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**Dissertação de Mestrado**

**ESTRUTURA ESPACIAL DO FITOPLÂNCTON EM LAGOS RASOS  
SUBTROPICAIS: ABORDAGEM COMPARATIVA SOB UMA PERSPECTIVA  
TAXONÔMICA E FUNCIONAL**

**LUCAS VINICIUS STELA**

**Porto Alegre, março de 2020**

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Lucas Vinicius Stela

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*“Nada é impossível para uma mente disposta.”*

Livros da Dinastia Han

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## RESUMO

Recentemente, passou-se a aceitar que microrganismos têm padrões biogeográficos e que comunidades microbianas podem ser estruturadas por outros processos, e não apenas por fatores ambientais, como contingência histórica, deriva ecológica e dispersão. Traços morfológicos influenciam diretamente na dispersão, processos metabólicos e interações entre espécies, sendo que dependendo do traço analisado pode-se chegar a distintos efeitos de fatores espaciais (dispersão) e ambientais na estruturação de metacomunidades. Nosso objetivo foi destacar e comparar as diferenças na influência de fatores espaciais e ambientais em uma metacomunidade fitoplanctônica, utilizando uma abordagem taxonômica e diferentes métricas de abordagem funcional, em um sistema de lagos rasos subtropicais. Esperávamos que a influência ambiental e espacial fosse melhor mensurada por métricas funcionais do que pela abordagem taxonômica. Também esperávamos que métricas funcionais compostas por um único traço denotassem uma influência maior dos fatores espaciais e ambientais. Além disso, esperávamos que espécies menores apresentassem maior influência do processo espacial em pequena escala através de *mass effects* (assumindo altas taxas de dispersão, e a distância máxima entre os sistemas de 58 km) do que espécies maiores. Em geral, o fator ambiental foi mais influente na variação da comunidade que o espacial, contudo ambos estruturaram de forma significativa a metacomunidade, com influências que variam de acordo com a abordagem utilizada. A abordagem taxonômica denotou uma maior influência de fatores ambientais que a maioria das métricas funcionais, ainda esta abordagem apresentou significância com fatores espaciais em distintas escalas, apesar das métricas funcionais denotarem maior influência em geral de fatores espaciais. As métricas funcionais de múltiplos traços apresentaram maior influência dos componentes espaciais e ambientais do que as métricas de traços únicos. Maior influência de variáveis espaciais em organismos menores foi evidente em todas métricas funcionais. Em conclusão, para fornecer respostas diretas aos padrões observados na natureza, precisamos avaliar e entender a relevância funcional da métrica utilizada, bem como a relação dos traços com variáveis ambientais e processos de dispersão em estudos de metacomunidade fitoplanctônica.

**Palavras-chave:** Tamanho do organismo, traços funcionais, grupos funcionais, microorganismos, dispersão.



## ABSTRACT

Recently, it has come to be accepted that microorganisms have biogeographic patterns and that microbial communities can be structured by other processes rather than environmental factors, such as historical contingency, ecological drift and dispersion. Morphological traits directly influence dispersion, metabolic processes and interactions between species, depending on the analyzed trait, different effects of spatial (dispersion) and environmental factors can structure metacommunities. Our objective was to highlight and compare the differences in the influence of spatial and environmental factors in a phytoplankton metacommunity, using a taxonomic approach and different metrics of functional approach, in a subtropical shallow lake system. We expected that environmental and spatial influence would be better measured by functional metrics than by the taxonomic approach. We also expected that functional metrics composed of a single trait would denote a greater influence of spatial and environmental factors. In addition, we expected smaller species to have greater influence of the small-scale space process through mass effects (assuming high dispersion rates, and the maximum distance between systems of 58 km) than larger species. In general, the environmental factor was more influential in the variation of the community than the spatial one, however both structured the metacommunity significantly, with influences that vary according to the approach used. The taxonomic approach denoted a greater influence of environmental factors than most functional metrics and showed significance with spatial factors at different scales, although functional metrics denote greater influence in general of spatial factors. The functional metrics of multiple traits showed greater influence of spatial and environmental components than the metrics of single trait. Greater influence of spatial variables on smaller organisms was evident in all functional metrics. In conclusion, in order to provide direct answers to the patterns observed in nature, we need to evaluate and understand the functional relevance of the metric used, as well as the relationship of the traits with environmental variables and dispersion processes in phytoplankton metacommunity studies.

**Keywords:** Organism size, functional traits, functional groups, microorganisms, dispersal.

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## REVISÃO BIBLIOGRÁFICA

### Ecologia de comunidades

A busca por entender qualquer sistema complexo é uma procura por um padrão que reduza a complexidade deste sistema a algumas regras de maior simplicidade (Levin, 1988). A identificação e explicação de padrões de distribuição, abundância e interações entre espécies são os objetivos centrais da ecologia de comunidades (Leibold *et al.*, 2004). Contudo, os padrões observados podem ser explicados por distintos processos que atuam em diferentes escalas espaço-temporais (Levin, 1988; Vellend *et al.*, 2014). Assim, não é surpreendente que os mecanismos e processos que explicam padrões de biodiversidade permaneçam altamente controversos (Zhou & Ning, 2017).

A teoria tradicional de nicho supõe que processos determinísticos, como interações interespecíficas e condições ambientais, governem a estrutura das comunidades (Adler *et al.*, 2007; Zhou & Ning, 2017). Esta teoria se baseia no pressuposto de que as diferenças entre os nichos fundamentais das espécies são grandes o bastante para que a competição ocorra majoritariamente de forma intraespecífica, promovendo a coexistência das espécies (Chesson, 2000; Adler *et al.*, 2007). Em contraste, a teoria neutra assume que são as similaridades entre as espécies, e não as diferenças, que explicam a coexistência e estruturam as comunidades naturais (Hubbel, 2001, Thompson & Townsend, 2006; Adler *et al.*, 2007). Os modelos neutros supõem que os padrões de biodiversidade são explicados por processos estocásticos como taxas demográficas (natalidade, mortalidade, imigração, emigração), extinção, colonização e especiação. Assim, as espécies são tidas como equivalentes em seu *fitness* e influência interespecífica (Adler *et al.*, 2007; Zhou & Ning, 2017).

Apesar das duas teorias terem sido abordadas por muito tempo como mutualmente exclusivas, a concepção atual é de que elas são complementares (Thompson & Townsend, 2006; Adler *et al.*, 2007; Zhou & Ning, 2017), e que o uso conjunto destas provê aos pesquisadores maior poder na explicação de padrões ecológicos. Neste contexto, processos determinísticos são quaisquer processos, não aleatórios, que envolvem seleção de nicho, incluindo filtro ambiental e interações biológicas (Vellend *et al.*, 2014; Zhou & Ning, 2017). Já

processos estocásticos se referem a mudanças aleatórias na estrutura da comunidade, considerando a identidade das espécies e seus traços funcionais (Vellend *et al.*, 2014). Recentemente, Zhou & Ning (2017) propuseram que este termo fosse descrito como “estocasticidade ecológica”, de forma a abranger variações na taxa de natalidade, mortalidade, emigração, imigração, no espaço-tempo e representar a influência de contingência histórica e da deriva gênica (Zhou & Ning, 2017).

Embora muitos mecanismos estruturadores de comunidades sejam altamente relacionados à estocasticidade ecológica ou aos efeitos de nicho (determinismo), a segregação entre eles pode ser, também, ambígua (Adler *et al.*, 2007; Zhou & Ning, 2017). A dispersão, por exemplo, é comumente atribuída a efeitos de estocasticidade ecológica. Contudo, pode ser associada também a processos determinísticos, quando se considera que a capacidade dispersiva das espécies depende de traços funcionais e ou estratégias de vida (Adler *et al.*, 2007; Vellend *et al.*, 2014; Zhou & Ning, 2017). Desta forma, diferentes mecanismos se situam em um espectro determinístico-estocástico, onde a influência relativa de cada processo para explicar os padrões da biodiversidade dependendo da escala espacial e temporal analisada (Vellend *et al.*, 2014).

## **Metacomunidades**

Metacomunidade é o nome dado ao conjunto de comunidades locais conectadas pela dispersão de múltiplas espécies interativas (Leibold *et al.*, 2004). O estudo de metacomunidades é uma recente subdisciplina da ecologia (Heino *et al.*, 2017), onde a dispersão entre comunidades locais é considerada como chave para explicar padrões de biodiversidade a nível regional (Leibold *et al.*, 2004; Cottenie 2005; Winegardner *et al.*, 2012; Heino *et al.*, 2015; Vilmi *et al.*, 2017). A concepção clássica desta teoria foi proposta por Leibold *et al.* (2004), cujo estudo descreve quatro principais paradigmas (*Species sorting*, *mass-effects*, teoria neutra e *patch dynamics*), que podem afetar em conjunto a estrutura das comunidades (Cottenie, 2005; Heino *et al.*, 2015).

O paradigma da teoria neutra se baseia no trabalho de Hubbell (2001), conforme descrito anteriormente. Como, neste paradigma, as espécies são

descritas como ecologicamente equivalentes (Thompson & Townsend, 2006; Adler *et al.*, 2007; Zhou & Ning, 2017), não se espera que efeitos determinísticos exerçam influência nas comunidades locais (Heino *et al.*, 2015). Ainda, como este paradigma é altamente associado à estocasticidade ecológica (Zhou & Ning, 2017), espera-se que a similaridade na estrutura das comunidades decresça com o aumento da distância espacial (Cottenie, 2005; Heino *et al.*, 2015). Em *patch dynamics* assume-se que há um *trade-off* entre colonizadores e competidores, onde ambientes afastados ou recém perturbados são dominados por melhores colonizadores, enquanto os melhores competidores extinguem os colonizadores em locais estáveis (sem deturpações) ou altamente conectados (Leibold *et al.*, 2004; Heino *et al.*, 2015; Vilmi *et al.*, 2017). Já em *species sorting* considera-se que filtros bióticos e abióticos são os principais mecanismos que explicam a coexistência em cada local, supondo que as espécies tenham capacidade dispersiva o bastante para ocorrer nos locais com as condições ambientais adequadas (Cottenie, 2005; Vilmi *et al.*, 2017). Por último, em *mass-effects* o aumento da capacidade dispersiva entre ambientes heterogêneos resulta em populações locais sendo altamente afetadas por relação *source-sink*, onde a existência de determinada espécie em um local desfavorável ao seu crescimento e manutenção é possível apenas pelo aporte contínuo de imigrantes, que sobrepujam os efeitos de nicho (Leibold *et al.*, 2004; Heino *et al.*, 2015; Heino *et al.*, 2017).

Considerando o avanço nos estudos de metacomunidades, e a tendência dos pesquisadores em focar na importância relativa dos quatro paradigmas clássicos (Cottenie, 2005), Winegardner *et al.* (2012) sugeriram que os estudos de metacomunidades deveriam se ater na explicação dos efeitos da dispersão e da seleção de nicho na estrutura da metacomunidade. Para tanto, os autores propuseram que *mass-effects* e *patch dynamics* sejam consideradas como casos especiais de *species sorting*. A partir desta sugestão, Heino *et al.* (2015) propôs que diferentes efeitos de dispersão podem se tornar evidentes em diferentes escalas espaciais, sendo que em escalas espaciais pequenas os efeitos de dispersão podem ser associados a alta dispersão (*mass effects*), enquanto em maiores escalas há efeitos maiores da limitação de dispersão (Heino *et al.*, 2015; Vilmi *et al.*, 2016; Vilmi *et al.*, 2017). Assim, o principal desafio na ecologia de metacomunidades, a partir destas novas concepções, é



separar e explicar a influência dos processos dispersivos em relação à influência do ambiente (*species sorting*) na estrutura da metacomunidade (Lindström & Langenheder, 2012; Heino *et al.*, 2015; Vilmi *et al.*, 2016).

## **Dispersão**

A movimentação de um indivíduo ou propágulo no espaço com potenciais consequências ao fluxo gênico e à (re)colonização de novos habitats é chamada de dispersão (Sharma *et al.*, 2007; Naselli-Flores *et al.*, 2016; Zhou & Ning, 2017). Este mecanismo é fundamental na ecologia e na evolução, influenciando em taxas demográficas, colonização, especiação, extinção e, em casos especiais, na área de ocorrência biogeográfica da espécie (Lester *et al.*, 2007; Sharma *et al.*, 2007).

As diferenças interespecíficas nas capacidades de dispersão das espécies podem explicar padrões de diversidade e coexistência em nível local e regional (Thompson & Townsend, 2006; Grainger & Gilbert, 2016). As diferentes estratégias dispersivas são o resultado de processos filogenéticos, envolvendo conjuntos de atributos morfológicos, comportamentais, fisiológicos e aspectos bioquímicos (De Bie *et al.*, 2012). Dentro deste contexto, a adaptação das espécies à dispersão passiva e/ou ativa é altamente relevante para explicar padrões biogeográficos (Martiny *et al.*, 2006; Jenkins *et al.*, 2007; Sharma *et al.*, 2007; Grainger & Gilbert, 2016; Naselli-Flores *et al.*, 2016) e de metacomunidades (Cottenie, 2005; De Bie *et al.*, 2012; Lindström & Langenheder, 2012; Tolonen *et al.*, 2018).

A dispersão passiva ocorre quando o organismo ou propágulo é transportado por vetores sob o qual o mesmo não tem controle, como vento, água, e outros animais (Kristiansen, 1996; De Bie *et al.*, 2012; Incagnone *et al.*, 2015; Padisák *et al.*, 2016). Já na dispersão ativa, o organismo seleciona ativamente seu habitat, se deslocando até ele (Jenkins *et al.*, 2007; Tolonen *et al.*, 2018). Ambas formas de dispersão são altamente influenciadas por atributos morfológicos e fisiológicos (Jenkins *et al.*, 2007; De Bie *et al.*, 2012), denotando distintos padrões espaciais. Ainda, a influência da dispersão na estruturação das comunidades depende fortemente da escala espacial da observação e de traços

morfológicos e fisiológicos (Thompson & Townsend, 2006; Ng *et al.*, 2009; Lindström & Langenheder, 2012; Heino *et al.*, 2015; Vellend *et al.*, 2014).

Apesar da importância da capacidade dispersiva dos organismos em estudos ecológicos, este é um dado ainda incipiente para diversos grupos biológicos, em especial para organismos aquáticos e microorganismos (Lindström & Langenheder, 2012; Heino *et al.*, 2015). Por esta razão, a maioria dos estudos em metacomunidades e biogeografia se utiliza de *proxys* para avaliar a influência de processos dispersivos (Heino *et al.*, 2015). Considerando que a capacidade dispersiva é altamente relacionada à distância espacial entre o sítio de emigração e imigração (Kristiansen, 1996), diversos estudos se utilizam de coordenadas geográficas entre comunidades locais para inferir efeitos da dispersão (Ng *et al.*, 2009; De Bie *et al.*, 2012; Heino *et al.*, 2015; Padial *et al.*, 2014; Souffreau *et al.*, 2015; Vilmi *et al.*, 2016; Heino *et al.*, 2017; Vilmi *et al.*, 2017; Tolonen *et al.*, 2018). Contudo, ao se utilizar deste *proxy*, o sinal espacial se torna ambíguo, podendo indicar tanto *mass effects* como limitação de dispersão, além de poder indicar outros fatores ambientais espacialmente estruturados que não foram mensurados (Ng *et al.*, 2009; De Bie *et al.*, 2012; Souffreau *et al.*, 2015). Todavia, alguns pesquisadores demonstraram que é possível aferir o papel da dispersão através do sinal espacial, ao se utilizar de diferentes escalas espaciais (Ng *et al.*, 2009), e/ou traços funcionais como tamanho corpóreo e habilidade dispersiva (Thompson & Townsend, 2006; De Bie *et al.*, 2012; Padial *et al.*, 2014; Tolonen *et al.*, 2018). Neste sentido, em pequenas escalas espaciais, o sinal espacial é associado a *mass effects* (diante da alta dispersão). A medida que se aumenta a escala de observação, *species sorting* pode se tornar evidente, quando a dispersão é ainda viável e há filtro ambiental (Heino *et al.*, 2015). Já em maiores escalas, o filtro ambiental deixa de ser relevante, sendo evidente apenas os efeitos de limitação de dispersão (Heino *et al.*, 2015).

## **Ecologia de microrganismos**

Microrganismos (bactérias, arqueobactérias, fungos, protistas e algas unicelulares) correspondem ao grupo de vida mais diverso do planeta, habitando

quase qualquer ambiente imaginável (Zhou & Ning, 2017), e sustentando processos que são essenciais para a manutenção da vida no planeta (Souffreau *et al.*, 2015). Comunidades de microrganismos demonstram uma complexa variação estrutural no tempo e espaço (Louca *et al.*, 2016). Apesar de sua enorme diversidade e importância, os mecanismos que promovem a variação na estrutura destas comunidades ainda são pouco entendidos (Louca *et al.*, 2016; Zhou & Ning, 2017).

O pequeno tamanho corpóreo e a abundância astronômica de células vegetativas, bem como de propágulos, permite que estes organismos tenham grande potencial de dispersão passiva mesmo em largas escalas geográficas a nível continental e global (Finlay, 2002; Horner-Devine *et al.*, 2007; Sharma *et al.*, 2007; De Bie *et al.*, 2012; Incagnone *et al.*, 2015). Devido a estas características, os microrganismos foram durante muito tempo considerados ubíquos, denotando que sua ocorrência era influenciada apenas por efeitos de nicho (Incagnone *et al.*, 2015; Padisák *et al.*, 2016).

Esta hipótese foi amplamente difundida por Bass-Becking (1934), na clássica frase: “tudo está em todo lugar, mas o ambiente seleciona”, e apoiada por Finlay (2002), o qual propôs que organismos (ou propágulos) com tamanho menor que 1mm não apresentam restrições geográficas, sendo ubíquos (Finlay, 2002). Contudo, a literatura atual aponta fortes críticas a estes trabalhos e suas hipóteses (Sharma *et al.*, 2007; Incagnone *et al.*, 2015; Padisák *et al.*, 2016), indicando o reconhecimento de que muitos microrganismos apresentam padrões biogeográficos apesar de sua elevada capacidade de dispersão passiva (Kristiansen, 1996; Martiny *et al.*, 2006; Horner-Devine *et al.*, 2007; Jenkins *et al.*, 2007; Naselli-Flores & Padisák, 2016; Soininen *et al.*, 2016; Moresco *et al.*, 2017; Ribeiro *et al.*, 2018a). Desta forma, a concepção atual é de que as comunidades de microrganismos são estruturadas por diversos fatores além dos efeitos de nicho, como a contingência histórica, deriva gênica e dispersão (Beisner *et al.*, 2006; Martiny *et al.*, 2006; Vellend *et al.*, 2014; Souffreau *et al.*, 2015; Louca *et al.*, 2016; Zhou & Ning, 2017).

Se utilizando da teoria de metacomunidades e também de biogeografia, diversos estudos buscaram revelar a importância relativa de fatores ambientais e processos dispersivos na estruturação das comunidades de microrganismos. Para bactérias, a maioria dos estudos encontra sinais espaciais significantes,

que são relacionados aos efeitos dispersivos, contudo os fatores ambientais continuam sendo predominantes (Beisner *et al.*, 2006; De Bie *et al.*, 2012; Lindström & Langenheder, 2012; Souffreau *et al.*, 2015; Louca *et al.*, 2016). Já para fitoplâncton e perifíton, a importância relativa de fatores espaciais e ambientais na estruturação destas comunidades se mantém altamente controversa (Huszar *et al.*, 2015; Izaguirre *et al.*, 2015). Alguns estudos indicam a predominância de fatores ambientais na estruturação das comunidades (De Bie *et al.*, 2012; Padial *et al.*, 2014; Huszar *et al.*, 2015; Maloufi *et al.*, 2016; Soininen *et al.*, 2016; Heino *et al.*, 2017; Crossetti *et al.*, 2018; Wu *et al.*, 2018). Enquanto outros estudos apontam que o efeito conjunto dos fatores ambientais e espaciais representa a influência predominante na estruturação destas comunidades (Vilmi *et al.*, 2016, 2017; Feki-Sahnoun *et al.*, 2018), e que a influência relativa de cada fator varia conforme a escala geográfica analisada (Izaguirre *et al.*, 2015; Moresco *et al.*, 2017; Ribeiro *et al.*, 2018b; Xiao *et al.*, 2018). Contudo, há também resultados que indicam a ausência de fatores ambientais e espaciais significantes na estruturação destas comunidades (Beisner *et al.*, 2006). Assim, tem-se que a falta aparente de consenso nos resultados pode ser explicada por diferenças nas escalas geográficas dos estudos, o nível de eutrofização dos ambientes aquáticos analisados, a conectividade e a heterogeneidade ambiental destes locais (Maloufi *et al.*, 2016). Contudo, é evidente que ambos fatores ambientais e espaciais (dispersivos) são influentes na estruturação da comunidade destes organismos.

