



Universidade Federal do Rio Grande
Instituto de Ciências Biológicas
Pós-graduação em Biologia de
Ambientes Aquáticos Continentais



**Diatomáceas perifíticas como indicadoras:
abordagens para o monitoramento ambiental em
riachos**

Ana Paula Tavares Costa

Orientadora: Prof^a Dr^a Fabiana Schneck

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Tese apresentada ao Programa de Pós-graduação em Biologia de Ambientes Aquáticos Continentais como requisito parcial para a obtenção do título de Doutora em Biologia de Ambientes Aquáticos Continentais.

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Dedico aos meus pais e maior saudade: Paulo Roberto Costa e Amélia Maria Tavares Costa

(In memoriam)

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RESUMO

O Antropoceno é marcado por diversos impactos humanos nos mais variados ecossistemas, sendo considerado um período de extinções em massa. Os ecossistemas lóticos estão entre os mais afetados. Nesse sentido, a Tese aqui apresentada teve como principal objetivo testar métricas de biomonitoramento e avaliações ecológicas em riachos - utilizando diatomáceas - visando principalmente a redução de tempo em laboratório, especialmente em relação à identificação das comunidades em nível de espécie. O primeiro capítulo compõe uma revisão sistemática que teve por objetivo investigar as tendências sobre o uso de diatomáceas no biomonitoramento e avaliações ecológicas em ambientes lóticos. Os principais achados nesse capítulo foram: (i) a pesquisa sobre esse tema é consolidada na União Européia e América do Norte; (ii) a comunidade epilítica é a mais amostrada nos estudos; (iii) a eutrofização é o impacto mais avaliado; (iv) espécies como *Achnanthes minutissimum*, *Gomphonema parvulum* e *Nitzschia palea* são comumente citadas como indicadoras e, (v) algumas métricas, especialmente as não-taxonômicas, têm se destacado nos últimos anos. No segundo capítulo foi testado se a identificação em nível de gênero e o uso de classes de biovolume (potenciais grupos substitutos) são eficazes em representar a variação espacial da estrutura das comunidades de diatomáceas em nível de espécie. Também foi avaliada a importância relativa das variáveis espaciais e ambientais na comunidade em nível de espécie e grupos substitutos. Entre os achados desse capítulo estão: (i) a congruência entre as comunidades em nível de espécie e os substitutos foi elevada quando utilizado dados de abundância; (ii) o componente espacial foi importante para explicar a composição de comunidades apenas em nível de espécie e, (iii) o componente ambiental foi importante para explicar a composição de comunidades em nível de espécie e dos grupos substitutos. O terceiro capítulo teve por objetivo testar métricas taxonômicas (índices de diversidade e diatomológicos) e não-taxonômicas (morfologia de valvas) no biomonitoramento e avaliações ecológicas de riachos categorizados de acordo com as concentrações de nitrogênio. Aqui, apenas as métricas morfológicas de largura média e forma de *A. minutissimum* diferiram entre as categorias de riachos. As informações presentes nessa Tese permitiram o avanço no conhecimento acerca do uso de métricas de diatomáceas que buscam simplificar o biomonitoramento e avaliações ecológicas, especialmente em relação à necessidade de identificação das comunidades em nível de espécie.

Palavras-chave: Revisão sistemática, Grupos substitutos, Diatomácea bentônica, Bioma Pampa, Ambiente lótico, Morfometria

ABSTRACT

The Anthropocene is marked by several anthropogenic impacts on the most varied ecosystems and is considered a period of mass extinctions. Lotic ecosystems are among the most affected. In this sense, the main objective of the Thesis presented here was to test biomonitoring metrics and ecological assessments in streams - using diatoms - that were mainly aimed at reducing laboratory time, especially in relation to the identification of communities at the species level. The first chapter is a systematic review investigating trends in diatoms' use in biomonitoring and ecological assessments in lotic environments. The main findings in this chapter were: (i) research on this topic is consolidated in the European Union and North America; (ii) the epilithic community is the most sampled in studies; (iii) eutrophication is the most evaluated impact; (iv) species such as *Achnanthes minutissimum*, *Gomphonema parvulum* and *Nitzschia palea* are commonly cited as indicators and, (v) some metrics, especially non-taxonomic, have been highlighted in recent years. The second chapter tested whether genus-level identification and the use of biovolume classes (potential surrogates) are effective in representing the spatial variation of diatom community structure at the species level. We also assessed the relative importance of spatial and environmental variables in the community at the species and surrogate levels. Among the findings of this chapter are: (i) congruence between communities at the species level and surrogates was high when using abundance data; (ii) the spatial component was important in explaining community composition only at the species level and, (iii) the environmental component was important in explaining community composition at the species level and surrogates. The aim of the third chapter was to test taxonomic (diversity and diatom indices) and non-taxonomic (valve morphology) metrics in biomonitoring and ecological assessments of streams categorized according to the nitrogen concentrations. Here, only the morphological metrics of average width and shape of *A. minutissimum* differed between stream categories. Based on the information presented in this Thesis, it can be concluded that we have made some progress in our knowledge about the use of diatom metrics that seek to simplify biomonitoring and ecological assessments, especially in relation to the need to identify communities at the species level.

Key-words: Systematic review, Surrogates, Benthic diatom, Pampa biome, Lotic environment, Morphometry

APRESENTAÇÃO

Esta Tese de doutorado segue o modelo sugerido pelo Programa de Pós-Graduação em Biologia de Ambientes Aquáticos Continentais e está estruturada em cinco seções principais. A primeira seção compõe a **Introdução Geral** onde abordo os principais temas conceituais propostos por essa Tese, seguido pelos objetivos que nortearam o trabalho. Os principais tópicos da Introdução Geral são: (i) impactos antrópicos nos ecossistemas aquáticos; (ii) monitoramento ambiental em ambientes lóticos e as comunidades aquáticas; (iii) diatomáceas perifíticas; (iv) métricas taxonômicas de diatomáceas no monitoramento de ambientes lóticos; (v) métricas não taxonômicas de diatomáceas no monitoramento de ambientes lóticos; (vi) área de estudo e; (vii) objetivos. Essa seção está formatada de acordo as normas da Associação Brasileira de Normas Técnicas (ABNT). A segunda seção está apresentada como **capítulo 1** e é referente ao artigo baseado em uma revisão sistemática e análise cienciométrica que traz uma visão global sobre o uso de diatomáceas em estudos de biomonitoramento e avaliações ecológicas de ambientes lóticos entre 2000 e 2020. Esse capítulo está publicado e formatado de acordo com as normas da revista *Environmental Monitoring and Assessment*. A terceira seção está apresentada como **capítulo 2** e é referente ao artigo que buscou verificar se a identificação da comunidade em nível de gênero e o uso de classes de biovolume são eficazes em representar a variação espacial da estrutura das comunidades de diatomáceas, bem como a importância relativa das variáveis espaciais e ambientais nas comunidades em nível de espécie, gênero e classes de biovolume. Esse segundo capítulo está aceito na revista *Anais da Academia Brasileira de Ciências*. A quarta seção está apresentada como **capítulo 3** e é referente ao manuscrito que buscou verificar o desempenho de métricas não taxonômicas (tamanho, forma e deformidades nas valvas de diatomáceas) e taxonômicas (índices de diversidade e diatomológicos) para o monitoramento ambiental em riachos. Esse terceiro capítulo será submetido e está escrito de acordo com as normas da revista *Acta Limnologica Brasiliensis*. A quinta seção compõe as **Considerações Finais e Perspectivas** com as principais conclusões obtidas a partir dos trabalhos desenvolvidos durante a Tese, e com perspectivas de estudos futuros.

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INTRODUÇÃO GERAL

Impactos antrópicos nos ecossistemas aquáticos

Os ambientes aquáticos continentais comumente se encontram entre os ecossistemas mais negligenciados por políticas de preservação ambiental (e.g. CÉRÉGHINO *et al.*, 2008; AZEVEDO-SANTOS *et al.*, 2018). No Brasil, o cenário de degradação ambiental se agravou de forma mais intensa nos últimos quatro anos (2019-2022) em função de retrocessos consideráveis de políticas ambientais que fomentaram principalmente o avanço da mineração e do agronegócio sobre ecossistemas de relevância mundial (DIAS *et al.*, 2021; PELICICE; CASTELLO, 2021). A despeito disso, ecossistemas dulcícolas brasileiros seguem entre os mais biodiversos, com número elevado de espécies representantes de algas, poríferos, microcrustáceos e peixes (AGOSTINHO; THOMAZ; GOMES, 2005). No entanto, em consequência do acelerado processo de degradação ambiental, esses ecossistemas estão entre os ambientes com maiores taxas de extinção local e global de espécies (HE *et al.*, 2019).

Os principais impactos antrópicos responsáveis atual crise na biodiversidade aquática são: (i) introdução accidental e/ou deliberada de espécies exóticas; (ii) poluição por águas residuais (por exemplo, efluentes domésticos, agrícolas ou industriais liberados nos ecossistemas sem tratamento adequado); (iii) alterações no uso do solo e cobertura da terra para fins de atividades antrópicas, como agricultura extensiva; (iv) alterações hidrológicas, como para construções de barragens e hidrelétricas; (v) sobre-exploração de recursos e; (vi) mudanças climáticas (AGOSTINHO; THOMAZ; GOMES, 2005; DUDGEON, 2019; Figura 1). Além disso, é sabido que os ambientes aquáticos podem estar vulneráveis a dois ou mais impactos e os seus efeitos simultâneos podem potencializar os danos ecológicos. Por exemplo, como já evidenciado para a biomassa e estrutura de comunidades fitoplanctônicas, que podem ser alteradas em função da disponibilidade de nutrientes associado ao aumento da temperatura da água de lagos de pequeno porte (VERBEEK *et al.*, 2018). Em um outro estudo, as autoras simularam temperaturas elevadas associadas ao aumento do escoamento superficial em mesocosmos e verificaram que esses dois potenciais estressores causam alterações na diversidade taxonômica e funcional de diatomáceas perifíticas (DA SILVA; TORGAN; SCHNECK, 2019).

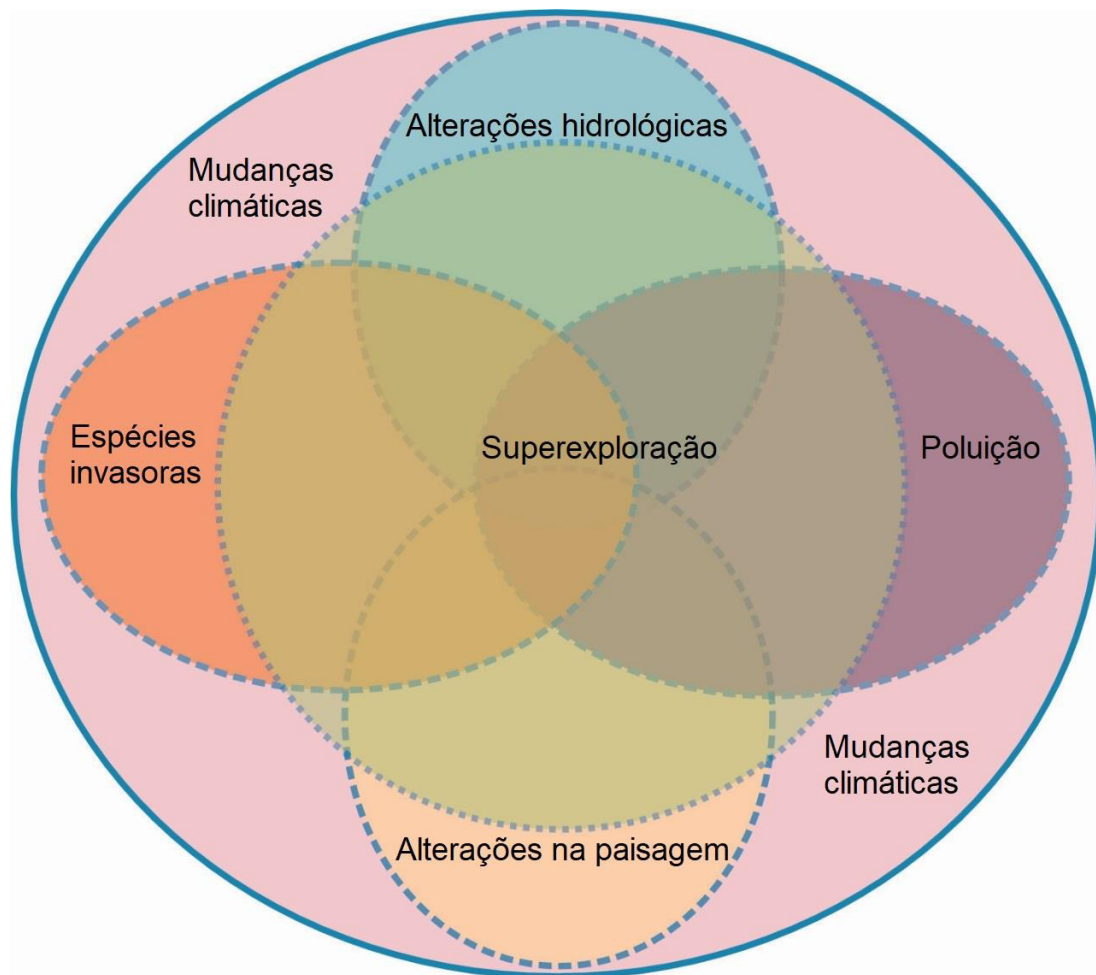


Figura 1. Esquema ilustrativo sobre as principais ameaças responsáveis pela perda de qualidade de habitat e consequente redução da diversidade em ambientes aquáticos continentais. Imagem adaptada de Dudgeon (2019).

Frente a essa problemática responsável pela perda da integridade ambiental e consequente redução da biodiversidade, o monitoramento biológico associado a avaliações de parâmetros químicos e físicos da água, é essencial para verificar a integridade ecológica dos ambientes aquáticos continentais. Ainda, vale ressaltar que estratégias de biomonitoramento e avaliações ecológicas que visem otimizar o tempo, assim como reduzir recursos financeiros, que são demandados em análises de laboratório e que possibilitem a avaliação de um volume maior de amostras no tempo e no espaço são cada vez mais necessárias devido ao avanço acelerado dos impactos antropogênicos sobre os ecossistemas aquáticos.

Monitoramento ambiental em ambientes lóticos e as comunidades aquáticas

A qualidade da água de ambientes lóticos pode ser verificada através da detecção de parâmetros limnológicos seguido de aplicação de índices, como o Índice de Qualidade da Água (IQA; CASTRO JÚNIOR; SOBREIRA; BORTOLOTI, 2007) e o Índice de Estado Trófico (IET; LAMPARELLI, 2004). Uma ressalva importante sobre índices baseados em caracterizações limnológicas é que eles fornecem informações ambientais acerca do momento da coleta. Por outro lado, a análise de comunidades aquáticas permite o entendimento sobre processos que podem ter ocorrido anteriormente à coleta, e que têm influência na integridade ambiental (LOBO; CALLEGARO; BENDER, 2002). Dessa forma, os parâmetros físicos e químicos da água e os parâmetros biológicos são considerados complementares e permitem avaliações adequadas sobre a integridade ambiental dos ecossistemas lóticos.

Países da União Europeia comumente se destacam (tanto em número como em qualidade) em estudos sobre monitoramento biológico e restauração de ambientes lóticos, como já evidenciado em revisões da literatura (FEIO *et al.*, 2021; COSTA; SCHNECK, 2022). Isso provavelmente está relacionado ao fato de que esses países estão comprometidos, através de políticas de gestão de recursos hídricos, a garantir um bom estado ecológico de águas superficiais pela *Water Framework Directive (WFD)* implementada em 2000 (veja HERING *et al.*, 2010 - uma revisão sobre as conquistas e os desafios após 10 anos de implementação da diretiva). Nos Estados Unidos existe a *Clear Water Act (CWA; 1972)* responsável por “restaurar e manter a integridade química, física e biológica das águas da Nação”, e cada estado é responsável por entregar relatórios periódicos acerca da qualidade ambiental dos ambientes aquáticos. Ainda, no continente africano existe uma série de programas implementados de biomonitoramento de ambientes lóticos (FEIO *et al.*, 2021). Em especial na África do Sul, existe a *National Water Act of South Africa* que é uma lei que tem por objetivo manter os parâmetros ambientais e biológicos (com foco em peixes, mata ciliar e macroinvertebrados) o mais próximo possível de ambientes classificados previamente como pouco perturbados (KLEYNHANS; LOUW, 2008; FEIO *et al.*, 2021).

