer can promote cyanoHABs (Naselli-Flores and Barone, 2005). Therefore, besides confronting water scarcity, the Brazilian population in the semiarid also has problems due to increasing frequency and amplitude of blooms.

With the high occurrence of cyanoHABs, some studies about biomanipulation were developed to minimize the biomass of this group in reservoirs. For example, the introduction of omnivorous fish reduced the biomass of large cladocerans in a study by Okun *et al.* (2008). However, this response was independent of the density of fish, and therefore, only a large-scale removal of these fish could lead to a significant reduction in the biomass of the phytoplankton community. On the other hand, research involving the biomanipulation of the zooplankton community showed that in the absence of nutrient limitation, limited by nitrogen or phosphorus, zooplankton could reduce the total biomass of phytoplankton, however, there was an increase in the biomass of *C. raciborskii* (Severiano *et al.*, 2017).

Toxic dominance events have been observed in the states of Pernambuco, Rio Grande do Norte, Paraíba, and Ceará, implying that there might be certain conditions at these places that favour the proliferation of toxic cyanobacteria. These blooms (and the consequent cases of human poisoning) have been registered on all continents since the 19th century (Carmichael, 1992). This excessive cyanobacterial growth has caused numerous problems to the environment, mainly due to the synthesis and release of toxins, such as hepatotoxins, neurotoxins, and dermatoxins (Chorus and Bartram, 1999). Therefore, monitoring these metabolites is essential for identifying their risks in continental waters, and ensuring the safety of the water supply for the human population (Brittain *et al.*, 2000).

Regarding the reservoirs evaluated in this literature review, we recorded the presence of microcystins, saxitoxins, cylindrospermopsin, and anatoxin-a(S). The most frequent cyanotoxin was the microcystins. The amounts of these toxins are expected to increase in eutrophic conditions, being related to high concentrations of nitrogen, turbity, and cyanobacterial biomass (Taranu *et al.*, 2017). However, we cannot specify which species produced these toxins, since the identification and quantification of these compounds were performed using bloom samples composed of more than one cyanobacterium species, making it impossible to associate the toxin with its producing species. Furthermore, studies have shown that toxin concentrations are not always related to the density or biomass of species that produce these compounds, as verified by Bittencourt-Oliveira *et al.* (2014) in Pernambuco reservoirs.

Among the toxins produced by cyanobacteria, microcystins were the most frequent in aquatic environments in Northeast Brazil. Microcystins are considered the most potent hepatotoxins (Romero-Oliva *et al.*, 2014), and more than 240 microcystin variants have been identified (Svirčev *et al.*, 2017; Spoof and Catherine, 2017) in several genera of cyanobacteria, such as *Microcystis*, *Planktothrix*, and *Anabaena*, among others (Paerl and Otten, 2013). Among these toxins, microcystin-LR (identified in various bloom events in the states of Pernambuco and Paraíba) stands out as the most toxic, frequently found in aquatic ecosystems (Brittain *et al.*, 2000) and associated with several cases of intoxication in both humans (Carmichael *et al.*, 2001; Azevedo *et al.*, 2002), and fish (Chellappa *et al.*, 2008). Microcystins can also be toxic to aquatic macrophytes (Amorim *et al.*, 2017), and crop plants (Bittencourt-Oliveira *et al.*, 2016), posing risks for the people and animals that feed on these plants. The main mode of action of microcystins is the inhibition of the proteins phosphatase 1 and 2A, resulting in the disruption of the enzymatic activities of hepatocytes, which are highly sensitive to these toxins (Falconer, 2008). Saxitoxins, or paralytic shellfish poison (PSP) (Wiegand and Pflugmacher, 2005), were present in blooms registered in the states of Pernambuco, Rio Grande do Norte, and Ceará. These neurotoxins are produced by cyanobacteria of the genera *Anabaena*, *Aphanizomenon* Bornet & Flahault, *Cylindrospermopsis* and *Planktothrix* (Paerl and Otten, 2013) and are potent antagonists of neuronal voltage-dependent sodium channels. These toxins have been shown to cause paralysis or even death by respiratory arrest (Humpage, 2008). Cylindrospermopsin and anatoxin-a(S) were only identified in blooms registered in the state of Pernambuco. Cylindrospermopsin is a hepatotoxin that is synthesized by species belonging to the *Aphanizomenon*, *Cylindrospermopsis*, and *Raphidiopsis* Fritsch & Rich genera (Paerl and Otten, 2013); this toxin has been shown to inhibit protein synthesis in aquatic animals and plants (Kinnear, 2010). The neurotoxin anatoxin-a(S), which is produced by *Anabaena* species, interrupts acetylcholine esterase activity (Chorus and Bartram, 1999), causing respiratory arrest and death (Falconer, 2008).

CONCLUSIONS

Northeast Brazil is experiencing a high occurrence of cyanobacterial blooms that are composed of several species, many of which are potentially toxic. The high frequency and intensity of blooms can be explained by increased eutrophication and climate change (warming and drought) which affect many freshwater bodies in the tropics. Further studies are particularly needed in the tropical Southern hemisphere, including the North-eastern region of Brazil, where there are still few records of these metabolites in the states of Ceará and Paraíba, and no reports in the states of Maranhão, Piauí, Bahia, Alagoas, and Sergipe. The records of cyanotoxins in drinking water supply reservoirs in Northeast Brazil poses serious risks to human health, highlighting the continued importance to monitor their presence and develop effective mitigation measures.

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SUPPLEMENTARY MATERIAL

Cyanobacterial blooms in freshwater bodies from a semiarid region, Northeast Brazil: A review

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Supplementary Tab. 1. List of the 67 articles showing cyanobacterial dominance events and the dominant species in aquatic environments in the states of northeastern Brazil.

Reference	Reservoir	Year of sampling	Cyanobacteria dominant species
Pernambuco state (n = 35)			
Bouvy *et al.* (1999)	Ingazeiras	1997 and 1998	*Cylindrospermopsis raciborskii* (Woloszynska) Seenayya & Subba Raju and *C. acuminatocrispa* Couté & Bouvy
Domingos *et al.* (1999)	Tabocas	1996	*Aphanocapsa cumulus* Komárek & Cronberg
Bouvy *et al.* (2000)	39 eutrophic reservoirs	1998	*C. raciborskii*
Huszar *et al.* (2000)	Chapéu, Algodões, Poço da cruz, Pão de Açúcar and Ingazeiras	1995 and 1996	*C. raciborskii*, *C. phillipensis* (W.R.Taylor) Komárek, *C. catemaco* Komárková-Legnerová & Tavera and *Phormidium* sp.
Carmichael *et al.* (2001)	Tabocas	1996	*Microcystis* spp., *Anabaena* spp. and *Cylindrospermopsis* spp.
Molica *et al.* (2002)	Tabocas	1998	*C. raciborskii*
Bittencourt-Oliveira (2003)	Tabocas, Jucazinho, Duas Unas and Tapacurá	2003	*Microcystis* spp.
Bouvy *et al.* (2003)	Tapacurá	1998 to 2000	*C. raciborskii*
Molica *et al.* (2005)	Tapacurá	2002	*Anabaena spiroides* Klebahn, *Pseudanabaena* sp., *C. raciborskii* and *Microcystis aeruginosa* (Kützing) Kützing
Aragão *et al.* (2007)	Carpina	2006	*Raphidiopsis curvata* Fritsch & M.F.Rich
Bittencourt-Oliveira *et al.* (2007)	Carpina, Jucazinho and Tapacurá	2002	*Geitlerinema unigranulatum* (R.N.Singh) Komárek & M.T.P.Azevedo
Moura *et al.* (2007a)	Carpina	2001 and 2002	*Planktothrix agardhii* (Gomont) Anagnostidis & Komárek
Moura et al. (2007b)	Duas Unas	2000 and 2001	*Oscillatoria* sp.
Moura *et al.* (2007c)	Mundaú	2005	*C. raciborskii*
Andrade *et al.* (2009)	Tapacurá	2006 and 2007	*C. raciborskii*

Reference	Reservoir	Year of sampling	Cyanobacteria dominant species
Bittencourt-Oliveira *et al.* (2009)	Tabocas	1997 and 1998	*M. protocystis* W.B.Crow
Campeche *et al.* (2009)	Federação	2007	*O. splendida* Gomont (= *G. splendidum*)
Bittencourt-Oliveira *et al.* (2010)	Arcoverde, Botafogo, Carpina, Duas Unas, Jazigo, Mundaú and Tapacurá	2004 to 2006	*M. aeruginosa*, *M. novacekii* (Komárek) Compère, *M. panniformis* Komárek et al., *A. constricta* (Szafer) Geitler, *P. agardhii*
Bittencourt-Oliveira *et al.* (2011a)	Jucazinho, Arcoverde and Duas Unas	2009	*P. agardhii*, *M. novacekii*, *M. panniformis* and *C. raciborskii*
Bittencourt-Oliveira *et al.* (2011b)	Mundaú	2004 and 2005	*C. raciborskii*
Dantas *et al.* (2011)	Arcoverde and Pedra	2007 and 2008	*C. raciborskii*
Lira *et al.* (2011)	Carpina	2006	*C. raciborskii*, *G. amphibium* and *P. agardhii*
Moura *et al.* (2011)	Carpina	2007 and 2008	*P. agardhii*, *C. raciborskii*, *G. amphibium* and *P. catenata* Lauterborn
Bittencourt-Oliveira *et al.* (2012a)	Carpina and Mundaú	2006 and 2008	*C. raciborskii*, *M. novacekii*, *M. panniformis*, *M. botrys* Teiling and *G. amphibium*
Bittencourt-Oliveira *et al.* (2012b)	Arcoverde	2007	*C. raciborskii*
Dantas *et al.* (2012)	Duas Unas, Tapacurá and Jucazinho	2007 and 2008	*M. aeruginosa*, *M. flosaquae* (Wittrock) Kirchner and *Woronichinia botrys* (Skuja) Komárek & Hindák
Moura *et al.* (2012)	Jucazinho	2001 and 2002	*P. agardhii*, *C. raciborskii* and *P. catenata*
Piccin-Santos and Bittencourt-Oliveira (2012)	Carpina and Mundaú	2007	*M. panniformis* and *C. raciborskii*
Silva *et al.* (2013)	Mundaú	2010 and 2011	*Synechocystis aquatilis* Sauvageau, *C. raciborskii*, *M. panniformis* and *M. protocystis*
Bittencourt-Oliveira *et al.* (2014)	10 eutrophic reservoirs	2008 to 2010	*C. raciborskii*, *M. aeruginosa*, *M. panniformis*, *P. agardhii*, *P. isothrix* (Skuja) Komárek & Komárková, *Sphaerospermopsis aphanizomenoides* (Forti) Zapomelová et al., *G. amphibium* and *Merismopedia tenuissima* Lemmermann

Reference	Reservoir	Year of sampling	Cyanobacteria dominant species
Góis and Oliveira (2014)	Mororó	2007 and 2008	*Cylindrospermopsis* spp.
Lira *et al.* (2014)	Mundaú	2006	*C. raciborskii*
Oliveira *et al.* (2014)	Apipucos	2011 and 2012	*P. agardhii*
Moura *et al.* (2015)	Mundaú	2008 and 2009	*C. raciborskii*, *M. panniformis*, *G. amphibium* and *M. punctata* Meyen
Oliveira *et al.* (2015)	Carpina and Mundaú	2013 and 2014	*C. raciborskii*, *G. amphibium* and *P. agardhii*
Rio Grande do Norte state (n = 17)			
Costa *et al.* (1998)	Armando Ribeiro Gonçalves	1996	*Coelosphaerium naegelianum* Unger
Araújo *et al.* (2000)	Extremoz lake	1996 and 1997	*P. fragile* Gomont (= *Leptolyngbya fragilis* (Gomont)
Chellappa and Costa (2003)	Marechal Dutra	1999 and 2000	*C. raciborskii*, R. curvata, M. aeruginosa and Oscillatoria sp.
Costa *et al.* (2006)	Armando Ribeiro Gonçalves	2000	*C. raciborskii*, *M. panniformis*, *M. protocystis*, *M. novacekii*, *Aphanizomenon gracile* Lemmermann, *A.* cf. *issatschenkoi* (Usacev) Proshkina-Lavrenko and *A.* cf. *manguinii* Bourrelly
Chellappa *et al.* (2008a)	Marechal Dutra	2003 and 2004	*C. raciborskii* and *M. aeruginosa*
Chellappa *et al.* (2008b)	Cruzeta	2004 and 2005	*C. raciborskii*
Chellappa *et al.* (2009)	Armando Ribeiro Gonçalves	2006	*A. planctonica* Brunnthaler*, M. aeruginosa, M. protocystis, M. panniformis, Oscillatoria* sp. and *P. agardhii*
Marcon *et al.* (2010)	Lucrécia	2007	*S. brasiliense* and *Sphaerocavum* sp.
Menezes *et al.* (2010)	ESEC	2006	*Cylindrospermopsis* spp. and *Aphanocapsa* spp.
Eskinazi-Santana *et al.* (2013)	Armando Ribeiro Gonçalves, Gargalheiras, Boqueirão de Parelhas, Itans, Passagem das Traíras and Sabugi	2002 to 2004	*M. protocystis*, *M. aeruginosa*, *M. panniformis*, *C. raciborskii*, *G. unigranulatum*, *A. incerta* (Lemmermann) Cronberg & Komárek, A. delicatissima West & G.S.West, *Aphanocapsa* sp., *Aphanizomenon* sp., *A. circinalis* Bornet & Flahault, *Anabaena* sp., *P. agardhii* and *Sphaerocavum brasiliense* Azevedo & Sant' Anna
Silva Pinto and Becker (2014)	Extremoz lake	2012 and 2013	*Merismopedia glauca* (Ehrenberg) Kützing and *Planktolyngbya limnetica* (Lemmermann) Komárková-Legnerová & Cronberg

Reference	Reservoir	Year of sampling	Cyanobacteria dominant species
Câmara *et al.* (2015)	Armando Ribeiro Gonçalves	2008 and 2009	*M. protocystis*, *M. aeruginosa*, *M. panniformis*, *C. raciborskii*, *G. splendidum* (Gomont) Anagnostidis, *A. circinalis*, *P. agardh* and *Oscillatoria* sp.
Fonseca *et al.* (2015)	Armando Ribeiro Gonçalves, Passagem das Traíras, Itans and Gargalheiras	2009 to 2011	*P. agardhii*, *M. aeruginosa, M. panniformis*, *M. protocystis*, *C. raciborskii*, *A. circinalis* and *A. gracile*
Medeiros *et al.* (2015)	Cruzeta	2010 to 2012	*C. raciborskii*, *M. pannifomis*, *S. brasiliense*, *P. isothrix* and *A. gracile*
Silva and Costa (2015)	Pau dos Ferros and Santa Cruz do Apodi	2011 and 2012	*C. raciborskii*, *P. limnetica*, *A. gracile*, *A. delicatissima* and *A. incerta*
Vieira *et al.* (2015)	Armando Ribeiro Gonçalves	2009 to 2011	*P. agardhii*
Costa *et al.* (2016)	Pocinhos and ESEC	2012 to 2014	*P. limnetica* and *C. raciborskii*
Paraíba state (n = 7)			
Costa and Dantas (2011)	Solon de Lucena Lake and Águas Minerais reservoir	2009 and 2010	*A. nubila* Komárek & Kling
Vasconcelos *et al.* (2011)	20 reservoirs	2006 to 2009	*M. aeruginosa, C. raciborskii, P. agardhii* and *O. tenuissima* Gomont
Vasconcelos *et al.* (2013)	Acauã, Cordeiro and Camalau	2009	*C. raciborskii*, *M. protocystis* and *P. agardhii*
Azevedo *et al.* (2015)	Poções and Camalaú	2012	*C. raciborskii*
Felix *et al.* (2015)	Alagoa Grande	2012	*P. agardhii*
Lins *et al.* (2016)	Agemiro de Figueiredo	2007 to 2009	*P. agardhii, C. raciborskii, Dolichospermum circinale* (Bornet & Flahault) Wacklin et al. and *M. aeruginosa*
Mendes *et al.* (2016)	Acauã, Araçagi, Boqueirão do Cais, Cacimba da Várzea and Cordeiro	2012	*C. raciborskii* and *M. protocystis*
Ceará state (n = 5)			
von Sperling *et al.* (2008)	Gavião	1990 to 2006	*C. raciborskii* and *P. agardhii*
Molisani *et al.* (2010)	Castanhão	2006 and 2007	*C. raciborskii*
França *et al.* (2013)	Miranda	2012	*C.* raciborskii, *Planktothrix* sp. and *P. agardhii*