## **Ecologia funcional aplicada a microrganismos**

O termo “funcional” em ecologia funcional é relacionado ao papel (função) dos organismos dentro de comunidades, o qual por sua vez é definido pelas características (morfológicas, fisiológicas ou fenológicas) dos próprios organismos (Calow, 1987). Estas características podem ser consideradas traços funcionais, se impactarem direta ou indiretamente no *fitness* individual, por efeitos em taxas de crescimento, reprodução e sobrevivência (Violle *et al.*, 2007). Os traços funcionais são o resultado de processos adaptativos dentro da história de vida das espécies (Calow, 1987), sendo que a plasticidade fenotípica destas

espécies permite que cada indivíduo apresente variações de traços funcionais, dependendo do ambiente em que se encontra (Petchey & Gaston, 2006; Violle *et al.*, 2007). Assim, na ecologia funcional a identidade das espécies deixa de ser relevante, sendo considerado apenas as características ou funções de indivíduos no sistema (Marquet *et al.* 2004; Petchey & Gaston, 2006; Adler *et al.*, 2013). Esta abordagem pode aprimorar nossa capacidade de explicar a distribuição de comunidades biológicas em gradientes ambientais, temporais e espaciais, e inferir predições mais acuradas sobre a reorganização destas comunidades frente a mudanças globais (Petchey & Gaston, 2006; Litchman & Klausmeier, 2008; Adler *et al.*, 2013; Soininen *et al.*, 2016; Leruste *et al.*, 2018; Wu *et al.*, 2018; Weithoff & Beisner, 2019). Desta forma, pode-se detectar padrões e processos implícitos, que podem não ser detectáveis em abordagens taxonômicas clássicas (Huszar *et al.*, 2015; Vilmi *et al.*, 2017).

As análises funcionais podem se basear em mensurações diretas de traços funcionais de indivíduos, utilizando este dado na matriz funcional ou se utilizando de um valor médio de traço por espécie (Petchey & Gaston, 2006; Violle *et al.*, 2007; Villéger *et al.*, 2008). Esta forma de análise normalmente é aplicada para aferência de índices de diversidade funcional (vide Villéger *et al.*, 2008), possibilitando também a dedução de *trade-offs* entre traços funcionais (Weithoff & Beisner, 2019). Outra forma de análise amplamente empregada, agrupa espécies com traços funcionais similares em distintos grupos (Marquet *et al.* 2004). Nesta análise, o conceito é de que espécies com traços funcionais similares tendem a responder da mesma forma a alterações ambientais, temporais e espaciais (Marquet *et al.*, 2004; Huszar *et al.*, 2015; Vilmi *et al.*, 2017). Assim, variações de grupos funcionais entre ambientes podem indicar mecanismos estruturadores de comunidades.

Para os microorganismos, diferentes métricas funcionais vêm sendo amplamente aplicadas (Weithoff & Beisner, 2019), havendo uma clara preferência para métricas que utilizam agrupamentos de múltiplos traços funcionais, como os grupos funcionais de fitoplâncton (para uma revisão vide Salmaso *et al.*, 2015) ou como grupos metabólicos para bactérias (ex. Louca *et al.*, 2016). O uso desta forma de análise para o fitoplâncton, remonta a descrição das “formas de vida” e “morfologias funcionais” de Margalef (1978), cujo trabalho serviu como pilar para o desenvolvimento de agrupamentos funcionais (Salmaso

*et al.*, 2015; Weithoff & Beisner, 2019), bem como para o avanço de nosso entendimento sobre a relação de traços morfológicos, fisiológicos e ecológicos, com fatores de crescimento, reprodução e sobrevivência (ex. Weithoff, 2003; Litchman & Klausmeier, 2008; Litchman *et al.*, 2010; Iatskiu *et al.*, 2018; Pančić & Kiørboe, 2018).

## **Grupos e traços funcionais do fitoplâncton**

Fitoplâncton é o conjunto de microrganismos fotossintéticos, adaptados a viver parte ou toda sua vida em suspensão na coluna da água de mares, lagos, poças e rios (Reynolds, 2006). Este termo designa um universo de microrganismos altamente distintos em relação à sua ecologia, reprodução, fisiologia, morfologia e filogenia (Reynolds, 2006; Litchman & Klausmeier, 2008). O fitoplâncton forma a base das redes tróficas aquáticas (Scheffer *et al.*, 2003; Reynolds, 2006; Pančić & Kiørboe, 2018), afeta profundamente o ciclo bioquímico de diversos elementos (Reynolds, 2006; Litchman & Klausmeier, 2008), e é responsável por cerca de 60% da produção primária da Terra (Chisti, 2004). Como a estrutura e a distribuição espaço-temporal do fitoplâncton afeta o funcionamento dos ecossistemas aquáticos e o clima mundial (Litchman & Klausmeier, 2008), os estudos buscando elucidar mecanismos estruturadores destas comunidades são fundamentais.

A ampla gama de variedades de vida que compõem o fitoplâncton é normalmente analisada através da identificação de espécies e posterior classificação em agrupamentos funcionais (Salmaso *et al.*, 2015). Uma das classificações funcionais mais aplicadas ao fitoplâncton é o *Functional Group* (Reynolds *et al.*, 2002; Padisák *et al.*, 2009) a qual se propõem a agrupar espécies de acordo com sua morfologia, fisiologia e características ecológicas (Reynolds *et al.*, 2002; Huszar *et al.*, 2015; Salmaso *et al.*, 2015). Esta classificação é altamente associada a gradientes ambientais (Salmaso *et al.*, 2015), sendo utilizada como base para índices de biomonitoramento (ex. Padisák *et al.*, 2006). Desta forma a aplicação deste agrupamento funcional pode limitar estudos cujas hipóteses ecológicas não se baseiam apenas na influência de efeitos de nicho.

Outro agrupamento funcional chamado de *Morphologically Based Functional Group* (MBFG), se baseia principalmente em traços morfológicos, segregando as espécies de fitoplâncton em sete distintos grupos (Kruk *et al.*, 2010), os quais foram posteriormente ampliados por Reynolds *et al.* (2014), pela adição um oitavo grupo. Como traços morfológicos (volume, máxima dimensão linear, presença de mucilagem, razão superfície/área, etc..) são altamente associados à ecologia do fitoplâncton (Litchman & Klausmeier, 2008; Litchman *et al.*, 2010; Iatskiu *et al.*, 2018; Allende *et al.*, 2019; Weithoff & Beisner, 2019), podendo influenciar na limitação de dispersão (De Bie *et al.*, 2012) e também em mecanismos defensivos (Pančić & Kiørboe, 2018), esta abordagem de agrupamento funcional pode ser utilizada para formulação de hipóteses ecológicas relacionadas a processos que ocorrem associados à escalas geográficas (Salmaso *et al.*, 2015), como a dispersão. Ainda, outro agrupamento funcional altamente relacionado à morfologia, é o grupo de estrategistas de Reynolds (1988, 2006). Esta classificação é uma adaptação do trabalho clássico de estratégias ecológicas de plantas de Grime (1977), o qual segregava as espécies de acordo com respostas a distúrbios e estresse (CSR). Apesar do termo “estratégias de vida” denotar uma forte influência de efeitos de nicho, a classificação de espécies de fitoplâncton nestes grupos é baseada na relação de traços morfológicos, como máxima dimensão linear e da razão superfície/volume (Reynolds, 2006). Desta forma este agrupamento também denota potencial para acessar influências de processos dispersivos.

Além de agrupamentos funcionais pré-estabelecidos, há uma ampla gama de traços funcionais já descritos (Weithoff, 2003; Litchman & Klausmeier, 2008; Litchman *et al.*, 2010; Iatskiu *et al.*, 2018; Pančić & Kiørboe, 2018), dentre os quais destaca-se o tamanho do organismo, que é considerado a característica morfológica mais influente (Litchman & Klausmeier, 2008; Litchman *et al.*, 2010; Iatskiu *et al.*, 2018; Leruste *et al.*, 2018; Weithoff & Beisner, 2019). Um pequeno tamanho celular implica em um metabolismo mais ativo, com eficiência de aquisição de nutrientes, alta taxa reprodutiva, e menor taxa de sedimentação (Reynolds, 2006; Litchman & Klausmeier, 2008; Litchman *et al.*, 2010; Iatskiu *et al.*, 2018). Contudo, os pequenos organismos são mais influenciados por interações biológicas (competição e predação) do que os organismos maiores (para uma revisão em traços de defesa, veja Pančić & Kiørboe, 2018). Ao

contrário, organismos maiores tendem a ter uma menor taxa metabólica, alta capacidade de armazenamento de nutrientes, maior resistência à predação e maior tolerância a variações ambientais (Iatskiu *et al.*, 2018). Além disso, conforme já citado, o tamanho celular é considerado um traço chave na estruturação de metacomunidades, sendo relacionado à habilidade dispersiva (De Bie *et al.*, 2012; Tolonen *et al.*, 2018).

Considerando que diferentes respostas na estrutura da comunidade podem ser obtidas ao se aplicar distintas análises (Huszar *et al.*, 2015; Leruste *et al.*, 2018), a seleção de uma métrica funcional deve ser realizada com cuidado (Weithoff & Beisner, 2019), se baseando na relação dos traços com os fatores estruturadores de comunidades que o estudo objetiva elucidar. Neste quesito, a aplicação de agrupamentos funcionais pode ser prejudicial por nublar a identificação e relação dos traços funcionais com os fatores estruturadores da comunidade. Este é um problema especialmente relevante na interpretação de dados provenientes de agrupamentos formados por múltiplos traços funcionais, uma vez que cada traço tem diversas relações com características morfológicas, fisiológicas e ecológicas, bem como respectivos *trade-offs* (Weithoff, 2003; Litchman & Klausmeier, 2008; Litchman *et al.*, 2010; Iatskiu *et al.*, 2018; Pančić & Kiørboe, 2018).

Como forma de contornar este problema, diversos estudos aplicam e comparam a resposta de distintas métricas funcionais em relação a fatores estruturadores de comunidades (Soininen *et al.*, 2016; Vilmi *et al.*, 2016, 2017; Xiao *et al.*, 2018; Bortolini *et al.*, 2019). Há também estudos que comparam o uso da clássica abordagem taxonômica frente a análises funcionais (Huszar *et al.*, 2015; Wojciechowski *et al.*, 2017; Leruste *et al.*, 2018). Assim, se constroem um arcabouço sobre as potencialidades de aplicação de determinados agrupamentos funcionais na exposição de fatores estruturadores de comunidades, sem necessariamente inferir o papel intrínscio dos traços funcionais os quais formam estes agrupamentos. Considerando que a descrição de múltiplos traços funcionais em relação a respostas ambientais, temporais e espaciais é altamente complexa, pode-se realizar análises apenas com categorias de um único traço funcional (ex. Crossetti & Bicudo 2008; Crossetti *et al.*, 2018; Wu *et al.*, 2018). Apesar de permitirem uma relação direta com o traço funcional, serem relativamente simples de aplicar, e denotarem pouco ou



nenhum conhecimento taxonômico, o uso destas categorizações ainda é incipiente, pela falta de comparações destas análises com as métricas funcionais que se utilizam de múltiplos traços. Desta forma, faz-se necessários estudos comparativos entre estas distintas análises (métricas funcionais que utilizam agrupamentos determinados por mais de um traço funcional x métricas utilizando um único traço funcional), descrevendo as diferentes respostas da comunidade frente às variações ambientais e espaciais.

## OBJETIVO E EXPECTATIVAS

O presente estudo objetiva comparar as diferenças na influência dos fatores espaciais (assumindo dispersão) e ambientais em uma metacomunidade fitoplanctônica, utilizando uma abordagem taxonômica (Sp), métricas funcionais utilizando múltiplos traços (MBFG e CRS) e métricas de um único traço funcional (Volume e máxima dimensão linear), em um sistema de quatorze lagoas subtropicais rasas, no sul do Brasil). Ao fazer isso, esperamos contribuir com a discussão sobre o uso de diferentes abordagens para acessar padrões de biodiversidade em microorganismos, bem como incentivar a interpretação do papel ecológico de traços nas classificações funcionais do fitoplâncton. Esperávamos que a influência ambiental e espacial fosse melhor acessada pelas métricas funcionais do que pela abordagem taxonômica, pois espécies dentro de grupos funcionais (que combinam múltiplos traços) ou categorias (baseadas em traço único) teriam respostas semelhantes à variação ambiental e espacial. Com base nisso, também esperávamos que a métrica funcional de traço único revelasse uma maior influência de fatores ambientais e espaciais do que as grupos funcionais baseadas em múltiplas características. E, finalmente, devido à influência do tamanho da célula na funcionalidade dos organismos, esperávamos que grupos funcionais e categorias relacionadas às espécies menores apresentassem maior influência do processo espacial em pequena escala através de *mass effects* (assumindo altas taxas de dispersão), enquanto grupos relacionados a espécies maiores, mostrariam uma maior influência do ambiente (*species sorting*, assumindo que a dispersão está ocorrendo e as espécies ocorrem em todos habitats adequados), dada a escala geográfica considerada (58 km).

## **ARTIGO**

**Artigo nas normas para publicação no periódico Freshwater Biology.**

### **Title page**

Spatial and environmental influence in phytoplankton metacommunity can be captured differently by functional metrics and taxonomic approach

Keywords: Organism size, functional trait, functional groups, microorganism, dispersal.

## Summary

1. Recently, it has come to be accepted that microorganisms have biogeographic patterns and that microbial communities can be structured by other processes rather than environmental factors, such as historical contingency, ecological drift and dispersion. Morphological traits directly influence dispersion, metabolic processes and interactions between species, depending on the analyzed trait, different effects of spatial (dispersion) and environmental factors can structure metacommunities.

2. Our objective was to highlight and compare the differences in the influence of spatial and environmental factors in a phytoplankton metacommunity, using a taxonomic approach and different metrics of functional approach, in a subtropical shallow lake system. We expected that environmental and spatial influence would be better measured by functional metrics than by the taxonomic approach. We also expected that functional metrics composed of a single trait would denote a greater influence of spatial and environmental factors. In addition, we expected smaller species to have greater influence of the small-scale space process through mass effects (assuming high dispersion rates, and the maximum distance between systems of 58 km) than larger species.

3. In general, the environmental factor was more influential in the variation of the community than the spatial one, however both structured the metacommunity significantly, with influences that vary according to the approach used. The taxonomic approach denoted a greater influence of environmental factors than most functional metrics and showed significance with spatial factors at different scales, although functional metrics denote greater influence in general of spatial factors. The functional metrics of multiple traits showed greater influence of spatial and environmental components than the metrics of single trait. Greater influence of spatial variables on smaller organisms was evident in all functional metrics. In conclusion, in order to provide direct answers to the patterns observed in nature, we need to evaluate and understand the functional relevance of the metric used, as well as the relationship of the traits with environmental variables and dispersion processes in phytoplankton metacommunity studies.

## 1. Introduction

Dispersal is a fundamental process in ecology and evolution, influencing demographic rates, colonization success, speciation, extinction and, in some cases, even in the geographical distribution of species (Lester *et al.*, 2007; Sharma *et al.*, 2007). In microbial ecology, dispersal processes were, until recently, overlooked (Guo *et al.*, 2019), because it was commonly assumed that passive dispersal was so high that microorganism (smaller than 1mm) had no geographical barrier (Finlay, 2002), and that environmental control was the only factor driving communities. However, to the date, it has been accepted that even microorganisms have biogeographical patterns (Kristiansen, 1996; Martiny *et al.*, 2006; Horner-Devine *et al.*, 2007; Jenkins *et al.*, 2007; Souffreau *et al.*, 2015; Naselli-Flores & Padisák, 2016; Soininen *et al.*, 2016; Moresco *et al.*, 2017; Ribeiro *et al.*, 2018a), and that microbial communities may be structured by other processes, rather than environmental factors alone, such as historical contingency, ecological drift and dispersal (Martiny *et al.*, 2006; Vellend *et al.*, 2014; Zhou & Ning, 2017). Therefore, the metacommunity framework, in which dispersal between local communities is considered a key factor to understand and explain regional biodiversity patterns (Leibold *et al.*, 2004; Cottenie 2005; Winegardner *et al.*, 2012; Heino *et al.*, 2015; Vilmi *et al.*, 2017), can be applied in microbial studies (Lindström & Langenheder, 2012; Heino *et al.*, 2015; Padial *et al.*, 2014; Maloufi *et al.*, 2016; Heino *et al.*, 2017; Ribeiro *et al.*, 2018b; Vilmi *et al.*, 2017; Wojciechowski *et al.*, 2017, Wu *et al.*, 2018; Xiao *et al.*, 2018; Bortolini *et al.*, 2019; Guo *et al.*, 2019).

Since dispersal is distant-dependent (Kristiansen, 1996; Ng *et al.*, 2009), it can be expected a larger influence of dispersal limitation over a broad spatial

scale. At smaller scales the high dispersal rates homogenise communities determining a spatial influence which may be related to mass effects (Ng *et al.*, 2009; Heino *et al.*, 2015). At adequate dispersion rates, species can reach localities and are susceptible to the environmental filtering, being sorted to the favorable habitats (species sorting) (Leibold *et al.*, 2004; Heino *et al.*, 2015). However, dismantling the influence of dispersal into limited or high is often not straightforward, because both can be detected as spatial significance in ecological data (Ng *et al.*, 2009). Addressing this problem, some authors have succeeded in extracting the influence of limited and high dispersal in metacommunities using analysis at different spatial scales (Ng *et al.*, 2009), while others managed to directly address the dispersal mechanism through ecological hypothesis based on functional traits and their relation with dispersal processes (Thompson & Townsend, 2006; De Bie *et al.*, 2012; Padial *et al.*, 2014; Tolonen *et al.*, 2018; Guo *et al.*, 2019). However, when dealing with microorganisms, assuming high dispersal rates, the issue now lay on the appropriate biological trait to track the influence of dispersion in the arrangement of these communities.

A functional trait is any morphological, physiological or phenological feature measurable at the individual level, which impacts fitness (Violle *et al.*, 2007). Using traits to group distinct organisms in a functional classification facilitates the understanding of patterns and processes along environmental, spatial and temporal gradients (Litchman & Klausmeier, 2008; Soininen *et al.*, 2016; Leruste *et al.*, 2018; Wu *et al.*, 2018; Guo *et al.*, 2019; Weithoff & Beisner, 2019). This approach may detect underlying patterns that are not explicit when using classic species identification (Huszar *et al.*, 2015; Vilmi *et al.*, 2017). For phytoplankton, the most considered functional traits are the morphological (e.g.

size, form of life, mucilage), the physiological (e.g. myxotrophy, resting stages, nitrogen fixation) and the behavioral traits (e.g. flagella, aerotopes) (Litchman & Klausmeier, 2008). Based on that, several measures and grouping systems have been proposed so far and, depending on the selected traits, some metrics may differently capture community assembly, structure and dynamics, ecosystem function and biogeographic aspects (Guo *et al.*, 2019; Wheithoff & Beisner, 2019). That is the case of the Morphological Based Functional Group system (MBFG) proposed by Kruk *et al.* (2010), and enhanced by Reynolds *et al.* (2014), which groups phytoplankton species into eight groups based on morphological (cell volume, surface area, maximum linear dimension, presence of mucilage and siliceous exoskeletal structure), physiological (presence of heterocytes) and behavioural traits (presence of flagella and aerotopes), and have presented strong relation to environmental variation (Huszar *et al.*, 2015; Salmaso *et al.*, 2015; Xiao *et al.*, 2018; Allende *et al.*, 2019). Another grouping system, much less used nowadays, that may be also highly related to environmental variation, is the life strategy system (CSR – Competitive, Stress-Tolerant and Ruderal strategies) proposed by Reynolds (1988) for the phytoplankton, based on the concepts introduced by Grime (1977) for terrestrial vegetation. Here, the species are sorted into each category based on relations between morphological traits (surface area, volume and maximum linear dimension) which refer to nutrient uptake, light harvesting, growth rates, loss rates (sinking and grazing) in an environmental spectrum of habitat productivity (nutrient availability) and habitat duration (water column mixing depth and euphotic zone). Then, in this perspective, functional process-driven approaches may be useful if correctly applied in the research context, and the misuse of functional classifications, i.e.

ignoring the applicability of the functional approach used and the ecological role of the traits, can lead to serious mistakes in interpreting ecological processes (Salmaso *et al.*, 2015; Weithoff & Beisner, 2019).