Em contrapartida, no Brasil existe a resolução 357 de 2005 do CONAMA (Conselho Nacional de Meio Ambiente) que categoriza os ambientes dulcícolas aquáticos em diferentes classes de uso com base em diversos parâmetros físicos, químicos, e também biológicos - como densidade de cianobactérias, coliformes fecais, *Escherichia coli* e concentrações de clorofila *a* na água. Apesar de ser um país detentor de grande diversidade de ecossistemas aquáticos continentais (ABELL *et al.*, 2008), não existe no Brasil uma política ambiental

específica que vise monitorar periodicamente a integridade dos corpos de água através de avaliação de parâmetros ambientais associados às comunidades biológicas. Dessa forma, estudos de biomonitoramento e avaliações ecológicas em ambientes lóticos brasileiros são comumente realizados frente a demandas específicas, como avaliar e/ou mitigar possíveis impactos originados a partir de grandes empreendimentos (e.g. construções de hidrelétricas) nos ecossistemas lóticos (FEIO *et al.*, 2021). O Programa de Pesquisa Ecológica de Longa Duração (PELD/CNPq) existe no Brasil há 25 anos e possui sítios associados a Instituições Federais distribuídos em todo o Brasil. Dessa forma, os PELDs apresentam grande contribuição científica acerca dos padrões que regem a biodiversidade brasileira nos mais variados ecossistemas, bem como sobre potenciais estressores, naturais e antropogênicos que afetam esses ecossistemas (e.g. BRITO *et al.*, 2020). Ainda, vale ressaltar o importante papel das Universidades Federais na produção científica brasileira (MCMANUS *et al.*, 2021), que é refletido também na produção de trabalhos de biomonitoramento e avaliações ecológicas de ambientes lóticos (FEIO *et al.*, 2021). No entanto, é sabido que a ciência nas Universidades Federais é construída principalmente através de projetos de pesquisa realizados por mestrands e doutorandos (e que desenvolvem seus trabalhos entre dois e quatro anos) (MCMANUS *et al.*, 2021), assim uma ressalva importante que ainda existe em relação a estudos de biomonitoramento e avaliações ecológicas no Brasil é a falta de amostragens e avaliações temporais das comunidades aquáticas (FEIO *et al.*, 2021).

Em estudos de biomonitoramento e avaliações ecológicas em ambientes aquáticos continentais é comum encontrar os termos bioindicador e biomarcador, e eventualmente esses dois conceitos podem gerar certa confusão. A principal diferença entre esses conceitos está no tipo de resposta que os organismos-modelo podem fornecer ao biomonitoramento. De acordo com Johnson *et al.* (1993) uma espécie (ou um conjunto de espécies) considerada indicadora “apresenta requerimentos específicos para um conjunto de variáveis físicas e químicas, de forma que alterações na sua presença/ausência indicam que atuais condições físicas químicas estão fora do limite de tolerância daquela espécie”. Nesse sentido, espécies tolerantes tendem a manter suas populações frente a impactos ambientais em detrimento do desaparecimento de espécies consideradas sensíveis. Por outro lado, um biomarcador refere-se geralmente a medidas em nível de organismo, como alterações bioquímicas, morfológicas e/ou fisiológicas que podem indicar um potencial estresse ambiental (BARTELL, 2006). Como já evidenciado, táxons de diatomáceas como *Pinnularia* sp. e *Achnanthyidium minutissimum* são consideradas

bons biomarcadores pois desenvolvem teratologias nas valvas em resposta a altas concentrações de metais pesados e pesticidas na água (LAVOIE *et al.*, 2017).

Algumas comunidades aparecem com frequência em estudos de biomonitoramento e avaliações ecológicas em ecossistemas lóticos. Dentre essas comunidades podem ser destacadas as diatomáceas (COSTA; SCHNECK, 2022), macrófitas aquáticas (e.g. UMETSU *et al.*, 2018), macroinvertebrados (e.g. KARAOUZAS *et al.*, 2019) e peixes (HERING; JOHNSON; BUFFAGNI, 2006). Algumas divergências acerca de qual comunidade é a mais adequada para avaliar os ambientes lóticos ainda são encontradas. Enquanto alguns autores defendem que apenas as comunidades de diatomáceas seriam suficientes para a avaliação da integridade ambiental de riachos (KARAOUZAS *et al.*, 2019), outros afirmam que comunidades de peixes e macroinvertebrados são mais sensíveis e, consequentemente respondem melhor a alterações de origem antrópica em ambientes lóticos (HERING; JOHNSON; BUFFAGNI, 2006; MARZIN *et al.*, 2012). Por fim, um outro estudo defende que as comunidades pertencentes a diferentes táxons (como diatomáceas e macroinvertebrados) mostram respostas complementares ao biomonitoramento, especialmente em relação à especificidade do estressor no ambiente (PACE *et al.*, 2012).

Diversas métricas taxonômicas são comumente aplicadas às comunidades a fim de avaliar os ambientes aquáticos. Dentre essas métricas estão a verificação da diversidade alfa, como riqueza de espécies e índices de Shannon, Equitabilidade de Pielou e Simpson (e.g. XUE *et al.*, 2019). Análises de composição de comunidades, bem como de diversidade beta, também são comumente avaliadas (e.g. CHEN *et al.*, 2018). Além disso, índices bióticos específicos (em geral baseados em presença/ausência e diferenças na abundância de espécies) para determinar a qualidade ambiental são frequentemente utilizados. Dentre esses índices se pode destacar o *Iberian Biomonitoring Working Party* para macroinvertebrados (IBMWP; ALBA-TERCEDOR *et al.*, 2002), *Macrophyte Index of Biotic integrity in Itanhaém* para macrófitas aquáticas (MIBI-ITA; UMETSU *et al.*, 2018) e o *Specific Pollution sensitivity Index* para diatomáceas (SPI; CEMAGREF, 1982).

Diatomáceas perifíticas

É denominado perifiton o biofilme que se forma aderido a qualquer substrato submerso (seja natural ou artificial) em ambientes aquáticos (WETZEL, 1983). Esse biofilme forma-se em função da colonização de diferentes comunidades como bactérias, cianobactérias, fungos, pequenos invertebrados e algas (WETZEL, 1983). Dentre essas comunidades, as diatomáceas

comumente se destacam em função da abundância e diversidade do grupo, especialmente em ecossistemas de riachos (ALLAN; FLECKER, 1993). Ainda, a comunidade perifítica pode ser classificada de acordo com o substrato no qual está aderida. Por exemplo: (i) comunidade epilítica – caracterizada pela comunidade aderida a rochas; (ii) comunidade epifítica – aderida a macrófitas aquáticas; (iii) comunidade epizóica – aderida a carapaças de animais (e.g. conchas de moluscos) e; (iv) comunidade epipélica – comunidade que cresce sobre o sedimento (SLÁDECKOVÁ, 1962).

Diatomáceas são algas microscópicas pertencentes ao grupo Bacillariophyta. Apesar de ainda não aceito universalmente, estudos apontam para a existência de três classes de diatomáceas: Coscinodiscophyceae, Mediophyceae (espécies com simetria radial e comumente denominadas cêntricas) e Bacillariophyceae (espécies com simetria bilateral e comumente denominadas penadas) (MEDLIN; KACZMARSKA, 2004; MEDLIN, 2016). As diatomáceas são caracterizadas pela presença de um pigmento específico no grupo denominado fucoxantina, responsável pela coloração amarronzada observada (tanto no plâncton como no perifiton) quando esses organismos estão em abundância no ambiente (LOBO; CALLEGARO; BENDER, 2002). A crisolaminarina (um polissacarídeo) é a principal fonte de reserva energética desse grupo, porém corpos lipídicos são comumente encontrados no interior das células desses organismos (LOBO; CALLEGARO; BENDER, 2002). Uma das principais características que define o grupo é a presença de uma carapaça no entorno da célula, denominada frústula (formada por duas valvas unidas pelo cingulo), composta predominantemente de sílica (REVIERS, 2006). Na frústula das diatomáceas existe uma série de aréolas – estruturas que permitem as trocas desses organismos com o meio externo (REVIERS, 2006). Além disso, a forma das frústulas e a disposição das aréolas criam diferentes ornamentações nos indivíduos, o que permite a identificação em nível de espécie por meio de microscopia óptica (MANN, 1999). No entanto, em alguns casos, essas diferenças são diminutas e a identificação em nível de espécie só é possível em microscopia eletrônica de varredura, como é o caso de algumas espécies de Fragilariales (MORALES; SIVER; TRAINOR, 2001).

Estimativas sugerem que existem pelo menos 12.000 espécies de diatomáceas descritas, e pelo menos 8.000 espécies ainda não descritas (GUIRY, 2012), distribuídas em diferentes formas de vida que podem ser unicelulares, coloniais ou filamentosas (REVIERS, 2006). Esses organismos podem ser encontrados tanto no plâncton como no perifiton de ambientes aquáticos continentais (HOEK; MANN; JAHNS, 1995). As classes

Coscinodiscophyceae e Mediophyceae predominam no plâncton (HOEK; MANN; JAHNS, 1995), mas ocorrem eventualmente no perifíton, como o é caso de *Cyclotella meneghiniana* (e.g. MACIEL; CAVALCANTE; LUDWIG, 2022). Por outro lado, a classe Bacillariophyceae ocorre comumente no perifíton em função de diversas características que permitem a aderência desses organismos ao substrato, como a formação de tubos ou pedúnculos de mucilagem, além do hábito de vida prostrado com forte aderência ao substrato (RIMET; BOUCHEZ, 2012).

É sabido que algumas espécies de diatomáceas podem crescer em meio heterotrófico, como a *Fistulifera pelliculosa* (LEWIN, 1953), e outras podem apresentar o metabolismo mixotrófico, ou seja, alternando entre heterotrofia e autotrofia dependendo das condições ambientais (e.g. VILLANOVA *et al.*, 2017). No entanto, a maior parte do grupo é fotossintetizante (ROUND; CRAWFORD; MANN, 1990), o que torna as diatomáceas perifíticas essenciais para a produção primária (servindo de alimento para pequenos invertebrados e peixes) de origem autóctone de ambientes aquáticos continentais, especialmente ambientes rasos e pouco sombreados pela vegetação ciliar (BIGGS, 1996). Ainda, as diatomáceas perifíticas desempenham importante papel na ciclagem de nutrientes, especialmente ao absorver e reter nutrientes disponíveis na água, como nitrogênio, fósforo e sílica (FERNANDES; ESTEVES, 2011). Dessa forma, a comunidade perifítica também é responsável por transformar formas inorgânicas de nutrientes em formas orgânicas (FERNANDES; ESTEVES, 2011).

As diatomáceas perifíticas são comumente utilizadas em estudos de biomonitoramento e avaliações ecológicas de ambientes lóticos, especialmente as epilíticas (COSTA; SCHNECK, 2022). A capacidade da comunidade perifítica de acumular poluentes (como agrotóxicos, por exemplo) é frequentemente destacada em estudos que visam avaliar a qualidade de ambientes aquáticos (FERNANDES; ESTEVES, 2011). Ainda, respostas rápidas às alterações ambientais, ampla distribuição, rápido ciclo de vida, facilidade de amostragem e possibilidade de confecção de lâminas permanentes são características fundamentais que tornam as diatomáceas bons organismos-modelo em estudos de monitoramento e avaliações ecológicas em ambientes lóticos (STEVENSON; PAN; VAN DAM, 2010).

Entender a relação entre comunidades de diatomáceas e variáveis ambientais é interesse de pesquisadores da área há mais de um século (e.g. KOLKWITZ; MARSSON, 1908). Desde então muitas métricas são comumente utilizadas com o intuito de avaliar a integridade de ambientes lóticos. Nas últimas décadas, por exemplo, métricas taxonômicas

como avaliações através de índices de diversidade e diatomológicos, bem como análises de composição de comunidades são as mais utilizadas em estudos em vários países (COSTA; SCHNECK, 2022). Atualmente, o uso de DNA *metabarcoding* a fim de detectar alterações ambientais vem recebendo destaque (COSTA; SCHNECK, 2022). Mais recentemente métricas não taxonômicas vêm recebendo destaque na literatura, especialmente porque não há necessidade de identificação da comunidade em nível de espécie, otimizando assim o tempo que é demandado em laboratório (PANDEY *et al.*, 2018a; COSTA; SCHNECK, 2022). Dentre essas métricas se pode destacar análises morfofisiológicas, como mensurações de corpos lipídicos da célula, tamanho e deformidades (também denominadas teratologias) da valva.

Métricas taxonômicas de diatomáceas no monitoramento de ambientes lóticos

Métricas baseadas em diversidade taxonômica e diversidade funcional de diatomáceas são comumente utilizadas em estudos que buscam a avaliação de ambientes lóticos, uma vez que é esperado que a diversidade seja menor em ecossistemas perturbados por atividades antrópicas. Como já evidenciado em riachos costeiros, a riqueza de espécies de diatomáceas possui relação negativa com o aumento das concentrações de fósforo total na água, provável consequência de um gradiente urbano existente (COSTA *et al.*, 2022). Ainda, a diversidade avaliada através do índice de Shannon tende a diminuir em função do aumento de concentrações de metais pesados (como cobre e zinco), como verificado em um estudo *in situ* com substratos difusores de metais (PANDEY; BERGEY, 2018). No entanto, vale ressaltar que os índices de diversidade nem sempre respondem de forma esperada aos estressores ambientais. Por exemplo, ao avaliar impactos do uso do solo no entorno de ambientes lóticos, índices diatomológicos se mostraram mais eficazes em detectar efeitos de estressores ambientais na água quando comparados ao índice de equabilidade de Pielou (KARAOUZAS *et al.*, 2018).

Análises de composição de espécies utilizando dados de presença e ausência ou de abundância são também utilizadas para entender a relação das comunidades biológicas com variáveis ambientais. Algumas análises de ordenação multivariadas são utilizadas para sumarizar um grande número de informações disponíveis sobre variáveis ambientais e visualizar possíveis relações com os componentes bióticos (GAUCH, 1982). Uma das análises amplamente utilizada é a Análise de Correspondência Canônica (CCA; TER BRAAK, 1986) a qual visa explorar possíveis relações entre dois conjuntos de variáveis (e.g.

bióticas e abióticas). Uma outra análise muito utilizada é a Análise de Redundância (*RDA*; MAKARENKOV; LEGENDRE, 2002), que se assemelha à *CCA* pois explora duas matrizes de variáveis, porém as distâncias utilizadas são diferentes (enquanto a *CCA* utiliza distância qui-quadrado a *RDA* utiliza distância euclidiana). Além disso, a *RDA* é considerada uma extensão da regressão múltipla de forma que relaciona a matriz multivariada de composição de espécies (variável resposta) com a matriz de variáveis ambientais (variáveis preditoras).

A diversidade beta, bem como seus componentes (substituição e aninhamento) é uma outra forma de entender como a composição e estrutura das comunidades respondem aos estressores ambientais de origem antrópica. Por exemplo, em riachos costeiros, além da redução da riqueza de espécies de diatomáceas em função de um gradiente de fósforo, também foi evidenciado um aninhamento de espécies, ou seja, comunidades mais pobres em número de espécies foram subconjuntos de comunidades mais ricas, localizadas em locais com menores concentrações de fósforo na água (COSTA *et al.*, 2022). Em outro estudo, foi observado que a diversidade beta à jusante de um rio fortemente influenciado pelo incremento de nutrientes e metais pesados sendo significativamente menor em comparação à região à montante (MORIN *et al.*, 2015). Além disso, nesse mesmo estudo verificou-se que as espécies que predominaram à jusante do rio foram aquelas consideradas tolerantes a ambientes perturbados.

A verificação de espécies indicadoras de diatomáceas é comumente utilizada em estudos de avaliações ecológicas e biomonitoramento. Dentre as análises para verificar espécies indicadoras se pode destacar o procedimento de *BioEnv* (CLARKE; AINSWORTH, 1993), que correlaciona a dissimilaridade biótica com a dissimilaridade abiótica determinando assim quais variáveis abióticas influenciam a matriz biótica e a *Two-way Indicator Species Analysis* (*TWINSPAN*; HILL, 1979) que seleciona grupos de espécies indicadoras semelhantes entre si. Ainda, uma das análises mais utilizadas é o Índice do Valor de Indicação (IndVal; DUFRÊNE; LEGENDRE, 1997) que combina especificidade e fidelidade das espécies para determinada unidade amostral, sendo a especificidade baseada na abundância relativa e a fidelidade na frequência relativa. Uma outra análise utilizada em estudos mais recentes é a *Threshold Indicator Taxa Analysis* (*TITAN*; BAKER; KING, 2010) que foi elaborada baseada no IndVal. No entanto, uma das diferenças é que o IndVal determina as espécies indicadoras baseado em unidades amostrais já determinadas, enquanto o *TITAN* agrupa as espécies independente da unidade amostral. Vale ressaltar que a faixa de tolerância de diferentes

espécies de diatomáceas pode alterar dependendo do tipo de impacto que está sendo avaliado (e.g. COSTA; SCHNECK, 2022).