Reference	Reservoir	Year of sampling	Cyanobacteria dominant species
Lopes *et al.* (2015)	Sítios Novos	2010 and 2011	*C. raciborskii and P. agardhii*
Lucas *et al.* (2016)	Rosário	2013	*Microcystis* sp.
Bahia state (n = 3)			
Teixeira *et al.* (1993)	Itaparica	1988	*Anabaena* spp. and *Microcystis* spp.
Fuentes *et al.* (2010)	Funil	2007 and 2008	*P. agardhii*
Moura *et al.* (2013)	Pedra and Funil	2007 to 2010	*P. agardhii*

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Seasonal variations of morpho-functional phytoplankton groups influence the top-down control of a cladoceran in a tropical hypereutrophic lake

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Abstract Shallow lakes are often affected by the increase in nutrients and global climate change, with frequent occurrences of cyanobacterial blooms. In this context, the biomanipulation of the higher trophic levels, such as zooplankton, can efficiently control these blooms. Based on this, this study aimed to verify the potential of a medium-sized cladoceran, *Macrothrix spinosa*, to control the phytoplankton biomass from the Apipucos reservoir, a shallow tropical hypereutrophic lake. For this, grazing experiments were carried out during different seasonal periods, which were characterized by the dominance of cyanobacteria in the dry season and of chlorophytes in the rainy season. The experiments were carried out in 250-mL Erlenmeyer flasks containing filtered water from the environment and different densities of the cladocerans: 0 (control), 100, 200 and 300 ind L^{-1}. The results varied between the seasonal periods; however, *M. spinosa* reduced phytoplankton biomass mainly during the rainy season. In the dry season, *M. spinosa* significantly reduced the biomass of the morpho-functional groups IV and VII, while in the rainy season all groups were significantly reduced, except for group V. The cladoceran was also able to reduce the mean length of filamentous cyanobacteria with aerotopes (group III); one possible explanation for this result is that *M. spinosa* could cut the filaments before consuming them. In this way, the seasonal variations in the phytoplankton structure influenced the top-down control of *M. spinosa* in a shallow hypereutrophic lake, showing its potential to control algal blooms, especially of chlorophytes, and could be used in biomanipulation strategies in eutrophic freshwater environments.

Keywords Algal blooms · Biomanipulation · Chlorophytes · Cyanobacteria · Grazing experiments · Zooplankton

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Introduction

Freshwater aquatic environments, especially shallow lakes, are globally threatened by anthropic actions and climate change (Jeppesen et al. 2014). On a global scale, there are many more shallow lakes environments than deep ones; therefore, they are greatly important for the functioning of ecosystems and surrounding areas, as many of them are used for human supply (Chen et al. 2003; Verpoorter et al. 2014). Since they are sensitive to climate variations and respond quickly to modifications, shallow lakes are considered sentinels of climate change, because they are located within more vulnerable environments (Adrian et al. 2009). In addition, eutrophication has deteriorated the water quality of these ecosystems, reducing their multiple uses and ecosystem services (Hilt et al. 2017).

The aquatic communities in shallow lakes, especially phytoplankton and zooplankton, are intensely affected by variations in climate and eutrophication levels. In temperate environments, lakes have two alternative main stable states, one with clear waters and low phytoplankton biomass, and another with turbid water and frequent algal blooms (Scheffer et al. 1993). For the tropical region, eutrophic and hypereutrophic lakes can undergo alternating periods of dominance of submerged or floating aquatic plants, depending on the trophic levels of the environment (Scheffer et al. 2003). These seasonal changes significantly affect the composition and structure of the phytoplankton community, which can be dominated by cyanobacteria or chlorophytes in situations of extreme eutrophication (Jensen et al. 1994).

Currently, one of the main problems for water management is the proliferation of cyanobacterial blooms, which can jeopardize the other aquatic communities and human populations due to their potential production of cyanotoxins (e.g., Carmichael et al. 2001; Ger et al. 2014; Amorim et al. 2017). The situation becomes increasingly worrying since global climate change, associated with the eutrophication of water bodies, can further promote the growth of potentially toxic cyanobacteria (O'Neil et al. 2012; Paerl and Otten 2013; Moura et al. 2018). In search of solutions to these problems, research has been developed using biomanipulation of the main components of aquatic trophic webs, such as macrophytes and zooplankton, to reduce the biomass of cyanobacteria (e.g., Vanderstukken et al. 2011; Ekvall et al. 2014; Severiano et al. 2018; Diniz et al. 2019).

Research on the increasing of the zooplankton to control phytoplankton growth presents controversial results between temperate and (sub)tropical environments. While phytoplankton is efficiently controlled in temperate environments (e.g., Ha et al. 2013; Ekvall et al. 2014), in (sub)tropical environments, zooplankton has little or no effect in reducing phytoplankton (e.g., Lacerot et al. 2013; Severiano et al. 2017, 2018). The absence of trophic cascade between zooplankton and phytoplankton in tropical regions can be explained by the predominance of small-sized organisms found in these environments when compared to temperate regions, where zooplankton is often dominated by large cladocerans, as *Daphnia* (Jeppesen et al. 2005; Ger et al. 2014, 2016). Cladocerans are often used in biomanipulation studies because they present a high predation capacity on phytoplankton, with high rates of non-selective ingestion, which allows them to filter and consume particles of varying sizes (Ger et al. 2014).

Despite failed attempts of biomanipulation using the increasing of zooplankton in situ in tropical lakes and reservoirs, studies have experimentally demonstrated that zooplankton in this region is able to efficiently consume cyanobacteria in laboratory experiments (Kâ et al. 2012). Therefore, the absence of top-down control of cyanobacteria in situ may be related to their adaptive strategies that prevent zooplankton consumption, such as large colony size and filaments, nutrient deficiency and their ability to produce cyanotoxins (Ger et al. 2016). In addition, some zooplankton species that are dominant in tropical lakes and reservoirs, such as calanoid copepods, present food selectivity and can avoid consuming unpalatable cyanobacteria (Ger and Panosso 2014; Leitão et al. 2018).

The use of functional groups based on phytoplankton morphology is an important tool for understanding how the interactions between phytoplankton and zooplankton occur in aquatic environments. In this context, an important morpho-functional classification was developed by Kruk et al. (2010), which grouped the phytoplankton species into seven groups based on size, surface–area ratio, the presence of siliceous structures, flagella and aerotopes, and some physiological characteristics, such as the production of cyanotoxins. More recently, Colina et al. (2016)

improved the original classification to encompass the existing interactions between phytoplankton and zooplankton, showing their degree of palatability.

Accordingly, the aim of the present study was to verify the capacity of a medium-sized cladoceran to control the phytoplankton biomass of a tropical hypereutrophic shallow lake. We tested the hypothesis that the seasonal differences in phytoplankton groups dominance can interfere significantly on top-down control of a medium-sized cladoceran. For this, laboratory experiments were carried out to verify the potential control of the cladoceran *Macrothrix spinosa* King (1853) on the phytoplankton in the Apipucos reservoir during two seasonal periods, which were characterized by the dominance of cyanobacteria in the dry season and chlorophytes in the rainy season.

Materials and methods

Phytoplankton and cladoceran used in the experiment

The phytoplankton community used in the present study was collected from the Apipucos reservoir, a shallow hypereutrophic lake located in the municipality of Recife, Pernambuco, Brazil. This lake has an area of approximately 2.9 km^2, an estimated volume of 556,375 m^3 and an average depth of 2.5 m (Neumann-Leitão et al. 1989). It is considered a hypereutrophic reservoir with a significant presence of the floating macrophyte *Eichhornia crassipes* (Mart.) (Almeida et al. 2012). The collection of phytoplankton for the taxonomic study was carried out by using a plankton net with a mesh opening of 25 µm on the surface of the water, after that the samples were fixed with 4% formalin. The species were identified under optical microscopy using specialized bibliography and subsequently classified in the morphology-based functional groups (MBFG) proposed by Kruk et al. (2010).

The cladoceran used in the experiments was a clone of *M. spinosa,* a medium-sized phytophile microcrustacean with an average length of 955.2 µm (± 19.6), which was isolated from a single female from the Duas Unas reservoir, a eutrophic environment with cyanobacterial blooms and cyanotoxins (Bittencourt-Oliveira et al. 2014). This clone was chosen because of its lower sensitivity to cyanobacteria and cyanotoxins showed in previous experiments, one factor that could influence the results. Before the experiments, *M. spinosa* was cultivated in Erlenmeyer flasks with reconstituted water (30% of filtered and autoclaved water from the environment, with 70% of commercial mineral water and 250 µL of BlackWater® humic acid extracts), under controlled conditions of temperature (26 °C), light intensity (40 µmol m^{-2} s^{-1}) and photoperiod of 12 h. The organisms were fed with the chlorophyte *Ankistrodesmus falcatus* (Corda) Ralfs (provided by the Culture Collection of Microalgae from the Federal Rural University of Pernambuco, strain BMIUFRPE-01) every other day.

Grazing experiments

The grazing experiments were carried out during the dry (October 2016) and rainy (July 2017) seasons, since the phytoplankton structure is influenced by seasonal changes in the environmental conditions. Five liters of water were collected from the subsurface of the coastal region of the Apipucos reservoir with a Van Dorn bottle. This water was processed by filtering it through a 60-µm mesh-opening plankton net to remove larger grazers, which could exert some effect on phytoplankton, and was subsequently transported to the laboratory in plastic bottles.

Before the experiments, adult organisms of *M. spinosa* were packaged in 10-mL vials containing reconstituted water and maintained without food for 2 h. Subsequently, the test organisms were transferred to 250-mL Erlenmeyer flasks containing 200 mL of processed water from the Apipucos reservoir with the natural phytoplankton community.

The experiment was conducted in an aseptic climatized room with the same conditions described for the cultivation of *M. spinosa*. The treatments were divided into three different cladoceran densities, with three replicates each: 100 ind L^{-1} (20 individuals added), 200 ind L^{-1} (40 individuals added) and 300 ind L^{-1} (60 individuals added). The control treatment consisted of Erlenmeyer flasks without the addition of *M. spinosa*. The experiment lasted 8 h, and 2 mL aliquots were collected at the beginning of the experiment and at intervals of 2 h to analyze density, biomass and maximum linear dimension (MLD) of algae. The samples were preserved with 1% acetic Lugol and placed at 4 °C until the analyses took place.

Phytoplankton density was determined by counting the organisms in sedimentation chambers under an inverted Zeiss Axiovert 135 M microscope following the methodology of Utermöhl (1958). The biomass determination of phytoplankton species was performed by multiplying the density by the mean biovolume of the species and expressed in mg L^{-1}. The biovolume was determined using the geometric formulas proposed by Sun and Liu (2003), which consider the specific shape of the species to calculate the mean biovolume. The maximum linear dimension was evaluated by the maximum length of cells, colonies or filaments of the phytoplankton organisms present in the samples.

Phytoplankton and morpho-functional groups growth rates (μ) were calculated based on the biomass values at the beginning and end of the experiments. The formula proposed by Wood et al. (2005) was used: $\mu\ (h^{-1}) = (\ln(Nt)\ \ln(Nt0))/t - t0$, where N represents the biomass values after 8 h of the experiment (t) and at the beginning ($t0$).

Statistical analyses

A one-way ANOVA was used to verify the differences in biomass and growth rates of phytoplankton and each morpho-functional group between treatments for each sampling hour. Before the analyses of variances, the data were tested for normality using the Kolmogorov–Smirnov test and homoscedasticity using the Bartlett test. When significant differences were verified, the analyses were followed by a Tukey post hoc test. To verify the dependence of phytoplankton biomass (dependent variable) with the time of the experiments and the density of *M. spinosa* (independent variables), generalized linear models were generated (GLM) for treatments with 200 and 300 ind L^{-1}, and for 6 h and 8 h, which presented the most significant reductions. All statistical analyses were performed in the R program, and the significance level was set at $p < 0.05$ (R Core Team 2016).

Results

The phytoplankton community from the Apipucos reservoir was represented by 42 species (40 in the dry period and 38 in the rainy season). During the dry period, the filamentous cyanobacteria *Planktothrix agardhii* (Gomont) Anagnostidis and Komárek (group III) was the most abundant species, while in the rainy season the chlorophyte *Pediastrum duplex* Meyen (group IV) presented higher biomasses. Details about the phytoplankton community from the Apipucos reservoir, such as species richness and biomass of the species, are described in the Supplementary Table S1.

The initial biomasses of phytoplankton during the experiments were 18.19 mg L^{-1} (± 2.78; n = 12) in the dry season and 25.09 mg L^{-1} (± 2.5; n = 12) in the rainy season. After phytoplankton was exposed to the cladoceran *M. spinosa*, there were significant reductions in total biomass in the dry season after 2 h of experiment and after 6 h and 8 h in the rainy season. During the dry period, when there was a dominance of filamentous cyanobacteria (group III), the cladoceran reduced the phytoplankton biomass to 29.2% and 47% in the treatments with 200 and 300 ind L^{-1} after 8 h, respectively. However, no significant differences were observed between the biomass values for the treatments after 8 h during the experiment (Fig. 1a). For the rainy season, when phytoplankton showed a higher abundance of colonial green algae (group IV), there were significant reductions in phytoplankton biomass after 6 h in the treatment with 300 ind L^{-1} and after 8 h in all treatments with addition of *M. spinosa*, reaching a reduction of 69.1% with addition of 300 ind L^{-1} of the cladoceran (Fig. 1b).

The reductions in phytoplankton biomass depended significantly on the densities of *M. spinosa* during the dry (8 h) and rainy seasons (6 and 8 h). In addition, reductions were also time-dependent in the treatment with 300 ind L^{-1} in the dry season, and in 200 and 300 ind L^{-1} treatments in the rainy season (Table 1).

Regarding the functional groups, results varied according to the seasonal period. During the dry season, the addition of *M. spinosa* significantly reduced the biomass of groups III (200 and 300 ind L^{-1} after 2 h), IV (100, 200 and 300 ind L^{-1} after 2 and 8 h), V (200 ind L^{-1} after 2 h) and VII (100 ind L^{-1} after 4 h; and 100, 200 and 300 ind L^{-1} after 8 h). On the other hand, groups I (8 h), III (2 h) and VI (6 h) were stimulated with the addition of 100 ind L^{-1}. After 6 and 8 h, group III showed a considerable decrease in biomass in treatments with 200 and 300 ind L^{-1}; however, these differences were not significant (Fig. 2).