As most phytoplankton researchers keep working on explaining responses of different functional metrics on community variation (Soininen *et al.*, 2016; Vilmi *et al.*, 2016, 2017; Xiao *et al.*, 2018; Bortolini *et al.*, 2019), or comparing functional and taxonomic approaches (Huszar *et al.*, 2015; Wojciechowski *et al.*, 2017; Leruste *et al.*, 2018), few have tried to assess community variation using unique functional traits categorizations to test their hypothesis. By doing so, it is possible to unveil the role of species functional traits in the community structuring over environmental and spatial gradients (e.g. Crossetti & Bicudo 2008; Crossetti *et al.*, 2018; Wu *et al.*, 2018; Guo *et al.*, 2019). In these sense, some master traits were highlighted for further ecological tests (Litchman & Klausmeier, 2008; Iatskiu *et al.*, 2018; Weithoff & Beisner, 2019), such as the cell size because of its direct relation to nutrient acquisition, growth rates, reproduction, sedimentation rates, grazing susceptibility (Reynolds, 2006; Litchman & Klausmeier, 2008; Litchman *et al.*, 2010; Iatskiu *et al.*, 2018), biological interactions, such as predation (for a review on the topic see Pančić & Kiørboe, 2018), and dispersal capability (Jenkins *et al.*, 2007; De Bie *et al.*, 2012).

On the phytoplankton metacommunity perspective, recent findings demonstrated that species identification (taxonomic approach) better revealed the spatial processes than the functional grouping systems (Xiao *et al.*, 2018), and that species composition and its functional groups are better shaped by environmental variation (Huszar *et al.*, 2015). Despite comparisons between taxonomic approaches and functional groupings, recent studies have shown that



the influence of spatial and environmental variation in phytoplankton metacommunities may be trait-dependent (Guo *et al.*, 2019). Then, given the lack of studies integrating taxonomic, multiple and unique functional trait grouping in phytoplankton structuring, in the present study our goal was to highlight and compare differences in the influence of spatial (assuming dispersal) and environmental factors in a phytoplankton metacommunity, using a taxonomic approach (Sp), functional grouping systems (MBFG and CSR), and the functional traits volume and maximum linear dimension as size measures, in a fourteen subtropical shallow lakes' system, in south Brazil. By doing so, we hope to contribute with the discussion upon using different approaches to access biodiversity patterns in microorganisms, as well to encourage the interpretation of the ecological role of traits in phytoplankton functional classifications. We expected that both environmental and spatial influence would be better accessed by functional classifications than the taxonomic approach, as species within functional groups (that combine multiple traits) or categories (unique trait, as volume and maximum linear dimension) would have similar responses to environmental and spatial variation. Based on that, we also expected that unique trait functional metric would reveal a higher influence of environmental and spatial factors, than functional matrices based on multiple traits. And finally, due to the influence of the cell size, we expected that functional groups and categories related to the smaller species would present higher influence of spatial process in a small scale through mass effects (assuming high dispersal rates), while groups related to larger species, would show a higher influence of environment (environment filtering, assuming that dispersion is occurring and species will be

sorted to the environmental suitable sites), given the geographic scale considered (58 km).

## **2. Material and Methods**

### **2.1. Study area and sampling**

The study was conducted in 14 coastal lakes located within the Tramandaí River Basin at the northern and medium coast of Rio Grande do Sul – Brazil (Figure 1). These water bodies vary widely in shape, size, and connectivity (Guimarães *et al.*, 2014), but are shallow freshwater lakes formed by oscillations in ocean water levels that occurred between Pleistocene and Holocene (Schwarzbold & Schäfer, 1984; Schäfer *et al.*, 2017). Also, these lakes are highly influenced by northeast and southwest winds (Bohnenberger *et al.*, 2018), in a humid subtropical climate with hot summers (Cfa; Alvares *et al.*, 2013). Furthermore, many of these coastal lakes have been suffering the effects of increasing regional urbanization, promoting a trophic gradient, that can be related to waste water pollution (Pedrozo & Rocha, 2007).

In this study, 14 coastal lakes with distinct trophic status and with maximum linear distance of 58 km between lakes were analyzed in February of 2018, during austral summer period. In each lake, we collected samples for biotic and abiotic analyses at the subsurface of the water column at three sampling stations, comprising equidistance of 10m in the pelagic zone, which amounted 42 sampling points. We also obtained the geographical coordinates at each sampling station using a Garmin Etrex 10 GPS (Table 1).

The abiotic sampling encompasses 23 environmental variables measured both in situ or in laboratory. The temperature of the water (T), electrical conductivity (Cond), dissolved oxygen (DO) and pH were measured in situ by means of a multiparameter probe (Manta 2 Water-Quality; Eureka, Austin, TX, USA). In addition, in field we also measured depth (Depth), water transparency (SD) (using a Secchi disk) and color (Color, i.e. absorbance at 450 nm, using a Digimed DM-COR colorimeter Digimed Instrumentação Analítica, São Paulo, SP, Brazil). Water was sampled for analysis in laboratory of total suspended, fixed and volatile solids (TSS, FSS and VSS, respectively), soluble reactive silicon (Sil), total nitrogen (TN), total dissolved nitrogen (TDN), total ammonia nitrogen (N-NH<sub>3</sub><sup>+</sup>, N-NH<sub>4</sub><sup>+</sup>), nitrate (N-NO<sub>3</sub><sup>-</sup>), nitrite (N-NO<sub>2</sub><sup>-</sup>), total phosphorus (TP) and soluble reactive phosphorus (SRP), following APHA (2012). We also obtained the dissolved inorganic nitrogen (DIN) by summing N-NH<sub>3</sub><sup>+</sup>, N-NH<sub>4</sub><sup>+</sup>, N-NO<sub>3</sub><sup>-</sup>, N-NO<sub>2</sub><sup>-</sup>. Finally, total carbon (TOC), dissolved organic carbon (DOC), dissolved inorganic carbon (DIC) and particulate organic carbon (POC) were evaluated using TOC V equipment (Shimadzu VCPH).

Phytoplankton quantification followed the Utermöhl method (1958) and Lund *et al.* (1958) for settling time and quantification accuracy (95%). We estimated the biovolume (mm<sup>3</sup>.L<sup>-1</sup>) of each species found in the 42 sampling stations, based on measurements of 20 individuals (whenever possible) in each sample, by using geometric solid formulas that were closest to the cellular form (Hillebrand *et al.*, 1999; Fonseca *et al.*, 2014, Calvacante *et al.*, 2018). All phytoplankton species were identified at the lowest taxonomic classification possible, following the available literature (Komárek & Fott, 1983; Castro *et al.*, 1991; Xavier, 1994; Azevedo *et al.*, 1996; Bicudo *et al.*, 2003; Bicudo, 2004;

Araújo & Bicudo 2006; Fernandes & Bicudo, 2009; Hentcheke & Torgan, 2010; Silva *et al.*, 2010; Silva *et al.*, 2013; Bicudo & Menezes, 2017).

## 2.2. Biotic datasets

We used traditional taxonomic composition and four functional metrics based on biovolume data from the 42 samples. The taxonomic composition (Sp) included the estimated biovolume of 169 phytoplankton species registered in all the sampling stations. The functional metrics were divided in multiple trait grouping classifications, and unique trait categories. For the multiple trait grouping system we used MBFG groups according to Kruk *et al.* (2010) and Reynolds *et al.* (2014), where: I – Small organism with high S/V; II – Small flagellated organisms with siliceous exoskeletal structures; III – Large filaments with aerotopes; IV – Organisms of medium size lacking specialized traits; V – Unicellular flagellates of medium to large size; VI – Non-flagellated organisms with siliceous exoskeletons; VII – Large mucilaginous colonies and VIII – Nitrogen fixing cyanobacteria. We also classified phytoplankton species in CSR multiple trait grouping system (Reynolds, 1988, 2006), as: C – Invasive opportunists/competitors; R – Disturbance-tolerant/ruderals; S – Acquisitive/stress-tolerant; and SS – Chronic stress-tolerant. As unique trait categories, we used volume (V), estimated by the mean volume obtained for each species, and the maximum linear dimension (MLD), estimated by the mean greatest linear dimension measured for each species). Then, phytoplankton species were classified in four volume categories, following Crossetti *et al.* (2018), and in four maximum linear dimension categories, as described by Sieburth *et al.* (1978), where: I – volume < 10  $\mu\text{m}^3$ ; II – volume between 11 and

1000  $\mu\text{m}^3$ ; III - volume between 1001 and 10000  $\mu\text{m}^3$ ; IV – volume > 10000  $\mu\text{m}^3$ ; picophytoplankton – MLD = 0.2 to 2  $\mu\text{m}$ ; nanophytoplankton – MLD = 2 to 20  $\mu\text{m}$ ; microphytoplankton – MLD = 20 to 200  $\mu\text{m}$  and mesophytoplankton - MLD = 200 to 2000  $\mu\text{m}$ . To meet our third expectation, we considered small related body size the following groups and categories: I and II (MBFG); C and R (CSR); I and II (volume); picophytoplankton and nanophytoplankton (MLD). Large body size were related to: III and VII (MBFG); S and SS (CSR); III and IV (volume); microphytoplankton and mesophytoplankton (MLD). Within the MBFG, as the groups IV, V, VI and VIII may comprise both small and large body size organisms, they may be considered as small or large body related, depending on the species composition.

After classification of species based on MBFG, CSR, Vol and MLD, we then summed the species biovolume at each functional group/category to construct four functional biotic response datasets. Before analyses, all biotic datasets were Hellinger-transformed (Legendre & Legendre, 2012), in order to reduce the influence of common species and to avoid biased data, thus allowing the use of linear multivariate methods (Peres-Neto et al., 2006).

### **2.3. Environmental and spatial variables**

All 23 environmental variables were used to construct an environmental explanatory matrix (E), which was standardized in order to compare the variables with distinct scales (Legendre & Legendre, 2012). We also tested the collinearity of these variables, maintaining only variables with variance inflation factor (VIF) less than 20 (ter Braak & Smilauer, 2012). Hence, after selection, 12

environmental variables were retained: Depth, SD, T, pH, DO, Cond, Sil, TSS, TP, SRP, DIC and DIN (Table 1), which were used in the following analysis.

For the spatial matrix, we used distance-based Moran's Eigenvector Maps (dbMEMs, Dray *et al.*, 2012; Legendre & Legendre, 2012), to generate spatial variables representing geographic positions at different spatial scales. dbMEMs were calculated from the geographical coordinates of the 42 sampling stations, assuming geographical distances based on soil and not on hydrological connections. This assumption was made considering that in our small study area highly influenced by wind, phytoplankton distribution will not be limited by aquatic connectivity, since species can disperse through air (Kristiansen, 1996; Finlay, 2002; Sharma *et al.*, 2007; Incagnone *et al.*, 2015). This procedure generated 41 spatial variables, 6 with positive spatial autocorrelation (Moran's I), and 35 with negative spatial autocorrelation. Since species spatial patterns may be describe as aggregated (individuals tend to be close together) for positive spatial autocorrelation, or regularity (individuals tend to avoid each other) for negative spatial autocorrelation (Dray *et al.*, 2012), and that dispersal may be an underlying mechanism in these spatial variables, we test the significance of both positive and negative spatial variables using ANOVA with 999 permutations, after a redundancy analysis (RDA) with all 5 biotic datasets as response. Since the negative spatial variables showed no significance with any biotic dataset, we exclude them and used only positive spatial autocorrelation as explanatory variables (S) for further analysis.

Furthermore, we divided our positive dbMEMs into submodels corresponding to different spatial scales (Dray *et al.*, 2012; Legendre & Legendre, 2012). By concentrating most of the spatial variation, the first eigenvector usually

describes broad spatial scales, while the last eigenvectors (with lower eigenvalues) describe fine spatial structures. Since we obtained only 6 positive MEMs, we classified MEM 1-3 into broad spatial scale ( $S_b$ ) and MEM 4-6 into fine spatial scales ( $S_f$ ).

Lastly, we implemented a forward selection procedure with double stopping criteria (Legendre & Legendre, 2012) to only select spatial variables (MEMs) that significantly explained the variance in each biotic dataset. Therefore, for each response matrix (biotic dataset) distinct MEMs were selected for representing broad and fine spatial scales, as spatial explanatory matrices (S) (Table 2).

#### **2.4. Data analysis**

The relative importance of environmental and spatial variables in the structuring of our biotic datasets was assessed by partial redundancy analyses (pRDA) in association with variation-partitioning procedures (Borcard *et al.*, 1992; Legendre & Legendre, 2012). This analysis decomposes the total variation into fractions that indicate the relative importance of the pure environmental (E|S) and pure spatial (S|E) components, as well the influence of unique broad and fine spatial scales, shared fractions, and the unexplained variation (Residuals). The analysis was performed separately for each of the five response matrices, using the environmental variables (E) and the selected MEM (S), decomposed into broad ( $S_b$ ) and fine ( $S_f$ ) spatial scales. The explained variation at each fraction was estimated using adjusted  $R^2$  values (Peres-Neto *et al.*, 2006) and the significance of the pure fractions was tested through an ANOVA, with 999 permutations (Legendre & Legendre, 2012).

All analyses were performed in the R environment (R Core Team, 2019) using the following packages: *adespatial* (Dray *et al.*, 2019), to construct spatial variables (dbMEMs) and to perform the forward selection procedures; *vegan* (Oksanen *et al.*, 2019) for the multivariate analyses and VIF selection; and *ggplot2* (Wickham *et al.*, 2019) to plot the data.

### 3. Results

A total of 169 phytoplankton species was identified. *Dolichospermum* sp2, and *Melosira varians* showed the highest mean abundance in all samples (Figure 2). Only *Plagioselmis lacustris*, *Synechococcus* spp. and *Synechocystis* spp. occurred in all sampling stations. Overall, larger species of nitrogen-fixing cyanobacteria (Group VIII – MBFG), disturbance-tolerant species (Group R – CSR), species with greater length (Mesophytoplankton – MLD = 200 to 2000  $\mu\text{m}$ ) and with higher volume (Category IV – Vol > 10000  $\mu\text{m}^3$ ) attained a higher biomass, over other multiple trait groups and unique trait categories (Figure 3).

The variance partitioning revealed that the phytoplankton metacommunity was influenced by both pure environmental (E) and spatial (S) components, varying according to the biotic dataset used (Table 3, Figure 4). The fine spatial scale (Sf) showed a higher contribution to the S component than broad spatial scale (Sb), for most of the metrics used (Figure 5). We also found that the joint effect of environmental and spatial components, called spatially structured environment (E+S) had a high explanation power for most of the analysis and between functional groups/categories.

The taxonomic composition matrix (Sp) was significantly explained by all explanatory variables, showing a higher influence of pure environmental



component (adjusted  $R^2 = 0.2241$ ,  $p < 0.001$ ). This approach had also the highest value of unexplained variation comparing all other functional approaches (residuals = 0.6652).

The MBFG system revealed a higher influence of pure environmental component (adjusted  $R^2 = 0.2615$ ,  $p < 0.001$ ). However, the spatial component also showed significance, regarding broad spatial scales (adjusted  $R^2 = 0.0667$ ,  $p < 0.005$ ). Comparing other approaches, MBFG was the only in which broad spatial scale (Sb) was the main contributor of the spatial component (Figure 5). When analyzing between groups, most of the MBFG groups showed significance with both environmental and spatial (as broad and fine scales) components (Table 3 and Figure 4), with exception of the Group III (large filaments with aerotopes) which was almost unexplained by our variables (residuals = 0.9202). While some groups were significantly explained only by environmental component (Groups IV, V and VII), others were highly influenced by spatial components at different scales (Groups I and VI). Also Group II (small flagellated organisms with siliceous exoskeletal structures) and VIII (nitrogen fixing cyanobacteria) were significantly explained both by environmental and spatial components, being fine spatial scale for the former, and broad spatial scale for the latter.

Differently from MBFG and Sp approach, grouping species in CSR displayed a stronger influence of spatial component at fine spatial scale, which was the only component of community variance that showed significance (adjusted  $R^2 = 0.2275$ ,  $p < 0.001$ ). When analyzed between groups (Figure 4), fine spatial scale continued to explain data variation in Group C (Invasive opportunists/competitors) and R (Disturbance-tolerant/ruderals) ( $p < 0.005$ , for both). Yet, environmental component also became influential for Group C

(adjusted  $R^2 = 0.1833$ ,  $p < 0.05$ ). Group S (Acquisitive/stress-tolerant) was unexplained by any variable, showing a higher residual value = 0.7356. Species belonging to Group SS (Chronic stress-tolerant) were not found in the sampling stations and, therefore, were not considered in the analysis.

MLD metric displayed similar results with CSR grouping system, showing that the metacommunity was significantly explained only by spatial component, as fine scale (adjusted  $R^2 = 0.0653$ ,  $p < 0.05$ ). In this metric, environmental component was not influential, and fine scale spatial significance was found only for the smaller length categories, such as Picophytoplankton (MLD = 0.2 to 2  $\mu\text{m}$ ) (adjusted  $R^2 = 0.0806$ ,  $p < 0.05$ ) and Nanophytoplankton (MLD = 2 to 20  $\mu\text{m}$ ) (adjusted  $R^2 = 0.1370$ ,  $p < 0.005$ ). The data variation of the bigger length categories (Microphytoplankton and Mesophytoplankton) were not explained by the explanatory variables (Table 3). However, for Mesophytoplankton (MLD = 200 to 2000  $\mu\text{m}$ ) the spatially structured environment (E+S) component explained data variability, as it showed a high value of adjusted  $R^2$  (0.5611).

Lastly, volume (Vol) displayed the higher explanation power of the variables comparing all other approaches (Table 3, Figure 4), resulting in a lower residual value (residuals = 0.3424). Vol was significantly explained by all components of data variation at both large and fine spatial scales, showing a higher influence of pure environmental component (adjusted  $R^2 = 0.1772$ ,  $p < 0.001$ ). Fine scale spatial variance showed a higher influence in structuring the community through volume than broad spatial scale (Figure 5). All volume categories revealed significance with both environment and spatial (at different scales) components, with exception of the larger category (IV - Vol > 10000  $\mu\text{m}^3$ ), which was explained only by fine scale spatial component (Table 3, Figure 4).

By comparing approaches, the results demonstrated that the influence of environmental and spatial variables in structuring metacommunities were better ascertained by the volume (Vol), multiple trait system MBFG, and also by taxonomic composition (Sp) (Figure 4). From these, volume categorization displayed a higher spatial signal, and MBFG functional metrics showed a higher influence of environmental variables. Taxonomic composition (Sp) was less explained by our explanatory variables than functional metrics such as Vol and MBFG. When accessing differences between groups from distinct functional metrics, the following groups and categories were better predicted by environmental and spatial variables: Groups II (Small flagellated organisms with siliceous exoskeletal structures) and VIII (Nitrogen fixing cyanobacteria) of MBFG; Categories I (volume < 10  $\mu\text{m}^3$ ), II (volume between 11 and 1000  $\mu\text{m}^3$ ) and III (volume between 1001 and 10000  $\mu\text{m}^3$ ) of volume unique trait metric, and Group C (Invasive opportunists/competitors) from CSR multiple functional trait grouping. From these, CSR Group C revealed a higher influence of spatial variables, and Category III of volume unique functional trait categorization (Vol) displayed higher environmental influence than other categories/groups, despite MBFG had an overall higher environmental influence.