Dezenas de índices diatomológicos baseados na abundância e valores atribuídos às espécies dependendo de suas respectivas faixas de tolerância foram desenvolvidos e possuem aplicação em todo o planeta, tanto em ambientes lóticos como em ambientes lênticos. Alguns como o *SPI* e o *Trophic Diatom Index (TDI)* (KELLY; WHITTON, 1995) são amplamente utilizados (e.g. TAN *et al.*, 2017; YIRIGUI; LEE; NEJADHASHEMI, 2019; BIGHIU *et al.*, 2020). Além disso, índices para aplicação em escala regional também são comumente utilizados, como o *Pampean Diatom Index (IDP)* (GÓMEZ; LICURSI, 2001), que é um índice desenvolvido para avaliar rios e riachos do bioma Pampa. Especificamente o *SPI* é considerado um dos índices mais robustos, pois abrange mais de 2000 espécies e é frequentemente utilizado para criar e calibrar outros índices (e.g. DESCY; COSTE, 1991). Além dos índices diatomológicos, índices que combinam diferentes aspectos da comunidade como resposta para avaliar a integridade dos ecossistemas, como o *Diatom Multimetric Index (MDIAT)* (DELGADO; PARDO; GARCÍA, 2010) são frequentemente aplicados em ambientes lóticos.

Paralelamente o DNA *metabarcoding* é uma ferramenta promissora em estudos de biomonitoramento e avaliações ecológicas de ambientes aquáticos com diatomáceas (PAWLOWSKI *et al.*, 2016). Com a extração do DNA ambiental ocorrente na comunidade periférica é possível amplificar fragmentos de genes através da reação em cadeia da polimerase (PCR) e assim, obter sequências de DNA das espécies que estão na amostra (TAPOLCZAI *et al.*, 2019). Dessa forma, o emprego dessa técnica permite a análise de um volume grande de amostras (espaciais e temporais da comunidade) em curto espaço de tempo quando comparado às demais métricas taxonômicas anteriormente mencionadas. Além disso, essa abordagem já se mostrou eficiente para detectar alterações em comunidades de diatomáceas ao longo de um gradiente de nitrogênio e fósforo (SMUCKER *et al.*, 2020), e também para detectar efeitos de uso agrícola do solo em ambientes lóticos (TAPOLCZAI *et al.*, 2021). Ainda, quando comparada a índices tradicionalmente utilizados (diatomológicos e diversidade, por exemplo), a resposta ao biomonitoramento é semelhante (VASSELON *et al.*, 2017). Porém, o alto custo demandado para a implementação do DNA *metabarcoding* é uma ressalva importante dessa técnica. Além disso, informações importantes sobre as comunidades são perdidas, como por exemplo grupos e traços funcionais, a presença de teratologias e medidas lineares de tamanho.

A utilização de resoluções taxonômicas menos refinadas, como em nível de gênero ou família, é considerada uma boa forma de simplificar estratégias de biomonitoramento e avaliações ecológicas e elucidar padrões de diversidade em ambientes aquáticos continentais (e.g. HEINO; SOININEN, 2007; VASCONCELOS; MELO; SCHWARZBOLD, 2013; MISSIAS *et al.*, 2017; PIRES *et al.*, 2021). Ainda, vale destacar que a verificação das comunidades através de uma resolução taxonômica mais grosseira pode ser considerada um bom substituto de espécies em estudos de biomonitoramento, caso ambos os níveis respondam de forma similar aos padrões espaciais, temporais e/ou ambientais responsáveis pela estruturação das comunidades aquáticas. Para diatomáceas, por exemplo, já foi demonstrado que a identificação de gêneros é mais eficiente que de famílias como substituto de espécies para detectar padrões de diversidade e estrutura de comunidades em riachos (HEINO; SOININEN, 2007).

Por fim, outra estratégia comumente mencionada de simplificação do biomonitoramento é através do emprego da resolução numérica, por exemplo com dados de abundância e de presença e ausência (e.g. CARNEIRO; BINI; RODRIGUES, 2010). É sabido que avaliações através de dados de abundância favorecem as espécies mais comuns na comunidade, por outro lado, avaliações com dados de presença e ausência tendem a favorecer as espécies raras da comunidade (ANDERSON *et al.*, 2011), provendo assim informações diferentes sobre a estrutura dessas comunidades. Estudos evidenciaram congruência entre as respostas fornecidas por dados de presença e ausência e de abundância para comunidades de invertebrados (SOUZA *et al.*, 2019), peixes (BARBOSA *et al.*, 2019), fitoplanctônicas (CARNEIRO; BINI; RODRIGUES, 2010) e perifíticas (FAQUIM *et al.*, 2021), de forma que uma resolução numérica menos refinada (baseada em dados de presença e ausência) pode ser considerada um substituto em estudos de biomonitoramento e avaliações ecológicas.

Métricas não-taxonômicas de diatomáceas no monitoramento de ambientes lóticos

A necessidade de taxonomistas (especialidade cada vez mais rara entre os cientistas; WÄGELE *et al.*, 2011) com experiência em identificação de diatomáceas e de literatura atualizada são dificuldades já relatadas em estudos de avaliações de integridade de ambientes lóticos (TAYLOR *et al.*, 2005; LAW; ELLIOTT; THACKERAY, 2014). Frente a essas dificuldades, métricas baseadas em biomarcadores, como características morfológicas e fisiológicas (comumente denominadas métricas não-taxonômicas; PANDEY *et al.*, 2018a) de uma ou algumas poucas espécies presentes em abundância nas comunidades, têm ganhado

destaque na literatura nos últimos anos (COSTA; SCHNECK, 2022). Dentre essas métricas se pode destacar: (i) avaliação de corpos lipídicos no interior das células, (ii) mensurações de medidas lineares da valva, como comprimento e largura, e (iii) detecção de valvas teratológicas (ou deformadas) nas populações.

O aumento no número e tamanho de corpos lipídicos no conteúdo intracelular de diatomáceas frente a fatores estressores já é bem estabelecido na literatura (e.g. PANDEY *et al.*, 2018a; PANDEY; BERGEY; 2018; PARK *et al.*, 2020), apesar do mecanismo fisiológico ainda não estar bem esclarecido na literatura (PANDEY *et al.*, 2017). Pode-se destacar a relação positiva do biovolume de corpos lipídicos com a presença de metais pesados (PANDEY; BERGEY; 2018; PANDEY *et al.*, 2018b; PARK *et al.*, 2020) e com o aumento nas concentrações de nitrogênio e fósforo (PANDEY *et al.*, 2018a; PANDEY *et al.*, 2018b). Por outro lado, não foi detectada influência de lixiviados de bitucas de cigarro nos lipídios (número ou biovolume) de diatomáceas (WERDEL; PANDEY; BERGEY, 2021). Vale ressaltar que a mensuração de corpos lipídicos com o intuito de verificar a integridade ambiental de ecossistemas lóticos aparece recentemente na literatura, conforme destacado por Costa e Schneck (2022), sendo ainda necessário testar a eficiência dessa métrica frente a outros estressores, como poluição orgânica, alterações de uso do solo e alterações hidrológicas.

Métricas baseadas em tamanho celular ou tamanho da valva de diatomáceas são potencialmente implementáveis em estudos de avaliações ecológicas devido a sua metodologia relativamente simples, rápida e de custo reduzido (e.g. PANDEY *et al.*, 2017). Além disso, alguns autores já demonstraram a eficiência de tais métricas frente a alterações ambientais. Por exemplo, a sazonalidade (e consequentemente diferenças na temperatura e nível da água) afeta diretamente o tamanho das diatomáceas de forma que células maiores predominam no inverno e primavera, enquanto células menores são mais abundantes no verão e outono (SUN *et al.*, 2018). Ainda, como demonstrado para ambientes lênticos temperados, a largura média da valva de *Achnanthes minutissimum* possui relação positiva com o aumento das concentrações de nitrogênio e fósforo na água, sendo a obtenção dessas medidas eficientes para detectar a eutrofização artificial, inclusive durante a fase inicial do processo de incremento de nutrientes no ambiente (VILMI *et al.*, 2015). No entanto, ainda é necessário verificar o desempenho dessa abordagem em ambientes lóticos e em regiões tropicais e subtropicais.

Deformidades em valvas de diatomáceas podem surgir em função de deficiências durante a captação e absorção de sílica na frústula (PANDEY *et al.*, 2014) ou devido a problemas no arcabouço estrutural microtubular durante a fase mitótica da célula (DEBENEST *et al.*, 2008). São identificadas pelo menos quatro classes de teratologias: (i) no contorno da valva; (ii) na rafe; (iii) nas estrias e; (iv) mista (associação de pelo menos duas das deformidades mencionadas) (PANDEY *et al.*, 2014). A figura abaixo ilustra algumas deformidades encontradas no contorno da valva em diatomáceas de riachos do bioma Pampa (Figura 2).

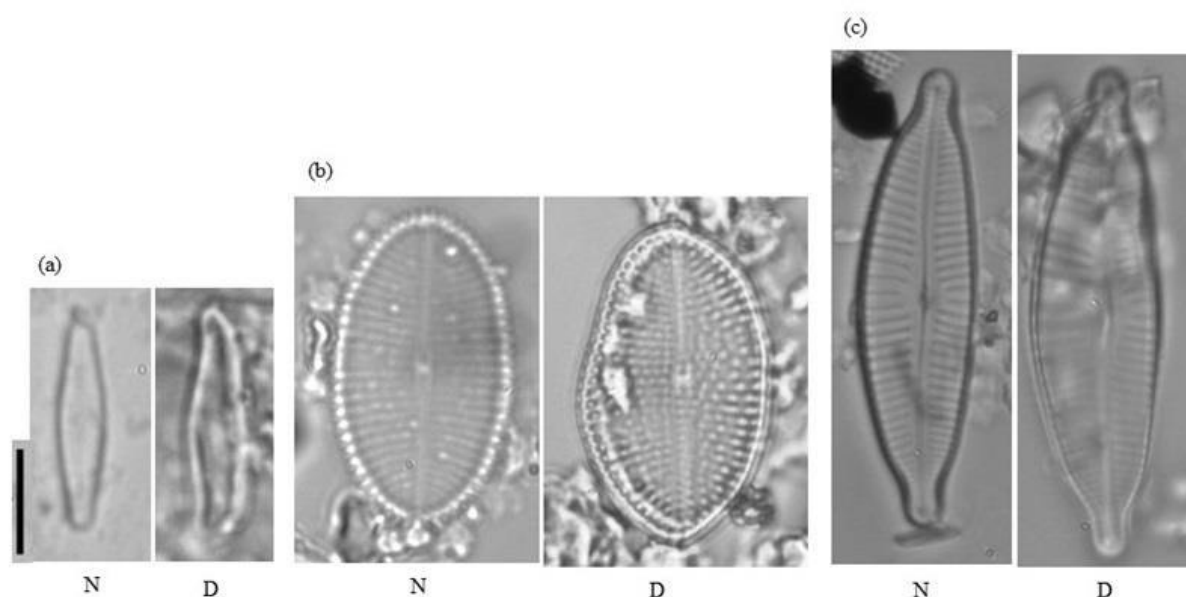


Figura 2. Imagens obtidas em microscópio óptico com ampliação de $1000\times$ das espécies (a) *Achnanthidium minutissimum*, (b) *Cocconeis fluviatilis*, (c) *Cymboppleura naviculiformis*. Barra de escala = $10\ \mu\text{m}$. “N” ilustra uma frústula normal e “D” ilustra uma frústula deformada. Imagens de autoria própria.

Deformidades podem ser encontradas em comunidades de ambientes preservados, porém a frequência é comumente baixa ($<0,5\%$) (LAVOIE *et al.*, 2017). Por outro lado, ambientes que sofrem com impactos antrópicos, especialmente contaminação por metais (e.g. LAVOIE *et al.*, 2017), apresentam aumento na frequência de ocorrência de valvas deformadas, o que despertou interesse na avaliação de deformidades como biomarcadores para estudos de biomonitoramento e avaliações ecológicas. Nesse sentido, já foi evidenciado que a porcentagem de valvas deformadas possui relação positiva com a condutividade elétrica (variável *proxy* de salinidade no estudo), bem como com o tempo de exposição ao estressor ao

qual a comunidade é submetida em mesocosmos (NOUNE *et al.*, 2023). Em estudos *in situ* já foi demonstrado que a frequência de deformidades também tende a aumentar em função de metais pesados (PANDEY *et al.*, 2019), drenagem ácida de minas (OLENICI *et al.*, 2017) e efeitos de metais pesados e eutrofização associados (PANDEY *et al.*, 2018a).

Em alguns casos, a morfometria geométrica é empregada com o intuito de acessar deformidades em valvas de diatomáceas frente a contaminantes. Por exemplo, em um estudo experimental com populações de culturas foram evidenciadas deformidades através da morfometria geométrica em *Achnantheidium minutissimum*, *Gomphonema gracile* e *Nitzschia palea* em função da exposição ao cádmio e ao zinco (CERISIER *et al.*, 2018). Ainda, foram encontradas variações na forma das valvas atribuídas a alometria de espécies de *Frustulia* devido a diferenças de habitat atribuídas às turfeiras (KULICHOVÁ; FIALOVÁ, 2016). Em um outro exemplo, os autores utilizaram a morfometria geométrica para detectar deformidades em populações de *A. minutissimum* expostas a efluentes de mineração (OLENICI *et al.*, 2017). Vale destacar que estudos que visam monitorar alterações na forma da valva (com desenvolvimento ou não de deformidades) por meio de morfometria geométrica em populações de diatomáceas expostas a diferentes condições ambientais ainda são escassos na literatura. Dessa forma, é necessário ainda validar essa ferramenta frente a populações expostas a eutrofização artificial, alterações hidrológicas e mudanças no uso do solo.

Área de estudo

O bioma Pampa ocorre no Brasil, Argentina e Uruguai, sendo que no Brasil o bioma compreende 63% do Estado do Rio Grande do Sul (RS) e 2,07% do território brasileiro (ROESCH *et al.*, 2009). O clima do Pampa brasileiro é subtropical, com as estações do ano bem definidas (ROESCH *et al.*, 2009). O predomínio da vegetação rasteira com ocorrência de arbustos e pequenas florestas espaçadas é característico do bioma (OVERBECK *et al.*, 2007). Além disso, especificamente no RS há uma grande diversidade de ecossistemas aquáticos, como áreas úmidas permanentes (comumente denominadas banhados) e intermitentes, lagos rasos (MALTCHIK, 2003), além das extensas regiões hidrográficas do Uruguai, Guaíba e Litoral (e.g. GUERRA, 2015).

O Pampa abriga elevada diversidade de espécies, com destaque para gimnospermas, insetos, aracnídeos, briófitas e algas (incluindo as diatomáceas) (ANDRADE *et al.*, 2023). Ainda, estimativas sugerem que a biodiversidade real da região é maior do que a

documentada, uma vez que várias espécies ainda não foram devidamente descritas e documentadas para o Pampa (ANDRADE *et al.*, 2023). Apesar da sua biodiversidade, ocorrência de espécies endêmicas (OVERBECK *et al.*, 2007) e serviços ecossistêmicos prestados à população humana (ALTMANN; FILHO, 2020), o bioma Pampa é considerado um dos mais negligenciados devido à escassez de políticas de conservação adequadas para a região, especialmente quando comparado a biomas florestados no Brasil (OVERBECK *et al.*, 2007; OVERBECK *et al.*, 2015; ANDRADE *et al.*, 2023). Esse cenário provavelmente está relacionado ao fato de que o Pampa é um dos biomas brasileiros com menor visibilidade no Sistema Nacional de Unidades de Conservação (SNUC). Um dos grandes problemas relacionados à degradação do Pampa é a conversão da paisagem natural em áreas de monocultura, especialmente para o cultivo de soja, arroz e para silvicultura (OLIVEIRA *et al.*, 2017). Essas atividades afetam negativamente tanto os ecossistemas terrestres como os aquáticos no entorno, facilitando a lixiviação do solo para dentro dos corpos de água, além de favorecer o estabelecimento de espécies exóticas invasoras no bioma (OLIVEIRA *et al.*, 2017).