On the other hand, during the rainy season, all morpho-functional phytoplankton groups were

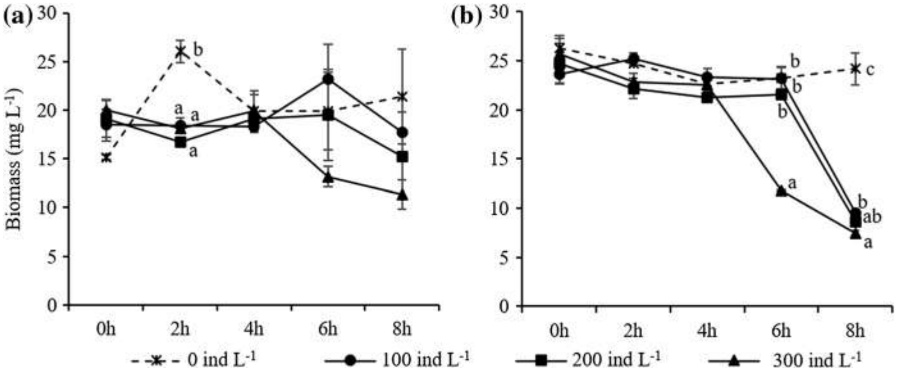

Fig. 1 Total biomass of the phytoplankton community from the Apipucos reservoir submitted to the grazing experiments by *Macrothrix spinosa* at different densities in the dry (**a**) and rainy (**b**) seasons. Lines represent means and standard errors. Different letters (a, b, and c) represent significant differences between the treatments (Tukey: $p < 0.05$)

Table 1 Generalized linear models to explain the dependence of phytoplankton biomass on the *Macrothrix spinosa* density (density) and on time (h) during the grazing experiments in the dry and rainy seasons

	Linear models	AIC	r^2
Based on *M. spinosa* density			
Dry period			
6 h	22.521–0.12 (density)^{ns}	81.592	0.1835
8 h	21.31–0.164 (density)*	74.852	0.4241
Rainy period			
6 h	25.346–0.18 (density)**	64.735	0.6733
8 h	20.093–0.255 (density)**	72.926	0.6775
Based on time			
Dry period			
200 ind L^{-1}	18.957–0.258 (h)^{ns}	89.017	0.0346
300 ind L^{-1}	21.004–1.12 (h)***	76.511	0.609
Rainy period			
200 ind L^{-1}	26.272–1.645 (h)***	86.695	0.6303
300 ind L^{-1}	27.545–2.369 (h)***	77.962	0.8635

All models were significant ($p < 0.0001$). Significances of independent variables are as follow: ^{ns}non-significant; $*p < 0.05$; $**p < 0.01$; $***p < 0.001$

significantly reduced after exposure to *M. spinosa*. After 8 h of the experiment, all functional groups, except for group V, showed significant reductions in all treatments with the addition of cladocerans. Furthermore, *M. spinosa* also reduced the biomass of groups III (200 and 300 ind L^{-1} after 4 h; and 100 and 300 ind L^{-1} after 6 h), IV and VII (300 ind L^{-1} after 6 h), V (200 and 300 ind L^{-1} after 2 h; and 300 ind L^{-1} after 4 h) and VI (200 and 300 ind L^{-1} after 6 h). Only group III showed a significant increase in biomass after 2 h at exposure to 100 ind L^{-1} (Fig. 3).

The species in group III, represented by *P. agardhii* and *Cylindrospermopsis raciborskii* (Woloszynska) Seenayya and Subba Raju, showed a reduction in the average length under exposure to *M. spinosa* in both seasonal periods after 4 h of the experiment in treatments with 200 and 300 ind L^{-1}. In the dry season, in addition to group III, groups IV and VI also showed a reduction in the maximum linear dimension (Fig. 4).

The growth rate of phytoplankton in the dry season was positive in the control treatment (0.04 h^{-1}) and became negative in all treatments with the addition of *M. spinosa,* reaching − 0.07 h^{-1} (300 ind L^{-1}), with lower values observed in treatments with 200 and 300 ind L^{-1} (Fig. 5a). In this period, the cladocerans reduced the growth rates of groups I, IV, V, and VII (Fig. 5b). In the rainy season, phytoplankton growth rates were negative in all treatments, reaching up to − 0.15 h^{-1} (300 ind L^{-1}), all treatments with *M. spinosa* differed significantly from the control (Fig. 5a). In this period, there were significant reductions in the growth rates of groups I, III, IV, VI, and VII (Fig. 5c).

Discussion

The cladoceran *M. spinosa* could exert effective top-down control on phytoplankton biomass in the Apipucos reservoir. This control was dependent on

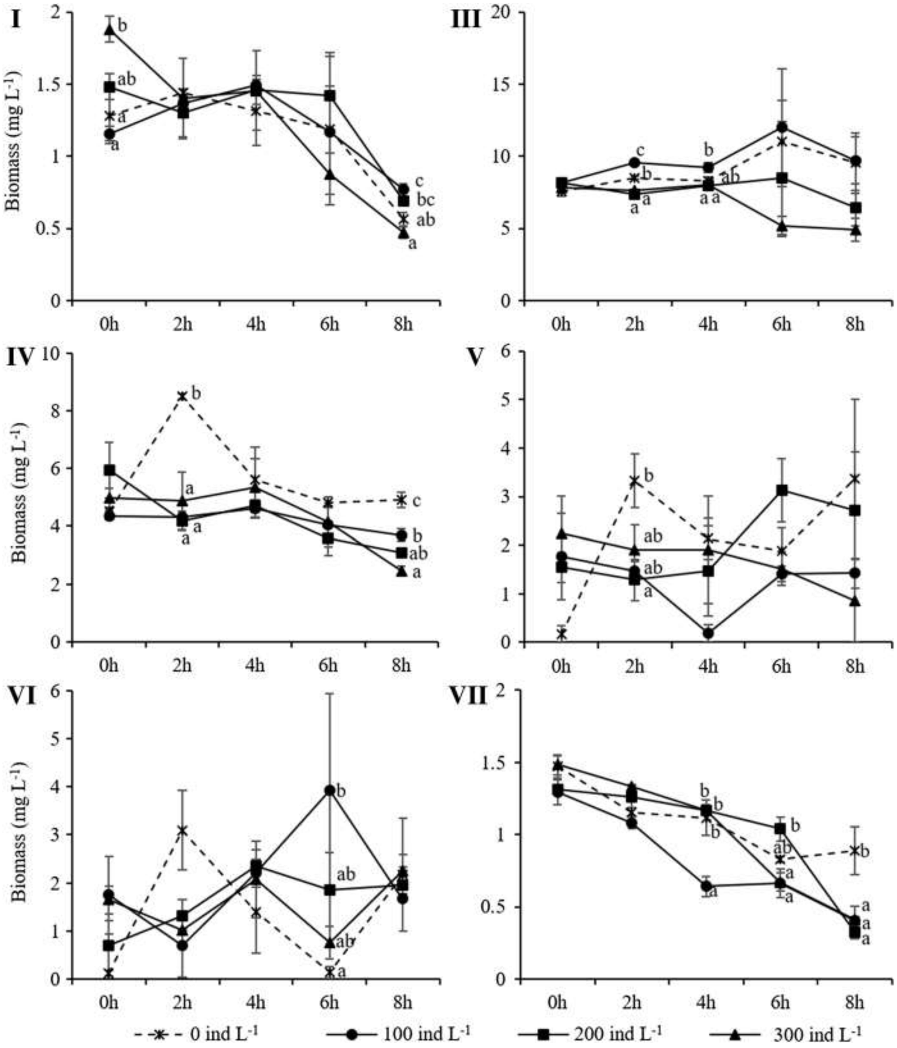

Fig. 2 Biomass of the morpho-functional phytoplankton groups **I**, **III**, **IV**, **V**, **VI**, and **VII** from the Apipucos reservoir submitted to the grazing experiment by *Macrothrix spinosa* at different densities in the dry season. Lines represent means and standard errors. Different letters (a, b, and c) represent significant differences between the treatments (Tukey: $p < 0.05$)

the density of cladocerans used, which reduced the biomass of phytoplankton after 6 h of incubation. In addition, this cladoceran was able to consume various phytoplankton groups, especially chlorophytes (group IV), as well as filamentous and colonial cyanobacteria (groups III and VII, respectively). In addition to modifying the size of filamentous cyanobacteria, whose filaments probably were cut to facilitate the consumption of smaller parts.

The use of morpho-functional phytoplankton groups was adequate to explain the predation relationships between phytoplankton and *M. spinosa* in the studied lake. Diniz et al. (2019) also verified the correlation of the morpho-functional phytoplankton groups with zooplankton in two tropical reservoirs, showing their applicability in studies involving these two communities. In this sense, the use of phytoplankton classifications based on morphology is important for understanding the palatability and susceptibility of phytoplankton to predation by zooplankton (Colina et al. 2016). In this way, different morphological characteristics, such as shape or size, can prevent or favor predation (Frau et al. 2017).

The Apipucos reservoir, a tropical shallow hypereutrophic lake, proved to be a good ecosystem model for studying the phytoplankton–zooplankton interaction since it contains a diverse community of algae and cyanobacteria and alternating periods of filamentous cyanobacteria (group III) and colonial green algae (group IV) blooms. The great diversity of species in this ecosystem provided a wide variety of morphological groups for consumption by the cladoceran.

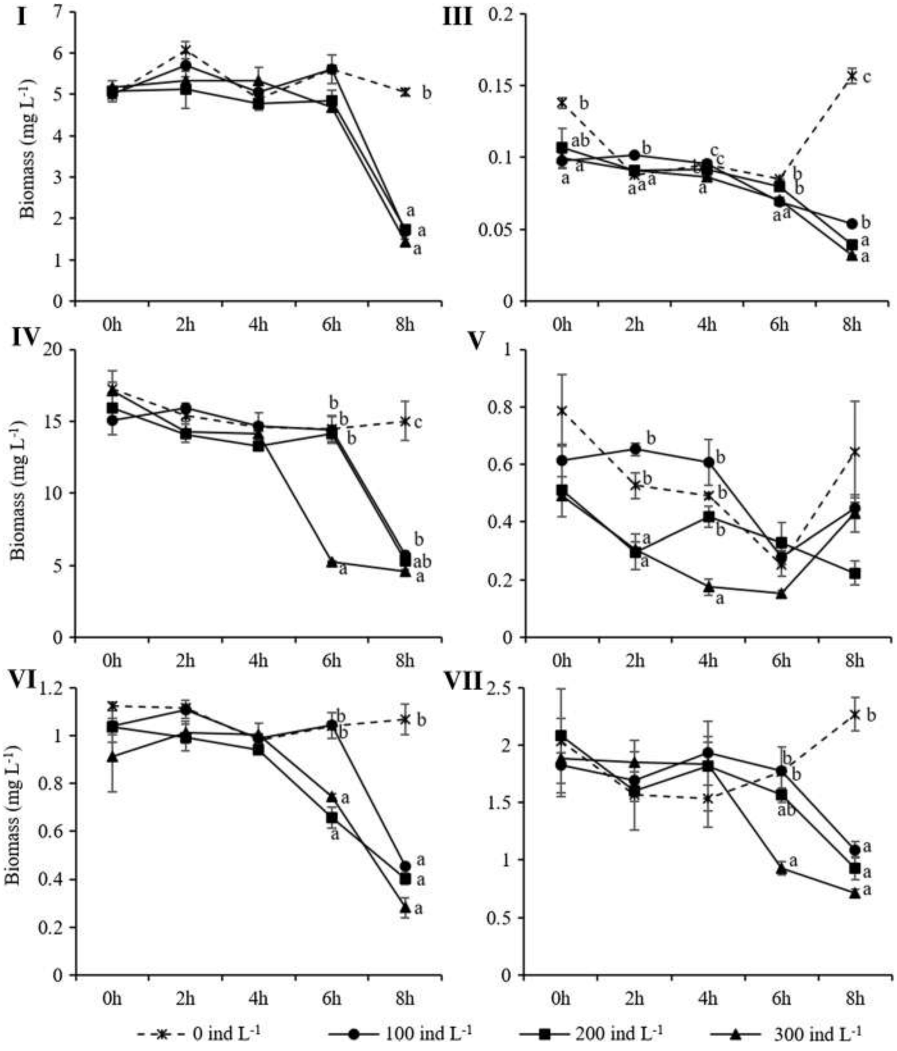

Fig. 3 Biomass of the morpho-functional phytoplankton groups **I**, **III**, **IV**, **V**, **VI**, and **VII** from the Apipucos reservoir submitted to the grazing experiment by *Macrothrix spinosa* at different densities in the rainy season. Lines represent means and standard errors. Different letters (a, b, and c) represent significant differences between the treatments (Tukey: $p < 0.05$)

In eutrophic lakes, the dominance of filamentous bloom-forming cyanobacteria is common, constituting a stable alternative state of phytoplankton (Scheffer et al. 1997). More specifically, the dominance of the Oscillatoriales order (e.g., the genus *Planktothrix*) may be associated with its ability to tolerate low luminosities, reaching high biomasses in turbid eutrophic environments. However, increased water transparency favors the growth of other algae, such as chlorophytes, since they have higher growth rates than cyanobacteria (Scheffer and van Nes 2007). This fact justifies the seasonal alternation of *P. agardhii* dominance in the dry season and chlorophytes in the rainy season, since the rains may have favored the increased luminosity in the Apipucos reservoir.

The results of the grazing experiments varied seasonally, showing control over phytoplankton biomass in both periods, but more efficiently in the rainy season. Even though the reduction of phytoplankton biomass was not significant in the dry period, *M. spinosa* was able to significantly reduce phytoplankton growth rates in this period, showing its potential to control algal biomass even with cyanobacterial dominance. Although *M. spinosa* did not present food selectivity, it mainly consumed the most abundant morpho-functional groups, especially IV. Group III showed high biomass in the dry period; however, the cladoceran was not able to significantly reduce its biomass, despite reducing the size of the filaments. On the other hand, in the rainy season, when group III had

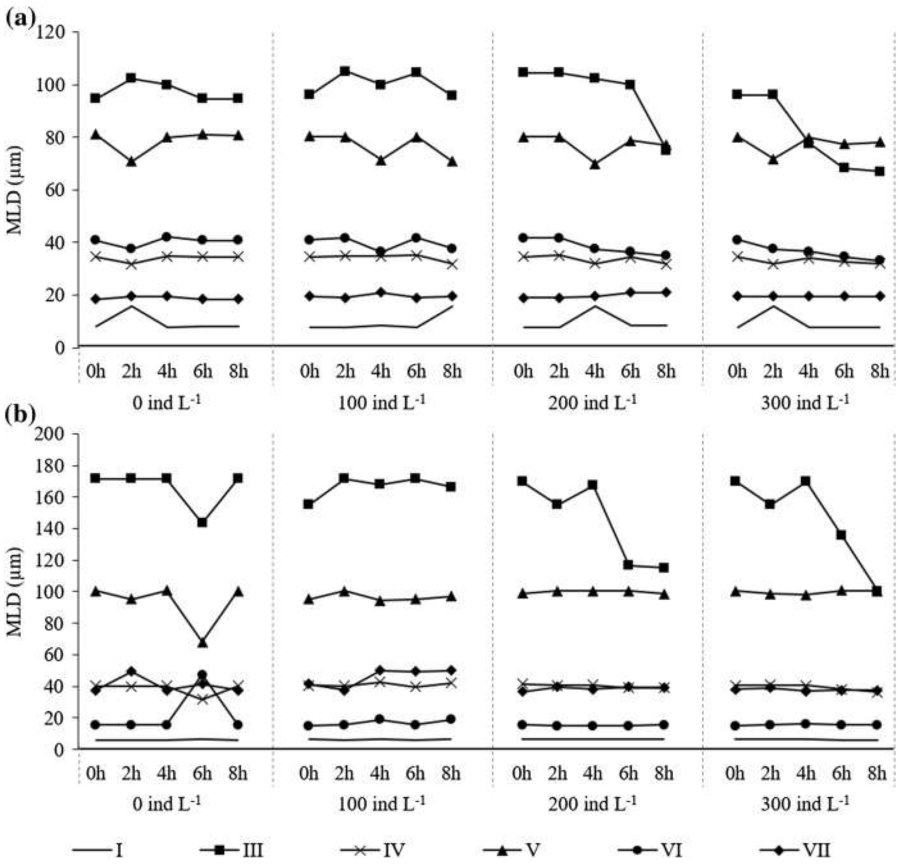

Fig. 4 Maximum linear dimension (MLD) of the morpho-functional phytoplankton groups from the Apipucos reservoir submitted to the grazing experiments with *Macrothrix spinosa* at different densities in dry (**a**) and rainy (**b**) seasons

low biomass values, the cladoceran was able to reduce the biomass of this group in all treatments.