#### **4. Discussion**

Phytoplankton metacommunity in the studied area (58 km) was explained by both environmental and spatial variables. However, environmental and spatial signs were differently evidenced depending on the functional metric.

By comparing the classical taxonomic approach with multiple functional trait grouping (MBFG and CSR) and with unique functional trait categorizations

(volume and MLD), we expected that the former would be, comparatively, less explained by the environmental and spatial variables. As functional metrics group species with similar ecological abilities (Padisák *et al.*, 2009), they would better summarize the environmental and spatial influence than species as individual entities (Huszar *et al.*, 2015; Leruste *et al.*, 2018). The present results showed that the overall MBFG metric displayed a higher influence of environmental variables than taxonomic approach (Sp), as well as the fine spatial variables explained better volume and CRS overall variations. This result partially corroborated our first expectation, since the Sp matrix was better predicted by environmental variables than MLD, volume and CSR and, except by volume overall metric, Sp was the only approach to present significant influence of both broad and fine spatial variables. Recently, Xiao *et al.* (2018) also demonstrated that, for phytoplankton metacommunity of Chinese three lakes regions (1285–3232 km), species-based classification revealed better spatial processes than functional approaches, including MBFG, pointing out that despite common responses of species within functional groups, species-specific approach comprises dispersal abilities, mechanisms, and strategies that cannot be neglected in phytoplankton patterns at spatial scales. By the other side, Huszar *et al.* (2015) demonstrated that species composition and MBFG were influenced by environment, but spatial influence on species were not seen in a large subtropical Brazilian river basin (1150 km). Interestingly, Sp approach had the higher residual value from all other functional metrics in the present study. The low explanatory power of Sp may reflect the large multiplicity of factors that affects phytoplankton species variation (Scheffer *et al.*, 2003; Wojciechowski *et al.*, 2017), and that hinder the explanation power of community structuring

variables in this approach. Despite the scale differences observed between our results and the study of Xiao *et al.* (2018), the taxonomic approach significantly captured the spatial signal and should not be ruled out in phytoplankton metacommunity studies. The lack of other comparative studies in this regard suggests the need for further research, especially comprising different scales.

Our second expectation laid on the ability of our functional metrics to be sensitive to spatial and environmental variations. In this case, the use of multiple functional trait metrics, would difficult the interpretation of dispersal influence and environmental filtering, which might show higher influence by using unique traits direct inferring processes in the metacommunity context (e.g. dispersion variation regarding cell size). Our results showed that, in fact, the influence of the spatial and environment varied according the functional metric used, but not as we expected. The environmental signal was significantly better captured by MBFG > volume, despite the higher influence of dispersive processes in some functional groups/categories. The higher influence of environmental factors, over dispersal, in structuring the phytoplankton metacommunity is corroborated in several microorganisms' studies (Beisner *et al.*, 2006; De Bie *et al.*, 2012; Lindström & Langenheder, 2012; Padial *et al.*, 2014; Huszar *et al.*, 2015; Souffreau *et al.*, 2015; Louca *et al.*, 2016; Maloufi *et al.*, 2016; Soininen *et al.*, 2016; Heino *et al.*, 2017; Crossetti *et al.*, 2018; Wu *et al.*, 2018).The spatial signal were better captured in fine scale by CSR > volume > MLD, and in broad scale by MBFG > volume. This unexpected result may be explained by the intrinsic characteristics of each tested metric. Among the functional metrics here tested, the overall volume was the only to be significantly explained by both environmental and spatial variables (fine and broad scales). This may be a result of the strong

association that the trait volume, a direct measurement of size, has with both environmental and dispersal factors (Reynolds, 2006; Jenkins *et al.*, 2007; Litchman & Klausmeier, 2008; Litchman *et al.*, 2010; De Bie *et al.*, 2012; Iatskiu *et al.*, 2018; Leruste *et al.*, 2018). Since volume is also a functional trait used in MBFG multiple functional trait grouping (Kruk *et al.*, 2010), the divergences observed between MBFG and volume metrics, specially regarding fine scale spatial component, may be related to the influence of other traits in MBFG classification, which are highly related to environmental conditions, such as presence of mucilage and aerotopes (Litchman & Klausmeier, 2008). Further, MBFG multiple functional trait grouping is known to be a better predictor of larger scale variations (Salmaso *et al.*, 2015), which may also be the cause of the absence of significance for fine scale spatial component. Regarding the spatial signal, the overall CSR metric presented the highest explanation in fine scale, probably as an outcome arising from the dispersion-related ability comprised in the relation between the morphological traits (surface, volume ratio and maximum linear dimension) considered in this functional metric (Reynolds, 1997). This same thought might be applicable to MLD. For MLD, the low explanatory power of environmental and absence of significance may be because measured environmental variables displayed high spatial structure, when considering length categories. This functional metric reflected most the influence of spatially structured environment variables (E+S) and we could not test the significance of this shared fraction. Besides, the maximum linear dimension of the organisms may not capture most of the environmental variability, being rather used to track grazing susceptibility (e.g. Cardoso *et al.*, 2019).

For phytoplankton, the small cell size and enormous abundance of vegetative cells and propagules, allows a huge passive dispersal potential (Finlay, 2002; Sharma *et al.*, 2007; De Bie *et al.*, 2012; Incagnone *et al.*, 2015). Hence, since dispersal is distant-dependent (Kristiansen, 1996; Ng *et al.*, 2009) and that a gradual increase in dispersal limitation with body size in aquatic passive dispersers are expected (De Bie *et al.*, 2012), we presumed that, given the small geographic area of our study (58 km) and no dispersal limitation, functional groups and traits related to the smaller species would present higher influence of spatial process through mass effects and the larger related ones would be sorted by environmental filtering, as stated by Ng *et al.* (2009) and Heino *et al.*, (2015). Based on the results, our expectations were also partially corroborated. Within MBFG, we found fine scale spatial significance for small body related groups I (small organism with high S/V) and II (small flagellated organisms with siliceous exoskeletal structures) which encourage our initial expectation. But we also found spatial signal (broad and fine scales) for the groups VIII (Nitrogen fixing cyanobacteria) and VI (non-flagellated organisms with siliceous exoskeletons - diatoms), which are usually strongly associated to environmental gradients (Reynolds 2006; Vilmi *et al.*, 2016). As in MBFG, size is just one of the multiple traits that group species, assumptions of dispersal processes over body size are not straight forward. Hence, the spatial signal observed in groups VIII (Nitrogen fixing cyanobacteria) and VI (diatoms) may be explained the combined effects of other traits influencing in the analysis. For cyanobacteria (MBFG – Groups III, VII and VIII) Ribeiro *et al.* (2018b) found that, at broader spatial scales, the community distribution was mainly structured by spatial (assumed dispersal limitation) and spatially structured environment components, whereas other

studies indicated dispersal limitation is only at continental scales (Martiny *et al.*, 2006; Izaguirre *et al.*, 2015). Although we do not assume dispersal limitation because of the small scale of our study, the results showed that nitrogen fixing cyanobacteria reflected the influence of both environmental and dispersal processes even at small geographical extents, and that this relation is not strictly related to cell size, as this group is formed by several species with distinct morphologies (see Appendix). For diatoms (MBFG - Group VI), our finding was also interesting, because spatial signal was only recorded for this taxon over continental scales (Soininen *et al.*, 2016), and because it showed no significance with environmental component, despite being a group used for bioassessment (e.g. Vilmi *et al.*, 2016). The lack of significance of pure environmental component, may be explained by a singularity in our data set. *M. varians* was found in only one sampling station but its high abundance probably influenced the analysis, undermining the explanatory power of environmental component. Moreover, the spatial significance found in Group VI (diatoms) may also be explained by the wide body size temporal variation within species' populations (Hense & Beckmann, 2015).

Withing CSR system, the small species categories C (invasive opportunists/competitors) and R (disturbance-tolerant/ruderals) showed significant fine scale spatial signals, whereas the larger organisms of S (acquisitive/stress-tolerant) were not explained by neither spatial nor environmental variables. The absence of spatial significance only in the larger species (S - acquisitive/stress-tolerant), may be a indicative that for this group, high dispersal mechanism, such as mass effects, may not be a driving force as it is seen for smaller organism which are more easily suspended in air (Sharma *et*



*al.*, 2007), and hence, dispersed. In fact, studies with several taxa shows that smaller organism normally have higher dispersal capability, except when larger sizes mean that the organism can locomote better (De Bie *et al.*, 2012; Tolonen *et al.*, 2018). For smaller species in group C (invasive opportunists/competitors) we did find spatial and environmental significance, which is reasonable since these species are resource constrained (Reynolds 2006; Salmaso *et al.*, 2015), and that smaller organism tend to display higher spatial signal than larger ones (De Bie *et al.*, 2012). As group R (disturbance-tolerant/ruderals) species generally need water turbulence to be suspended in water, and that is a common feature in our study area (Bohnenberger *et al.*, 2018), the lack of environmental signal may be caused by the absence of environmental constraint for this group. While the spatial signal may indicate that dispersal processes do overcome environmental filtering, at least for group R (disturbance-tolerant/ruderals), in our study sites.

As found for CSR, MLD the smaller sized categories picophytoplankton (0.2 to 2  $\mu\text{m}$ ) and nanophytoplankton (2 to 20  $\mu\text{m}$ ), displayed fine scale spatial significance. As described above, smaller organisms do display higher spatial signal than larger ones (De Bie *et al.*, 2012). However, for the larger species categories microphytoplankton (20 to 200  $\mu\text{m}$ ) and mesophytoplankton (200 to 2000  $\mu\text{m}$ ), the results did not show significance with any explanatory variable. The absence of environmental influence for larger microorganisms may be caused by their higher in-cell nutrient storage capacity, lower metabolic rate, and greater resistance to predation, all factors that assist organism to withstand environmental constraints (Litchman & Klausmeier, 2008; Litchman *et al.*, 2010; Iatskiu *et al.*, 2018; Pančić & Kjørboe, 2018).

Lastly, regarding the volume categories I (volume < 10  $\mu\text{m}^3$ ), II (volume between 11 and 1000  $\mu\text{m}^3$ ) and IV (volume > 10000  $\mu\text{m}^3$ ) showed fine spatial scale significance, while groups II and III (volume between 1001 and 10000  $\mu\text{m}^3$ ) were significantly explained by broad spatial scale. In this metric, only the larger species group IV (volume > 10000  $\mu\text{m}^3$ ) showed no significance with the environmental component, yet it presented the higher significance with the Sf component. As larger organisms may have higher tolerances to environmental constraints (Iatskiu *et al.*, 2018), which undermine explanation power of environmental variables, one may wonder if in a gradual size increase, environmental and spatial influence are being replaced by weaker or absence of environmental influence and a dispersal limitation for the larger organisms (here > 10000  $\mu\text{m}^3$ ).

In a general view, based on the presented results, the spatial signal found for the overall functional metrics can be interpreted as high dispersal effects, or mass effects (Heino *et al.*, 2015, Vilmi *et al.*, 2017), given the scale comprised in the present study. As spatial signals in phytoplankton are found at larger geographical scales and commonly interpreted as limited dispersal (Beisner *et al.*, 2006; Martiny *et al.*, 2006; Padial *et al.*, 2014; Huszar *et al.*, 2015; Izaguirre *et al.*, 2015; Soininen *et al.*, 2016; Moresco *et al.*, 2017; Ribeiro *et al.*, 2018b; Wu *et al.*, 2018; Xiao *et al.*, 2018), our findings do imply that dispersal processes are significant drivers of phytoplankton metacommunity variation even at smaller spatial scales. Since dispersal mechanisms were historically ignored factors structuring microorganism metacommunities (Huszar *et al.*, 2015; Incagnone *et al.*, 2015; Padisák *et al.*, 2016), our results contribute to the actual understanding that microorganism community may be structured by other processes, rather than

environmental factors alone (Beisner *et al.*, 2006; Martiny *et al.*, 2006; Vellend *et al.*, 2014; Louca *et al.*, 2016; Zhou & Ning, 2017).

Summing up, we evaluate different influences of community structuring factors not only when using distinct approaches, but also when analyzing data variation between groups/categories of the same functional metric. We showed that both spatial and environmental are important drivers of community variation in a small scale, but their influence varies according to the approach used, as seen by Guo *et al.* (2019). In our study, taxonomic composition displayed better the influence of environmental and spatial components than some functional classification systems and should not be neglected in phytoplankton community assembly researches. Our findings also indicated that, by using fewer organism groups/categories that are highly influenced by environment and dispersal, researches can also access the influence of community driving factors. Considering functional metrics, volume and MBFG seemed to be fitting classifications to access microorganism structuring factors, because of its higher explanatory power and sensitivity to spatial and environmental signals. However, when deconstructing the overall classifications and evaluating the functional groups (usually composed by multiple traits), it may not be easy to relate the ecological mechanisms of interest in metacommunity studies. That is the case, for example, of the group VI of MBFG, that englobe all diatoms, in which regardless of their highly recognized environmental fidelity, no environmental signal was found. Also, the influence of spatial variables in structuring this group, should be performed carefully as diatoms natural populations have an acknowledged wide body size range.

Using size traits to assess dispersal processes improved our spatial signal, as all functional metrics used were related to morphology and displayed spatial significance at least in one scale. Nonetheless, higher influence of spatial variables in smaller organisms, with higher dispersal ability, in a small geographic scale, were only evident in all functional metrics, however bigger sized species didn't display environmental influence, as expected. In conclusion, in order to provide a direct and more concise answers to patterns observed in nature we need to evaluate and understand the functional relevance of the metric used, as well the relationship of the traits to environmental variables and dispersal processes in phytoplankton metacommunity studies. As future perspectives, comparative approaches upon functional classifications and species composition must be encouraged in different spatial scales to strengthen the understanding of these metrics in the context of microorganism metacommunities assembly.

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## **6. References**

Allende L., Fontanarrosa M.S., Murno A. & Sinistro R. (2019). Phytoplankton functional group classifications as a tool for biomonitoring shallow lakes: a case study. *Knowledge & Management of Aquatic Ecosystems*, 420(5).

Alvares C.A., Stape J.L., Sentelhas P.C., De Moraes Gonçalves J.L., & Sparovek G. (2013). Köppen's climate classification map for Brazil. *Meteorologische Zeitschrift*, 22: 711–728.

American Public Health Association (APHA). (2012). *Standard Methods for the Examination of Water and Wastewater*, 21st ed. American Public Health Association, Washington, DC.

Araújo A., & Bicudo C.E.M. (2006). Criptógamos do Parque Estadual das Fontes do Ipiranga, São Paulo, SP. Algas, 22: Zygnemaphyceae (gêneros *Actinotaenium*, *Cosmarium* e *Heimansia*). *Hoehnea*, 33(2): 219-237.

Azevedo M.T.P., Nogueira N.M.C., & Sant'Anna C.L. (1996). Criptógamos do Parque Estadual das Fontes do Ipiranga, São Paulo, SP. Algas, 8: Cyanophyceae. *Hoehnea*, 23(1): 1-38.

Beisner B.E., Peres-Neto P.R., Lindström E.S., Barnett A. & Longhi M.L. (2006). The Role of Environmental and Spatial Processes in Structuring Lake Communities from Bacteria to Fish. *Ecology*, 87: 2985–2991.

Bicudo C.E.M. (2004). Criptógamos do Parque Estadual das Fontes do Ipiranga, São Paulo, SP. Algas, 18: Chlorophyceae (Volvocales). *Revista Brasileira de Botânica*, 27(1): 85-102.

Bicudo C.E.M., Bicudo D.C., Ferragut C., Lopes M.R.M., & Pires P.R. (2003). Criptógamos do Parque Estadual das Fontes do Ipiranga, São Paulo, SP. Algas, 17: Chrysophyceae. *Hoehnea*, 30(2): 127-153.

Bicudo C. & Menezes M. (2017). Gêneros de algas de águas continentais do Brasil (chave para identificação e descrições). São Carlos: RiMa, 3º ed, 554p.

Bohnenberger J.E., Schneck F., Crossetti L.O., Lima M.S. & Motta-Marques D. (2018). Taxonomic and functional nestedness patterns of phytoplankton communities among coastal shallow lakes in southern Brazil. *Journal of Plankton Research*, 40: 555–567.

Borcard D., Legendre P. & Drapeau P. (1992). Partialling out the spatial component of ecological variation. *Ecology*, 73: 1045–1055.

Bortolini J.C., Silva P.R.L., Baumgartner G. & Bueno N.C. (2019). Response to environmental, spatial, and temporal mechanisms of the phytoplankton metacommunity: comparing ecological approaches in subtropical reservoirs. *Hydrobiologia*, 830: 45-61.

Calvacante K.P., Becker V. & Cardoso L.S. (2018). A proxy for estimating the cell volume of *Ceratium furcoides* (Dinophyceae): basis for monitoring Brazilian reservoirs. *Lakes & Reservoirs*, 23:168–171.

Cardoso, L.S., Faria D.M., Crossetti, L.O., & Da Motta Marques, D. (2019). Phytoplankton, periphyton, and zooplankton patterns in the pelagic and littoral regions of a large subtropical shallow lake. *Hydrobiologia*, 831: 119-132.

Castro A.A.J., Bicudo C.E.M. & Bicudo D.C. (1991). Criptógamos do Parque Estadual das Fontes do Ipiranga, São Paulo, SP. Algas, 2: Cryptophyceae. *Hoehnea*, 18(1): 87-106.

Cottenie K. (2005). Integrating environmental and spatial processes in ecological community dynamics. *Ecology Letters*, 8: 1175–1182.

Crossetti L.O., Freitas-Teixeira L.M., Bohnenberger J.L., Schulz U.H., Rodrigues L.R. & and Motta-Marques D. (2018). Responses of the phytoplankton functional structure to the spatial and temporal heterogeneity in a large subtropical shallow lake. *Acta Limnologica Brasiliensia*, 30(214).

De Bie T., De Meester L., Brendonck L., Martens K., Goddeeris B., Ercken D., Hampel H., Denys L., Vanhecke L., Van der Gucht K., Van Wichelen J., Vyverman W. & Declerck S.A.J. (2012). Body size and dispersal mode as key traits determining metacommunity structure of aquatic organisms. *Ecology Letters*, 15: 740–747.

Dray S., Bauman D., Blanchet G., Borcard D., Clappe S., Guenard G., Jombart T., Larocque G., Legendre P., Madi N. & Wagner H.H. (2019). adespatial: Multivariate Multiscale Spatial Analysis. R package version 0.3-7.

Dray S., Péliissier R., Couteron P., Fortin M.J., Legendre P., Peres-Neto P.R., Bellier E., Bivand R., Blanchet F.G., De Cáceres M., Dufour A.B., Heegaard E., Jombart T., Munoz F., Oksanen J., Thioulouse J. & Wagner H.H., 2012. Community ecology in the age of multivariate multiscale spatial analysis. *Ecological Monographs*, 82: 257–262.

Fernandes S., & Bicudo C.E.M. (2009). Criptógamos do Parque Estadual das Fontes do Ipiranga, São Paulo, SP. Algas, 26: Chlorophyceae (famílias Chlorococcaceae e Coccomyxaceae). *Hoehnea*, 36(1): 173-191.

Finlay B.J. (2002). Global Dispersal of Free-Living Microbial Eukaryote Species. *Science*, 296: 1061-1063.

Fonseca B.M., Ferragut C., Tucci A., Crossetti L.O., Ferrari F., Bicudo D.C., Sant'Anna C.L. & Bicudo C.E.M. (2014). Biovolume de cianobactérias e algas de reservatórios tropicais do Brasil com diferentes estados tróficos. *Hoehnea*, 41(1): 9-30.