Realizei a amostragem para este estudo em 25 riachos localizados no Pampa (Figura 3), distribuídos nos municípios de São Gabriel, Rosário do Sul e Alegrete no Estado do Rio Grande do Sul.

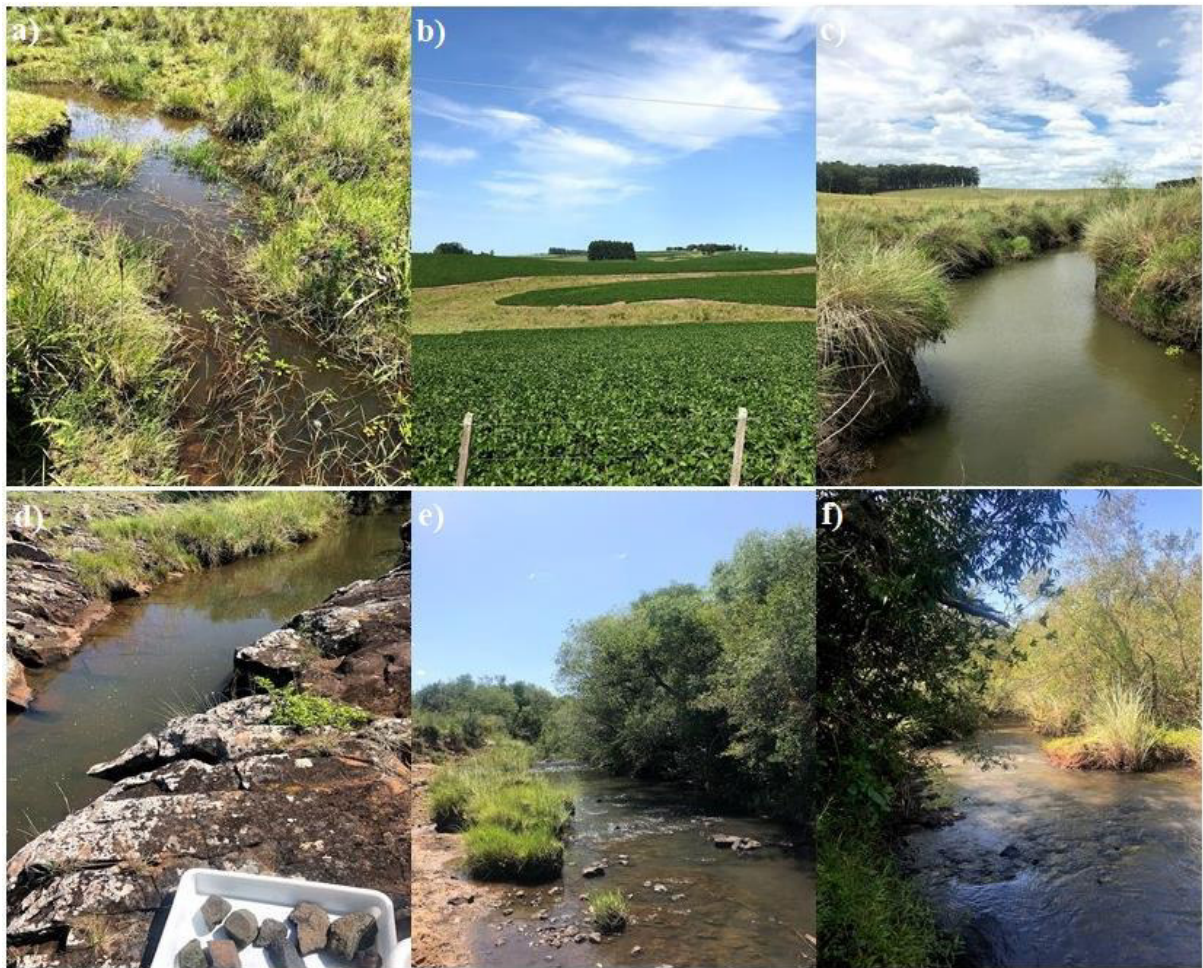


Figura 3. Riachos com forte influência agrícola no seu entorno (a, b, c) e com predominância de pastagens naturais (d, e, f). Imagens de Cássia Fernanda Martins da Silva e Ana Paula Tavares Costa.

Objetivos

Objetivos gerais

Desenvolvi a presente Tese de doutorado com base em dois objetivos principais: (i) entender como se dá o uso de diatomáceas em estudos de biomonitoramento e avaliações ecológicas de ambientes lóticos nos últimos 20 anos através de uma análise cienciométrica e revisão sistemática, e (ii) testar diferentes métricas de biomonitoramento e avaliações ecológicas em riachos - utilizando diatomáceas como organismos modelo - que visassem a redução de tempo e custo em laboratório, especialmente em relação à triagem e identificação do material biológico em nível de espécie.

Objetivos específicos

O primeiro capítulo foi desenvolvido com os seguintes objetivos: (i) fornecer uma visão global sobre o uso de diatomáceas em estudos de biomonitoramento e avaliações ecológicas em ambientes lóticos através de revisão sistemática e análise cienciométrica; (ii) identificar quais espécies são comumente consideradas como indicadoras de ambientes impactados e preservados; (iii) identificar quais tipos de substratos são comumente amostrados para comunidades de diatomáceas e; (iv) verificar quais impactos antrópicos são mais comuns nos ambientes lóticos, bem como quais são as métricas diatomológicas comumente aplicadas para avaliá-los.

O segundo capítulo foi desenvolvido com os seguintes objetivos: (i) verificar se a identificação em nível de gênero e o uso de classes de biovolume são eficazes em representar a variação espacial da estrutura das comunidades de diatomáceas; (ii) verificar a importância relativa das variáveis espaciais e ambientais na comunidade utilizando dados em nível de espécie, gênero e classes de biovolume; e (iii) verificar se os grupos substitutos às espécies (gênero e classes de biovolume) respondem de maneira semelhante às variáveis ambientais e espaciais.

O terceiro capítulo foi desenvolvido com o principal objetivo de verificar métricas taxonômicas (índices de diversidade e diatomológicos) e não-taxonômicas (biomarcadores) de diatomáceas (porcentagem de valvas teratológicas nas comunidades, largura média e forma da valva de *Achnantheidium minutissimum*) para avaliações ecológicas em riachos inseridos no bioma Pampa.

REFERÊNCIAS

- ABELL, R.; THIEME, M. L.; REVENGA, C.; BRYER, M.; KOTTELAT, M.; BOGUTSKAYA, N.; COAD, B.; MANDRAK, N.; BALDERAS, S. C.; BUSSING, W.; STIASSNY, M. L. J.; SKELTON, P.; ALLEN, G. R.; UNMACK, P.; NASEKA, A.; NG, R.; SINDORF, N.; ROBERTSON, J.; ARMIJO, E.; HIGGINS, V.; HEIBEL, T. J.; WIKRAMANAYAKE, E.; OLSON, D.; LÓPEZ, H. L.; REIS, R. E.; LUNDBERG, J. G.; PÉREZ, M. H. S.; PETRY, P. Freshwater ecoregions of the world: a new map of biogeographic units for freshwater biodiversity conservation. **BioScience**, v. 58, n. 5, p. 403–414, 2008.
- AGOSTINHO, A. A.; THOMAZ, S. M.; GOMES, L. Conservation of the biodiversity of Brazil's inland waters. **Conservation Biology**, v. 19, n. 3, p. 646–652, 2005.
- ALBA-TERCEDOR, J.; JÁIMEZ-CUÉLLAR, P.; ÁLVAREZ, M.; AVILÉS, J.; BONADA, M.; CASAS, J.; MELLADO, A.; ORTEGA, M.; PARDO, I.; PRAT, N.; RIERADEVALL, M.; ROBLES, S.; SÁINZ-CANTERO, C. E.; SÁNCHEZ-ORTEGA, A.; SUÁREZ, M. L.; TORO, M.; VIDAL-ABARCA, M. R.; VIVAS, S.; ZAMORA-MUÑOZ, C. Caracterización del estado ecológico de ríos mediterráneos ibéricos mediante el índice IBMWP (antes BMWP'). **Limnetica**, v. 21, p. 175–185, 2002.
- ALLAN, J. D.; FLECKER, S. Biodiversity conservation in running waters. **BioScience**, v. 43, n. 1, p. 32–43, 1993.
- ALTMANN, A.; FILHO, A. G. B. certification and labeling for conservation of ecosystem services in the Pampa Biome: Case study of the Aliança do Pastizal scheme. **Ecosystem Services**, v. 46, p. 10129, 2020.
- ANDERSON, M. J.; CRIST, T. O.; CHASE, J. M.; VELLEND, M.; INOUE, B. D.; FREESTONE, A. L.; SANDERS, N. J.; CORNELL, H. V.; COMITA, L. S.; DAVIES, K. F.; HARRISON, S. P.; KRAFT, N. J. B.; STEGEN, J. C.; SWENSON, N. G. Navigating the multiple meanings of β diversity: a roadmap for the practicing ecologist. **Ecology Letters**, v. 14, n. 1, p. 19–28, 2011.
- ANDRADE, B. O.; DRÖSE, W.; AGUIAR, C. A. *et al.* 12,500 + and counting: biodiversity of the Brazilian Pampa. **Frontiers of Biogeography**, v. 15, n. 2, p. e59288, 2023.

AZEVEDO-SANTOS, V. M.; FREDERICO, R. G.; FAGUNDES, C. K.; POMPEU, P. S.; PELICICE, F. M.; PADIAL, A.A.; NOGUEIRA, M. G.; FEARNESIDE, P. M.; LIMA, L. B.; DAGA, V. S.; OLIVEIRA, F. J. M.; VITULE, J. R. S.; CALLISTO, M.; AGOSTINHO, A. A.; ESTEVES, F. A.; LIMA-JUNIOR, D. P.; MAGALHÃES, A. L. B.; SABINO, J.; MORMUL, R. P.; GRASEL, D.; ZUANON, J.; VILELLA, F. S.; HENRY, R. Protected areas: A focus on Brazilian freshwater biodiversity. **Diversity and Distributions**, v. 25, n. 3, p. 442–448, 2018.

BAKER, M. E.; KING, R. S. A new method for detecting and interpreting biodiversity and ecological community thresholds. **Methods in Ecology and Evolution**, v. 1, n. 1, p. 25–37, 2010.

BARBOSA, H. O.; BORGES, K.; VIEIRA, M. C.; PEREIRA, H. R.; GOMES, L. F.; NABOUT, J. C.; TERESA, F. B.; VIEIRA, L. C.G. Alternatives for the biomonitoring of fish and phytoplankton in tropical streams. **Neotropical Biology and Conservation**, v. 14, n. 4, p. 361–380, 2019.

BARTELL, S. M. Biomarkers, bioindicators and ecological risk assessment – a brief review and evaluation. **Environmental Bioindicators**, v. 1, n. 1, p. 60–73, 2006.

BIGGS, B. J. F. Patterns in benthic algal in streams. In: STEVENSON, J.; BOTHWELL, J.; LOWE, R. L. Orgs. **Algal Ecology: Freshwater Benthic Ecosystems**. London: Academic Press, 1996. P. 31–56.

BIGHIU, M. A.; HOSS, S.; TRAUNSPURGER, W.; KAHLERT, M.; GOEDKOOP, W. Limited effects of pesticides on stream macroinvertebrates, biofilm nematodes, and algae in intensive agricultural landscapes in Sweden. **Water Research**, v. 174, p. 11640, 2020.

BRASIL. Ministério do Meio Ambiente. Resolução CONAMA nº 357, de 15 de junho de 2005. Dispõe sobre a classificação dos corpos de água e diretrizes ambientais para o seu enquadramento, bem como estabelecer as condições e padrões de lançamento de efluentes, e dá outras providências. Disponível em: https://www.icmbio.gov.br/cepsul/images/stories/legislacao/Resolucao/2005/res_conama_357_005_classificacao_corpos_agua_rtfda_altrd_res_393_2007_397_2008_410_2009_430_2011.pdf Acesso em: 05 fevereiro 2023.

- BRITO, M. A.; OLIVEIRA, D.; MAMEDE, M. A.; RANDIG, O.; LACERDA, F. S. Programa de Pesquisa Ecológica de Longa Duração – PELD/CNPq – desafios da gestão, avanços e perspectivas. **Oecologia Australis**, v. 24, n. 2, p. 259–265, 2020.
- CARNEIRO, F. M.; BINI, L. M.; RODRIGUES, L. C. Influence of taxonomic and numerical resolution on the analysis of temporal changes in phytoplankton communities. **Ecological Indicators**, v. 10, n. 2, p. 249–255, 2010.
- CASTRO JUNIOR, R. M.; SOMBREIRA, F. G.; BORTOLOTTI, F. D. Modelagem geoestatística a partir de parâmetros de qualidade da água (IQA-NSF) para a sub-bacia hidrográfica do Rio Castelo (ES) usando Sistema de Informações Geográficas. **Revista Brasileira de Cartografia**, v. 59, n. 3, p. 241–253, 2007.
- CEMAGREF, A. Etude des methodes biologiques d’appréciation quantitative de la qualité des eaux. **Rapport Division Qualité des Eaux Lyon Agence de l’Eau Rhône-Méditerranéen-Corse**, p. 218, 1983.
- CÉRÉGHINO, R.; BIGGS, J.; OERTLI, B.; DECLERCK, S. The ecology of European ponds: defining the characteristics of a neglected freshwater habitat. **Hydrobiologia**, v. 597, p. 1–6, 2008.
- CERISIER, A.; VEDRENNE, J.; LAVOIE, I.; MORIN, S. Assessing the severity of diatom deformities using geometric morphometric. **Botany Letters**, v. 166, n. 1, p. 32–40, 2018.
- CHEN, K.; SUN, D.; RAJPER, A. R.; MULATIBIEKE, M.; HUGHES, R. M.; PAN, Y.; TAYIBAZAER, A.; CHEN, Q.; WANG, B. Concordance in biological condition and biodiversity between diatom and macroinvertebrate assemblages in Chinese arid-zone streams. **Hydrobiologia**, v. 829, p. 245–263, 2018.
- CLARKE, R. K.; AINSWORTH, M. A method of linking multivariate community structure to environmental variables. **Marine Ecology-Progress Sciences**, v. 92, p. 205–205, 1993.
- COSTA, A. P. T.; SCHNECK, F. Diatoms as indicators in running waters: trends of studies on biological assessment and monitoring. **Environmental Monitoring and Assessment**, v. 194, n. 10, p. 695, 2022.

COSTA, A. P. T.; CASTRO, E.; DA SILVA, C. F. M.; SCHNECK, F. Eutrophication changes community composition and drives nestedness of benthic diatoms from coastal streams. **Acta Limnologica Brasiliensia**, v. 34, p. e14, 2022.

DA SILVA, C. F. M.; TORGAN, L. C.; SCHNECK, F. Temperature and surface runoff affect the community of periphytic diatoms and have distinct effects on functional groups: evidence of a mesocosms experiment. **Hydrobiologia**, v. 839, p. 37–50, 2019.

DEBENEST, T.; SILVESTRE, J.; COSTE, M.; DELMAS, F.; PINELLI, E. Herbicide effects on freshwater benthic diatoms: Induction of nucleus alterations and silica cell wall abnormalities. **Aquatic Toxicology**, v. 88, n. 1, p. 88–94, 2008.

DESCY, J. P.; COSTE, M. A test of methods for assessing water quality based on diatoms. **Internationale Vereinigung für theoretische und angewandte Limnologie: Verhandlungen**, v. 24, n. 4, p. 2112–2116, 1991.

DIAS, R. M.; PELICICE, F. M.; TÓFOLI, R. M.; ALVES, G. H. Z.; LOPES, T. M.; CASTELLO, L.; TONELLA, L. H.; ESPÍNOLA, L. A.; AGOSTINHO, A. A. Brazil's leading environmental agency and aquatic biodiversity threatened by federal decree. **Anais da Academia Brasileira de Ciências**, v. 93, p. e20210175, 2021.

DELGADO, C.; PARDO, I.; GARCÍA, L. A multimetric diatom index to assess the ecological status of coastal Galician rivers (NW Spain). **Hydrobiologia**, v. 644, n. 1, p. 371–384, 2010.

DUDGEON, D. Multiple threats imperil freshwater biodiversity in the Anthropocene. **Current Biology**, v. 29, n. 19, p. R960–R967, 2019.