During the dry season, the dominance of cyanobacteria of the group III made its ingestion difficult by *M. spinosa*, which did not significantly reduce the phytoplankton biomass or the biomass of group III. Such reduced top-down control in this period may be associated with the defenses of cyanobacteria against zooplankton predation, such as the potential toxicity, unpalatability, and morphology (Ger et al. 2014, 2016). Several cyanobacteria can produce cyanotoxins, such as hepatotoxins and neurotoxins, which can function as a defense against zooplankton predation (Rohrlack et al. 2005). In addition, deficiency in sterols and polyunsaturated fatty acids make cyanobacteria a poor source of nutrition for zooplankton (Martin-Creuzburg and von Elert 2009). Morphology is also a limiting factor for the consumption by zooplankton since the large size of the filaments and colonies of cyanobacteria can clog and damage the filtration apparatus of the cladocerans (Fulton and Paerl 1987; DeMott et al. 2001).

For filamentous cyanobacteria with aerotopes (group III), which showed greater lengths, the cladoceran was able to reduce their biomasses in both periods, although not intensely in the dry period. This reduction in biomass was favored by decreasing the size of the filaments, which decreased the mean biovolume of the species and consequently the biomass. These results show that *M. spinosa* can cut the large filaments and consume the smaller parts, this mechanism, despite reducing the average biovolume of the species, can also increase the density of filaments, since the larger filaments are divided into two or more parts, as a result of the cutting performed by zooplankton. Such results agree with other experimental studies (e.g., Kâ et al. 2012), which also

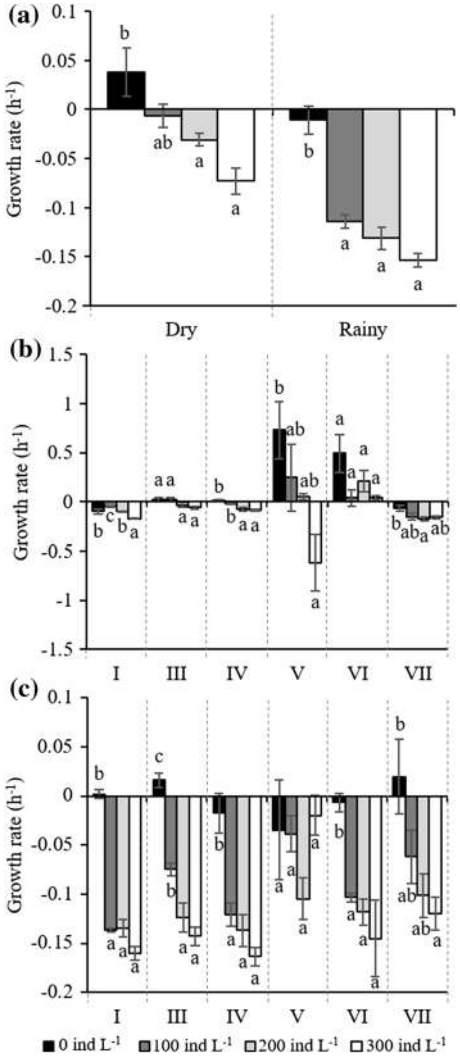

Fig. 5 Phytoplankton growth rates during the grazing experiments by the cladoceran *Macrothrix spinosa* under the total phytoplankton community from the Apipucos reservoir in the dry and rainy seasons (**a**) and morpho-functional phytoplankton groups in the dry (**b**) and rainy (**c**) seasons. Bars represent means and standard errors. Different letters (a, b, and c) represent significant differences between the treatments (Tukey: $p < 0.05$)

showed a size reduction for filamentous cyanobacteria under zooplankton grazing pressure.

Filamentous and colonial cyanobacteria are known to present low palatability to zooplankton due to their low nutritional quality (Kâ et al. 2012). Specifically for *Microcystis aeruginosa* (Kützing) Kützing (group VII), the large size of the colonies may be a factor that prevents predation by zooplankton, which prefers consuming smaller colonies (Panosso et al. 2003). In the Apipucos reservoir, the *M. aeruginosa* colonies presented small size (maximum linear dimension around 20 µm), falling within the sizes that are consumable by zooplankton; so, their biomasses were reduced in the dry period.

The morpho-functional group IV showed a significant reduction in its biomass in both seasonal periods. This group is represented by chlorophytes without structures that may prevent their ingestion by the zooplankton, as thorns and mucilage, which makes them susceptible to predation (Kruk et al. 2010; Colina et al. 2016). In addition, species belonging to group IV are usually medium-sized and considered a good nutritional source (Sterner and Esler 2002), which favored their consumption by *M. spinosa*. Similarly, group V, represented by flagellated organisms, also constitutes a great nutritional source and are medium or large sized (Kruk et al. 2010). However, the mobility and large size of some species may hinder the ability of zooplankton to capture and ingest this group (Reynolds 1997). Such characteristics may have prevented the ingestion of group V by *M. spinosa*, which was not able to reduce the biomass of this group in both seasonal periods.

Regarding group I, small organisms with a high surface–volume ratio, even being highly susceptible to predation by zooplankton (Kruk et al. 2010), can recover its biomass quickly due to efficient uptake of nutrients and high growth rates (Sommer et al. 2003; Colina et al. 2016), which justifies the absence of predation of this group in the dry season. Similarly, group VI, represented by diatoms, was also not efficiently predated by *M. spinosa*, which may have been due to seasonal changes in the size of the group, composed of species of larger linear dimension in the dry period when compared to the rainy season, as *Ulnaria ulna* (Nitzsch) Compère, which may have hindered the ingestion of the cladoceran. Diatoms generally present an average susceptibility to zooplankton predation (Colina et al. 2016), which is mainly influenced by the presence of silica in their frustules that prevent their ingestion by the zooplankton (Van Donk et al. 2011).

The zooplankton community of tropical eutrophic environments is commonly dominated by small cladocerans or rotifers, which justifies the absence of top-down control in tropical regions, since phytoplankton in these regions often present dominance of large-sized filamentous and colonial cyanobacteria, preventing the consumption by zooplankton (Ger et al. 2014). In temperate regions, several studies have shown the potential of zooplankton to control phytoplankton from eutrophic environments, especially those with cyanobacterial blooms (e.g., Ekvall et al. 2014; Søndergaard et al. 2008, 2017). This efficient control is justified by the zooplankton composition in these regions, which is mainly represented by large-sized cladocerans (up to 5 mm), especially *Daphnia* (Sarma et al. 2005).

The zooplankton community of the Apipucos reservoir is composed mainly of small-sized rotifers and omnivorous cyclopoid copepods (Severiano et al. 2017), which are not efficient phytoplankton consumers, providing high algal biomass in this environment. However, with the experiments herein, the addition of cladocerans in different densities, even with medium body size (955.2 µm), reduced the phytoplankton growth in the Apipucos reservoir, especially during the rainy season, with the dominance of chlorophytes.

Conclusions

Macrothrix spinosa efficiently controlled the phytoplankton biomass in the Apipucos reservoir, mainly during the rainy season. Even with no dietary selectivity, *M. spinosa* consumed mostly chlorophytes, in addition to filamentous and colonial cyanobacteria, which potentially form toxic blooms. This cladoceran also reduced the size of the cyanobacteria filaments, facilitating the ingestion of smaller parts. Therefore, *M. spinosa* could potentially be used in biomanipulation strategies to control algal biomass in tropical eutrophic aquatic ecosystems. The seasonal variations in the phytoplankton structure played a fundamental role in the variability of the morpho-functional groups, providing alternations in the dominance of palatable or unpalatable groups for zooplankton. Moreover, the use of morpho-functional groups of phytoplankton accurately reflected the dietary preferences of the cladoceran, as well as the levels of susceptibility of algae to zooplankton predation.

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Data availability Data, associated metadata, and calculation tools will be made available on request to the corresponding author (ariadne_moura@hotmail.com).

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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Supplementary Table S1

Journal: Aquatic Ecology

Article title: Seasonal variations of morpho-functional phytoplankton groups influence the top-down control of a cladoceran in a tropical hypereutrophic lake

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Table S1. Floristic composition and average biomass (n=12) ± standard deviation (SD±) of the phytoplanktonic species in the Apipucos Reservoir during the grazing experiments performed in the dry and rainy seasons.

Species	Dry period		Rainy period	
	Mean	SD±	Mean	SD±
MBFG I				
Chlorococcum infusionum (Schrank) Meneghini	1.190	0.34	4.357	0.27
Chroococcus minimus (Keissler) Lemmermann	0.016	0.01	0.000	0.00
Chroococcus minutus (Kützing) Nägeli	0.122	0.07	0.051	0.00
Crucigenia fenestrata (Schmidle) Schmidle	0.010	0.01	0.046	0.00
Crucigeniella crucifera (Wolle) Komárek	0.063	0.03	0.558	0.08
Tetraëdron regulare Kützing	0.002	0.01	0.021	0.00
Tetraëdron trigonum (Nägeli) Hansgirg	0.009	0.00	0.001	0.00
Tetrastrum elegans Playfair	0.037	0.03	0.024	0.01
MBFG III				
Cylindrospermopsis raciborskii (Woloszynska) Seenayya & Subba Raju	0.019	0.03	0.099	0.02
Planktothrix agardhii (Gomont) Anagnostidis & Komárek	7.908	0.44	0.012	0.01
MBFG IV				
Actinastrum hantzschii Lagerheim	0.371	0.08	0.358	0.01
Ankistrodesmus falcatus (Corda) Ralfs	0.019	0.02	0.045	0.01
Coelastrum microporum Nägeli	0.018	0.02	0.006	0.00
Desmodesmus communis (E.Hegewald) E.Hegewald	0.000	0.00	0.008	0.01
Desmodesmus denticulatus (Lagerheim) SSAn, T. Friedl & E.Hegewald	0.007	0.01	0.061	0.01
Desmodesmus opoliensis (P.G.Richter) E.Hegewald	0.014	0.02	0.419	0.02
Desmodesmus protuberans (F.E.Fritsch & M.F.Rich) E.Hegewald	0.058	0.06	0.068	0.01
Scenedesmus quadricauda (Turpin) Brébisson	0.073	0.02	0.045	0.00
Geitlerinema amphibium (C.Agardh ex Gomont) Anagnostidis	1.034	0.25	0.626	0.02
Kirchneriella lunaris (Kirchner) Möbius	0.499	0.03	1.403	3.92
Merismopedia tenuissima Lemmermann	1.486	0.07	0.734	0.05
Micractinium bornhemiense (W.Conrad) Korshikov	0.000	0.00	0.009	0.01
Micractinium pusillum Fresenius	0.032	0.03	2.136	0.57
Monoraphidium arcuatum (Korshikov) Hindák	0.390	0.13	0.003	0.00
Monoraphidium griffithii (Berkeley) Komárková-Legnerová	0.177	0.08	0.370	0.02
Pediastrum duplex Meyen	0.250	0.87	9.595	2.91
Pediastrum tetras (Ehrenberg) Ralfs	0.087	0.16	0.196	0.06
Scenedesmus acuminatus (Lagerheim) Chodat	0.281	0.09	0.073	0.03
Scenedesmus arcuatus (Lemmermann) Lemmermann	0.132	0.07	0.152	0.02
Treubaria schmidlei (Schröder) Fott & Kovácik	0.017	0.03	0.035	0.01
MBFG V				
Lepocinclis acus (O.F.Müller) B.Marin & Melkonian	0.932	0.74	0.002	0.00
Lepocinclis ovum (Ehrenberg) Lemmermann	0.423	0.83	0.000	0.00
Phacus longicauda (Ehrenberg) Dujardin	0.010	0.02	0.404	0.14
Phacus tortus (Lemmermann) Skvortzov	0.011	0.02	0.000	0.00
Synura sp.	0.055	0.19	0.195	0.04
MBFG VI				
Aulacoseira granulata (Ehrenberg) Simonsen	0.027	0.04	0.470	0.15
Cyclotella meneghiniana Kützing	0.090	0.08	0.010	0.01
Cyclotella sp.	0.723	0.93	0.546	0.06
Ulnaria ulna (Nitzsch) Compère	0.213	0.53	0.000	0.00
MBFG VII				
Botryococcus sp.	0.053	0.10	1.642	0.43
Microcystis aeruginosa (Kützing) Kützing	1.336	0.09	0.000	0.00
Sphaerocystis planctonica (Korshikov) Bourrelly	0.000	0.00	0.312	0.03

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Allelopathic effects of the aquatic macrophyte *Ceratophyllum demersum* L. on phytoplankton species: contrasting effects between cyanobacteria and chlorophytes

Efeitos alelopáticos da macrófita aquática *Ceratophyllum demersum* L. sobre espécies fitoplanctônicas: efeitos contrastantes entre cianobactérias e clorófitas

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Abstract: Aim: To assess the allelopathic effects of the submerged macrophyte *Ceratophyllum demersum* on four strains of phytoplankton species: two cyanobacteria (*Microcystis aeruginosa* - microcystin producing and *M. panniformis* - non-microcystin producing), and two chlorophytes (*Ankistrodesmus falcatus* and *Raphidocelis subcapitata*). **Methods:** A coexistence experiment between *C. demersum* and the four strains was carried out for six days, with eight treatments and three replicates. The strains were cultivated in ASM1 culture medium, under controlled laboratory conditions. Two treatments were assigned for each strain, one with 6 $g.L^{-1}$ of the macrophyte, and the control without the plant. Biomasses and growth rates of the strains were evaluated every two days, which were compared through the T-test and two-way ANOVA, respectively. **Results:** The results varied among the strains, with toxic *M. aeruginosa* being intensely inhibited by *C. demersum*, with a decrease of 99.5% in its biomass ($p<0.001$), while non-toxic *M. panniformis* was less affected by the allelochemicals, with a reduction of 86.2% ($p<0.001$). *Ankistrodesmus falcatus* delayed its growth when in coexistence with the macrophyte, decreasing its biomass in 50.4% ($p<0.01$), while *R. subcapitata* was not altered ($p>0.05$). In coexistence with *C. demersum*, *M. aeruginosa* exhibited the lowest growth rates (-0.65 d^{-1}), followed by *M. panniformis* (-0.15 d^{-1}), *A. falcatus* (0.19 d^{-1}), and *R. subcapitata* (0.34 d^{-1}), with significant differences between all strains ($p<0.001$). *Microcystis aeruginosa* presented higher inhibition rates than *M. panniformis* ($p<0.001$), as well as, *A. falcatus* was more inhibited than *R. subcapitata* ($p<0.05$). **Conclusions:** The presence of microcystins could influence the allelopathic responses of *C. demersum*, that may release more allelochemicals in coexistence with toxic strains of *M. aeruginosa*. Accordingly, *C. demersum* can be used in biomanipulation strategies to control toxic and non-toxic cyanobacterial blooms, without damaging other phytoplankton species, like chlorophytes.