Grime J.P. (1977). Evidence for the existence of three primary strategies in plants and its relevance to ecological and evolutionary theory. *American Naturalist*, 111(982): 1169–1194.

Guimarães T.F.R., Hartz S.M. & Becker F.G. (2014). Lake connectivity and fish species richness in southern Brazilian coastal lakes. *Hydrobiologia*, 740: 207-217.

Guo K., Wu N., Wang C., Yang D., He Y., Luo Y., Chai Y., Duan M., Huang X. & Riis T. (2019). Trait dependent roles of environmental factors, spatial processes and grazing pressure on lake phytoplankton metacommunity. *Ecological Indicators*, 103: 312-320.

Heino J., Melo A.S., Siqueira T., Soininen J., Valanko S. & Bini L.M. (2015). Metacommunity organisation, spatial extent and dispersal in aquatic systems: patterns, processes and prospects. *Freshwater Biology*, 60(5): 845-869.

Heino J., Soininen J., Alahuhta J., Lappalainen J. & Virtanen R. (2017). Metacommunity ecology meets biogeography: effects of geographical region, spatial dynamics and environmental filtering on community structure in aquatic organisms. *Oecologia*, 183(1): 121–137.

Hense I. & Beckmann A. (2015). A theoretical investigation of the diatom cell size reduction–restitution cycle. *Ecological Modelling*, 317: 66-82.

Hentcheke G.S. & Torgan L.C. (2010). *Desmodesmus* and *Scenedesmus* (Scenedesmaceae, Sphaeropleales, Chlorophyceae) in aquatic environments from the Coastal Plain of Rio Grande do Sul, Brazil. *Rodriguésia*, 61(4): 585-601.

Hillebrand H., Dürseken D., Kirschiel D., Pollinger U. & Zohary T. (1999). Biovolume calculation for pelagic and benthic microalgae. *Journal of Phycology*, 35: 403–424.

Horner-Devine M.C., Silver J.M., Leibold M.A., Bohannan B.J.M., Colwell R.K., Fuhrman J.A., Green J.L., Kuske C.R., Martiny J.B.H., Muyzer G., Øvreås L., Reysenbach A.L. & Smith V.H. (2007). A comparison of taxon co-occurrence patterns for macro- and microorganisms. *Ecology* 88: 1345–1353.

Huszar V.L.M., Nabout J.C., Appel M., Santos J.B.O., Abe D.S. & Silva L.H.S. (2015). Environmental and not spatial processes (directional and non-directional) shape the phytoplankton composition and functional groups in a large subtropical river basin. *Journal of Plankton Research*, 37: 1190–1200.

Incagnone G., Marrone F., Barone R., Robba L. & Naselli-Flores L. (2015). How do freshwater organisms cross the “dry ocean”? A review on passive dispersal and colonization processes with a special focus on temporary ponds. *Hydrobiologia*, 750: 103–123.

Iatskiu P., Bovo-Scomparin V.M., Segovia B.T., Velho L.F.M., Lemke M.J. & Rodrigues L.C. (2018). Variability in mean size of phytoplankton in two floodplain lakes of different climatic regions. *Hydrobiologia*, 823: 135-151.

Izaguirre I., Saad J.F., Romina-Schiaffino M., Vinocur A., Tell G., Sánchez M.L., Allende L. & Sinistro R. (2015). Drivers of phytoplankton diversity in patagonian and antarctic lakes across a latitudinal gradient (2150 km): The importance of spatial and environmental factors. *Hydrobiologia*, 764: 157–170.

Jenkins D.G., Brescacin C.R., Duxbury C.V., Elliott J.A., Evans J.A., Grablow K.R., Hillegass M., Lyon B.N., Metzger G.A., Olandese M.L., Pepe D., Silvers G.A., Suresch H.N., Thompson T.N., Trexler C.M., Williams G.E., Williams N.C. & Williams S.E. (2007). Does size matter for dispersal distance?. *Global Ecology and Biogeography*, 16: 415-425.

Komárek J., & Fott B. (1983). Das Phytoplankton des Süßwassers (Systematik und Biologie). Stuttgart: E. Schweizerbart'sche Verlagsbuchhandlung (Nägele u. obermiller).

Kristiansen J. (1996). Dispersal of freshwater algae – a review. *Hydrobiologia*, 336: 151-157.

Kruk C., Huszar V.L.M., Peeters E.T.H.M., Bonilla S., Costa L., Lürling M., Reynolds C.S. & Scheffer M. (2010). A morphological classification capturing functional variation in phytoplankton. *Freshwater Biology*, 55: 614-627.

Legendre P. & Legendre L. (2012). *Numerical Ecology*, 3rd ed. Elsevier, Oxford.

Lester S.E., Ruttenberg B.I., Gaines S.D. & Kinlan B.P. (2007). The relationship between dispersal ability and geographic range size. *Ecology Letters*, 10: 745-758.

Leibold, M. A., Holyoak M., Mouquet N., Amarasekare P., Chase J.M., Hoopes M.F., Holt R.D., Shurin J.B., Law R., Tilman D., Loreau M., & Gonzalez A. (2004). The metacommunity concept: A framework for multi-scale community ecology. *Ecology Letters*, 7: 601–613.

Leruste A., Villéger S., Malet N., De Wit R. & Bec B. (2018). Complementarity of the multidimensional functional and the taxonomic approaches to study phytoplankton communities in three Mediterranean coastal lagoons of different trophic status. *Hydrobiologia*, 815: 207–227.

Lindström, E.S. & Langenheder S. (2012). Local and regional factors influencing bacterial community assembly. *Environmental Microbiology*, 4: 1–9.

Litchman E. & Klausmeier C.A. (2008). Trait-Based Community Ecology of Phytoplankton. *Annual Review of Ecology, Evolution, and Systematics*, 39: 615-639.

Litchman E., Pinto P.T., Klausmeier C.A., Thomas M.K. & Yoshiyama K. (2010). Linking traits to species diversity and community structure in phytoplankton. *Hydrobiologia*, 653: 15-28.

Louca, S., Jacques S.M.S., Pires A.P.F., Leal J.S., Srivastava D.S., Parfrey L.W., Farjalla V.F. & Doebeli M. (2016). High taxonomic variability despite stable functional structure across microbial communities. *Nature Publishing Group Nature Publishing Group*, 1: 1–12.



Lund J.W.G., Kipling C. & LeCren E.D. (1958). The invert microscope method of estimating algal numbers and the statistical basis of estimations by counting. *Hydrobiologia*, 11: 143–170.

Maloufi S., Catherine A., Mouillot D., Louvard C., Couté A., Bernard C. & Troussellier M. (2016). Environmental heterogeneity among lakes promotes hyper  $\beta$ -diversity across phytoplankton communities. *Freshwater Biology*, 61: 633-645.

Marquet P.A., Fernández M., Navarrete A.S. & Valdovinos C. (2004). Diversity emerging: towards a deconstruction of biodiversity patterns. In Lomolino, M. V. and Heaney, L. (eds), *Frontiers of Biogeography: New Directions in the Geography of Nature*. Cambridge University Press, Cambridge, pp. 191–209.

Martiny, J.B.H., Bohannon B.J.M., Brown J.H., Colwell R.K., Fuhrman J.A., Green J.L., Horner-Devine M.C., Kane M., Krumins J.A., Kuske C.R., Morin P.J., Naeem S., Øvreås L., Reysenbach A.L., Smith V.H., & Staley J.T. (2006). Microbial biogeography: Putting microorganisms on the map. *Nature Reviews Microbiology*, 4: 102–112.

Moresco GA., Bortolini J.C., Dias J.D. Pineda A., Jati S. & Rodrigues L.C. (2017). Drivers of phytoplankton richness and diversity components in Neotropical floodplain lakes, from small to large spatial scales. *Hydrobiologia*, 799(1): 203-215.

Naselli-Flores L. & Padisák J. (2016). Blowing in the wind: how many roads can a phytoplankton walk down? A synthesis on phytoplankton biogeography and spatial processes. *Hydrobiologia*, 764(1): 303-313.

Ng I.S.Y., Carr C.M. & Cottenie K. (2009). Hierarchical zooplankton metacommunities: distinguishing between high and limiting dispersal mechanisms. *Hydrobiologia*, 619: 133-143.

Oksanen J.F., Blanchet G., Friendly M., Kindt R., Legendre P., McGlinn D., Minchin P.R., O'Hara R.B., Simpson G.L., Solymos P., Stevens M.H.H., Szoecs E. & Wagner H. (2019). *vegan: Community Ecology Package*. R Package Version 2.5-6.

Padial, A.A., Ceschin F., Declerck S.A.J., De Meester L., Bonecker C.C., Lansac-Tôha F.A., Rodrigues L., Rodrigues L.C., Train S., Velho L.F.M., & Bini L.M. (2014). Dispersal ability determines the role of environmental, spatial and temporal drivers of metacommunity structure. *PLoS ONE*, 9: 1–8.

Padisák J., Crossetti L.O. & Naselli-Flores L. (2009). Use and misuse in the application of the phytoplankton functional classification: A critical review with updates. *Hydrobiologia*, 621: 1–19.

Padisák, J., Vasas G., & Borics G., (2016). Phycogeography of freshwater phytoplankton: Traditional knowledge and new molecular tools. *Hydrobiologia*, 764: 3–27.

- Pančić M. & Kiørboe T. (2018). Phytoplankton defence mechanisms: traits and trade-offs. *Biological Reviews*, 93(2):1269-1303.
- Pedrozo S.C., & Rocha O. (2007). Environmental Quality Evaluation of Lakes in the Rio Grande do Sul Coastal Plain. *Brazilian Archives of Biology and Technology*, 50: 673–685.
- Peres-Neto P.R., Legendre P., Dray S. & Borcard D. (2006). Variation partitioning of species data matrices: estimation and comparison of fractions. *Ecology*, 87: 2614–2625.
- R Core Team (2019). R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. <http://www.Rproject.org>.
- Reynolds C.S. (1988). Functional morphology and the adaptive strategies of freshwater phytoplankton. In: *Growth and Reproductive Strategies of Freshwater Phytoplankton* (Ed. C.D. Sandgren), pp. 388–433. Cambridge University Press, Cambridge.
- Reynolds C.S. (2006) *The Ecology of Phytoplankton*. Cambridge University Press, Cambridge.
- Reynolds C.S., Elliott J.A., Frassl M.A. (2014). Predictive utility of trait-separated phytoplankton groups: A robust approach to modeling population dynamics. *Journal of Great Lakes Research*, 40: 143-150.
- (a) Ribeiro, K.F., Duarte, L. & Crossetti, L.O. (2018). Everything is not everywhere: a tale on the biogeography of cyanobacteria. *Hydrobiologia*, 820: 23-49.
- (b) Ribeiro K.F., da Rocha C.M., de Castro D., Rodrigues L.R. & Crossetti L.O. (2018). Distribution and coexistence patterns of phytoplankton in subtropical shallow lakes and the role of niche-based and spatial processes. *Hydrobiologia*, 814(1): 233-246.
- Salmaso N., Naselli-Flores L. & Padisák J. (2015). Functional classifications and their application in phytoplankton ecology. *Freshwater Biology*, 60: 603–619.
- Schäfer A., Lanzer R. & Scur L. (2017). Atlas Socioambiental do Município de Osório. Caxias do Sul, RS: EducS, 237p.
- Scheffer M., Rinaldi S., Huisman J. & Weissing F.J. (2003). Why plankton communities have no equilibrium: solutions to the paradox. *Hydrobiologia*, 491: 9-18.
- Schwarzbold A. & Schäfer A. (1984). Gênese e morfologia das lagoas costeiras do Rio Grande do Sul, Brasil. *Amazoniana*, 9: 87–104.
- Sharma N.K., Rai A.K., Singh S. & Brown Jr. R.M. (2007). Airborne algae: their present status and relevance. *Journal of Phycology*, 43: 615-627.

Sieburth J.McN., Smetacek V. & Lenz J. (1978). Pelagic ecosystem structure: heterotrophic compartments of the plankton and their relationship to plankton size fractions. *Limnology and Oceanography*, 23, 1256–63.

Silva A.M., Ludwig T.A.V., Tremarin P.I., & Vercellino I.S. (2010). Diatomáceas perifíticas em um sistema eutrófico brasileiro (Reservatório do Iraí, estado do Paraná). *Acta Botânica Brasílica*, 24(4): 997-1016.

Silva S.M.A., Cabreira J.C., Voos J.G., & Lobo E.A. (2013). Species richness of the genera *Trachelomonas* and *Strombomonas* (pigmented Euglenophyceae) in a subtropical urban lake in the Porto Alegre Botanical Garden, RS, Brazil. *Acta Botanica Brasílica*, 27(3): 526-536.

Soininen J., Jamoneau A., Rosebery J. & Passy S.I. (2016). Global patterns and drivers of species and trait composition in diatoms. *Global Ecology and Biogeography*, 25: 940–950.

Souffreau, C., Van Der Gucht K., Van Gremberghe I., Kosten S., Lacerot G., Lobão L.M., Lúcia V., Huszar D.M., Roland F., Jeppesen E., Vyverman W. & De Meester L. (2015). Environmental rather than spatial factors structure bacterioplankton communities in shallow lakes along a > 6000 km latitudinal gradient in South America. *Environmental Microbiology*, 17: 2336–2351.

ter Braak, C. J. F. & Smilauer, P. (2012). Canoco reference manual and user's guide: software for ordination, version 5.0. Ithaca USA: Microcomputer Power.

Thompson R. & Townsend C. (2006). A truce with neutral theory: local deterministic factors, species traits and dispersal limitation together determine patterns of diversity in stream invertebrates. *Journal of Animal Ecology*, 75: 476:484.

Tolonen K. T., Yongjiu C., Vilmi A., Karjalainen S.M., Sutela T. & Heino J. (2018). Environmental filtering and spatial effects on metacommunity organisation differ among littoral macroinvertebrate groups deconstructed by biological traits. *Aquatic Ecology*, 52: 119-131.

Utermöhl H. (1958). Zur Vervollkomnung der quantitative Phytoplankton-Methodik. *Mitteilungen der Internationale Vereinigung für Theoretische und Angewandte Limnologie*, 9: 1–38.

Vellend, M., Srivastava D.S., Anderson K.M., Brown C.D., Jankowski J.E., Kleynhans E.J., Kraft N.J.B., Letaw A.D., Macdonald A.A.M., Maclean J.E., Myers-smith I.H., Norris A.R., & Xue X. (2014). Assessing the relative importance of neutral stochasticity in ecological communities. *Oikos*, 0: 1–11.

Villéger S., Mason N.W.H. & Mouillot D. (2008). New multidimensional functional diversity indices for a multifaceted framework in functional ecology. *Ecology*, 89(8):2290-2301.

Vilmi A., Tolonen K.T., Karjalainen S.M. & Heino J. (2017). Metacommunity structuring in a highly-connected aquatic system: effects of dispersal, abiotic environment and grazing pressure on microalgal guilds. *Hydrobiologia*, 790: 125.

Weithoff G. (2003). The concepts of 'plant functional types' and 'functional diversity' in lake phytoplankton – a new understanding of phytoplankton ecology?. *Freshwater Biology*, 48: 1669–1675.

Weithoff G. & Beiner B.E. (2019). Measures and Approaches in Trait-Based Phytoplankton Community Ecology – From Freshwater to Marine Ecosystems. *Frontiers in Marine Science*. 6:40. doi: 10.3389/fmars.2019.00040.

Wickham H., Chang W., Henry L., Pedersen T.L., Takahashi K., Wilke C., Woo K. & Yutani H. (2019). ggplot2: Create Elegant Data Visualisations Using the Grammar of Graphics. R Package Version 3.2.1.

Winegardner A.K., Jones B.K., Ng I.S.Y., Siqueira T. & Cottenie K. (2012). The terminology of metacommunity ecology. *Trends in Ecology and Evolution (Letters)*, 27(5).

Wojciechowski J., Heino J., Bini L.M. & Padial A.A. (2017). The strength of species sorting of phytoplankton communities is temporally variable in subtropical reservoirs. *Hydrobiologia*, 800(1): 31-43.

Wu N., Qu Y., Guse B., Makarevičiūtė K., To S., Riis T. & Fohrer N. (2018). Hydrological and environmental variables outperform spatial factors in structuring species, trait composition, and beta diversity of pelagic algae. *Ecology and Evolution*, 8: 2947–2961.

Xavier M.B. (1994). Criptógamos do Parque Estadual das Fontes Do Ipiranga, São Paulo, Sp. Algas, 5: Euglenophyceae (Euglenophyceae Pigmentadas). *Hoehnea*, 21(1/2): 47-73.

Xiao L.J., Zhu Y., Yang Y., Lin Q., Han B.P. & Padišák J. (2018). Species-based classification reveals spatial processes of phytoplankton meta-communities better than functional group approaches: a case study from three freshwater lake regions in China. *Hydrobiologia*, 811: 313–324.

Zhou, J., & Ning D. (2017). Stochastic Community Assembly: Does It Matter in Microbial Ecology?. *Microbiology and Molecular Biology Reviews*, 81: 1–32.

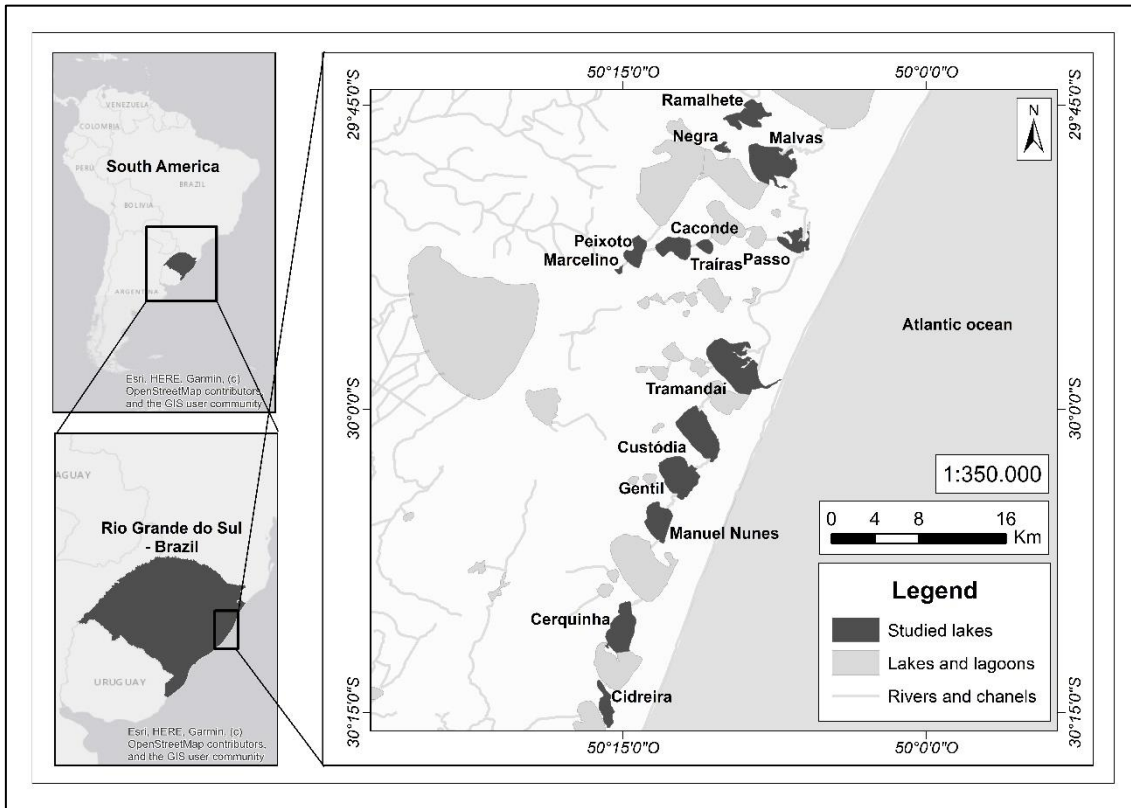


Figure 1. Study region, located at the coastal plain of southern Brazil, highlighting the 14 studied lakes. Datum: SIRGAS 2000, 22S.