DUFRÊNE, M.; LEGENDRE, P. Species assemblages and indicator species: The need for a flexible asymmetrical approach. **Ecological Monographs**, v. 67, n. 3, p. 345–366, 1997.

FAQUIM, R. C. P.; MACHADO, K. B.; TERESA, F. B.; OLIVEIRA, P. H. F.; GRANJEIRO, G. F.; VIEIRA, L. C. G.; NABOUT, J. C. Shortcuts for biomonitoring programs of stream ecosystems: Evaluating the taxonomic, numeric, and cross-taxa congruence in phytoplankton, periphyton, zooplankton, and fish assemblages. **PlosOne**, v. 16, n. 10, p. e0258342, 2021.

FEIO, M. J.; HUGHES, R. M.; CALLISTO, M.; NICHOLS, S. J.; ODUME, O. N.; QUINTELLA, B. R.; KUEMMERLEN, M.; AGUIAR, F. C.; ALMEIDA, S. F. P.; ALONSO-EGUÍALIS, P.; ARIMORO, F. O.; DYER, F. J.; HARDING, J. S.; JANG, S.; KAUFMANN, P. R.; LEE, S.; LI, J.; MACEDO, D. R.; MENDES, A.; MERCADO-SILVA, N.; MONK, W.; NAKAMURA, K.; NDIRITU, G. G.; OGDEN, R.; PEAT, M.; REYNOLDSON, T. B.; RIOS-TOUMA, B.; SEGURADO, P.; YATES, A. G. The biological assessment and rehabilitation of the world's rivers: an overview. **Water**, v. 13, n. 3, p. 371, 2021.

FERNANDES, V. O.; ESTEVES, F. A. Comunidade Perifítica. In: ESTEVES, F. A. Org. **Fundamentos de Limnologia**. Rio de Janeiro: Editora Interciência, 2011. P. 447–460.

GAUCH, H. G. **Multivariate Analysis in Community Ecology**. Cambridge: Cambridge University Press, 1982.

GÓMEZ, N.; LICURSI, M. The Pampean diatom Index (IDP) for assessment of rivers and streams in Argentina. **Aquatic Ecology**, v. 35, n. 2, p. 173–181, 2001.

GUERRA, T. **Educação Ambiental: Contribuição para a Gestão Socioambiental na Bacia Hidrográfica do Rio Gravataí**. Porto Alegre: MC&G Editorial, 2015.

GUIRY, M. D. How many species of algae are there? **Journal of Phycology**, v. 48, n. 5, p. 1057–1063, 2012.

HE, F.; ZARFL, Z.; BREMERICH, V.; DAVID, J. N. W.; HOGAN, Z.; KALINKAT, G.; TOCKNER, K.; JÄHNIG, S. C. The global decline of freshwater megafauna. **Global Change Biology**, v. 25, n. 11, p. 3883–3892, 2019.

HEINO, J.; SOININEN, J. Are higher taxa adequate surrogates for species-level assemblage patterns and species richness in streams organisms? **Biological Conservation**, v. 137, n. 1, p. 78–89, 2007.

HERING, D.; JOHNSON, R. K.; BUFFAGNI, A. Liking organisms groups – major results and conclusions from the STAR project. **Hydrobiologia**, v. 566, p. 109–113, 2006.

HERING, D.; BORJA, A.; CARSTENSEN, J.; CARVALHO, L.; ELLIOTT, M.; FELD, C. K.; HEISKANEN, A.S.; JOHNSON, R. K.; MOE, J.; PONT, D.; SOLHEIM, A. L.; BUND,

W. V. The European Water Framework Directive at the age of 10: A critical review of the achievements with recommendations for the future. **Science of the Total Environment**, v. 408, n. 10, p. 4007–4019, 2010.

HILL, M. O. **TWINSPAN – A FORTRAN Program for arranging multivariate data in an ordered two-way table by classifications oh the individuals and attributes**. Ithaca: Cornell University, 1979.

HOEK, C. V. D.; MANN, D. G.; JAHNS, M. **Algae: an introduction to phycology**. Cambridge: Cambridge University Press, 1995.

JOHNSON, R. K.; WIEDERHOLM, T.; ROSENBERG, D. M. Freshwater biomonitoring using individual organisms, populations, and species assemblages of benthic macroinvertebrates. In: ROSENBERG, D. M.; RESH, V. H. Org(s). **Freshwater Biomonitoring and Benthic Macroinvertebrates**. London: Chapman & Hall, 1993. P. 40–125.

KARAOUZAS, I.; SMETI, E.; VOURKA, A.; VARDAKAS, L.; MENTZAFΟΥ, A.; TORNÉS, E.; SABATER, S.; MUÑOZ, I.; SKOULIKIDIS, N. T.; KALOGGIANI, E. Assessing the ecological effects of water stress and pollution in a temporary river – Implications for water management. **Science of the Total Environment**, v. 618, n. 15, p. 1591–1604, 2018.

KARAOUZAS, I.; SMETI, E.; KALOGIANNI, E.; SKOULIKIDIS, N. T. Ecological status monitoring and assessment in Greek rivers: Do macroinvertebrate and diatom indices indicate same responses to anthropogenic pressures? **Ecological Indicators**, v. 101, p. 126–132, 2019.

KELLY, M. G.; WHITTON, B. A. The Trophic Diatom Index: A new index for biomonitoring eutrophication in rivers. **Journal of Applied Phycology**, v. 7, n. 4, p. 433–444, 1995.

KLEYNHANS, C. J.; LOUW, M. D. **Module A: EcoClassification and EcoStatus determination in River EcoClassification: Manual for EcoStatus Determination (Version 2)**. Water Research Commission and Department of Water Affairs and Forestry: Pretoria, South Africa, v. 333, n. 08, 2008.

KOLKWITZ, R.; MARSSON, M. Ökologie der pflanzlichen saprobien. **Berichte der Deutschen Botanischen Gesellschaft**, v. 26, p. 505–519, 1908.

KULICHOVÁ, J.; FIALOVÁ, M. Correspondence between morphology and ecology: morphological variation of the *Frustulia crassinervia-saxonica* species complex (Bacillariophyta) reflects the ombro-minerotrophic gradients. **Cryptogamie Algologie**, v. 37, n. 1, p. 15–28, 2016.

LAMPARELLI, M. C. Graus de trofia em corpos d'água do Estado de São Paulo: avaliação dos métodos de monitoramento. Tese (Doutorado em Ciências) – Departamento de Ecologia, 309 Universidade de São Paulo. São Paulo, p. 238. 2004.

LAVOIE, I.; HAMILTON, P. B.; MORIN, S.; TIAM, S. K.; KAHLERT, M.; GONÇALVES, S.; FALASCO, E.; FORTIN, C.; GONTERO, B.; HEUDRE, D.; KOJADINOVIC-SIRINELLI, M.; MONOYLOV, K.; PANDEY, L. K. TAYLOR, J. C. Diatom teratologies as biomarkers of contamination: Are all deformities ecologically meaningful? **Ecological Indicators**, v. 82, p. 539–550, 2017.

LAW, R. J.; ELLIOTT, A.; THACKERAY, S. J. Do functional or morphological classifications explain stream phytobenthic community assemblages? **Diatom Research**, v. 29, n. 4, p. 309–324, 2014.

LEWIN, J. C. Heterotrophy in diatoms. **Microbiology**, v. 9, n. 2, p. 305–313, 1953.

LOBO, E. A.; CALLEGARO, V. L. M.; BENDER, E. P. **Utilização de Algas Diatomáceas Epilíticas como Indicadoras da Qualidade da Água em Rios e Arroios da Região Hidrográfica do Guaíba, RS, Brasil**. Santa Cruz do Sul: EDUNISC, 2002.

MACIEL, M. G. R.; CAVALCANTE, K. P.; LUDWIG, T. A. V. Unraveling centric diatoms from the Caatinga: Coscinodiscophyceae and Mediophyceae in northwestern Ceará, Brazil. **Rodriguésia**, v. 73, p. e00222021, 2022.

MAKARENKOV, V.; LEGENDRE, P. Nonlinear redundancy analysis and canonical correspondence analysis based on polynomial regression. **Ecology**, v. 83, n. 4, p. 1146–1161, 2002.

- MALTCHIK, L. Three new wetlands inventories in Brazil. **Interciencia**, v. 28, n. 7, p. 421–423.
- MCMANUS, C.; NEVES, A. A. B.; FILHO, J. A. D.; MARANHÃO, A. Q.; FILHO, A. G. C. Profiles not metrics: the case of Brazilian universities. **Anais da Academia Brasileira de Ciências**, v. 93, n. 4, p. e20200261, 2021.
- MANN, D. G. The species concept in diatoms. **Phycologia**, v. 38, n. 6, p. 437–495, 1999.
- MARZIN, A.; ARCHAIMBAULT, V.; BELLIARD, J.; CHAUVIN, C.; DELMAS, F.; PONT, D. Ecological assessment of running waters: Do macrophytes, macroinvertebrates, diatoms and fish show similar responses to human pressures? **Ecological Indicators**, v. 23, p. 56–65, 2012.
- MEDLIN, L. K.; KACZMARSKA, I. Evolution of the diatoms: V. morphological and cytological support for the major clades and taxonomic revision. **Phycologia**, v. 43, n. 3, p. 245–270, 2004.
- MEDLIN, L. K. Evolution of the diatoms: major steps in their evolution and a review of the supporting molecular and morphological evidence. **Phycologia**, v. 55, n. 1, p. 79–103, 2016.
- MISSIAS, A. C. A.; GOMES, L. F.; PEREIRA, H. R.; SILVA, L. C. F.; ANGELINI, R.; VIEIRA, L. C. G. Is it possible to simplify environmental monitoring? Approaches with zooplankton in a hydroelectric reservoir. **Acta Limnologica Brasiliensia**, v. 29, p. e8, 2017.
- MORALES, E. A.; SIVER, P. A.; TRAINOR, F. R. Identification of diatoms (Bacillariophyceae) during ecological assessments: Comparison between light microscopy and scanning electron microscopy techniques. **Proceedings of the Academy of Natural Sciences of Philadelphia**, v. 151, n. 1, p. 95–103, 2001.
- MORIN, S.; BONET, B.; CORCOLL, N.; GUASCH, H.; BOTTIN, M.; COSTE, M. Cumulative stressors trigger increased vulnerability of diatom communities to additional disturbances. **Microbial Ecology**, v. 70, p. 585–595, 2015.
- NOUNE, F.; CHAIB, N.; KADDECHE, H.; DZIZI, S.; METALLAOUI, S.; BLANCO, S. Effects of salinity on valves morphology in freshwater diatoms. **Environmental Monitoring and Assessment**, v. 195, n. 1, p. 159, 2023.

OLENICI, A.; BLANCO, S.; BORREGO-RAMOS, M.; MOMEU, L.; BACIU, C. Exploring the effects of acid mine drainage on diatom teratology using geometric morphometric. **Ecotoxicology**, v. 26, p. 1018–1030, 2017.

OLIVEIRA, T. E.; FREITAS, D. S.; GIANEZINI, M.; RUVIARO, C. F.; ZAGO, D.; MÉRCIO, T. Z.; DIAS, E. A.; LAMPERT, V. N.; BARCELLOS, J. O. J. Agricultural land use changes in the Brazilian Pampa Biome: The reduction of natural grasslands. **Land Use Policy**, v. 63, p. 394–400, 2017.

OVERBECK, G. E.; MULLER, S. C.; FIDELIS, A.; PFADENHAUER, J.; PILLAR, V. D.; BLANCO, C. C.; BLANCO, C. C.; BOLDRINI, I. I.; BOTH, R.; FORNECK, E. D. Brazil's neglected biome: The South Brazilian *Campos*. **Perspectives in Plant Ecology, Evolution and Systematics**, v. 9, n. 2, p. 101–116, 2007.

OVERBECK, G. E.; VÉLEZ-MARTIN, E.; SCARANO, F. R.; LEWINSOHN, T. M.; FONSECA, C. R.; MEYER, S. T.; MULLER, S. C.; CEOTTO, P.; DADALT, L.; DURIGSN, G.; GANADE, G.; GOSSNER, M. M.; GUADAGNIN, D. L.; LORENZEN, K.; JACOBI, C. M.; WEISSER, W. W.; PILLAR, V. D. Conservation in Brazil needs to include non-forested ecosystems. **Diversity and Distributions**, v. 21, p. 1455–1460, 2015.

PACE, G.; DELLA BELLA, V.; BARILE, M.; ANDREANI, P.; MANCINI, L.; BELFIORE, C. A comparison of macroinvertebrate and diatom responses to anthropogenic stress in small sized volcanic siliceous streams of Central Italy (Mediterranean Ecoregion). **Ecological Indicators**, v. 23, p. 544–554, 2012.

PANDEY, L. K.; KUMAR, D.; YADAV, A.; RAI, J.; GAUR, J. P. Morphological abnormalities in periphytic diatoms as a tool biomonitoring of heavy metal pollution in a river. **Ecological Indicators**, v. 36, p. 272–279, 2014.

PANDEY, L. K.; BERGEY, E. A. Exploring the status of motility, lipid bodies, deformities and size reduction in periphytic diatom community from chronically metal (Cu, Zn) polluted waterbodies as a biomonitoring tool. **Science of the Total Environment**, v. 550, p. 372–381, 2016.

PANDEY, L. K.; BERGEY, E. A.; LYU, J.; PARK, J.; CHOI, S.; LEE, H.; DEPUYDT, S.; OH, Y. T.; LEE, S. M.; HAN, T. The use of diatoms in ecotoxicology and bioassessment: Insights, advances and challenges. **Water Research**, v. 118, n. 1, p. 39–58, 2017.

PANDEY, L. K.; BERGEY, E. A. Metal toxicity and recovery response of riverine periphytic algae. **Science of the Total Environment**, v. 642, v. 15, p. 1020–1031, 2018.

PANDEY, L. K.; LAVOIE, I.; MORIN, S.; PARK, J.; LYU, J.; CHOI, S.; LEE, H.; HAN, T. River water quality assessment based on a multi-descriptor approach including chemistry, diatom assemblage structure, and non-taxonomical diatom metrics. **Ecological Indicators**, v. 84, p. 140–151, 2018a.

PANDEY, L. K.; SHARMA, Y. C.; PARK, J.; CHOI, S.; LEE, H.; LYU, J.; HAN, T. Evaluating features of periphytic diatom communities as biomonitoring tools in fresh, brackish and marine waters. **Aquatic Toxicology**, v. 194, p. 67–77, 2018b.

PANDEY, L. K.; LAVOIE, I.; MORIN, S.; DEPUYDT, S.; LYU, J.; LEE, H.; JUNG, J.; YEOM, D. H.; HAN, T.; PARK, J. Towards a multi-bioassay-based index for toxicity assessment of fluvial waters. **Environmental Monitoring and Assessment**, v. 191, p. 1–19, 2019.

PARK, J.; BERGEY, E. A.; HAN, T.; PANDEY, L. K. Diatoms as indicators of environmental health on Korean islands. **Aquatic Toxicology**, v. 227, p. 105594, 2020.

PAWLOWSKI, J.; LEJZEROWICZ, F.; GENTIL, L. A. P.; VISCO, J.; ESLING, P. Protist metabarcoding and environmental biomonitoring: Time for change. **European Journal of Protistology**, v. 55, p. 12–25, 2016.

PELICICE, F. M.; CASTELLO, L. A political tsunami hits Amazon conservation. **Aquatic Conservation**, v. 31, n. 5, p. 1221–1229, 2021.

PIRES, M. M.; GRECH, M. G.; STENERT, C.; MALTCHIK, L.; EPELE, L. B.; MCLEAN, K. I.; KNEITEL, J. M.; BELL, D. A.; GREIG, H. S.; GAGNE, C. R.; BATZER, D. P. Does taxonomic and numerical resolution affect the assessment of invertebrate community structure in New World freshwater wetlands? **Ecological Indicators**, v. 125, n. 107437, 2021.

REVIERS, B. **Biologia e Filogenia das Algas**. Porto Alegre: Artmed, 2006.

RIMET, F.; BOUCHEZ, A. Life-forms, cell-sizes and ecological guilds of diatoms in European rivers. **Knowledge and Management of Aquatic Ecosystems**, v. 406, p. 1–12, 2012.

ROESCH, L. F. W.; VIEIRA, F. C. B.; PEREIRA, V. A.; SCHUNEMANN, A. L.; TEIXEIRA, I. F.; SENNA, A. J. T.; STEFENON, V. M. The Brazilian Pampa: a fragile biome. **Diversity**, v. 1, n. 2, p. 182–198, 2009.