Keywords: allelopathy; biomanipulation; coexistence experiments; control of cyanobacteria; submerged macrophytes.

Resumo: Objetivo: Avaliar os efeitos alelopáticos da macrófita submersa *Ceratophyllum demersum* sobre quatro cepas de espécies fitoplanctônicas: duas cianobactérias (*Microcystis aeruginosa* - produtora de microcistinas e *M. panniformis* - não produtora) e duas clorófitas (*Ankistrodesmus* *falcatus* e *Raphidocelis subcapitata*). **Métodos:** Foi realizado um experimento de coexistência entre *C. demersum* e as quatro cepas, durante seis dias, com oito tratamentos e três réplicas. As cepas foram cultivadas em meio ASM1, sob condições laboratoriais controladas. Foram designados dois tratamentos para cada cepa, um com 6 g.L^{-1} da macrófita, e um controle sem a planta. Biomassas e taxas de crescimento das cepas foram avaliadas a cada dois dias e comparadas pelo teste-T e ANOVA two-way, respectivamente. **Resultados:** Os resultados variaram entre as cepas, sendo a cepa tóxica de *M. aeruginosa* intensamente inibida por *C. demersum*, com uma redução de 99,5% na sua biomassa (p<0,001), enquanto a cepa não tóxica de *M. panniformis* foi menos afetada pelos aleloquímicos, com uma redução de 86,2% (p<0,001). *Ankistrodesmus falcatus* retardou seu crescimento em coexistência com a macrófita, reduzindo sua biomassa em 50,4% (p<0,01), enquanto que *R. subcapitata* não foi afetada (p>0,05). Em coexistência com *C. demersum*, *M. aeruginosa* apresentou as menores taxas de crescimento (-0,65 d^{-1}), seguida de *M. panniformis* (-0,15 d^{-1}), *A. falcatus* (0,19 d^{-1}) e *R. subcapitata* (0,34 d^{-1}), com diferenças significativas entre todas as cepas (p<0,001). *Microcystis aeruginosa* apresentou maiores taxas de inibição que *Microcystis panniformis* (p<0,001), bem como, *A. falcatus* foi mais inibida que *R. subcapitata* (p<0,05). **Conclusões:** A presença de microcistinas pode afetar as respostas alelopáticas de *C. demersum*, que pode liberar mais aleloquímicos em coexistência com cepas tóxicas de *M. aeruginosa*. Portanto, *C. demersum* pode ser utilizada em estratégias de biomanipulação para controle de florações de cianobactérias tóxicas e não tóxicas, sem causar danos às demais espécies fitoplanctônicas, como as clorófitas.

Palavras-chave: alelopatia; biomanipulação; experimentos de coexistência; controle de cianobactérias; macrófitas submersas.

1. Introduction

In the last few decades, the rising temperatures linked to an excessive input of nutrients in the water bodies have supported the occurrence of cyanobacterial blooms (Kosten et al., 2012; Paerl & Otten, 2013). These blooms have become a frequent global problem for the public supply reservoirs, which can be composed of species that produce cyanotoxins, such as hepatotoxins, neurotoxins, and dermatotoxins (Wiegand & Pflugmacher, 2005). Among the most frequent and harmful cyanobacteria, the genus *Microcystis* presents bloom records in 108 countries, 79 of which report the production of microcystin (Harke et al., 2016).

Microcystins are the most extensively studied cyanotoxins in the world, which are considered the most frequent and lethal. Therefore, the World Health Organization (WHO) and the Brazilian Ministry of Health established a tolerable limit of 1.0 μg.L^{-1} of microcystins in waters destined for public supply (Chorus & Bartram, 1999; Brasil, 2011). These regulations were created after the "Tragedy of Caruaru", known worldwide as the largest case of human poisoning by microcystins, in which 76 renal patients died after using water contaminated with microcystins at a hemodialysis clinic in Caruaru, Pernambuco, in 1996 (Carmichael et al., 2001). In the Brazilian Semiarid region, the occurrence of microcystin-containing cyanobacteria blooms is still more recurrent (Bittencourt-Oliveira et al., 2014; Lorenzi et al., 2018), which is certainly due to the climatic and eutrophication conditions of the water bodies in this region that favor the occurrence and establishment of these blooms (Moura et al., 2018).

One potential solution for the control of cyanobacterial blooms includes the use of submerged aquatic macrophytes as a biomanipulation alternative since they can efficiently decrease phytoplankton growth (Zuo et al., 2012). These plants help to maintain clear conditions in shallow lakes (Scheffer et al., 1993, 2003; Hilt & Gross, 2008). Their mechanisms of action are related to the reduction of nutrient concentrations in the water column, which is essential for phytoplankton growth; reduction of sediment resuspension; and supply of refuge to zooplankton and macroinvertebrates (Scheffer et al., 1993; Mulderij et al., 2007), which are efficient phytoplankton consumers (Amorim et al., 2019). In addition, these plants can also release allelochemicals in the water, acting on the inhibition of planktonic and epiphytic algae (Erhard & Gross, 2006; Hilt & Gross, 2008).

Several studies have shown the allelopathic potential of aquatic macrophytes on cyanobacteria and microalgae in laboratory studies, with submerged macrophytes being more efficient (Mohamed, 2017). In this context, cyanobacteria are more sensitive to allelochemicals when compared to chlorophytes (Körner & Nicklisch, 2002; Erhard & Gross, 2006; Zhu et al., 2010). However, Chang et al. (2012) pointed out that *Myriophyllum verticillatum* L. is able to inhibit the growth of *Microcystis aeruginosa* (Kützing) Kützing in pure cultures, however, this cyanobacterium is stimulated when co-cultivated with the green algae *Desmodesmus armatus* (Chodat) E.Hegewald. Besides, Švanys et al. (2016) showed that non-toxic strains of *M. aeruginosa* are more sensitive to tannic acid, an allelochemical isolated from aquatic macrophytes. However, Amorim (2017) found that when coexisting, toxic strains of *Microcystis* are more affected by submerged macrophytes, as the stress caused by the microcystins promotes a greater release of allelochemicals by aquatic plants.

Considering the high occurrence of cyanobacterial blooms in the Brazilian Semiarid region, in addition to the expectation of increasing of the blooms for the coming years due to climate change and eutrophication, more studies aiming to control cyanobacterial blooms using aquatic macrophytes are needed. Thus, the aim of this study was to evaluate the allelopathic potential of the aquatic macrophyte *Ceratophyllum demersum* L. on the growth of cyanobacteria species, with one toxic and another non-toxic strain, and chlorophytes to understand the role of allelochemicals on different phytoplankton species.

2. Material and Methods

2.1. Phytoplankton organisms, submerged macrophyte and culture conditions

During the experiments, four strains of phytoplankton species were used: two cyanobacteria and two chlorophytes. The strain of *M. aeruginosa* (NPLJ-4) was obtained from the cyanobacteria culture collection at the Laboratory of Ecophysiology and Toxicology of Cyanobacteria, Federal University of Rio de Janeiro. In previous experiments with the same conditions of the present study, this strain was found to produce four variants of microcystins, mainly [D-Leu^{1}] microcystin-LR, with about 90% of the total microcystins, in addition to three other unknown variants (for details of toxin detection by High-Performance Liquid Chromatography see Amorim et al., 2017). A strain of *M. panniformis* Komárek et al. (BCCUSP29) was provided by the Brazilian Cyanobacteria Collection at the University of São Paulo and does not produce microcystins (Bittencourt-Oliveira, 2003). The chlorophyte strains, *Ankistrodesmus falcatus* (Corda) Ralfs (BMIUFRPE-01) and *Raphidocelis subcapitata* (Korshikov) Nygaard et al. (BMIUFRPE-02), were obtained from the Culture Collection of Microalgae from the Federal Rural University of Pernambuco – BMIUFRPE.

These strains were cultivated in ASM1 nutrient medium (Gorham et al., 1964), in a climatic chamber with controlled temperature (25 °C ± 1.5), light intensity (40 µmol photons m^{-2}.s^{-1}), pH (7.5), photoperiod (12 h), and the cultures were homogenized three times a day. The cultivations were kept until a biomass of approximately 50 mg.L^{-1} for cyanobacterial strains and 5 mg.L^{-1} for chlorophyte strains, during the exponential growth phase. These culture conditions were tested at the laboratory with higher growth for all tested phytoplankton species (e.g. Amorim, 2017; Amorim et al., 2017, 2019).

Young and photosynthetically active plants of *C. demersum* were collected from the Carpina Reservoir (latitude 7°53’08” S, and longitude 35°20’42” W), municipality of Lagoa do Carro, Pernambuco, Brazil. After collection, the plants were washed several times with a soft brush and distilled water jets to remove sediment, epiphyte microalgae, and zooplankton/zoobenthos, and cultivated in 8 L aquaria containing tap water, which was renewed weekly. Subsequently, they were cultivated in an aseptic and climatized room under the same conditions described for the culture of the strains, with constant aeration.

2.2. Experimental design

The coexistence experiment was carried out in an aseptic climatized room with the same conditions previously described for the culture of the strains. Eight treatments were used, each one with three replicates, totaling 24 experimental units, consisting of 1,000 mL Erlenmeyer flasks filled with 500 ml of ASM1 cultivation medium. The coexistence treatments consisted of the cultivation of each strain with the addition of a young and apical branch of *C. demersum* to achieve biomass of 6.0 gFW.L^{-1} (g of fresh weight per liter). Three days before the beginning of the experiment, the plants were washed five times with ultrapure water to remove algae and adhered animals and were subsequently kept in ASM1 medium for acclimatization. The control treatments consisted of the cultivation of each strain without *C. demersum* branches.

The experiment lasted six days and samples were taken to quantify the biomass of the strains every two days. For each sampling day, 2 mL aliquots were taken, which were fixed with 4% formalin for further density determination (cells.mL^{-1}), by counting cells in a Fuchs-Rosenthal chamber. At least 400 cells per sample were counted to obtain a 90% reliability degree (Lund et al., 1958). Then, the biomass (mg.L^{-1}) was determined by multiplying the density by the average biovolume of the strains, using geometric formulas proposed by Sun & Liu (2003).

2.3. Growth and inhibition rates

Growth rates (μ) were calculated according to Wood et al. (2005): $\mu\ (d^{-1}) = (\ln(N_t) - \ln(N_{t0})) / t - t0$, where N represents the biomass values on the sixth day of the experiment (t) and at the initial time (t0). The inhibition rate (IR%) was calculated as follows: $IR\% = ((Nm - Nc) / Nc) \times 100$; where N_m represents the biomass of the strains in the coexistence treatments with the macrophyte in each replicate, and N_c represents the mean biomass of the strains in the controls.

2.4. Statistical analyses

Significant differences in the biomasses of the strains were verified by the T-test between the treatments of coexistence and controls, for each strain and day of the experiment. Before the analyses, the data were tested for normality by the Shapiro test. A two-way ANOVA was performed to compare the differences in the growth rates between the species and the treatments. For that, the data were tested for normality and homoscedasticity using the Kolmogorov-Smirnov and Bartlett tests, respectively. The inhibition rates were compared between the strains separately for cyanobacteria and for chlorophytes by the T-test. Statistical analyses were performed in the R program, with a level of significance set at $p<0.05$ (R Core Team, 2016).

3. Results

3.1. Effects of C. demersum on the biomass of the strains

The submerged macrophyte *C. demersum* induced varied responses between the strains, both for the coexistence treatments with the cyanobacteria strains (producer and non-producer of microcystins), as well as with the chlorophytes. The biomass of the toxic *M. aeruginosa* strain was inhibited from the second day of coexistence with *C. demersum* to the end of the experiment, reaching a biomass close to zero on the sixth day (Figure 1a). However, the non-toxic strain of *M. panniformis*

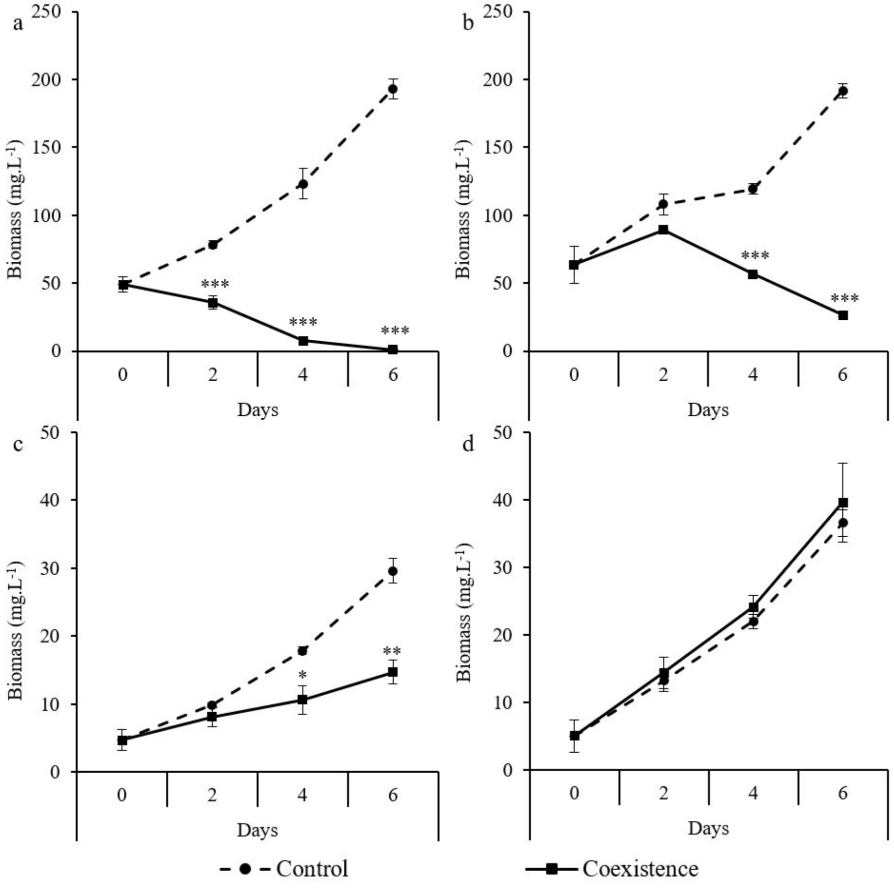

Figure 1. Biomasses of the strains of toxic *Microcystis aeruginosa* (a), non-toxic *M. panniformis* (b), *Ankistrodesmus falcatus* (c), and *Raphidocelis subcapitata* (d), submitted to the treatments of coexistence with *Ceratophyllum demersum* and control. Lines are the means and standard error. Significant differences between the coexistence and control treatments for each day are represented by asterisks (T-test, $^{*}p<0.05$; $^{**}p<0.01$; $^{***}p<0.001$).

showed a less pronounced inhibition, with reductions in growth from the fourth to the sixth day of the experiment (Figure 1b). Both chlorophyte strains were less affected in relation to cyanobacteria. The strain of *A. falcatus* presented a delay in its growth, showing biomass lower than the control from the fourth day (Figure 1c). On the other hand, the strain of *R. subcapitata* was not affected by *C. demersum* in coexistence, with biomass similar to the control during all experiment (Figure 1d).