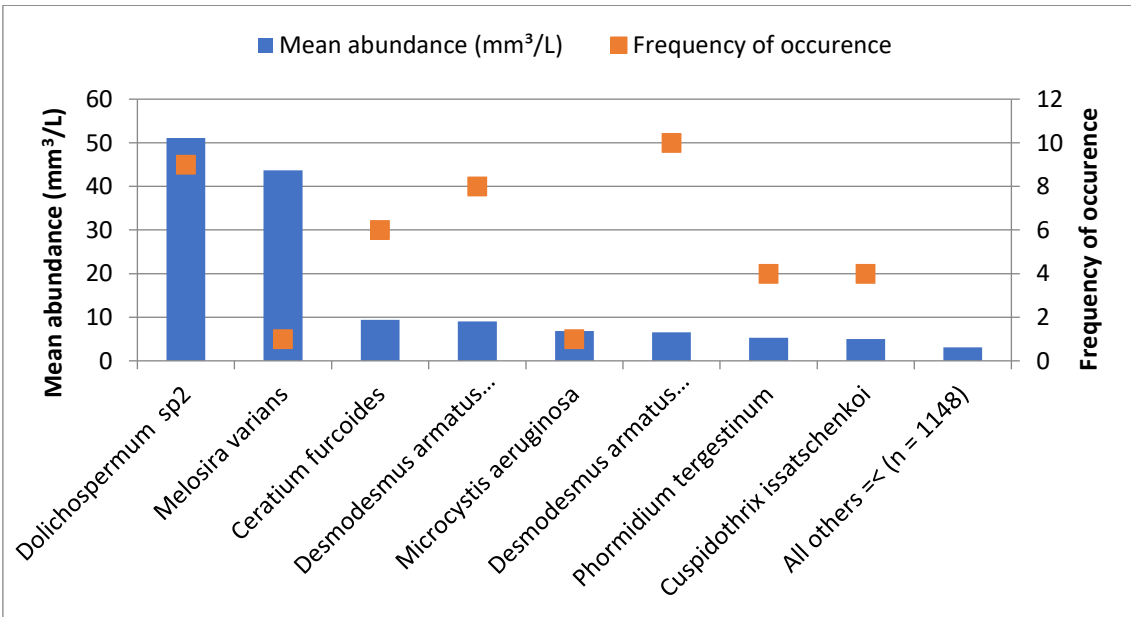
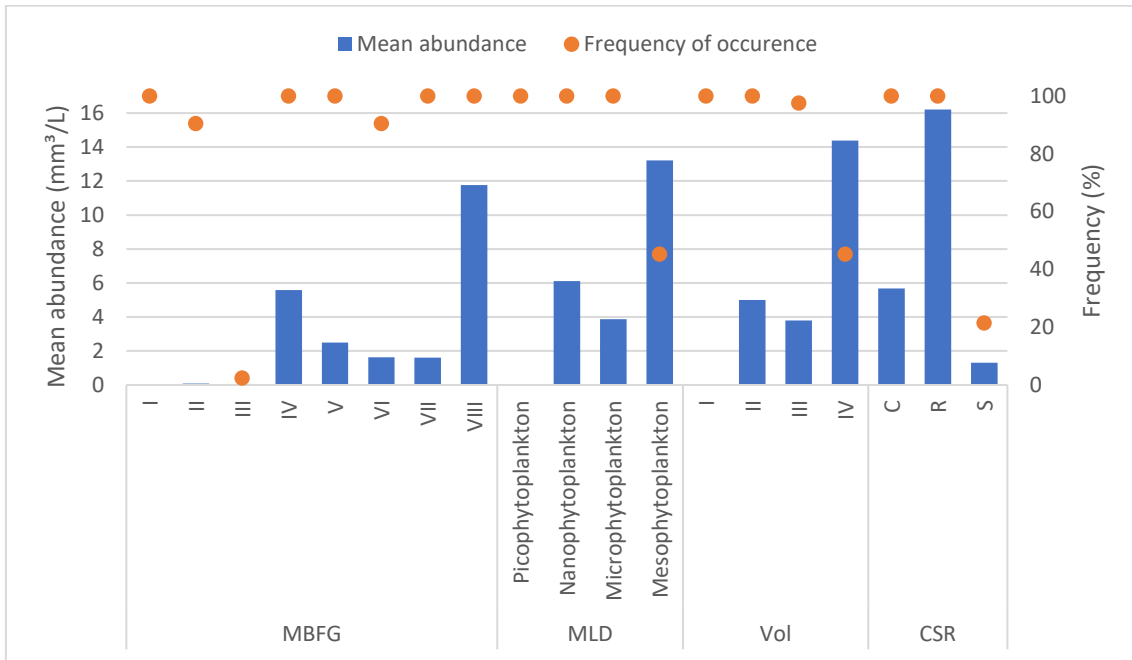
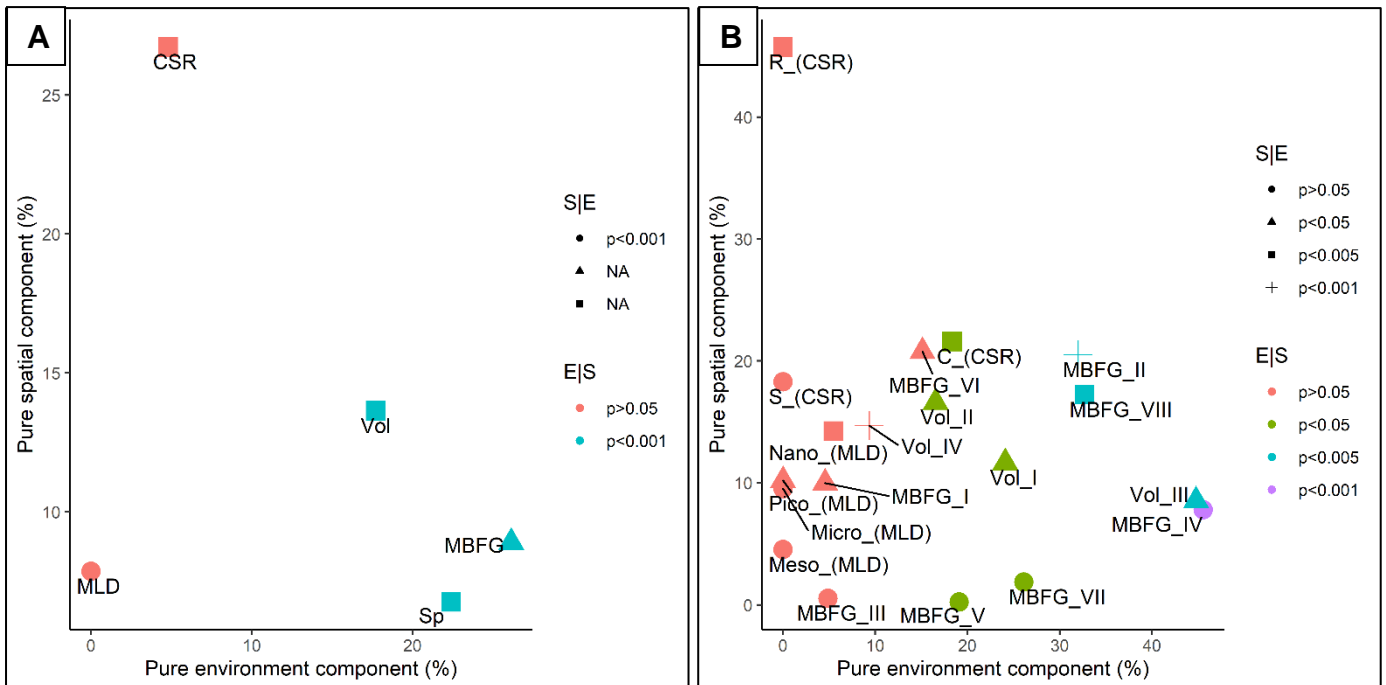


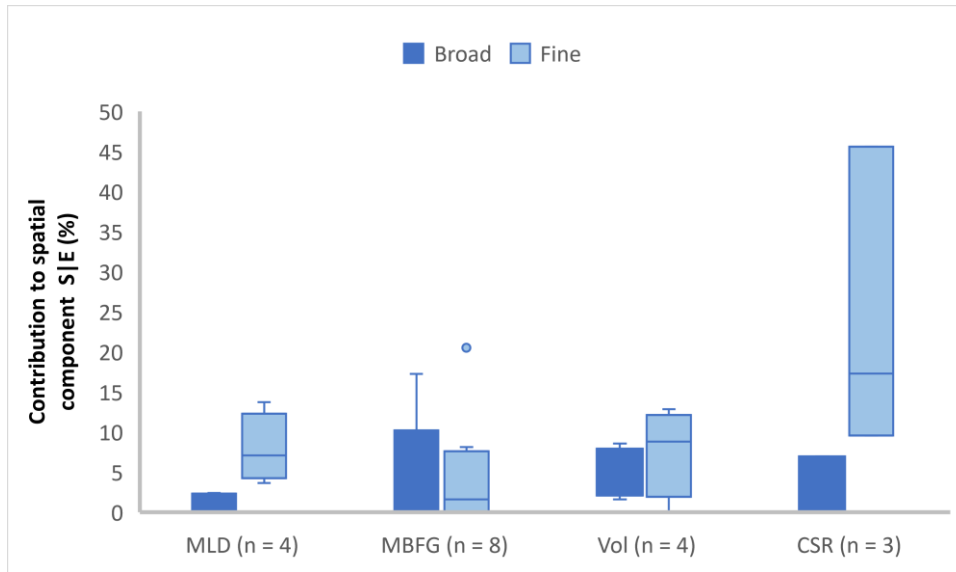
Figure 2 – Mean abundance (mm<sup>3</sup>/L) and frequency of occurrence of main phytoplankton species in the 42 sampled stations.



**Figure 3 – Mean abundance (mm<sup>3</sup>/L) and frequency of occurrence of functional and unique trait groups in the 42 sampled stations.**



**Figure 4 – Relation between the purely spatial and environment components of community variation between different approaches (A) and within functional metrics (B). Pure spatial and environmental components are described as adjusted R<sup>2</sup> values (%). Also, it is shown the spatial significance (S|E) with different codes, and environmental significance (E|S) with different colors.**



**Figure 5 – Mean contribution (n = number of groups/categories found for each functional metric) of broad and fine scale MEM variables to the variation that was uniquely explained by the spatial model at each functional metric.**

**Table 1 – Environmental variables selected and used as explanatory matrix (E), as well geographic coordinates used to compute the eigenvectors for spatial matrix (S). Data for all 42 sampling stations, with description of the lake. Where: SD = water transparency, T = temperature, DO = dissolved oxygen, Cond = electrical conductivity, Sil = soluble reactive silicon, TSS = total suspended solids, TP = total phosphorous, SRP = soluble reactive phosphorus, DIC = dissolved inorganic carbon and DIN = dissolved inorganic nitrogen.**

Lake	Sample	Depth (cm)	SD (cm)	T (°C)	pH	DO (mg/L)	Cond ( $\mu\text{S}\cdot\text{cm}^{-1}$ )	Sil (mg/L)	TSS (mg/L)	TP (mg/L)	SRP (mg/L)	DIC (mg/L)	DIN ( $\mu\text{g/L}$ )	Latitude	Longitude
Ramalhete	L1P1	180	50	24.6	7.96	7.1	68.5	8.8	94	0.008	0.007	2.607	0.053	-29.7626	-50.143517
	L1P2	190	40	25.8	8	7.3	67.6	8.6	53	0.034	0.003	3.728	0.062	-29.759433	-50.142833
	L1P3	190	45	24.7	7.86	7.2	68.1	10.8	52	0.024	0.001	1.998	0.054	-29.762217	-50.145217
Negra	L2P1	130	60	25	8.32	7.2	90.6	9.4	19	0.031	0.030	2.976	0.017	-29.785417	-50.169467
	L2P2	130	55	25.6	8.32	7.5	98.5	8.5	19	0.029	0.001	3.892	0.031	-29.786433	-50.169767
	L2P3	120	55	25.6	8.22	7.35	87.9	9.7	24	0.018	0.014	3.669	0.016	-29.784383	-50.169083
Passo	L3P1	140	70	25.5	8.29	8.7	77.6	7.7	35	0.078	0.078	3.446	0.015	-29.864633	-50.106017
	L3P2	130	60	25.3	8.25	8.2	75.1	8.6	29	0.004	0.001	5.074	0.019	-29.86615	-50.10525
	L3P3	150	70	25.1	8.34	8.45	82.5	6.5	29	0.247	0.214	3.285	0.026	-29.865267	-50.10765
Peixoto	L4P1	155	50	25.9	8.63	9.1	131.2	6.7	17	0.048	0.143	11.8	0.294	-29.86915	-50.237483
	L4P2	160	50	26	9.41	8.4	133.2	7.0	21	0.365	0.327	13.94	0.017	-29.869183	-50.237517
	L4P3	180	40	25.5	8.1	8.75	130.6	6.2	20	0.375	0.368	11.27	0.012	-29.870517	-50.23755
Marcelino	L5P1	60	20	25.2	10.81	7.1	294	13.2	17	0.851	0.296	31.57	2.840	-29.886217	-50.253067
	L5P2	40	10	25	10.84	6.8	292	12.4	29	0.865	0.322	31.18	1.429	-29.886967	-50.252667
	L5P3	55	12	25.7	10.93	6.95	308	12.8	26	0.897	0.301	30.19	2.086	-29.8865	-50.2538
Caconde	L6P1	240	150	28	8.22	8.7	59.8	1.9	16	0.542	0.201	4.846	0.059	-29.869117	-50.200917
	L6P2	250	130	27.3	8.21	7.8	60.6	1.8	5	0.073	0.045	3.872	0.004	-29.870767	-50.199683
	L6P3	320	150	27.1	8.3	8.25	61	1.8	8	0.063	0.013	4.461	0.056	-29.867217	-50.202017
Traira	L7P1	190	120	25.7	8.25	7.4	64.9	1	9	0.098	0.063	3.925	0.037	-29.8661	-50.18225
	L7P2	210	150	26.4	8.25	8.1	65.3	0.8	8	0.065	0.035	3.748	0.026	-29.86725	-50.180283
	L7P3	210	120	26.1	8.34	7.75	65.6	0.6	10	0.074	0.048	3.923	0.033	-29.864783	-50.18135



Lake	Sample	Depth (cm)	SD (cm)	T (°C)	pH	DO (mg/L)	Cond ( $\mu\text{S}\cdot\text{cm}^{-1}$ )	Sil (mg/L)	TSS (mg/L)	TP (mg/L)	SRP (mg/L)	DIC (mg/L)	DIN ( $\mu\text{g/L}$ )	Latitude	Longitude
Cerquinha	L8P1	140	80	26.1	7.95	7.2	155.8	4.8	15	0.097	0.088	2.746	0.031	-30.178517	-50.247867
	L8P2	140	60	26.1	7.95	7.8	156.1	4.7	23	0.073	0.067	2.891	0.011	-30.179267	-50.246
	L8P3	140	60	25.4	7.95	7.5	158.3	5.2	28	0.190	0.113	2.334	0.013	-30.177883	-50.249433
Cidreira	L9P1	90	60	26.6	7.79	8.2	190.7	7.0	21	0.093	0.088	2.775	0.035	-30.232333	-50.265317
	L9P2	90	60	26.4	7.76	7.9	191.6	6.8	24	0.081	0.058	3.032	0.045	-30.2326	-50.265983
	L9P3	90	60	26.6	7.62	8.05	191.5	5.0	22	0.077	0.051	1.629	0.046	-30.232	-50.264567
Custódia	L10P1	140	70	25.1	8.38	8.9	9.53	1.1	74	0.141	0.054	9.784	0.025	-30.014883	-50.187533
	L10P2	140	70	25.3	8.41	8.1	9.57	0.4	59	0.167	0.153	7.513	0.033	-30.01585	-50.18895
	L10P3	150	70	25.9	8.39	8.5	9.7	1.3	73	0.132	0.115	13.07	0.034	-30.014917	-50.1861
Gentil	L11P1	140	120	25.6	7.92	7.7	3.28	1.3	25	0.143	0.089	3.318	0.052	-30.05265	-50.201533
	L11P2	120	90	25.5	7.9	6.9	3.9	1.5	23	0.114	0.083	2.969	0.051	-30.05185	-50.200767
	L11P3	140	90	25.7	7.94	7.3	3.25	1.2	19	0.140	0.119	3.062	0.041	-30.052917	-50.202767
Manuel Nunes	L12P1	120	50	25.5	7.77	7.1	6.23	4.9	33	0.185	0.165	3.071	0.053	-30.093683	-50.217517
	L12P2	110	50	25	7.79	8.5	6.6	5.3	14	0.087	0.044	3.693	0.059	-30.094533	-50.216017
	L12P3	110	60	25.2	7.68	7.8	6.26	3.7	17	0.089	0.039	2.198	0.042	-30.094283	-50.21745
Tramandaí	L13P1	170	90	26	8.37	8.5	14.7	2.3	141	0.264	0.219	15.72	0.033	-29.964467	-50.164583
	L13P2	160	80	26	8.34	8	12.49	1.4	118	0.189	0.180	19.71	0.030	-29.96435	-50.16275
	L13P3	165	85	25.1	8.31	8.25	14.42	2.7	167	0.138	0.112	24.08	0.025	-29.964783	-50.161583
Malvas	L14P1	180	70	24	7.73	7.2	65.4	7.8	28	0.167	0.103	5.36	0.049	-29.794933	-50.1327
	L14P2	170	70	24.1	7.84	7.5	65.5	8.9	33	0.096	0.044	6.649	0.212	-29.794333	-50.13065
	L14P3	170	70	24.2	7.88	7.35	64.5	7.1	25	0.149	0.030	5.794	0.035	-29.795883	-50.131

**Table 2 - Environmental and spatial variables used as explanatory matrices (E and S, respectively) in the variation partitioning analysis, after selection procedures. Where MEM describes the positive spatial autocorrelation eigenvectors. Sb refers to broad spatial variables, and Sf to fine spatial variables.**

Approach	E	S	
		Sb	Sf
Sp	Depth, SD, T, pH, DO, Cond, Sil, TSS, TP, SRP, DIC and DIN	MEM 1 & 2	MEM 4 & 6
MBFG	Depth, SD, T, pH, DO, Cond, Sil, TSS, TP, SRP, DIC and DIN	MEM 1 & 2	MEM 6
CRS	Depth, SD, T, pH, DO, Cond, Sil, TSS, TP, SRP, DIC and DIN	MEM 1	MEM 4 & 6
V	Depth, SD, T, pH, DO, Cond, Sil, TSS, TP, SRP, DIC and DIN	MEM 1	MEM 6
M	Depth, SD, T, pH, DO, Cond, Sil, TSS, TP, SRP, DIC and DIN	MEM 1 & 2	MEM 6

**Table 3 – Adjusted R<sup>2</sup> values obtained after variation partitioning, representing the relative influence of environment (E) and spatial (S) variables on the structure of phytoplankton communities, based on different approaches. Where E|S = pure environment, S|E = pure spatial, Sb = broad pure spatial variables, Sf = fine pure spatial variables, E+S = Spatially structured environment, Sb|Sf = shared fraction of spatial matrices, Intercept = shared fraction of all explanatory matrices and Residuals = Community variation unexplained by explanatory variables.**