ROUND, F. E.; CRAWFORD, R. M.; MANN, D. G. **Diatoms: biology, and morphology of the genera**. Cambridge: Cambridge University Press, 1990.

SLÁDECKOVÁ, A. Limnological investigation methods for the periphyton (“Aufwuchs”) community. **The Botanical Review**, n. 25, n. 2, p. 286–350, 1962.

SMUCKER, N. J.; PILGRIM, E. M.; NIETCH, C. T.; DARLING, J. A.; JOHNSON, B. R. DNA metabarcoding effectively quantifies diatom responses to nutrient in streams. **Ecological Applications**, v. 30, n. 8, p. e02205, 2020.

SOUZA, C.A.; MACHADO, K. B.; NABOUT, J. C.; MUNIZ, D. H. F.; OLIVEIRA-FILHO, E. C.; KRAUS, C. N.; RIBEIRO, R. J. C.; VIEIRA, L. C. G. Monitoring simplification in plankton communities using different ecological approaches. **Acta Limnologica Brasiliensia**, v. 31, p. e20, 2019.

STEVENSON, R. J.; PAN, Y.; VAN DAM, H. Assessing environmental conditions in rivers and streams with diatoms. In: SMOL, J.; STOERMER, E. Orgs. **The Diatoms: Applications for the environmental and earth sciences**. Cambridge: Cambridge University Press, 2010. P. 57–85.

SUN, X.; WU, N.; FABER, C.; FOHRER, N. Effects of hydrological variables on structuring morphological trait (cell size) of diatom community in a lowlander river. **Ecological Indicators**, v. 94, p. 207–217, 2018.

TAN, X.; ZHANG, Q.; BURFORD, M. A.; SHELDON, F.; BUNN, S. E. Benthic diatom based indices for water quality assessment in two subtropical streams. **Frontiers in Microbiology**, v. 8, p. 601, 2017.

TAPOLCZAI, K.; KECK, F.; BOUCHEZ, A.; RIMET, F.; KAHLERT, M.; VASSELON, V. Diatom DNA metabarcoding for biomonitoring: strategies to avoid major taxonomical and bioinformatical biases limiting molecular indices capacities. **Frontiers in Ecology and Evolution**, v. 29, p. 409, 2019.

TAPOLCZAI, K.; SELMECZY, G. B.; SZABÓ, B.; B-BÉRES, V.; KECK, F.; BOUCHEZ, A.; RIMET, F.; PADISÁK, J. The potential of exact sequence variants (ESVs) to interpret and assess the impact of agricultural pressure on stream diatom assemblages revealed by DNA metabarcoding. **Ecological Indicators**, v. 122, p. 107322, 2021.

TAYLOR, J. C.; HARDING, W. R.; ARCHIBALD, C. G. M.; RENSBURG, L. V. Diatoms as indicators of water quality in the Jukskei-Crocodile river system in 1956 and 1957, a re-analysis of diatom count data generated by BJ Chohnoky. **Water SA**, v. 31, n. 2, p. 237–246, 2005.

TER BRAAK, C. J. F. Canonical correspondence analysis: A new eigenvector technique for multivariate direct gradient analysis. **Ecology**, v. 67, n. 5, p. 1167–1179, 1986.

UMETSU, C. A.; AGUIAR, F. A.; FERREIRA, M. T.; CANCIAN, L. F.; CAMARGO, A. F. M. Addressing bioassessment of tropical rivers using macrophytes: the case of Itanhaém Basin, São Paulo, Brazil. **Aquatic Botany**, v. 150, p. 53–63, 2018.

VASCONCELOS, M. C.; MELO, A. S. SCHWARZBOLD, A. Comparing the performance of different stream classification systems using aquatic macroinvertebrates. **Acta Limnologica Brasiliensia**, v. 25, n. 4, p. 406–417, 2013.

VASSELON, V.; DOMAIZON, I.; RIMET, F.; KAHLERT, M.; BOUCHEZ, A. Application of high-throughput sequencing (HTS) metabarcoding to diatom biomonitoring: Do DNA extraction methods matter? **Freshwater Science**, v. 36, n. 1, p. 162–177, 2017.

VERBEEK, L.; GALL, A.; HILLEBRAND, H.; STRIEBEL, M. Warming and oligotrophication causes shifts in freshwater phytoplankton communities. **Global Change Biology**, v. 24, n. 10, p. 4532–4543, 2018.

VILLANOVA, V.; FORTUNATO, A. E.; SINGH, D.; BO, D. D.; CONTE, M.; OBATA, T.; JOUHET, J.; FERNIE, A. R.; MARECHAL, E.; FALCIATORE, A.; PAGLIARDINI, J.; MONNIER, A. L.; POOLMAN, M.; CURIEN, G.; PETROUTSOS, D.; FINAZZI, G.

Investigating mixotrophic metabolism in the model diatom *Phaeodactylum tricornutum*. **Philosophical Transactions of the Royal Society B: Biological Sciences**, v. 372, p. 20160404, 2017.

VILMI, A.; KARJALAINEN, S. M.; LANDEIRO, V. L.; HEINO, J. Freshwater diatoms as environmental indicators: evaluating the effects of eutrophication using species morphology and biological indices. **Environmental Monitoring and Assessment**, v. 187, p. 1–10, 2015.

WÄGELE, H.; KLUSSMANN-KOLB, A.; KUHLMANN, M.; HASZPRUNAR, G.; LINDBERG, D.; KOCH, A.; WÄGELE, J. W. The taxonomist – an endangered race. A practical proposal for its survival. **Frontiers in Zoology**, v. 8, n. 1, p. 1–7, 2011.

WERDEL, G. M.; PANDEY, L. K.; BERGEY, E. A. Cigarette butt effects on diatom health in a stream ecosystem. **Aquatic Ecology**, v. 55, p. 999–1010, 2021.

WETZEL, R. G. **Periphyton of freshwater ecosystems**. Sweden: Dr W Junk Publishers, The Hage, 1983.

XUE, H.; ZHENG, B.; MENG, F.; WANG, Y.; ZHANG, L.; CHENG, P. Assessment of aquatic ecosystem health of the Wutong River based on benthic diatoms. **Water**, v. 11, n. 4, p. 727, 2019.

YIRIGUI, I.; LEE, S. W.; NEJADHASHEMI, A. P. Multi-scale assessment of relationships between fragmentation of riparian forests and biological conditions in streams. **Sustainability**, v. 11, n. 18, p. 5060, 2019.

CAPÍTULO 1

Diatoms as indicators in running waters: trends of studies on biological assessment and monitoring

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Abstract

Despite the biodiversity and ecosystem services provided by lotic ecosystems, they are strongly affected by anthropogenic activities. Therefore, biological monitoring and assessment strategies are crucial in helping maintain these ecosystems and developing mitigation policies. We provide a global overview of the use of benthic diatoms as bioindicators in lotic environments, by analyzing 764 articles published in the past 20 years. We analyzed the influence of substrate type on samplings, which species have been highlighted as indicators and for which type of impacts, which anthropogenic impacts have been most commonly evaluated, and which metrics have been commonly used in studies using diatoms to assess and monitor the quality of lotic environments. We found that the most studied anthropogenic impact is artificial eutrophication and that some species, especially *Nitzschia palea*, have been thoroughly mentioned as indicators of this impact. Indicator species related to other types of impact are less common, demonstrating the need for studies on this issue. Moreover, we verified that traditional taxonomic metrics, such as diversity and diatom indices, have been widely used. Some alternative metrics have been used recently, such as those based on teratological valves, lipid bodies, valve size, and DNA metabarcoding. The number of biomonitoring and assessment studies based on diatoms has increased considerably in the past 20 years. Nonetheless, the demand for natural resources and consequently the degradation of lotic ecosystems have accelerated significantly. Thus, the development of low-cost and time-efficient biological assessment and monitoring strategies is essential for evaluating the health of lotic environments.

Keywords: Global systematic review; Bacillariophyceae; *Nitzschia palea*; Freshwater ecosystems; Lotic environments

Introduction

Studies on biological assessment and monitoring are being carried out worldwide to verify the ecological status of running waters (Feeley et al., 2020; Harding & Taylor, 2014) and to assist in the development of strategies aimed at mitigating environmental impacts. Among the biological communities used for this purpose, benthic diatoms have gained prominence as environmental bioindicators since the beginning of the twentieth century (e.g., Kolkwitz & Marsson, 1908). This is because of their sensitivity to changes in environmental conditions (Potapova & Charles, 2007), fast life cycle, and easy sampling, as they can be found in any submerged substrate (Stevenson et al., 2010).

Diatoms are used to detect a great variety of anthropogenic impacts in running waters, such as organic pollution (Mbao et al., 2020), eutrophication (Salomoni et al., 2006), and acidification (Olenici et al., 2017). For this, a large number of metrics are applied, including widely used diversity metrics such as species richness and the Shannon index (e.g. Grimmett & Lebkuecher, 2017; Vilmi et al., 2017), and also diatom indices specifically developed for assessment and biomonitoring. Among these indices are the Trophic Diatom Index (TDI; Kelly & Whitton, 1995) which is used worldwide, as well as regional ones such as the Pampean Diatom Index (IDP; Gómez & Licursi, 2001) and the French Guiana Diatomic Index (FGDI; Carayon et al., 2020). Moreover, hundreds of species have already been considered indicators of different types of impacts around the world (see “Results”). However, there is still a need to summarize the knowledge related to indicator species as well as the trends and gaps in studies using benthic diatoms to assess the ecological status of lotic ecosystems (but see Rimet, 2012, for an overview on the use of diatoms to assess river pollution).

Here, we provide a global overview of the use of diatoms in assessment and monitoring studies in lotic environments. To this end, we carried out a broad analysis of articles published in peer-reviewed journals over the past 20 years (from 2000 to 2020). Specifically, we addressed which diatom species are commonly described as indicators of impacted and not-impacted environments and the substrate type used for sampling. We also verified the anthropogenic impacts most commonly assessed through the use of diatoms and the countries where this topic is most frequently being studied. Finally, we analyzed the temporal trends in the metrics applied to biological monitoring and assessment studies using diatoms.

Methods

We used the Web of Science database from Clarivate Analytics to search for articles published from January 2000 to July 2020 and including the following combinations of keywords: TS= (diatom* OR Bacillariophyceae OR Bacillariophyta) AND TS= (lotic* OR stream* OR river*) AND TS = (biomonitoring OR monitor* OR indicator* OR bioindicator* OR assessment* OR bioassessment*). We searched for these terms in articles' keywords, title, and abstract.

We obtained 1781 articles, of which 1017 were excluded from this study. The main exclusion criteria were as follows: (i) the studied environment was not of our interest (lentic, wetland, reservoir, estuarine, or oceanic ecosystems); (ii) the community used in the biomonitoring was not of our interest (phytoplankton, zooplankton, fish, macroinvertebrates, or macrophytes); (iii) paleolimnological studies; (iv) forensic studies; (v) experimental laboratory studies. We thus evaluated 764 articles (articles list available in Supplementary Information S1), focusing on the title, abstract, material and methods, results, and when necessary, the full text. For each article, we obtained the following main data: (i) year of publication; (ii) journal of publication; (iii) indicator species mentioned in the abstract or results and the type of anthropogenic impact; (iv) substrate used to sample diatoms; (v) anthropogenic impact assessed and monitored with diatoms in the study; (vi) the main diatom metrics used.

We used the VOSviewer software (van Eck & Waltman, 2010) to create a map of the international collaboration (with at least 20 articles per country). For indicator species data, we considered the information provided by authors through different analyses, such as threshold indicator taxa analysis (TITAN; Baker & King, 2010), the BIOENV procedure (Clarke & Ainsworth, 1993), individual indicator value (IndVal; Dufrêne & Legendre, 1997), or two-way indicator species analysis (TWINSPAN; Hill, 1979). We also used information provided by multivariate exploratory analyses that presented the authors' interpretation of the relationships between environmental variables and diatom species, such as redundancy analysis (RDA; Makarencov & Legendre, 2002), canonical correspondence analysis (CCA; ter Braak, 1986), detrended correspondence analysis (DCA; Hill & Gauch, 1980), and non-metric multidimensional scaling (NMDS; Coxon, 1982).

Finally, to check whether there was any pattern in the use of diatom metrics in the past 20 years, we quantified how many articles used each metric over time, and performed a principal component analysis (PCA; Legendre & Legendre, 1998) using logarithm-

transformed data ($\log x + 1$). We used the *stats* package (R Core Team, 2020) for PCA, and *ggplot2* (Whickham, 2016), *factoextra* (Kassambara & Mundt, 2020), and *viridis* (Garnier et al., 2021) to produce the figures in R (R Core Team, 2020).

Results and discussion

A brief scientometric study

We found a considerable increase in the number of publications involving the use of benthic diatoms for biological monitoring and assessment of lotic ecosystems in the past 20 years (Fig. 1). This was expected because during this time, several countries distributed across five continents (Africa, Asia, the Americas, Europe, and Oceania) have invested in assessment and rehabilitation studies of lotic environments (Feio et al., 2021). Furthermore, a total of 190 journals published the evaluated articles, but only 35 of them published at least five articles. *Ecological Indicators* published the most on this theme ($N = 94$), followed by *Hydrobiologia* ($N = 84$), and *Science of the Total Environment* ($N = 40$) (Table 1). All three of these journals present significant contributions to the area of freshwater biodiversity and biological assessment and monitoring (e.g., Ho & Goethals, 2020; Souza & Vianna, 2020), and as seen here, to freshwater science regarding the use of diatoms to assess the environmental quality of lotic ecosystems.

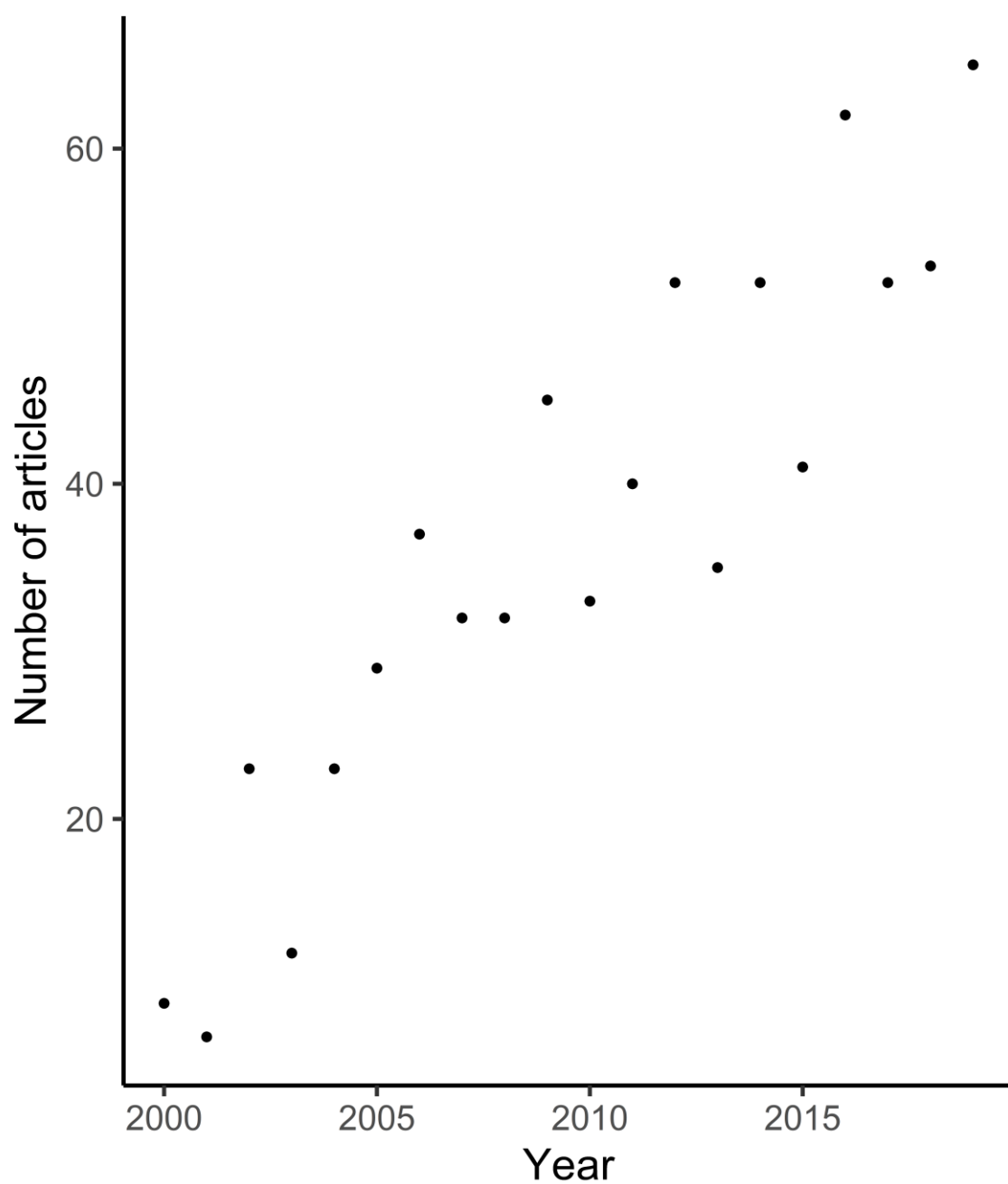


Figure 1. The number of articles published in the Web of Science database from Clarivate Analytics between 2000 and July 2020 using benthic diatoms for biological monitoring and assessment of lotic ecosystems.