3.2. Effects of C. demersum on the growth and inhibition rates of strains

Both strains of *Microcystis* showed similar growth rates in the control treatment, with the non-toxic strain of *M. panniformis* presenting lower values (0.18 d^{-1}) in relation to the toxic *M. aeruginosa* (0.23 d^{-1}). In coexistence with *C. demersum*, both strains presented negative values, and showed a significant reduction in growth, being more pronounced in the toxic strain (-0.65 d^{-1}) than in the non-toxic strain (-0.15 d^{-1}). Both strains showed significant differences in the growth rates for the coexistence and control treatments (p <0.001) (Figure 2).

Both chlorophyte strains showed positive growth rates when co-cultivated with *C. demersum* and in the control. The strain of *A. falcatus* presented a significantly lower growth rate in the coexistence (0.19 d^{-1}), when compared to the control (0.31 d^{-1}) (p <0.05). In contrast, *R. subcapitata* did not show significant differences in the growth rates between the control (0.33 d^{-1}) and coexistence (0.34 d^{-1}) treatments (p >0.05) (Figure 2).

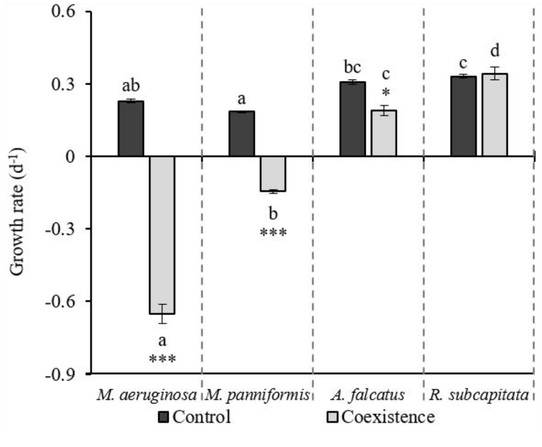

Figure 2. Growth rates of the strains of toxic *Microcystis aeruginosa*, non-toxic *M. panniformis*, *Ankistrodesmus falcatus*, and *Raphidocelis subcapitata*, submitted to the treatments of coexistence with *Ceratophyllum demersum* and control. Bars are the means and standard error. Different letters represent significant differences between the strains for each treatment (two-way ANOVA, $p < 0.05$). Significant differences between the coexistence and control treatments for each strain are represented by asterisks (two-way ANOVA, *p<0.05; ***p<0.001).

The growth rates of the strains showed significant differences between the treatments (F = 569.7, p <0.001) and the species (F = 316.7, p <0.001). In the control, both strains of cyanobacteria showed similar growth, as well as both chlorophyte strains. All tested strains presented different growth rates when in coexistence with *C. demersum*, with *M. aeruginosa* presenting lower values, followed by *M. panniformis*, *A. falcatus*, and *R. subcapitata* (Figure 2).

When evaluating the inhibition rates, the high sensitivity of the tested cyanobacteria to allelochemicals of *C. demersum*, especially the toxic strain of *M. aeruginosa*, is evident, while the strains of chlorophytes were less sensitive. The toxic strain of *M. aeruginosa* was inhibited in 99.5%, reaching biomass close to zero on the sixth day of the experiment in the coexistence treatment with *C. demersum*, while the non-toxic strain of *M. panniformis* was inhibited in 86.2%. The chlorophyte strains were less affected by the allelochemicals of *C. demersum*. *Ankistrodesmus falcatus* was inhibited only in 50.4% when in coexistence with the macrophyte, while *R. subcapitata* was stimulated in 8.3%. The inhibition rates of the strains showed significant differences between the strains for both cyanobacteria (t = 24.89, p <0.001) and chlorophytes (t = 3.43, p <0.05) (Figure 3).

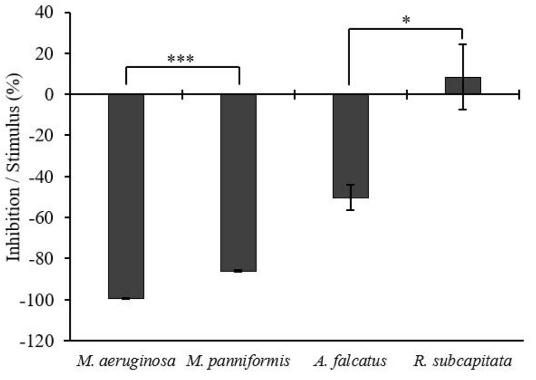

Figure 3. Inhibition rates of the strains of toxic *Microcystis aeruginosa*, non-toxic *M. panniformis*, *Ankistrodesmus falcatus*, and *Raphidocelis subcapitata*, submitted to the treatments of coexistence with *Ceratophyllum demersum* and control after six days. Bars are the means and standard error. Significant differences between the inhibition rates for both cyanobacteria strains and both chlorophyte strains are represented by asterisks (T-test, *p<0.05; ***p<0.001).

4. Discussion

The macrophyte *C. demersum* was able to allelopathically inhibit the growth of both cyanobacterial strains, more specifically the toxic *M. aeruginosa*, in relation to the non-toxic *M. panniformis*. In contrast, chlorophyte strains were less affected in coexistence with *C. demersum*, demonstrating lower sensitivity to the allelochemicals of this macrophyte. Nakai et al. (1999) also demonstrated the potential of this macrophyte in inhibiting allelopathically species of cyanobacteria, including *M. aeruginosa*. In addition, Dong et al. (2014) showed that *C. demersum* can alter the structure of phytoplankton community from a eutrophic lake, promoting the dominance of chlorophytes, as well as inhibiting the growth of *M. aeruginosa* and benefiting the restoration of water quality for the lake.

Allelopathy can be observed in both terrestrial and aquatic plants. In aquatic ecosystems, submerged and floating plants, in addition to algae, play a primordial role in the local dynamics (Pflugmacher, 2002). In these environments, allelopathy occurs in all groups of macrophytes and algae, including cyanobacteria, and its effects are usually negative for other living organisms, commonly inhibiting the growth and photosynthesis of their competitors (Żak et al., 2012). However, Li et al. (2016) emphasize that, in addition to allelopathy, other factors, such as competition for light and nutrients, can give to the macrophytes a greater advantage in relation to cyanobacteria.

Allelopathically active macrophytes can be used in the restoration process of eutrophic aquatic environments, since these plants can control algal growth, especially of cyanobacteria (Gross et al., 1996; Ghobrial et al., 2015). Recently, many studies aiming to restore the water quality in eutrophic environments have been performed, especially using submerged macrophytes as a biomanipulation strategy (e.g. Dong et al., 2014; Vanderstukken et al., 2014; Yu et al., 2016; Liu et al., 2018). These plants have various mechanisms of action in these environments, particularly through the release of allelopathic compounds (Scheffer et al., 1993; Gross et al., 1996).

However, most studies conducted in laboratory use extracts of aquatic plants or purified allelochemicals (e.g. Li et al., 2016; Gao et al., 2017; Švanys et al., 2016), which often exceed the concentrations released by aquatic macrophytes in natural environments (Nakai et al., 1999). Therefore, studies about coexistence between aquatic macrophytes and phytoplankton more accurately reflect the reality of aquatic environments, as they elucidate other mechanisms involved in the inhibition of phytoplankton, such as competition for nutrients, light, or mechanical interference of plants.

According to Mohamed (2017), more than 40 aquatic macrophytes can inhibit phytoplankton species, and most studies have been developed using cyanobacterial strains, in particular, *M. aeruginosa* (e.g. Chen et al., 2012; Zhu et al., 2014; Gao et al., 2017). Other studies show that cyanobacteria are more sensitive to allelochemicals from aquatic macrophytes, followed by chlorophytes (Körner & Nicklisch, 2002; Erhard & Gross, 2006), as verified in the present study. The main mode of action of allelochemicals on cyanobacteria is the inhibition of photosystem II, through damage caused to the electron transport chain during photosynthesis, in addition to oxidative stress (Leu et al., 2002; Zhu et al., 2010; Gao et al., 2017).

During the experiments, the toxic strain of *M. aeruginosa* presented a higher inhibition by *C. demersum* when compared to the non-toxic strain of *M. panniformis*. Few studies show the allelopathic effects of submerged macrophytes on toxic and non-toxic strains, for example, Mulderij et al. (2005) showed that a toxic strain of *M. aeruginosa* was more sensitive to the exudates of *Stratioides aloides* L. than the non-toxic lineage. However, when evaluating the effects of tannic acid on several toxic and non-toxic strains of *M. aeruginosa*, Švanys et al. (2016) verified that non-toxic strains are more sensitive to this allelochemical, showing that something related to the synthesis of microcystins confers higher tolerance to toxic strains.

However, these studies were carried out by using purified exudates or allelochemicals, and in coexistence, it is possible that other factors contribute to the allelopathic responses of aquatic macrophytes over toxic and non-toxic strains. For example, when studying the allelopathic effects of *Egeria densa* Planch. on the same strains of *Microcystis* used in the present study, Amorim (2017) found that the microcystin-producing strain was inhibited by macrophyte allelochemicals, while the non-toxic strain was stimulated. Therefore, the presence of microcystins may act as a stress factor for aquatic macrophytes, which are stimulated to release a greater amount of allelochemicals in the medium that are toxic to *Microcystis* (Amorim, 2017). This justifies the greater sensitivity of the toxic strain of *M. aeruginosa* in the present study, which may have been exposed to a greater amount of allelochemicals than the non-toxic strain.

Several studies have shown the adverse effects of microcystins on submerged macrophytes. For example, Amorim et al. (2017) showed that the co-cultivation of *E. densa* with the same strains of *Microcystis* from the present study caused serious damage to the plant when exposed to toxic strains. This damages caused by the toxic strain was the reduction of the plant length and biomass, inhibition of the emission of shoots and roots, alteration in the content of photosynthetic pigments and oxidative stress, with a higher production of malondialdehyde and greater activity of enzymes catalase, superoxide dismutase and ascorbate peroxidase, while none of these alterations were verified in the co-cultivation with the non-toxic strain of *M. panniformis* and in the control (Amorim et al., 2017). Other studies also showed the adverse effects of microcystins or extracts of cyanobacterial blooms containing these toxins on the growth and physiological performance of *C. demersum* (e.g. Pflugmacher, 2004; Romero-Oliva et al., 2014, 2015a, b).

Unlike in Amorim (2017), the non-toxic strain of *M. panniformis* was not stimulated but showed a delay in growth during the initial days of the experiment. However, the reduction in biomass was less pronounced than in the toxic strain of *M. aeruginosa*, since the absence of microcystins did not stimulate the plant to release allelochemicals. However, at the end of the experiment, this strain was inhibited, with a negative growth rate, since the plant may have started releasing allelochemicals in response to nutrient limitation caused by the non-toxic strain, or the presence of other compounds such as lipopolysaccharide.

Both strains of chlorophytes were less affected from the beginning of the experiment by *C. demersum*. This fact can be justified by the absence of toxins, which did not stimulate the plant to release potentially toxic allelochemicals. In addition, some studies report the lower sensitivity of chlorophytes to allelochemicals of aquatic macrophytes, correlated to their physiology and adaptations to these compounds (Hilt & Gross, 2008; Zhu et al., 2010).

In conclusion, the submerged macrophyte *C. demersum* inhibited both strains of cyanobacteria tested, being more markedly for the toxic *M. aeruginosa* than the non-toxic *M. panniformis*. Certainly, the greatest inhibition activity in the toxic strain was due to an intense release of allelochemicals by *C. demersum* in the treatment with this strain, since the presence of microcystins may have stressed the plant, resulting in higher production of allelochemicals. However, the chlorophyte strains were less affected in relation to cyanobacteria, showing delays in growth and demonstrating low sensitivity to the allelochemicals of aquatic macrophytes. In this sense, *C. demersum* could efficiently control toxic and non-toxic cyanobacterial blooms, without causing adverse effects to other phytoplankton organisms, such as chlorophytes.

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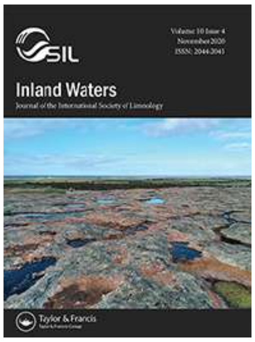

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Advances in limnological research in Earth's drylands

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EDITORIAL

Advances in limnological research in Earth's drylands

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ABSTRACT
Drought is an important driver of change in aquatic ecosystems and generally acts as a filter to select biological traits capable of persisting under severe environmental circumstances. Drylands are highly vulnerable to climate change and increases in climate variability. Consequently, an increase in the processes of eutrophication, salinization, and habitat desiccation may be observed in response to the loss of the hydrological connectivity. The International Network on Limnology of Drylands (INLD) was created with the overall goal of understanding the functioning and conservation status of aquatic ecosystems in drylands globally. INLD was made official in 2016 through the International Society of Limnology. Its objectives are to (1) assess the current state of biological diversity in dryland aquatic ecosystems, (2) evaluate the multiple environmental stressors acting in drylands, and (3) develop models to predict effects of global change on drylands. This special issue, *Limnology of Drylands*, consists of 10 manuscripts focused on the effects of environmental pressures, including global warming, on the biodiversity and distribution of aquatic communities in drylands.

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arid; biodiversity; climate change; dry-subhumid; hyper-arid; multiple stressors

Introduction

More than one-third of the human population lives in areas where there are drylands, and these areas cover more than 41% of Earth's land surface, including hyper-arid, arid, semiarid, and dry-subhumid areas (Millennium Ecosystem Assessment 2005). According to recent estimates, drylands may increase to 56% of the land surface by the end of the 21st century (Huang et al. 2016). This increase may cause several ecological and social problems, as well as climate change, affecting research, management, and policy decisions concerning drylands (Reynolds et al. 2007).

Several types of aquatic ecosystems are included in dryland regions, such as permanent and intermittent rivers, lakes, and rock pools, among others. Considering their elevated species richness and the high rate of endemism, aquatic ecosystems in semiarid and dry-subhumid areas are among the most biodiverse regions in the world (Gudka et al. 2014). They are strongly influenced by irregular rainfall patterns, where the spatial–temporal variability of precipitation increases with the level of aridity, influencing management and conservation practices (Williams 2000). Semiarid regions are considered more vulnerable to climate change, and some predictions show that an increased aridity trend is occurring more intensely in semiarid areas, as a function of a gradual decrease in precipitation and soil moisture, and increased drought frequency (Ji et al. 2014, Huang et al. 2016). This tendency, as well as rapid human population growth in dryland regions, will lead to an enhanced rate of desertification and increased vulnerability of aquatic ecosystems to deterioration (Rotenberg and Yakir 2010, Huang et al. 2016). Prolonged and extreme drought events have already threatened the biodiversity of dryland ecosystems and disrupted trophic interactions (Ledger et al. 2013).

Temporary lakes of dryland areas have different hydrological regimes, maintaining dry phases that can last a few days or months, with drying duration crucial for the establishment and maintenance of biodiversity (Naselli-Flores and Barone 2012). Frequent water level reductions, owing to prolonged droughts, pose a threat to systems through eutrophication and salinization in addition to leading to the proliferation of harmful algae (Brasil et al. 2016, Lins et al. 2016, Rocha Junior et al. 2018).