Approach	E S	S E		E+S	Sb Sf	Intercept	Residuals	
		Sb	Sf					
<b>Sp</b>	0.2241***	0.0352***	0.0324**	0.0485	-0.003	-0.0023	0.6652	
<b>Overall</b>	0.2615***	0.0667**	0.0223	0.1714	-0.002	-0.0069	0.4863	
I	0.0457	0.0341	0.0608*	0.4297	0.005	-0.0211	0.4457	
II	0.3199**	-0.0186	0.2051***	0.2129	-0.009	-0.0052	0.2944	
III	0.0487	-0.0367	0.0001	0.0685	0.0053	-0.0062	0.9202	
<b>MBFG</b>	0.4556***	0.0462	0.0318	0.1738	-0.011	0.0032	0.3006	
V	0.1911*	-0.032	-0.0055	0.3415	0.0024	-0.0095	0.5119	
VI	0.1512	0.1208*	0.0811*	0.1125	0.0059	-0.0136	0.5419	
VII	0.2612*	0.0179	-0.0219	0.1542	0.0009	-0.0076	0.5951	
VIII	0.327**	0.1725**	-0.0131	0.0783	-0.007	-0.0023	0.4451	
<b>Overall</b>	-0.0123	0.0093	0.0653*	0.3804	0.004	-0.0175	0.5706	
<b>MLD</b>	Picoplankton	-0.0448	0.0206	0.0806*	0.4332	0.0007	-0.0175	0.527
	Nanoplankton	0.0547	0.0017	0.137**	0.2974	0.0037	-0.0195	0.5247
	Microplankton	-0.039	0.024	0.0612	-0.042	0.0101	-0.0114	0.9974

Approach	E S	S E		E+S	Sb Sf	Intercept	Residuals	
		Sb	Sf					
Mesoplankton	-0.0306	0.0072	0.0362	0.5611	0.002	-0.0187	0.44274	
<b>V</b>	<b>Overall</b>	0.1772***	0.0389*	0.0952***	0.3579	0.0023	-0.0143	0.3424
	I	0.2409*	0.0352	0.0762*	0.1409	0.0052	-0.0113	0.5126
	II	0.1655*	0.0599*	0.0999*	0.23	0.0064	-0.0159	0.454
	III	0.4478**	0.0854*	-0.0126	0.0537	-0.004	0.001	0.4286
	IV	0.0936	0.016	0.1284***	0.5094	0.0027	-0.0187	0.2682
<b>CSR</b>	<b>Overall</b>	0.0481	0.0398	0.2275***	0.0583	-0.002	-0.0079	0.6366
	C	0.1833*	0.043	0.173**	0.1171	-0.005	-0.0062	0.495
	S	-0.0645	0.0697	0.0955	0.1746	0.0177	-0.0286	0.7356
	R	-0.0246	0.0016	0.4559**	-0.159	-0.021	0.0126	0.7341

\* p-value  $\leq$  0.05

\*\* p-value  $\leq$  0.005

\*\*\* p-value  $\leq$  0.001

## CONSIDERAÇÕES FINAIS

Em resumo, avaliamos diferentes influências dos fatores de estruturação da comunidade fitoplanctônica, não apenas ao usar abordagens taxonômicas e distintas abordagens funcionais, mas também ao analisarmos a variação de dados entre grupos/categorias do mesmo sistema de classificação funcional. Mostramos que tanto o espaço quanto o ambiente são fatores importantes da variação da comunidade em pequena escala, mas sua influência varia de acordo com a abordagem utilizada. Em nosso estudo, a composição taxonômica apresentou melhor influência dos componentes ambientais e espaciais do que alguns sistemas de classificação funcional e não deve ser negligenciada em pesquisas de estruturação de comunidades fitoplanctônicas. Nossas descobertas também indicaram que, ao usar menos grupos/categorias de organismos que são altamente influenciados pelo ambiente e pela dispersão, os pesquisadores também podem acessar a influência de fatores direcionadores da comunidade. Considerando as classificações funcionais, volume e MBFG parecem ser abordagens adequadas para acessar fatores estruturantes de microrganismos, devido ao seu maior poder explanatório e sensibilidade a sinais espaciais e ambientais. No entanto, ao desconstruir estas abordagens e avaliar os grupos funcionais que as compõem (geralmente compostos por múltiplos atributos), entende-se que não é trivial relacionar os mecanismos ecológicos de interesse nos estudos de metacomunidade. É o caso, por exemplo, do grupo VI do MBFG, que engloba todas as diatomáceas, nas quais, independentemente de sua reconhecida fidelidade a fatores ambientais, nenhum sinal ambiental foi encontrado. Além disso, a influência de variáveis espaciais na estruturação desse grupo deve ser realizada com cuidado, pois as populações naturais das diatomáceas têm uma reconhecida ampla faixa de tamanho corporal.

O uso de traços de tamanho para acessar os processos de dispersão melhorou nosso sinal espacial, pois todos os sistemas de classificação funcional utilizados estavam relacionados à morfologia e exibiram significância espacial pelo menos em uma escala. No entanto, maior influência de variáveis espaciais em organismos menores, com maior capacidade de dispersão, em pequena escala geográfica, só foi evidente em MLD, volume e parcialmente no agrupamento funcional de múltiplos traços do MBFG, o que pode refletir a

multiplicidade de *trade-offs* de traços funcionais de tamanho. Em conclusão, para fornecer respostas diretas e mais conceituais aos padrões observados na natureza, precisamos avaliar e entender a relevância funcional da abordagem utilizada, bem como a relação dos traços com variáveis ambientais e processos de dispersão em estudos de metacomunidade fitoplanctônica. Como perspectivas futuras, abordagens comparativas sobre classificações funcionais, composição de espécies e traços devem ser incentivadas em diferentes escalas espaciais para fortalecer o entendimento dessas métricas no contexto da estruturação de metacomunidades de microorganismos.

## REFERÊNCIAS BIBLIOGRÁFICAS

- Adler P.B., Hillerislambers J. & Levine J.M. (2007). A niche for neutrality. *Ecology Letters*, 10: 95-104.
- Adler P.B., Fajardo A., Kleinhesselink A.R. & Kraft N.J.B. (2013). Trait-based tests of coexistence mechanisms. *Ecology Letters*, 16:1294-1306.
- Allende L., Fontanarrosa M.S., Murno A. & Sinistro R. (2019). Phytoplankton functional group classifications as a tool for biomonitoring shallow lakes: a case study. *Knowledge & Management of Aquatic Ecosystems*, 420(5).
- Baas-Becking, L.G.M. (1934). Geobiologie of Inleiding Tot de Milieukunde. W.P. Van Stockum & Zoon, The Hague.
- Beisner B.E., Peres-Neto P.R., Lindström E.S., Barnett A. & Longhi M.L. (2006). The Role of Environmental and Spatial Processes in Structuring Lake Communities from Bacteria to Fish. *Ecology*, 87: 2985–2991.
- Bortolini J.C., Silva P.R.L., Baumgartner G. & Bueno N.C. (2019). Response to environmental, spatial, and temporal mechanisms of the phytoplankton metacommunity: comparing ecological approaches in subtropical reservoirs. *Hydrobiologia*, 830: 45-61.
- Calow P. (1987). Towards a definition of functional ecology. *Functional Ecology*, 1(1):57-61.
- Chesson, P. (2000). Mechanisms of maintenance of species diversity. *Annu. Rev. Ecol. Syst.*, 31: 343–66.
- Chisti Y. (2004). Microalgae: our marine forests. In: Richmond, A. (ed.). Handbook of microalgal culture: biotechnology and applied phycology. Oxford, Blackwell Science.
- Cottenie K. (2005). Integrating environmental and spatial processes in ecological community dynamics. *Ecology Letters*, 8: 1175–1182.
- Crossetti L.O., Freitas-Teixeira L.M., Bohnenberger J.L., Schulz U.H., Rodrigues L.R. & Motta-Marques D. (2018). Responses of the phytoplankton functional structure to the spatial and temporal heterogeneity in a large subtropical shallow lake. *Acta Limnologica Brasiliensia*, 30(214).
- De Bie T., De Meester L., Brendonck L., Martens K., Goddeeris B., Ercken D., Hampel H., Denys L., Vanhecke L., Van der Gucht K., Van Wichelen J., Vyverman W. & Declerck S.A.J. (2012). Body size and dispersal mode as key traits determining metacommunity structure of aquatic organisms. *Ecology Letters*, 15: 740–74.7

Feki-Sahnoun W., Hamza A., Béjaoui B., Mahfoudi M., Rebai A. & Bel-Hassen M. (2018). Multi-table approach to assess the biogeography of phytoplankton: ecological and management implications. *Hydrobiologia*, 815: 229–251.

Finlay B.J. (2002). Global Dispersal of Free-Living Microbial Eukaryote Species. *Science*, 296: 1061-1063.

Grainger T.N. & Gilbert B. (2016). Dispersal and diversity in experimental metacommunities: linking theory and practice. *Oikos*, 125: 1213-1223.

Grime J.P. (1977). Evidence for the existence of three primary strategies in plants and its relevance to ecological and evolutionary theory. *American Naturalist*, 111(982): 1169–1194.

Heino J., Melo A.S., Siqueira T., Soininen J., Valanko S. & Bini L.M. (2015). Metacommunity organisation, spatial extent and dispersal in aquatic systems: patterns, processes and prospects. *Freshwater Biology*, 60(5): 845-869.

Heino J., Soininen J., Alahuhta J., Lappalainen J. & Virtanen R. (2017). Metacommunity ecology meets biogeography: effects of geographical region, spatial dynamics and environmental filtering on community structure in aquatic organisms. *Oecologia*, 183(1): 121–137.

Horner-Devine M.C., Silver J.M., Leibold M.A., Bohannan B.J.M., Colwell R.K., Fuhrman J.A., Green J.L., Kuske C.R., Martiny J.B.H., Muyzer G., Øvreås L., Reysenbach A.L. & Smith V.H. (2007). A comparison of taxon co-occurrence patterns for macro- and microorganisms. *Ecology* 88: 1345–1353.

Hubbell S.P. (2001). *The Unified Neutral Theory of Biodiversity and Biogeography*. Princeton University Press, Princeton, NJ.

Huszar V.L.M., Nabout J.C., Appel M., Santos J.B.O., Abe D.S. & Silva L.H.S. (2015). Environmental and not spatial processes (directional and non-directional) shape the phytoplankton composition and functional groups in a large subtropical river basin. *Journal of Plankton Research*, 37: 1190–1200.

Incagnone G., Marrone F., Barone R., Robba L. & Naselli-Flores L. (2015). How do freshwater organisms cross the “dry ocean”? A review on passive dispersal and colonization processes with a special focus on temporary ponds. *Hydrobiologia*, 750: 103–123.

Iatskiu P., Bovo-Scomparin V.M., Segovia B.T., Velho L.F.M., Lemke M.J. & Rodrigues L.C. (2018). Variability in mean size of phytoplankton in two floodplain lakes of different climatic regions. *Hydrobiologia*, 823: 135-151.

Izaguirre I., Saad J.F., Romina-Schiaffino M., Vinocur A., Tell G., Sánchez M.L., Allende L. & Sinistro R. (2015). Drivers of phytoplankton diversity in patagonian and antarctic lakes across a latitudinal gradient (2150 km): The importance of spatial and environmental factors. *Hydrobiologia*, 764: 157–170.

Jenkins D.G., Brescacin C.R., Duxbury C.V., Elliott J.A., Evans J.A., Grablow K.R., Hillegass M., Lyon B.N., Metzger G.A., Olandese M.L., Pepe D., Silvers G.A., Suresch H.N., Thompson T.N., Trexler C.M., Williams G.E., Williams N.C. & Williams S.E. (2007). Does size matter for dispersal distance?. *Global Ecology and Biogeography*, 16: 415-425.

Kristiansen J. (1996). Dispersal of freshwater algae – a review. *Hydrobiologia*, 336: 151-157.

Kruk C., Huszar V.L.M., Peeters E.T.H.M., Bonilla S., Costa L., Lüring M., Reynolds C.S. & Scheffer M. (2010). A morphological classification capturing functional variation in phytoplankton. *Freshwater Biology*, 55: 614-627.

Lester S.E., Ruttenberg B.I., Gaines S.D. & Kinlan B.P. (2007). The relationship between dispersal ability and geographic range size. *Ecology Letters*, 10: 745-758.

Leibold, M. A., Holyoak M., Mouquet N., Amarasekare P., Chase J.M., Hoopes M.F., Holt R.D., Shurin J.B., Law R., Tilman D., Loreau M., & Gonzalez A. (2004). The metacommunity concept: A framework for multi-scale community ecology. *Ecology Letters*, 7: 601–613.

Leruste A., Villéger S., Malet N., De Wit R. & Bec B. (2018). Complementarity of the multidimensional functional and the taxonomic approaches to study phytoplankton communities in three Mediterranean coastal lagoons of different trophic status. *Hydrobiologia*, 815: 207–227.

Levin S.A. (1988). Pattern, Scale, and Variability: An Ecological Perspective. (In: Hastings A. (eds) *Community Ecology. Lecture Notes in Biomathematics*, vol 77). Springer, Berlin, Heidelberg.

Lindström, E.S. & Langenheder S. (2012). Local and regional factors influencing bacterial community assembly. *Environmental Microbiology*, 4: 1–9.

Litchman E. & Klausmeier C.A. (2008). Trait-Based Community Ecology of Phytoplankton. *Annual Review of Ecology, Evolution, and Systematics*, 39: 615-639.

Litchman E., Pinto P.T., Klausmeier C.A., Thomas M.K. & Yoshiyama K. (2010). Linking traits to species diversity and community structure in phytoplankton. *Hydrobiologia*, 653: 15-28.

Louca, S., Jacques S.M.S., Pires A.P.F., Leal J.S., Srivastava D.S., Parfrey L.W., Farjalla V.F. & Doebeli M. (2016). High taxonomic variability despite stable functional structure across microbial communities. *Nature Publishing Group Nature Publishing Group*, 1: 1–12.

Maloufi S., Catherine A., Mouillot D., Louvard C., Couté A., Bernard C. & Troussellier M. (2016). Environmental heterogeneity among lakes promotes



hyper  $\beta$ -diversity across phytoplankton communities. *Freshwater Biology*, 61: 633-645.

Margalef R. (1978). Life forms of phytoplankton as survival alternatives in an unstable environment. *Oceanologia Acta*, 1: 493–509.

Marquet P.A., Fernández M., Navarrete A.S. & Valdovinos C. (2004). Diversity emerging: towards a deconstruction of biodiversity patterns. In Lomolino, M. V. and Heaney, L. (eds), *Frontiers of Biogeography: New Directions in the Geography of Nature*. Cambridge University Press, Cambridge, pp. 191–209.

Martiny, J.B.H., Bohannon B.J.M., Brown J.H., Colwell R.K., Fuhrman J.A., Green J.L., Horner-Devine M.C., Kane M., Krumins J.A., Kuske C.R., Morin P.J., Naeem S., Øvreås L., Reysenbach A.L., Smith V.H., & Staley J.T. (2006). Microbial biogeography: Putting microorganisms on the map. *Nature Reviews Microbiology*, 4: 102–112.

Moresco GA., Bortolini J.C., Dias J.D. Pineda A., Jati S. & Rodrigues L.C. (2017). Drivers of phytoplankton richness and diversity components in Neotropical floodplain lakes, from small to large spatial scales. *Hydrobiologia*, 799(1): 203-215.

Naselli-Flores L. & Padisák J. (2016). Blowing in the wind: how many roads can a phytoplankton walk down? A synthesis on phytoplankton biogeography and spatial processes. *Hydrobiologia*, 764(1): 303-313.

Naselli-Flores L., Termine R. & Barone R. (2016). Phytoplankton colonization patterns. Is species richness depending on distance among freshwaters and on their connectivity?. *Hydrobiologia*, 764(1): 103-111.

Ng I.S.Y., Carr C.M. & Cottenie K. (2009). Hierarchical zooplankton metacommunities: distinguishing between high and limiting dispersal mechanisms. *Hydrobiologia*, 619: 133-143.

Padial, A.A., Ceschin F., Declerck S.A.J., De Meester L., Bonecker C.C., Lansac-Tôha F.A., Rodrigues L., Rodrigues L.C., Train S., Velho L.F.M., & Bini L.M. (2014). Dispersal ability determines the role of environmental, spatial and temporal drivers of metacommunity structure. *PLoS ONE*, 9: 1–8.

Padisák J., Borics G., Grigorszky I. & Soróczki-Pintér É. (2006). Use of phytoplankton assemblages for monitoring ecological status of lakes within the Water Framework Directive: the assemblage index. *Hydrobiologia*, 553: 1-14.

Padisák J., Crossetti L.O. & Naselli-Flores L. (2009). Use and misuse in the application of the phytoplankton functional classification: A critical review with updates. *Hydrobiologia*, 621: 1–19.

Padisák, J., Vasas G., & Borics G., (2016). Phycogeography of freshwater phytoplankton: Traditional knowledge and new molecular tools. *Hydrobiologia*, 764: 3–27.

Pančić M. & Kiørboe T. (2018). Phytoplankton defence mechanisms: traits and trade-offs. *Biological Reviews*, 93(2):1269-1303.

Petchey O.L. & Gaston K.J. (2006). Functional diversity: back to basics and looking forward. *Ecology Letters*, 9:741-758.

Reynolds C.S. (1988). Functional morphology and the adaptive strategies of freshwater phytoplankton. In: *Growth and Reproductive Strategies of Freshwater Phytoplankton* (Ed. C.D. Sandgren), pp. 388–433. Cambridge University Press, Cambridge.

Reynolds C.S., Huszar V., Kruk C., Nasselli-Flores L. & Melo S. (2002). Towards a functional classification of the freshwater phytoplankton. *Journal of Plankton Research*, 24(5): 417–428.

Reynolds C.S. (2006) *The Ecology of Phytoplankton*. Cambridge University Press, Cambridge.

Reynolds C.S., Elliott J.A., Frassl M.A. (2014). Predictive utility of trait-separated phytoplankton groups: A robust approach to modeling population dynamics. *Journal of Great Lakes Research*, 40: 143-150.

(a) Ribeiro, K.F., Duarte, L. & Crossetti, L.O. (2018). Everything is not everywhere: a tale on the biogeography of cyanobacteria. *Hydrobiologia*, 820: 23-49.

(b) Ribeiro K.F., da Rocha C.M., de Castro D., Rodrigues L.R. & Crossetti L.O. (2018). Distribution and coexistence patterns of phytoplankton in subtropical shallow lakes and the role of niche-based and spatial processes. *Hydrobiologia*, 814(1): 233-246.

Salmaso N., Naselli-Flores L. & Padisák J. (2015). Functional classifications and their application in phytoplankton ecology. *Freshwater Biology*, 60: 603–619.

Scheffer M., Rinaldi S., Huisman J. & Weissing F.J. (2003). Why plankton communities have no equilibrium: solutions to the paradox. *Hydrobiologia*, 491: 9-18.

Sharma N.K., Rai A.K., Singh S. & Brown Jr. R.M. (2007). Airborne algae: their present status and relevance. *Journal of Phycology*, 43: 615-627.

Soininen J., Jamoneau A., Rosebery J. & Passy S.I. (2016). Global patterns and drivers of species and trait composition in diatoms. *Global Ecology and Biogeography*, 25: 940–950.

Souffreau, C., Van Der Gucht K., Van Gremberghe I., Kosten S., Lacerot G., Lobão L.M., Lúcia V., Huszar D.M., Roland F., Jeppesen E., Vyverman W. & De Meester L. (2015). Environmental rather than spatial factors structure bacterioplankton communities in shallow lakes along a > 6000 km latitudinal gradient in South America. *Environmental Microbiology*, 17: 2336–2351.