Table 1. Journals indexed in the Web of Science database from Clarivate Analytics that published articles with benthic diatoms for biological monitoring and assessment of lotic ecosystems between 2000 and July 2020. Journal= journal names that have had at least five publications, N= total articles number, IF= impact factor of 2020.

Journal	N	IF
Ecological Indicators	94	4.95
Hydrobiologia	84	2.69
Science of the Total Environment	40	7.96
Freshwater Science (including J N AM Benthol Soc)	33	2.03
Environmental Monitoring and Assessment	24	2.51
Freshwater Biology	23	3.80
Diatom Research	16	1.81
Fundamental and Applied Limnology	14	1.14
Oceanological and Hydrological Studies	13	0.82
Fresenius Environmental Bulletin	12	0.48
Water SA	12	1.24
Journal of Applied Phycology	11	3.21
Environmental Science and Pollution Research	8	4.22
Journal of Freshwater Ecology	8	1.13
Limnetica	8	1.43
Limnologica	8	2.09
Water	8	3.10
Acta Botanica Croatica	7	0.94
Fottea	7	3.24
Journal of Phycology	7	2.92
Water Research	7	11.23
African Journal of Aquatic Science	6	1.00
Environmental Pollution	6	8.07
Journal of Limnology	6	1.58
River Research and Applications	6	2.44
Vie et Milieu-Life and Environment	6	0.23
Aquatic Toxicology	5	4.96
Ecological Applications	5	4.65
European Journal of Phycology	5	2.80
International Review of Hydrobiology	5	2.16
Marine and Freshwater Research	5	2.07
Nova Hedwigia	5	1.13
PLOS One	5	3.24
Water Air and Soil Pollution	5	2.52
Journals with $N < 5$	155	-

By analyzing the international collaboration map, we found that research using diatoms as bioindicators is consolidated in North America and Europe (Fig. 2). The

considerable number of publications found in both regions is undoubtedly related to the applicable countries' policies regarding the monitoring and assessment of lotic ecosystems, such as the Clean Water Act in the United States and the Water Framework Directive in Europe (Feio et al., 2021). To achieve the policies' goals, assessment with several model organisms (fish, macroinvertebrates, macrophytes, and diatoms) is performed periodically and, as a consequence, studies on this theme are constantly published (Fliedner et al., 2018; Kelly et al., 2008; Moore et al., 2012). Moreover, there has been a remarkable emergence of countries publishing studies on the use of benthic diatoms as bioindicators, including Hungary, Turkey, and China (Fig. 2).

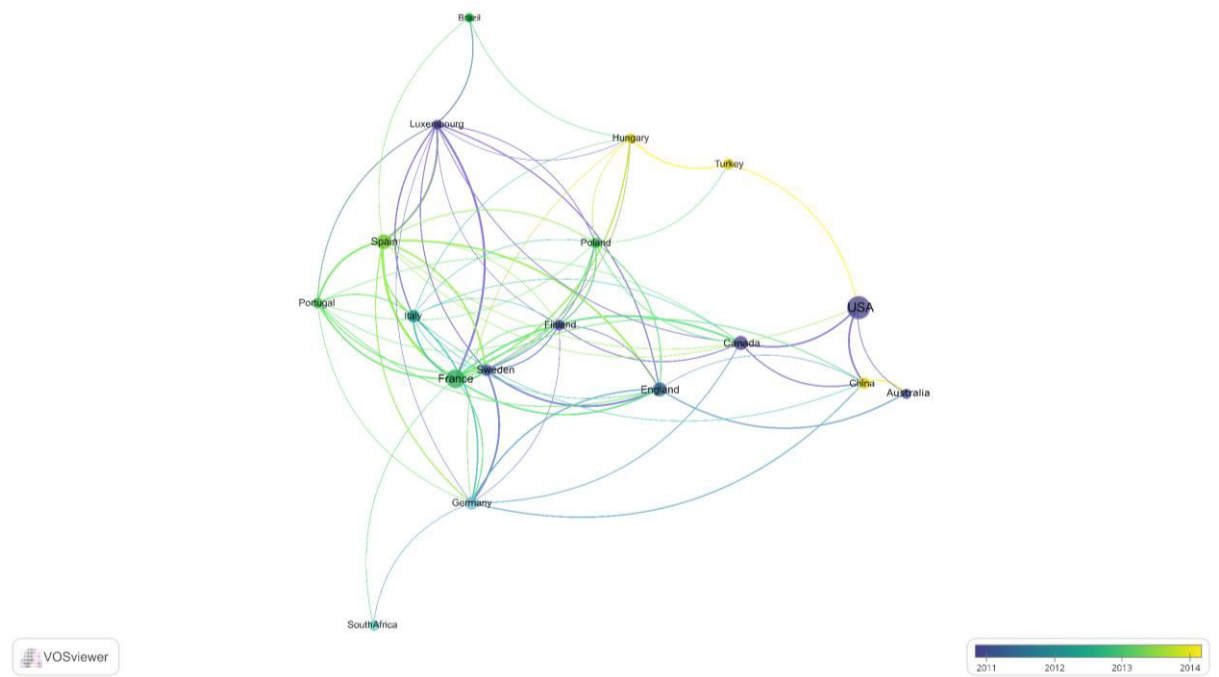


Figure 2. Map of the international collaboration among countries using benthic diatoms for biological monitoring and assessment in lotic ecosystems. The colors represent the year of published and the circle's size represents the number of articles per country.

Is the choice of the substrate for sampling relevant to biological assessment and monitoring?

Benthic diatom communities can be sampled by removing the biofilm adhered to natural or artificial submerged substrates, such as aquatic plants and macroalgae, stones, sand, fine sediment, wood, rope, and glass (Bere & Tundisi, 2011a; Duong et al., 2019; Richards et al., 2020). Our research revealed that in the period under study, epilithic communities (i.e., from stones) were sampled more often than epipsammic, epiphytic, or epipellic communities (Fig.

3). This is expected, considering that stones are common substrates in rivers and streams, being also easy to sample for benthic diatom communities. Moreover, 15% of the studies sampled two or more substrate types ($N = 117$), whereas 14% did not mention the type of substrate used for sampling ($N = 106$). However, it is worth noting that among the studies that used two or more substrates, only 37 made comparisons on how substrate choice influences metrics calculated for biomonitoring and assessment purposes. The most common comparison was between epilithic and epiphytic communities ($N=12$), followed by comparisons among epilithic, epiphytic, and epipelagic communities ($N = 6$), epilithic, epiphytic, epipelagic, and epipsammic communities ($N= 5$), and other substrate combinations (with $N<4$).

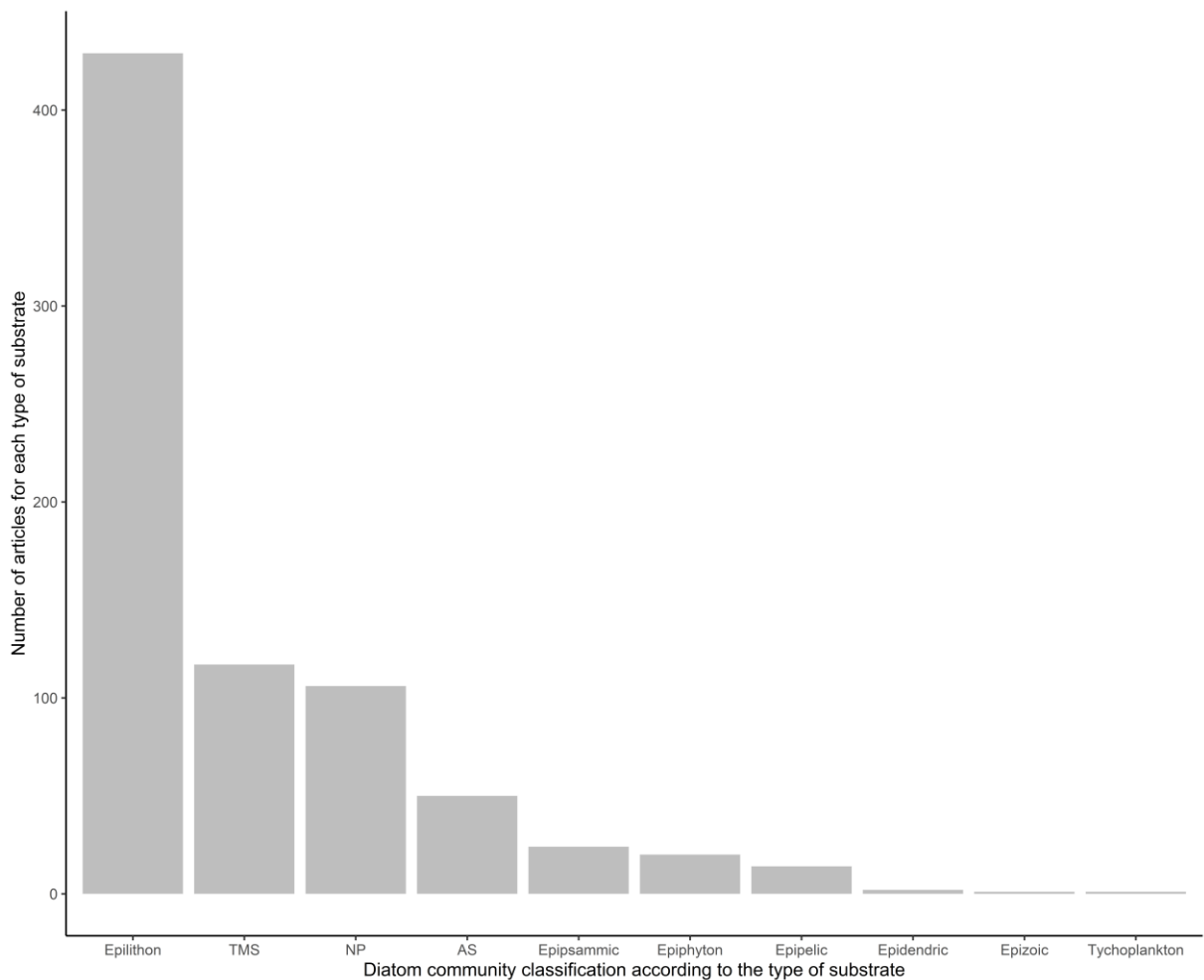


Figure 3. Number of articles in which each type of substrate was used to sample diatoms. NP articles that did not mention the sampling substrate, TMS articles in which sampling was performed in two or more substrates, AS artificial substrates

According to Vidaković et al. (2018), care should be taken with this preference for epilithic communities for biomonitoring and assessment. This is because the specificity of the substrate type can play an important role in monitoring and assessment results by presenting different responses to the water quality assessment. In fact, we found several studies presenting diverging results on what constitutes the best substrate for sampling benthic diatoms for assessing the ecological status of lotic ecosystems. For example, a study focusing on Australian streams found that the use of natural or artificial substrates did not interfere in water quality assessments when the Diatom Species Index for Australian Rivers was used (DSIAR; Chessman et al., 2007). On the other hand, diatom indices like the Specific Pollution Sensitive Index (IPS; Cemagref, 1982) and the Trophic Diatom Index (TDI; Kelly & Whitton, 1995) showed different responses due to substrate type (stones, macrophytes, and mosses) (Kahlert & Rašić, 2015). Furthermore, it can be seen that the values of the IPS and the Biological Diatom Index (IBD; Lenoir & Coste, 1996) are smaller for epiphytic than for epilithic diatoms, an issue that significantly interferes with the interpretation of water quality (Rusanov et al., 2012). Nonetheless, one study tested the IPS and two predictive diatom models (MoDI and DIATMOD) for epilithic, epiphytic, epipsammic, and epipellic communities, yielding similar results (Mendes et al., 2012).

In relation to the use of metrics based on diatom community diversity and composition, several studies have shown that communities from distinct substrate types are highly dissimilar, especially in the cases of epilithic and epiphytic communities (Beauger et al., 2014; Chen et al., 2017; Fisher & Dunbar, 2007; Kahlert & Rašić, 2015; Vidaković et al., 2018). An additional known problem pertains to epiphytic communities, due to the distinct diatom communities associated with specific aquatic plant species (Blindow, 1987; Guimarães & Garcia, 2016). As an example, Townsend and Gell (2005) compared natural substrates (rocks, macroalgae, macrophytes, wood, sand, and mud) and found that the species richness of epipsammic communities can be greater than or equal to epilithic communities, whereas the species richness of epiphytic communities can be highly variable depending on the macrophyte species used as the substrate. On the other hand, some authors have reported similar diatom communities among substrate types (Antoniades et al., 2009; Bere & Tundisi, 2011b; Falasco & Bona, 2011; Tudesque et al., 2012).

Despite stones being the most common substrates used worldwide and thus increasing comparability among studies, it is still necessary to better understand the effects of substrate types in biomonitoring and assessment results, especially when using traditional taxonomic

metrics, such as species diversity and diatom indices. This is particularly important when comparing lotic ecosystems dominated by different substrate types. An important gap associated with this issue that should be addressed in future studies is whether there are biases related to substrate type when morpho-physiological metrics are used instead of taxonomic ones. For example, analyses of lipid bodies or deformed valves in diatom populations may overcome the problems related to comparisons among substrate types.

The assessment of different anthropogenic impacts and indicator species

The most representative anthropogenic impact evaluated in the 764 articles we examined was artificial eutrophication (46% of the articles), followed by multiple stressors (when two or more impacts were measured; 20%), agricultural land use (8%), mining activities (6%), urbanization (5%), and hydrological changes (3%). Community composition, diatom and diversity indices, and pigments are metrics widely used to monitor different impacts compared to DNA metabarcoding or morpho-physiological analyses (Fig. 4).

Among the main consequences of eutrophication are changes in species composition, loss of water quality, and an increase in the frequency of water-related diseases (Smith & Schindler, 2009). Analyses of community composition and diatom indices (e.g., the TDI) are usually efficient in detecting eutrophication problems in lotic ecosystems (Besse-Lototskaya et al., 2011; Chambers et al., 2012; Wojtal & Sobczyk, 2012). Moreover, in eutrophic environments, it is common to observe the replacement of species sensitive to eutrophication by tolerant ones, especially by species of *Nitzschia* (e.g., Shen et al., 2018).

Multiple stressors can have a negative synergistic effect and, consequently, environmental damage may be enhanced (Dudgeon, 2019). The effects of different stressors are commonly detected using diatoms. For example, the TDI has proven to be an efficient metric for monitoring different sources of stressors in streams, such as urbanization and eutrophication (Glendell et al., 2019). Moreover, empirical models obtained from the diatom IPS, the Macroscopic Vegetation Index (IVAM; Moreno et al., 2006), and the Iberian Biomonitoring Working Party (IBMWP; Alba Tercedor et al., 2002) have been used to analyze future scenarios and have yielded promising results for modeling hydrological changes, eutrophication, and anthropogenic land use (Herrero et al., 2018). Recently, assessments based on biomarkers, such as the quantification of lipid bodies and teratologies (or deformities), have been shown to be promising metrics for detecting multiple stressors (Pandey et al., 2018).