Intermittent rivers are the main type of lotic ecosystem in drylands, composing about half of the global

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river network (Datry et al. 2014). Although these rivers have been considered to have low species richness, the increased habitat heterogeneity and terrestrial connectivity associated with the drying of the main channel is a source of biodiversity and consequently compensates for the decreased lotic biodiversity (Larned et al. 2010). Rock pools on the riverbed can act as aquatic refuges for species not adapted to desiccation in drylands (Sheldon et al. 2010). Nevertheless, intermittent rivers in dryland areas are fragile and threatened by climate change, land-use changes, and water abstraction (Datry et al. 2014).

This special issue of Inland Waters (Limnology of Drylands) focuses on the effects of climate change and environmental pressures (naturally and anthropogenically driven) on the biodiversity and structure of aquatic communities in several dryland regions around the world.

International Network on Limnology of Drylands (INLD): highlights, hypotheses, and targets

Historically, human population growth in dryland areas has led to chronic ecosystem degradation, often expressed by severe reductions in the quality and quantity of water resources. Global warming will cause severe and irreversible impacts on Earth's ecosystems (IPCC 2014). Empirical observations for the period of 1948–2008 show that the world's drylands have expanded and will continue to expand by the end of the 21st century (Feng and Fu 2013). For arid regions, the major expansion will take place over southwest North America and northern and southern Africa and Australia, and in semiarid regions it will occur in northern Mediterranean lands, southern Africa, and North and South America (Feng and Fu 2013). These shifts will increase water shortages and biodiversity loss at local, regional, and global scales and reduce ecosystem resilience to natural disturbances, leading to unprecedented changes in freshwater ecosystem services (e.g., McNeely 2003, Baho et al. 2017). Thus, the combined effects of global dryland expansion and increasing demand for water resources will magnify extreme droughts events in hyperarid, arid, semiarid, and dry-subhumid regions (Feng and Fu 2013).

In the face of these threats, INLD aims to understand how environmental stressors (i.e., water level reduction, eutrophication, salinization, and extreme climate events) affect community composition, distribution, and dynamics of dryland aquatic ecosystems. One of the main predictions is that climate change (e.g., rainfall reduction, water level reduction, and extreme drought events) and local human impacts (e.g., habitat destruction, deforestation, pollution, water abstraction, and land-use change) will synergistically change hydrological regimes, leading to a permanent state of low biodiversity and resilience in dryland aquatic ecosystems. The first steps of INLD are to elucidate how climate and anthropically driven environmental change affect drylands based on 3 assessments: (1) the current state of aquatic biological diversity; (2) how multiple environmental stressors affect aquatic communities; and (3) the development of predictive models for dryland aquatic ecosystems (Fig. 1).

Droughts have previously been considered a pulse disturbance of high intensity and, because of climate change, they can now be considered a "ramp disturbance," increasing in severity through time (Lake 2011). This ramp disturbance is likely to magnify the effects of eutrophication and salinization, among other impacts, selecting species best adapted to severe conditions. The assessment of these effects will require biological indicators (e.g., Chessman 2015). Thus, research on the life history of saline and freshwater species and their tolerance to prolonged drought, and other impacts, may help us to understand their biology and develop predictive models about their resilience and persistence of species in dry habitats (e.g., Chessman 2015, Kouba et al. 2016, Sarremejane et al. 2019).

Despite the large extension of dryland zones around the world, knowledge on the diversity of species and functioning of the temporary and permanent ecosystems is poorly distributed, creating gaps and preventing decisions regarding conservation plans in these areas. The persistence of the aquatic phase of waterbodies in dryland areas will decrease, causing direct mortality of the biota, leading to a reduction in the taxonomic and functional species richness. The gaps in knowledge and the increasing human and climate warming impacts on dryland aquatic ecosystems are the leading causes of failures in restoration practices, unraveling with ongoing decreases in ecosystem and organism resilience.

Contributions

Space-for-time substitution approach

Long-term data series are important tools to answer ecological and evolutionary questions that need broad spatial and temporal monitoring, especially when dealing with episodic and variable processes, such as extreme droughts and flood events (e.g., Dodds et al. 2012). The lack of temporal information (i.e., long-term data series) promotes serious misjudgments that can interfere not only with attempts to understand and predict changes but also with efforts to manage environments (Magnuson 1990).

On an evolutionary scale, the length of the dry phase is a critical filter, selecting biological traits (Matthews

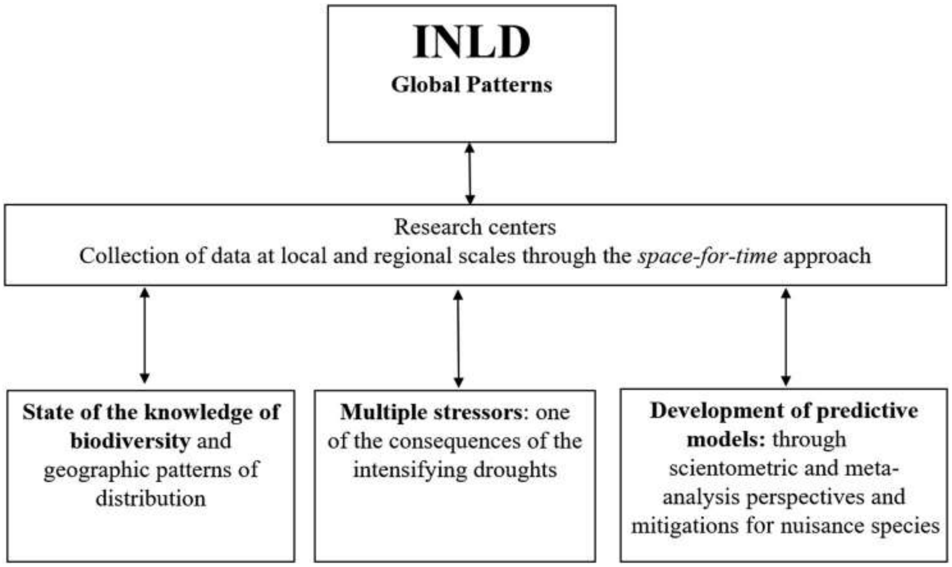

Figure 1. Schematic presentation of the International Network on Limnology of Drylands (INLD) indicating global patterns and the functioning of biodiversity in dryland freshwater ecosystems.

and Marsh-Matthews 2003). Species adaptations can be considered an ecological phenomenon, favoring physiological and morphological adjustments that amplify the competitive ability and compensate for severe changes occurring in dryland aquatic ecosystems (Williams 1985). Some morphological and physiological characteristics, in addition to some adaptations of organisms, can be associated with their ability to proliferate during the filling period and in other phases of the hydroperiod. Among these features, hatching, viability of resting eggs, high dispersal capacity of the adults, short life cycle, and generalist strategies enabling adaptation when habitat changes occur are more important for aquatic communities, such as zooplankton (Williams 1996).

Nevertheless, long-term studies assessing drought effects in temporary waterbodies are generally lacking, resulting in gaps in knowledge about the ecological memory of these ecosystems. Changes in the frequency and predictability of droughts caused by human activities and climate variations can be considered an “invisible place” (Magnuson 1990) or “invisible space” where the predictive potential is low (Swanson and Sparks 1990). In both cases, the absence of evolutionary and historical perspectives can produce results that range from highly uncertain to inconclusive.

Desiccation of rivers or wetlands can generate barriers and hinder biotic flows during drought (Bond and Cottingham 2008). During the wet season, however, the increased water level in these systems enhances their hydrological connections (Larned et al. 2010). With climate change, drylands are expected to experience extreme drought episodes, increasing habitat isolation, and hindering river flows and connections (Bonada et al. 2006).

Some metaphors may be used as a resource to show the relevance of the long-term studies in comprehending events with high interannual variability or high spatial variation, such as ice cover or drought events. Among these metaphors, the invisible present and the invisible place may indicate the relevance of historical databases in multiple and broad scales, preventing errors concerning data interpretation in the short term (Magnuson 1990, Swanson and Sparks 1990) and allowing distinction between natural and human-induced ecological events (Larson et al. 2007). The space-for-time substitution approach has been used to infer past or future trajectories of ecological systems (Blois et al. 2013). Patterns or trends in space are used to predict temporal changes, such as the role of macrophytes in shallow lakes in different climate zones (Meerhoff et al. 2007) or the effects of rainfall reduction on abiotic and biotic variables (Blois et al. 2013, Costa et al. 2019). From this insight, INLD aims to develop 3 main approaches: site monitoring (see Supplementary Material), implementation of experiments, and paleolimnological investigations.

Some contributions in this special issue address monitoring at a broad spatial scale to understand the distribution and abundance patterns of species and communities (e.g., Ilhéu et al. forthcoming 2020, Lanfranco et al. 2020, Silva et al. 2020a, 2020b). Other contributions use the space-for-time substitution approach for ecosystems located at different latitudes to explain the distribution of organisms along environmental gradients. In this context, Lanfranco (2020) investigated the influence of morphometric and geographic factors on the structure and composition of plant communities in 56 temporary freshwater rock pools in the Maltese Islands, Europe. In summary, the most influential predictors of community composition varied across the landscape suggesting that pool "location" can be an important determinant of community structure and composition because pool morphometry would favor some functional forms and species over others.

Submerged macrophytes play a crucial role in increasing habitat and food webs complexity in lakes (Manatunge et al. 2000, Thomaz and Cunha 2010), controlling nuisance species (Hilt and Gross 2008) and working as shelter and feeding sites for fish and zooplankton species (Jeppesen et al. 1997, Meerhoff et al. 2006). However, the vulnerability of these communities to global warming has not yet been clarified (Jeppesen et al. 2014). One of the gaps in ecosystem understanding for tropical arid and semiarid areas is the influence of submerged macrophytes on the attributes of algal communities, mainly concerning the colonization of periphytic algae on substrates, control of phytoplankton biomass, and maintenance of clear-water state (Hilt and Gross 2008). In this special section, Silva et al. (2020b) indicated that the morphological and architectural differences between the charophyte genera *Chara* and *Nitella* may induce differences in the colonization by periphyton in 19 freshwater ecosystems in the semiarid region of Brazil, indicating that the colonization process may be driven by the structure of the substrate, which selects different life forms and species.

State of knowledge of biodiversity and biogeographic patterns in dryland zones

Spatial patterns induced by drought

Aquatic ecosystems in dryland regions can be classified according to the length of their hydroperiod (Williams 2006). Droughts, as an unpredictable and unstable forcing, can favor resilient biota well adapted to deal with water loss following more predictable periods (Brock et al. 2003, Williams 2006). Therefore, aquatic fauna and flora with favorable physiological and morphological features will be better able to compensate for severe changes in these ecosystems (Williams 1985).

The length of the drought phase is a critical factor influencing the abundance and composition of species (Williams 2006). Intermittent flow may induce a fragmented distribution of resources and communities (Leibold et al. 2004). Farias et al. (2020) demonstrated that nonconnected temporary pools in dryland river segments and streams can be important for regional diversity under drought conditions. For some larger fish species, larger pools during periods of desiccation provide better environmental conditions (Ilhéu et al. 2020). Fish broodstock is assumed to migrate up tributaries when desiccation of waterbodies or reservoirs flushing begins, but broodstock may also bury itself in river sand/mud (Kolding et al. 2016) allowing its survival during unfavorable seasons.

Some studies showed the successful recruitment of fish in intermittent rivers even in dry years (Silva et al. 2020a). Therefore, despite the unpredictable regime, species from dryland rivers tend to have a rapid population recovery following droughts because of higher fecundity (Mallen-Cooper and Stuart 2003, Silva et al. 2020a), earlier breeding, and drought-resistant eggs (Williams 2006).

Multiple stressor analysis

The wide typological diversity of organisms from the humid areas in drylands can be considered hotspots sustained by the provision of environmental services (Williams 2006). Temporary wetlands are the most supersaturated in carbon dioxide (CO_2) among the aquatic ecosystems in the global carbon budget (Holgerson 2015). But, despite the relevance to the carbon fluxes at the local and global scale, temporary wetlands are neglected (Holgerson 2015). For a better understanding of carbon fluxes in drylands, more accurate estimates of the carbon and carbon greenhouse gases (CO_2 and CH_4) are needed. In this regard, Morant et al. (2020) showed that carbon fluxes, which can assist with the application of restoration and management practices in dryland ecosystems.

Climate change is affecting global hydrological cycles and influencing the dynamics of intermittent and temporary aquatic in dryland ecosystems, with severe impacts related to the reduction of water levels and changes in hydroperiod (Landkildehus et al. 2014). Expected reductions in water levels are likely to result in dramatic changes in aquatic communities because of increasing salinity and evapotranspiration, leading to high nutrient levels (Rocha Junior et al. 2018, Costa et al. 2019); however, the critical limit for those biological changes is still unknown. Some hypotheses indicate that Mediterranean (Jeppesen et al. 2015) and Australian (Nielsen et al. 2003) dryland lakes may have higher salinity levels in the future, a pattern expected to occur in other dryland regions around the world (Fig. 2).

Zadereev et al. (2020) assessed the conditions of saline lakes in Europe and Central Asia based on the anthropogenic and climate change drivers leading to reductions in water level. This synergy of drivers may be a great

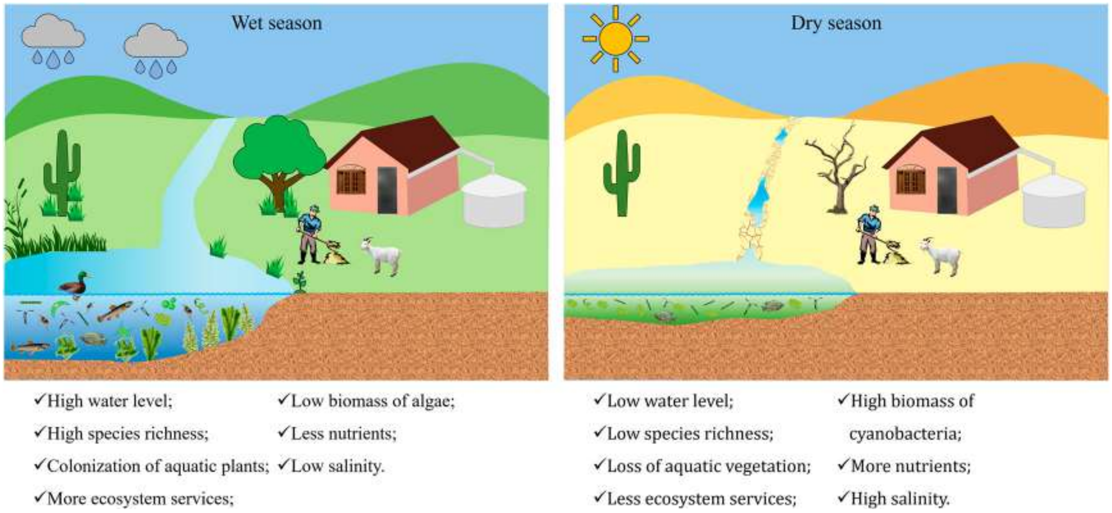

Figure 2. Potential changes in dryland aquatic ecosystems and associated landscape in response to severe droughts and related effects, especially drastic water level reduction of lakes.

challenge to the management and sustainable use of saline lakes in that region.

Hulot et al. (2020) analyzed 30 salt pans in Zimbabwe to show how water level fluctuations can influence the aquatic communities and food web structure by the replacement or loss of species. They found seasonal changes in the food webs associated with the simplification of networks during the dry period. Among the main drivers of this change, a drastic water level reduction induced an increase of potential pollutants, mainly ammonium. In this regard, sublethal concentrations of toxicants (e.g., copper, ammonium, or cyanotoxins) affect traits related to sexual reproduction and diapause egg production, as well as genetic diversity, in several zooplankton species (Hairston et al. 1999, Liang et al. 2018). This point highlights the ecological and evolutionary consequences of the interactive effects of multiple stressors (e.g., pollutants, toxins, higher temperatures) on aquatic biodiversity (Aránguiz-Acuña and Pérez-Portilla 2017).