- Thompson R. & Townsend C. (2006). A truce with neutral theory: local deterministic factors, species traits and dispersal limitation together determine patterns of diversity in stream invertebrates. *Journal of Animal Ecology*, 75: 476-484.
- Tolonen K. T., Yongjiu C., Vilmi A., Karjalainen S.M., Sutela T. & Heino J. (2018). Environmental filtering and spatial effects on metacommunity organisation differ among littoral macroinvertebrate groups deconstructed by biological traits. *Aquatic Ecology*, 52: 119-131.
- Vellend, M., Srivastava D.S., Anderson K.M., Brown C.D., Jankowski J.E., Kleynhans E.J., Kraft N.J.B., Letaw A.D., Macdonald A.A.M., Maclean J.E., Myers-smith I.H., Norris A.R., & Xue X. (2014). Assessing the relative importance of neutral stochasticity in ecological communities. *Oikos*, 0: 1–11.
- Villéger S., Mason N.W.H. & Mouillot D. (2008). New multidimensional functional diversity indices for a multifaceted framework in functional ecology. *Ecology*, 89(8):2290-2301.
- Vilmi A., Tolonen K.T., Karjalainen S.M. & Heino J. (2017). Metacommunity structuring in a highly-connected aquatic system: effects of dispersal, abiotic environment and grazing pressure on microalgal guilds. *Hydrobiologia*, 790: 125.
- Vilmi A., Karjalainen S.M., Hellsten S. & Heino J. (2016). Bioassessment in a metacommunity context: Are diatom communities structured solely by species sorting?. *Ecological indicators*, 62: 86-94.
- Violle C., Navas M.L., Vile D., Kazakou E., Fortunel C., Hummel I. & Garnier E. (2007). Let the concept of trait be functional!. *Oikos* 116: 882-892.
- Weithoff G. (2003). The concepts of 'plant functional types' and 'functional diversity' in lake phytoplankton – a new understanding of phytoplankton ecology?. *Freshwater Biology*, 48: 1669–1675.
- Weithoff G. & Beiner B.E. (2019). Measures and Approaches in Trait-Based Phytoplankton Community Ecology – From Freshwater to Marine Ecosystems. *Frontiers in Marine Science*. 6:40. doi: 10.3389/fmars.2019.00040.
- Winegardner A.K., Jones B.K., Ng I.S.Y., Siqueira T. & Cottenie K. (2012). The terminology of metacommunity ecology. *Trends in Ecology and Evolution (Letters)*, 27(5).
- Wojciechowski J., Heino J., Bini L.M. & Padial A.A. (2017). The strength of species sorting of phytoplankton communities is temporally variable in subtropical reservoirs. *Hydrobiologia*, 800(1): 31-43.
- Wu N., Qu Y., Guse B., Makarevičiūtė K., To S., Riis T. & Fohrer N. (2018). Hydrological and environmental variables outperform spatial factors in structuring

species, trait composition, and beta diversity of pelagic algae. *Ecology and Evolution*, 8: 2947–2961.

Xiao L.J., Zhu Y., Yang Y., Lin Q., Han B.P. & Padisák J. (2018). Species-based classification reveals spatial processes of phytoplankton meta-communities better than functional group approaches: a case study from three freshwater lake regions in China. *Hydrobiologia*, 811: 313–324.

Zhou, J., & Ning D. (2017). Stochastic Community Assembly: Does It Matter in Microbial Ecology?. *Microbiology and Molecular Biology Reviews*, 81: 1–32.

## APENDIX

### Classification of the 169 phytoplankton species in Morphology Based Functional Groups (MBFG), life strategy groups (CSR), volume categories (Vol) and maximum linear dimension categories (MLD).

Species	MBFG	MLD	Vol	CRS
<i>Acanthosphaera</i> cf. <i>tenuispina</i> Korshikov 1953	IV	Nanophytoplankton	II	C
<i>Ankistrodesmus arcuatus</i> Korshikov 1953	IV	Microphytoplankton	II	R
<i>Ankistrodesmus fusiformis</i> Corda 1838	IV	Microphytoplankton	II	C
<i>Ankistrodesmus spiralis</i> (W.B.Turner) Lemmermann 1908	IV	Microphytoplankton	II	C
<i>Aphanizomenon ovalisporum</i> Forti 1911	VIII	Microphytoplankton	III	R
<i>Aphanizomenon</i> sp.	VIII	Microphytoplankton	III	R
<i>Aphanocapsa incerta</i> (Lemmermann) G.Cronberg & Komárek 1994	VII	Microphytoplankton	III	R
<i>Aphanothece</i> sp.	VII	Microphytoplankton	III	R
<i>Aulacoseira ambigua</i> (Grunow) Simonsen 1979	VI	Microphytoplankton	II	R
<i>Aulacoseira granulata</i> (Ehrenberg) Simonsen 1979	VI	Mesophytoplankton	IV	R
<i>Capartogramma</i> sp.	VI	Nanophytoplankton	II	C
<i>Carteria</i> sp.	V	Nanophytoplankton	II	C
<i>Ceratium furcoides</i> (Levander) Langhans 1925	V	Microphytoplankton	IV	R
<i>Chlamydomonas</i> cf. <i>planctogloea</i> Skuja 1956	V	Nanophytoplankton	II	C
<i>Chlamydomonas debaryana</i> Goroschankin [Gorozhankin] 1891	V	Nanophytoplankton	II	C
<i>Chlorella minutissima</i> Fott & Nováková 1969	I	Nanophytoplankton	I	C
<i>Chlorella</i> sp.	I	Nanophytoplankton	II	C
<i>Chlorella vulgaris</i> Beyerinck [Beijerinck] 1890	I	Nanophytoplankton	II	C
Chlorococcales sp1	IV	Nanophytoplankton	II	C
Chlorococcales sp2	IV	Nanophytoplankton	III	C
Chlorococcales sp3	IV	Nanophytoplankton	II	C
Chlorococcales sp4	IV	Microphytoplankton	III	C
Chlorococcales sp5	IV	Nanophytoplankton	II	C
Chlorococcales sp6	I	Nanophytoplankton	II	C
Chlorococcales sp7	IV	Nanophytoplankton	II	C

Species	MBFG	MLD	Vol	CRS
<i>Chlorogonium</i> cf. <i>fusiforme</i> Matvienko 1938	IV	Nanophytoplankton	II	C
Chroococcales sp1	VII	Nanophytoplankton	II	C
Chroococcales sp2	VII	Nanophytoplankton	II	C
Chroococcales sp3	VII	Nanophytoplankton	II	C
Chroococcales sp4	I	Nanophytoplankton	I	C
Chroococcales sp5	VII	Nanophytoplankton	III	R
<i>Chroococcus</i> cf. <i>dispersus</i> (Keissler) Lemmermann 1904	VII	Microphytoplankton	III	R
<i>Chroococcus minutus</i> (Kützing) Nägeli 1849	VII	Nanophytoplankton	II	C
<i>Chrysophyceae</i> sp.	II	Microphytoplankton	III	R
<i>Closteriopsis</i> sp.	IV	Nanophytoplankton	II	R
<i>Closterium</i> cf. <i>setaceum</i> Ehrenberg ex Ralfs 1848	IV	Microphytoplankton	II	R
<i>Cocconeis</i> sp.	VI	Nanophytoplankton	II	C
<i>Coelastrum cruciatum</i> Schmidle 1900	IV	Nanophytoplankton	III	R
<i>Coelastrum microporum</i> Nägeli in A.Braun 1855	IV	Nanophytoplankton	III	R
<i>Coelastrum</i> sp.	IV	Nanophytoplankton	II	C
<i>Coelosphaerium punctiferum</i> Komárek & Komárková-Legnerová 1992	VII	Nanophytoplankton	II	C
<i>Cosmarium</i> cf. <i>contractum</i> var. <i>minutum</i> (Delponte) Coesel 1989	IV	Nanophytoplankton	II	C
<i>Cosmarium</i> sp1	IV	Microphytoplankton	III	R
<i>Cosmarium</i> sp2	IV	Microphytoplankton	III	S
<i>Craticula</i> sp.	VI	Microphytoplankton	II	R
<i>Crucigenia fenestrata</i> (Schmidle) Schmidle 1900	IV	Nanophytoplankton	II	C
<i>Crucigenia tetrapedia</i> (Kirchner) Kuntze 1898	I	Nanophytoplankton	II	C
<i>Cryptomonas</i> cf. <i>tenuis</i> Pascher 1913	V	Nanophytoplankton	II	R
<i>Cryptomonas curvata</i> Ehrenberg 1832	V	Microphytoplankton	III	R
<i>Cryptomonas erosa</i> Ehrenberg 1832	V	Nanophytoplankton	II	C
<i>Cryptomonas marssonii</i> Skuja 1948	V	Nanophytoplankton	II	R
<i>Cryptomonas phaseolus</i> Skuja 1948	V	Nanophytoplankton	II	C
<i>Cryptomonas</i> sp.1	V	Nanophytoplankton	II	C
<i>Cryptophyceae</i> sp. 1	V	Nanophytoplankton	III	R
<i>Cuspidothrix issatschenkoi</i> (Usachev) P.Rajaniemi, Komárek, R.Willame, P. Hrouzek, K.Kastovská, L.Hoffmann & K.Sivonen 2005	VIII	Microphytoplankton	III	R
Cyanophyceae sp1	VII	Microphytoplankton	II	C
<i>Cyclotella</i> sp.	VI	Nanophytoplankton	II	C
<i>Desmodesmus armatus</i> (Chodat) Hegewald var. <i>armatus</i>	IV	Nanophytoplankton	III	C
<i>Desmodesmus armatus</i> var. <i>bicaudatus</i> (Gugl.) Hegew	IV	Nanophytoplankton	II	C
<i>Desmodesmus</i> cf. <i>denticulatus</i> (Lagerheim) An, Friedl & Hegewald	IV	Microphytoplankton	III	R
<i>Desmodesmus</i> cf. <i>maximus</i> (W. & G. S. West) Hegew 2000	IV	Microphytoplankton	III	R
<i>Desmodesmus lunatus</i> (W. & G. S. West) Hegew 2000	IV	Nanophytoplankton	II	C
<i>Dinobryon</i> sp.	II	Microphytoplankton	II	R
Dinophyceae 1	V	Nanophytoplankton	III	R
Dinophyceae 2	V	Nanophytoplankton	II	R
Dinophyceae 3	V	Microphytoplankton	IV	S

Species	MBFG	MLD	Vol	CRS
Dolichospermum sp1	VIII	Microphytoplankton	III	R
Dolichospermum sp2	VIII	Mesophytoplankton	IV	R
<i>Elakatothrix gelatinosa</i> Wille 1898	IV	Microphytoplankton	II	R
<i>Elakatothrix</i> sp.	IV	Microphytoplankton	II	R
<i>Eucapsis parallelepipedon</i> (Schmidle) Komárek & Hindák 1989	VII	Nanophytoplankton	II	C
<i>Euglena</i> sp.	V	Microphytoplankton	IV	S
<i>Franceia ovalis</i> (Francé) Lemmermann 1898	IV	Nanophytoplankton	II	C
<i>Geitlerinema splendidum</i> (Greville ex Gomont) Anagnostidis 1989	III	Mesophytoplankton	III	R
<i>Gomphosphaeria</i> sp.	VII	Microphytoplankton	III	R
<i>Gyrosigma</i> sp.	VI	Microphytoplankton	III	R
<i>Hariotina reticulata</i> P.A.Dangeard 1889	IV	Microphytoplankton	IV	S
<i>Humidophila</i> cf. <i>contenta</i> (Grunow) Lowe, Kocielek, J.R.Johansen, Van de Vijver, Lange-Bertalot & Kopalová 2014	VI	Nanophytoplankton	II	C
<i>Kirchneriella obesa</i> (West) West & G.S.West 1894	IV	Nanophytoplankton	II	C
<i>Lemmermannia triangularis</i> (Chodat) C.Bock & Krienitz in C.Bock et al. 2013	IV	Nanophytoplankton	II	C
<i>Lepocinlis</i> sp.	V	Microphytoplankton	III	C
<i>Mallomonas</i> sp.	II	Microphytoplankton	III	R
<i>Melosira varians</i> C.Agardh 1827	VI	Mesophytoplankton	IV	S
<i>Merismopedia tranquila</i> (Ehrenberg) Trevisan 1845	VII	Nanophytoplankton	II	C
<i>Microcystis aeruginosa</i> (Kützing) Kützing 1846	VII	Microphytoplankton	IV	S
<i>Monallanthus</i> cf. <i>brevicylindrus</i> (Pascher) A.R. Loeblich III 1967	IV	Nanophytoplankton	II	C
<i>Monallanthus</i> sp1	IV	Nanophytoplankton	II	C
<i>Monoraphidium</i> cf. <i>irregulare</i> (G.M.Smith) Komárková-Legnerová 1969	IV	Microphytoplankton	II	R
<i>Monoraphidium contortum</i> (Thuret) Komárková-Legnerová in Fott 1969	I	Nanophytoplankton	II	C
<i>Monoraphidium flexuosum</i> Komárek 1974	I	Nanophytoplankton	II	C
<i>Monoraphidium griffithii</i> (Berkeley) Komárková-Legnerová 1969	IV	Microphytoplankton	II	C
<i>Monoraphidium kormarkovae</i> Nygaard 1979	IV	Microphytoplankton	II	C
<i>Monoraphidium minutum</i> (Nägeli) Komárková-Legnerová 1969	I	Nanophytoplankton	II	C
<i>Monoraphidium tortile</i> (West & G.S.West) Komárková-Legnerová 1969	IV	Microphytoplankton	II	C
<i>Mucidosphaerium pulchellum</i> (H.C.Wood) C.Bock, Proschold & Krienitz 2011	IV	Nanophytoplankton	II	C
<i>Navicula</i> sp.	VI	Microphytoplankton	II	R
<i>Neglectella solitaria</i> (Wittrock) Stenclová & Kastovsky in Stenclová et al 2017	IV	Nanophytoplankton	II	C
NI 01	IV	Nanophytoplankton	II	C
NI 02	IV	Nanophytoplankton	II	C
<i>Chrysochromulina</i> sp.	II	Nanophytoplankton	II	C
NI 04	IV	Nanophytoplankton	II	C
NI 05	IV	Nanophytoplankton	II	C
NI 06	IV	Nanophytoplankton	II	C
NI 07	V	Nanophytoplankton	II	C
NI 08	IV	Nanophytoplankton	II	C

Species	MBFG	MLD	Vol	CRS
NI 09	IV	Microphytoplankton	II	C
NI 10	IV	Nanophytoplankton	III	R
NI 11	IV	Nanophytoplankton	II	C
NI 12	IV	Microphytoplankton	III	R
NI 13	IV	Nanophytoplankton	II	C
NI 14	IV	Nanophytoplankton	II	C
NI 15	IV	Nanophytoplankton	II	C
<i>Nitzschia</i> sp.	VI	Microphytoplankton	II	R
<i>Nostocales</i> sp.	VIII	Nanophytoplankton	II	C
<i>Nupela</i> sp.	VI	Nanophytoplankton	II	C
<i>Oocystis lacustres</i> Chodat 1897	IV	Nanophytoplankton	III	R
<i>Oscillatoriales</i> sp.	VII	Nanophytoplankton	II	C
<i>Pectinodesmus</i> cf. <i>pectinatus</i> (Meyen) E.Hegewald, M.Wolf, Al.Keller, Friedl & Krienitz 2010	IV	Nanophytoplankton	III	R
<i>Pediastrum angulosum</i> Ehrenberg ex Meneghini 1840	IV	Nanophytoplankton	II	C
<i>Pediastrum boryanum</i> (Turpin) Meneghini 1840	IV	Nanophytoplankton	II	R
<i>Pediastrum duplex</i> Meyen 1829	IV	Microphytoplankton	III	R
<i>Pediastrum tetras</i> (Ehrenberg) Ralfs 1845	IV	Microphytoplankton	II	R
<i>Penales</i> sp.	VI	Nanophytoplankton	II	C
<i>Phacus</i> sp.	V	Nanophytoplankton	II	C
<i>Phormidium tergestinum</i> (Rabenhorst ex Gomont) Anagnostidis & Komárek 1988	VII	Mesophytoplankton	IV	R
<i>Pinnularia</i> sp.	VI	Microphytoplankton	III	R
<i>Placoneis</i> sp.	VI	Microphytoplankton	IV	S
<i>Plagioselmis lacustres</i> (Pascher & Ruttner) Javornicky 2001	V	Nanophytoplankton	II	C
<i>Planktolyngbya contorta</i> (Lemmermann) Anagnostidis & Komárek 1988	VII	Mesophytoplankton	III	R
<i>Planktolyngbya limnetica</i> (Lemmermann) Komárková-Legnerová & Cronberg 1992	VII	Microphytoplankton	II	R
<i>Pseudanabaena</i> cf. <i>catenata</i> Lauterborn 1915	IV	Microphytoplankton	II	R
<i>Pseudanabaena limnetica</i> (Lemmermann) Komárek 1974	IV	Microphytoplankton	II	R
<i>Pseudoschroederia antillarum</i> (Komárek) Hegewald & Schnepf 1986	IV	Microphytoplankton	II	R
<i>Scenedesmus acunae</i> Comas González 1980	IV	Microphytoplankton	III	C
<i>Scenedesmus</i> cf. <i>obtusus</i> f. <i>disciformis</i> (Chodat) Compère 1977	IV	Microphytoplankton	III	R
<i>Scenedesmus ecornis</i> (Ehrenberg) Chodat 1926	IV	Nanophytoplankton	II	C
<i>Scenedesmus</i> sp1	IV	Microphytoplankton	III	R
<i>Sellaphora</i> sp.	VI	Nanophytoplankton	II	C
<i>Sphaerocystis schroeteri</i> Chodat 1897	IV	Nanophytoplankton	III	C
<i>Staurastrum iversenii</i> var. <i>americanum</i> A.M.Scott & Grönblad	IV	Nanophytoplankton	II	C
<i>Staurastrum paradoxum</i> var. <i>parvum</i> (West) N.Carter 1923	IV	Nanophytoplankton	II	C
<i>Staurodesmus</i> sp1	IV	Nanophytoplankton	III	C
<i>Staurodesmus</i> sp2	IV	Nanophytoplankton	II	C
<i>Synechococcus</i> spp.	VIII	Nanophytoplankton	II	C
<i>Synechocystis</i> spp.	I	Picophytoplankton	I	C
<i>Synura</i> sp.	II	Nanophytoplankton	II	R

Species	MBFG	MLD	Vol	CRS
<i>Tetraëdron caudatum</i> (Corda) Hansgirg 1888	IV	Nanophytoplankton	II	C
<i>Tetraëdron cf. gracile</i> (Reinsch) Hansgirg 1889	IV	Nanophytoplankton	II	C
<i>Tetraëdron minimum</i> (A.Braun) Hansgirg 1888	IV	Nanophytoplankton	II	C
<i>Tetraëdron</i> sp.	IV	Nanophytoplankton	II	C
<i>Tetraëdron triangulare</i> Korshikov 1953	IV	Nanophytoplankton	II	C
<i>Tetrastrum glabrum</i> (Y.V.Roll) Ahlstrom & Tiffany 1934	IV	Nanophytoplankton	II	C
<i>Tetrastrum heteracanthum</i> (Nordstedt) Chodat 1895	IV	Nanophytoplankton	II	C
<i>Trachelomonas bernardii</i> Woloszynska 1912	V	Nanophytoplankton	II	C
<i>Trachelomonas cf. dybowskii</i> Drezepolski 1923	V	Nanophytoplankton	II	R
<i>Trachelomonas cf. stokesii</i> Drezepolski 1925	V	Nanophytoplankton	III	C
<i>Trachelomonas curta</i> var. <i>minima</i> Tell & Z.Domitrovic	V	Nanophytoplankton	II	C
<i>Trachelomonas pulcherrima</i> var. <i>minor</i> Playfair 1915	V	Nanophytoplankton	II	C
<i>Trachelomonas</i> sp1	V	Nanophytoplankton	II	C
<i>Trachelomonas</i> sp2	V	Nanophytoplankton	II	C
<i>Trachelomonas</i> sp3	V	Nanophytoplankton	II	C
<i>Trachelomonas</i> sp4	V	Nanophytoplankton	II	C
<i>Trachelomonas volvocina</i> (Ehrenberg) Ehrenberg 1834	V	Nanophytoplankton	II	C
<i>Trachelomonas volvocinopsis</i> Svirenko 1914	V	Nanophytoplankton	II	C
<i>Trachelomonas volzii</i> var. <i>intermedia</i> Playfair 1915	V	Nanophytoplankton	III	R
<i>Treubaria quadrispina</i> (G.M.Smith) Fott & Kováčik 1975	IV	Nanophytoplankton	II	C
<i>Treubaria setigera</i> (W.Archer) G.M.Smith 1933	IV	Nanophytoplankton	II	C
<i>Ulnaria ulna</i> (Nitzsch) Compère 2001	VI	Microphytoplankton	II	R
Zygnematophyceae 1	IV	Microphytoplankton	III	R