The transition from a clear to a turbid water state is associated with a decrease in submerged vegetation and changes in the structure of fish communities in shallow lakes (Scheffer et al. 1993, Scheffer and Carpenter 2003). However, droughts can induce a transient state that represents the "ghost of a stable state," keeping the lake in an unstable, clear state most of the time, despite high nutrient concentrations (Scheffer and van Nes 2007). Usually, water level reductions also have negative impacts, causing, for example, an increase of cyanobacterial biomass (Bakker and Hilt 2016), threatening the aquatic biota, enhancing eutrophication, and reducing ecosystem services. Among the main consequences of the severe water level reductions are increased total phosphorus concentrations, salinization, low water transparency, and loss of services (Jeppesen et al. 2015; Fig. 3).

Development of predictive models

Human-induced climate change is predicted to cause both an increase in temperature (resulting in increased evaporation) and a decrease in the amount of precipitation in most arid and semiarid regions worldwide (Ragab and Prudhomme 2002), which will increase aridity, frequency, and intensity of droughts. These shifts in local and regional climate are expected to reduce the flushing waterbodies and alter the local nutrient cycles, potentially resulting in periods of substantial eutrophication (Nielsen and Brock 2009).

In Mediterranean temporary and permanent dryland ponds, studies suggest that the temperature and precipitation patterns determine the structure and dynamics of phytoplankton on a regional scale (e.g., Naselli-Flores and Barone 2012). In these habitats, longer periods of relatively high temperatures and consequent water level reduction favor algal growth (Naselli-Flores and Barone 2005). Indeed, this pattern has also been demonstrated in several constructed lakes in northeast Brazil (e.g., Brasil et al. 2016). Nonetheless, with predictions of a warmer and drier world by the end of the 21st century (IPCC 2014), water resources will probably reduce in quantity and quality, increasing susceptibility to salinization and eutrophication, in addition to favoring cyanobacterial blooms in freshwaters (Jeppesen et al. 2015, Brasil et al. 2016).

In the last decade, global warming, increasingly contaminated waters, and illegal transportation have been the main elements favoring the expansion of invasive species (e.g., cyanobacteria) in arid and semiarid regions

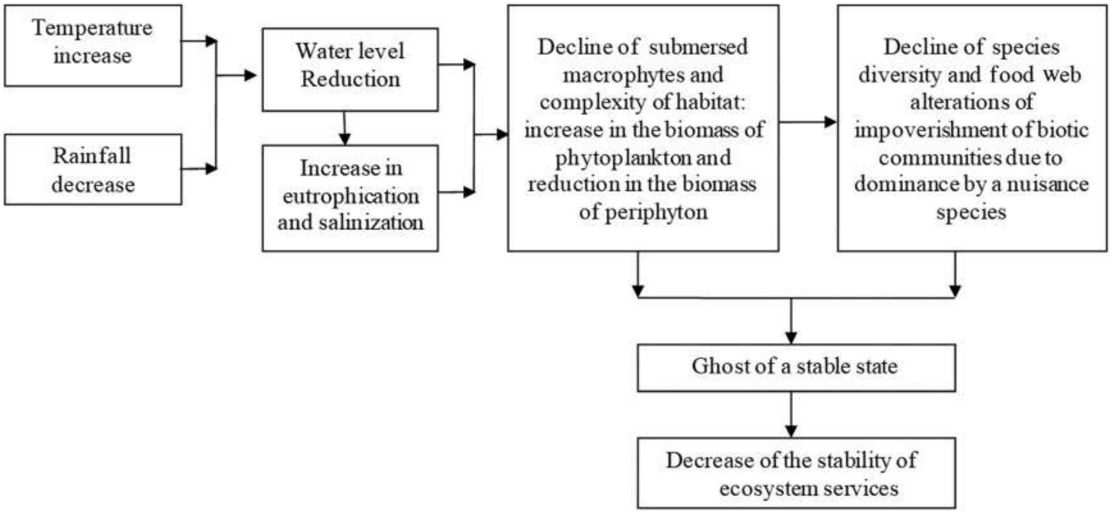

Figure 3. Schematic presentation of changes caused by the extreme water level changes in dry zones.

(e.g. Moura et al. 2018). This trend is expected for the coming years, considering that climate change will intensify the threats (Early et al. 2016), particularly in constructed reservoirs regarded as hotspots for invasive species (Rahel and Olden 2008). Naselli-Flores and Marrone (2019) showed how artificial lakes and ponds in a semiarid Mediterranean island are more susceptible to the establishment of non-native species because these environments are more recent than natural environments and have fewer of biological filters to prevent colonization by invaders. Also, the high number of constructed aquatic ecosystems in dryland regions facilitates invasive species by increasing the surface area for colonization (Naselli-Flores and Marrone 2019).

Predictive models would be useful to understand and implement restoration programs that include the interactive effect of environmental variables and aquatic communities. Recent studies have shown the influence of nutrients, temperature, and food quality on the abundance, composition, and diversity of the zooplankton during a long-term restoration program (Li et al. 2019).

In arid and semiarid zones, some studies have suggested a strong correlation between submerged macrophytes and flood intensity, demonstrating high resistance and resilience in the face of low-impact hydrological disturbances (Maltchik and Florin 2002, Pedro et al. 2006). By contrast, severe flooding may induce low resilience in these communities (Maltchik and Florin 2002). Aquatic communities of the temporary ecosystems are resilient because they can survive disturbances, recovering biomass through attributes that allow proliferation when the area is reflooded (Brock et al. 2003) and persisting through the production of dormant propagules or resistance structures (Brock et al. 2003, Williams 2006).

Over the last few decades, several models have been developed to prevent or reduce the effects of eutrophication (e.g., Mooij et al. 2010). The most widely accepted and applied conceptual model is associated with alternative states in shallow lakes (Scheffer and Carpenter 2003, Scheffer and Jeppesen 2007). These models predict that changes in the abiotic components would have little effect until a nutrient threshold is reached, represented by a rapid and potentially irreversible transition in the trophic structure and resilience of the system (Scheffer and Carpenter 2003). Barbosa et al. (2020) assessed the effects of allochthonous disturbances on phosphorus release in 2 shallow lakes by constructing models using partial least squares path modeling. Those models indicated that the submerged macrophyte cover acted as a stabilizing force, reducing the phosphorus released from the sediments and preventing shifts between the stable states.

Conclusions and perspectives: challenges for INLD

Ongoing climate change and global warming may be magnifying eutrophication and salinization in dryland aquatic ecosystems, owing to more extreme and more frequent drought events. Among the consequences linked to unpredictable and extreme drought events are (1) the advent of nuisance species (i.e., toxic cyanobacteria), (2) reduction of the complexity of the food webs, and (3) loss of ecosystem resilience.

INLD intends to expand the construction of predictive models through scientometric and metanalysis approaches, identifying patterns using already published databases. INLD also intends to address the consequences of climate change to help derive critical limits of water level reductions that do not result in the degradation of biodiversity and ecosystem services (e.g., Meerhoff et al. 2012). The development of initiatives to reduce eutrophication, for example, might help mitigate climate change effects (Moss et al. 2011). Therefore, INLD considers performing simultaneous standardized mesocosm experiments manipulating water level, biological communities, nutrients, and salinity in different dryland zones of the world to support the development of ecological models that infer future trajectories of freshwater systems as a function of rainfall reductions and worsening droughts. This approach may be useful to assess how critical changes in rainfall will affect water quality and freshwater community structure and composition in drylands, as well as to generate knowledge of the main mechanisms regulating those processes and develop strategies to mitigate the expected negative effects.

Under climate change scenarios, patch isolation through habitat drying is likely to occur, increasing vulnerability of rare and endemic species and leading to the loss of biodiversity. Endemic species vulnerability can also be magnified by the introduction of exotic species and eutrophication, which can lead to habitat homogenization. Strategies need to be developed that enhance biodiversity and ecosystem services as well as reduce fragmentation from drought in rivers and wetlands.

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Supplementary material

Advances in Limnological research in Earth's drylands

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Sampling protocol for the International Network on Limnology of Drylands (INLD) initiative

Monitoring of sites

The monitoring program will include lentic and lotic ecosystems in the main dryland regions of the world. A minimum of ten ecosystems will be selected based on their typology, aiming to achieve more diverse types of environments in the gradients of salinity, eutrophication, and temperature, among others. Samples will be taken at least twice a year from each ecosystem, including wet and dry periods.

PROTOCOL OF SAMPLING IN DRYLANDS ECOSYSTEMS (INLD)

Table 1. Label model for samplings of environmental, climatic and biological variables during INLD initiatives.

Identification	
Collector name:	
Contact (phone and email):	
Institution/Department:	
Address/Country:	
Sampling site:	
Sampling date:	
Geographical coordinates:	
Landscape observations:	
Climatic conditions:	
Any further observation:	
Lotic ecosystems	
Stream Order	
Catchment area	
Distance of source	
Water level range	
Lentic ecosystems	
Water level	WordClim (http://www.worldclim.org/)
Depth (m)	Echo-sounder, profundity-meter
Mixing depth (m)	Multiparameter probe at every 0.5 m of profundity
Storage volume (m^3)	Public agencies on water monitoring

Table 2. Environmental, climatic and biological variables, including units, methods, equipment and preservation substances for samples during INLD initiatives.

Environmental variables			
Variables	**Unity**	**Method/equipment**	
Water transparency	m	Secchi disk	
Water temperature	ºC	Multiparameter probe	
Dissolved oxygen	mg L^{-1}		
pH	-		
Redox potential	mV		
Electrical conductivity	µS cm^{-1}		
Total dissolved solids	mg L^{-1}		
Salinity	mg L^{-1}		
Turbidity	NTU		
Alkalinity	mgCaCO$_3$ L^{-1}	APHA (1998)	
Dissolved carbon	mgC m^{2}		
Total phosphorus	µg L^{-1}		
Total dissolved phosphorus	µg L^{-1}		
Total nitrogen	µg L^{-1}		
Total dissolved nitrogen	µg L^{-1}		
Chlorophyll *a*	µg L^{-1}		
Climatic variables			
Variables	**Unity**	**Method/equipment**	
Air temperature	ºC	Near meteorological stations	
Precipitation	mm		
Humidity	%		
Solar irradiation	kJ m^{-2}		
Wind speed	m s^{-1}		
Hydroperiod	Days	Sum of the days with precipitation	
Biotic communities			
Communities	**Type of sample**	**Equipment**	**Preserved**
Phytoplankton	Qualitative	Plankton net with a 25 µm mesh	4% formalin
	Quantitative	Plastic amber bottles	1% Lugol
Zooplankton	Qualitative/ Quantitative	Plankton net with a mesh size of 60 µm	4% formalin
Diatoms	Qualitative/Quantitative	Brushing at least 5 stones	4% formaldehyde solution
Macroinvertebrates	Qualitative/ Quantitative	Petersen grab sampler (0.0345 m^{2})	70% alcohol

Fish	Qualitative/ Quantitative	Gill and throw nets with a mesh size of 5, 10 and 15 mm	10% formalin and 70% alcohol
Aquatic macrophytes	Quantitative	1 x 1 m^2 squares in transects	-
	Qualitative	Taxonomic analyzes	-

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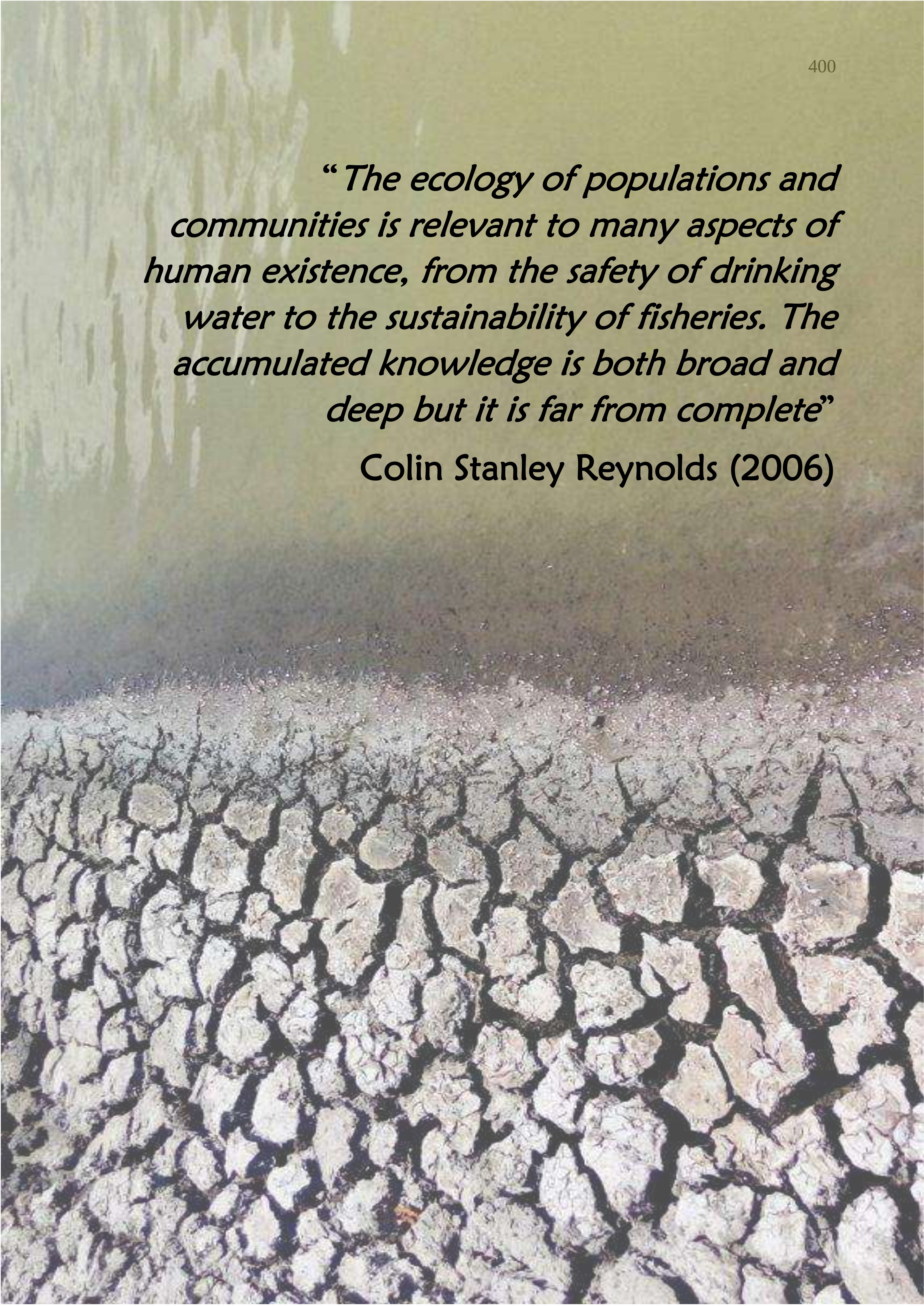

> “*The ecology of populations and communities is relevant to many aspects of human existence, from the safety of drinking water to the sustainability of fisheries. The accumulated knowledge is both broad and deep but it is far from complete*”
>
> Colin Stanley Reynolds (2006)