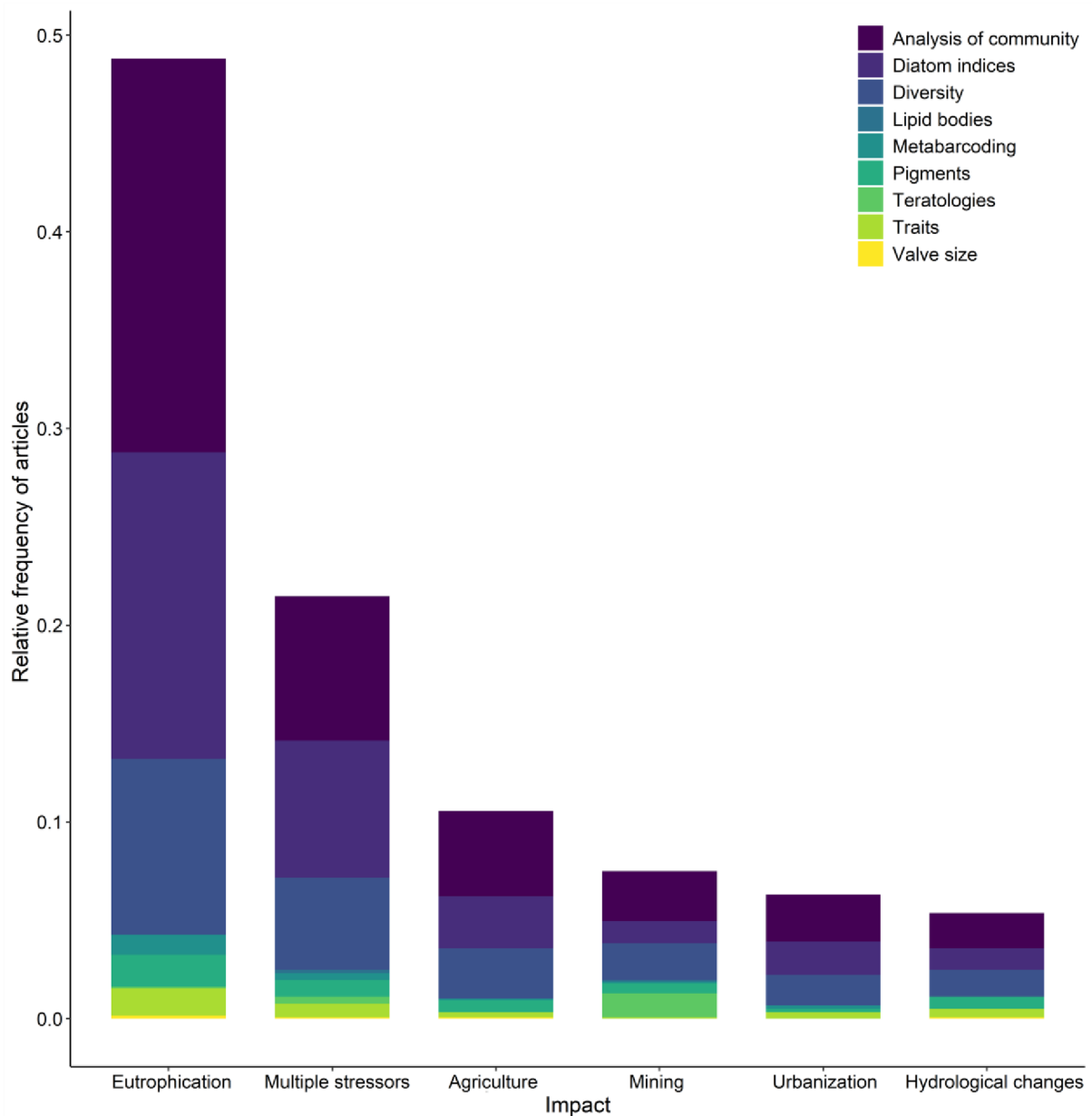


Figure 4. Main types of anthropogenic impacts (mentioned in at least 20 articles) assessed by using different benthic diatom metrics in lotic ecosystems.

Landscape modification for urbanization, agricultural, or livestock purposes has increased in recent years, impacting aquatic environments in a multitude of ways (Wen et al., 2017). For example, urbanization generates symptoms of the urban stream syndrome (Mbao et al., 2020; Walsh et al., 2005) and creates urban heat islands (Merckx et al., 2018), whereas agriculture and cattle raising increase pesticides, fertilizer inputs, and soil erosion in lotic ecosystems (Foley et al., 2005). Moreover, these impacts are responsible for generating the

sanitation crisis that is causing a variety of diseases in human populations around the world (Wen et al., 2017). These impacts in lotic environments are often monitored using various diversity and diatom indices, such as the Shannon and Simpson indices (Mbao et al., 2020), the TDI, the IPS, and the Generic Diatom Index (GDI; Rumeau & Coste, 1988). Also, diatom ecological guilds have been found to strongly respond to urbanization (Mbao et al., 2020). For example, Mbao et al. (2020) showed that the percentage of stalked diatoms was positively related to urban streams, whereas the percentage of prostate diatoms tended to diminish in these environments.

The environmental stress caused by mining activities or acid mine drainage (AMD) is associated with increases in sulfate and heavy metals (such as zinc, cadmium, and copper) in water and consequently pH reduction (Gray, 1998). Assessments of AMD's impacts are usually conducted using diatoms, with some species standing out due to their high tolerance to acidic environments, including *Pinnularia acidophila* (Kim et al., 2008; Schowe & Harding, 2014) and *Pinnularia aljustrellica* (Rivera et al., 2019, 2020). Some specific diatom indices have been designed to assess AMD's impacts, for instance, diatom model affinity (DMA; Passy & Bode, 2004) and the Diatom Index of Biotic Integrity (AMD-DIBI; Zalack et al., 2010). Moreover, diatom biomarkers (e.g., teratologies and lipid bodies) have yielded promising results for biomonitoring AMD waters (Olenici et al., 2017; Pandey & Bergey, 2016).

Finally, hydrological changes are commonly related to drought events (Stubbington et al., 2019), dam construction (Martina et al., 2013) and even invasive species, such as *Castor canadensis*, which is responsible for lentification in Argentinian streams (Rodríguez et al., 2020). Reductions in species richness and changes in the structure of diatom communities are commonly reported in lotic ecosystems that have undergone hydrological alterations (Rodríguez et al., 2020). Moreover, in order to detect the effect of dams, a Diatom-Based Index of Biotic Integrity (D-IBI; Wu et al., 2012) has been created. In addition to taxonomic metrics, pigments of benthic diatoms have been found to be good indicators of the effects of water scarcity. For example, Piano et al. (2017) found that the chlorophyll *a* of diatoms was lower than that of cyanobacteria and green algae during seasonal droughts.

We found 641 species classified as indicators, tolerant or sensitive to different anthropogenic impacts. Some species stood out in terms of the frequency with which they were mentioned as tolerant or sensitive, so that 11 species were mentioned in at least 30 articles that evaluated the most frequent anthropogenic impacts in the literature (Fig. 5). In

general, species of the genus *Nitzschia*, especially *Nitzschia palea*, are tolerant to different anthropogenic disturbances, such as mining activities (Archibald & Taylor, 2007; Morin et al., 2015), artificial eutrophication (Salomoni et al., 2006), and urbanization (Pham, 2020). *Gomphonema parvulum* was also mainly classified as tolerant, especially to eutrophication (Fig. 5). The few classifications of *G. parvulum* as sensitive to eutrophication, agriculture, and mining may be related to misidentifications (Law et al., 2014; Taylor et al., 2005). Species such as *Craticula subminuscula*, *Eolimna minima*, and *Cyclotella meneghiniana* were also mainly classified as tolerant (Fig. 5) and related to environments impacted by eutrophication (e.g., Bere & Tundisi, 2011a; Della Bella et al., 2012; Kókai et al., 2015).

We did not find species strictly classified as sensitive to anthropogenic impacts. However, we found inconsistencies regarding the tolerance range of some species, including *Achnanthes minutissimum*, *Cocconeis placentula*, *Amphora pediculus*, and *Ulnaria ulna* (Fig. 5), with the former species drawing particular attention due to the sheer quantity of such inconsistencies. For *A. minutissimum*, despite being strongly characterized as sensitive to eutrophication, some studies found that it was tolerant to the same impact type (Fig. 5). Furthermore, the species was similarly cited as sensitive or tolerant to impacts related to mining activities, multiple stressor, and urbanization. These different tolerance ranges found may be explained by *A. minutissimum* wide variation in both ecological and morphological characteristics (Potapova & Hamilton, 2007). It can also not be discarded that some inconsistencies may be related to misidentifications. In the case of *C. placentula*, for instance, there are frequent reports of misidentifications, especially among *C. placentula*, *C. placentula* var. *euglypta*, and *C. placentula* var. *lineata* (Prygiel et al., 2002).

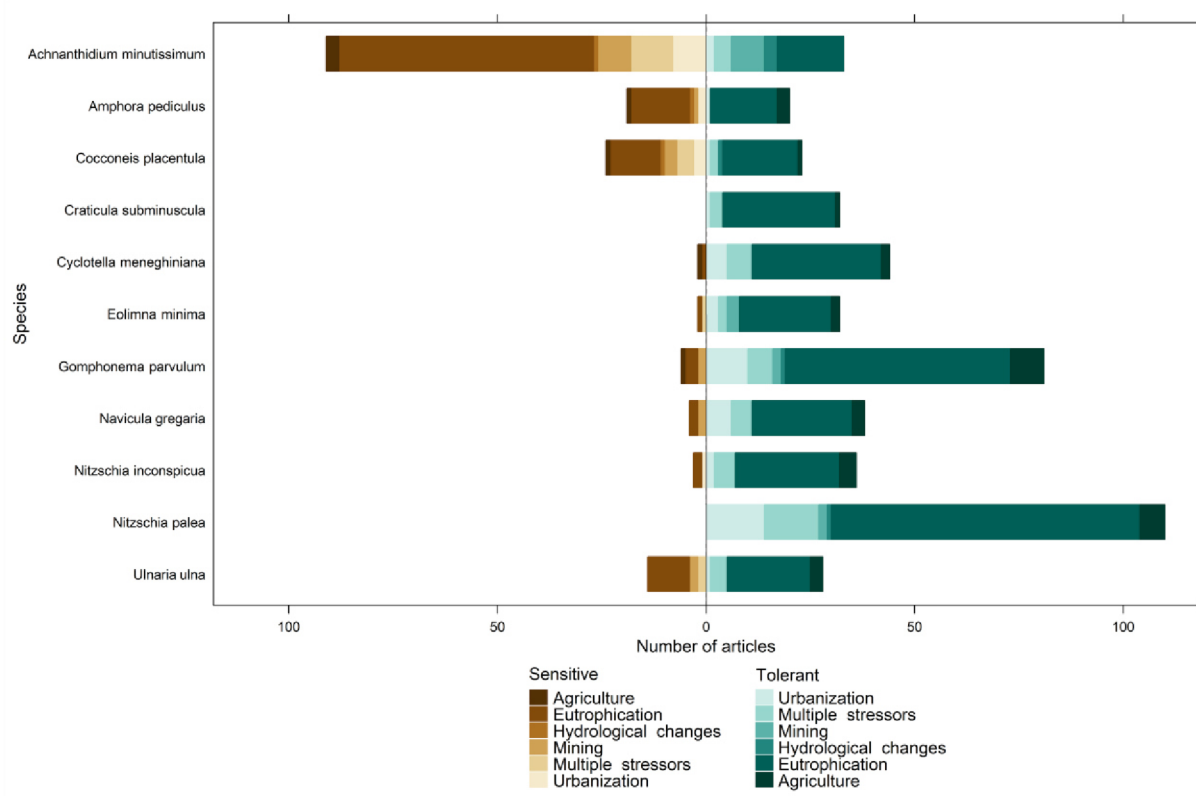


Figure 5. Species that were mentioned in at least 30 articles as indicators of impacted (tolerant) and preserved/reference (sensitive) environments.

Temporal trends in the metrics used for biomonitoring

A large number of metrics have been applied over time to assess the ecological status of streams and rivers. Among the most common metrics are indices specifically developed for biomonitoring and assessment, such as the IPS, the GDI, the TDI, and the Pampean Diatom Index (IDP; Gómez & Licursi, 2001). Furthermore, diversity metrics such as species richness, evenness, and the Shannon and Simpson indices are widely used (e.g., Chen et al., 2016). These traditional taxonomical metrics have been used for decades in biomonitoring (Kelly & Whitton, 1995; Kolkwitz & Marsson, 1908), yielding good results in the assessment of lotic ecosystems (Gray & Vis, 2013; Wojtal & Sobczyk, 2012). As an alternative to taxonomic metrics, benthic diatom life forms (e.g., motile and attached diatoms) have also long been used (e.g., Dickman et al., 2005). In fact, the use of traits (including ecological guilds) in biomonitoring and assessment studies increased from 2012, coinciding with Passy's (2007) development of diatom functional groups (high-profile, low-profile, and motile species) and Rimet and Bouchez's (2012) subsequent addition of the planktonic group. However, to

determine most diatom traits, it is still necessary to identify organisms to genus or species, and identifying and counting a large number of diatom valves demands time and a high level of taxonomic knowledge. Moreover, as mentioned before, substrate type can interfere with the results of diatom indices (Kahlert & Rašić, 2015) or diversity indices (Richards et al., 2020), potentially leading to incorrect assessments of environmental quality. Thus, it is important to develop and test metrics that are more easily obtained, reducing laboratory work time and financial costs. Widely implementing such metrics could increase the number of rivers and streams monitored and allow middle- and low- income countries to establish monitoring networks.

Recent advances in the use of diatoms to monitor running waters appeared in our mapping, with less conventional metrics gaining prominence in the literature. This was the case of morpho-physiological metrics, such as those based on valve sizes, deformities (or teratologies) in diatom valves, and the formation of lipid bodies (Fig. 6). In recent years, morphological alterations in diatoms have successfully been used as biomarkers, that is, organism-level measures that indicate environmental stress (Bartell, 2006; López-Barea, 1995). Among such alterations, valve deformities can occur due to deficiencies in silica uptake and absorption in the cell wall (Pandey et al., 2014), or because of problems in the microtubular system during mitosis (Debenest et al., 2008). Teratologies are often observed in the valve outline, striations, and raphe (Pandey et al., 2014). It has been reported that these deformities are good biomarkers of aquatic contamination owing to mining activities (Olenici et al., 2017), presence of heavy metals (Pandey, 2020; Pandey et al., 2018), and eutrophication (Dziengo-Czaja et al., 2008). A metric as simple as the percentage of teratological valves in a sample can be used as an indicator of contamination (Pandey et al., 2014). Furthermore, Cerisier et al. (2018) have suggested combining information on the number of teratologies with their severity, by using geometric morphometry to quantify how much the deformed valve deviates from the normal one. This approach requires more time for analysis but can greatly improve this biomarker's ability to assess impacts.

Contrary to teratologies, valve size is not widely recognized as an efficient metric to detect changes in environmental conditions, given that clear results are scarce. For instance, valve reduction has been observed in diatom species in environments contaminated by metals (Pandey & Bergey, 2016) and pesticides (Park et al., 2020), yet no relationship between diatom size and total phosphorus has been found (Lavoie et al., 2006, 2010). However,

approaches using the valve size of diatoms should be tested further for different types of stressors, because they are time- and cost-effective in biomonitoring.

Our overview has also revealed that the use of lipid bodies as biomarkers has been increasing in recent years (Fig. 6). According to the literature, the biochemical and physiological mechanisms involved in the formation of lipid bodies are unclear (Pandey et al., 2017). However, several studies have shown that lipid bodies increase both in number and in biovolume due to environmental stressors such as heavy metals (Pandey, 2020; Pandey et al., 2018; Park et al., 2020), pesticides from agricultural activities (Park et al., 2020), and mining activities (Pandey & Bergey, 2016). In this sense, lipid bodies are great candidates to be widely used in biomonitoring and assessment programs, but there is still a need to test this biomarker in lotic ecosystems impacted by other stressors, such as eutrophication and urbanization. Finally, DNA metabarcoding has been deemed a promising tool for biomonitoring studies. The use of high-throughput sequencing for the analysis of environmental quality together with the Diat.barcode library allows researchers to increase the production of spatial and temporal data for freshwater biomonitoring (Rimet et al., 2019; Tapolczai et al., 2019). In this context, Rivera et al. (2020) compared DNA metabarcoding with the traditional IPS metric, attaining similar results for the assessment of environmental quality. However, the overestimation of diatom species in a community constitutes a possible disadvantage of the metabarcoding method, because it can lead to wrong estimates of diversity (Mora et al., 2019). Moreover, morpho-physiological information about the studied community, such as ecological guilds, the development of teratologies, and the production of lipid bodies, is not detected. A final issue that needs to be considered is that this technique is still very expensive, hindering its definitive implementation, particularly in low- and middle-income countries.

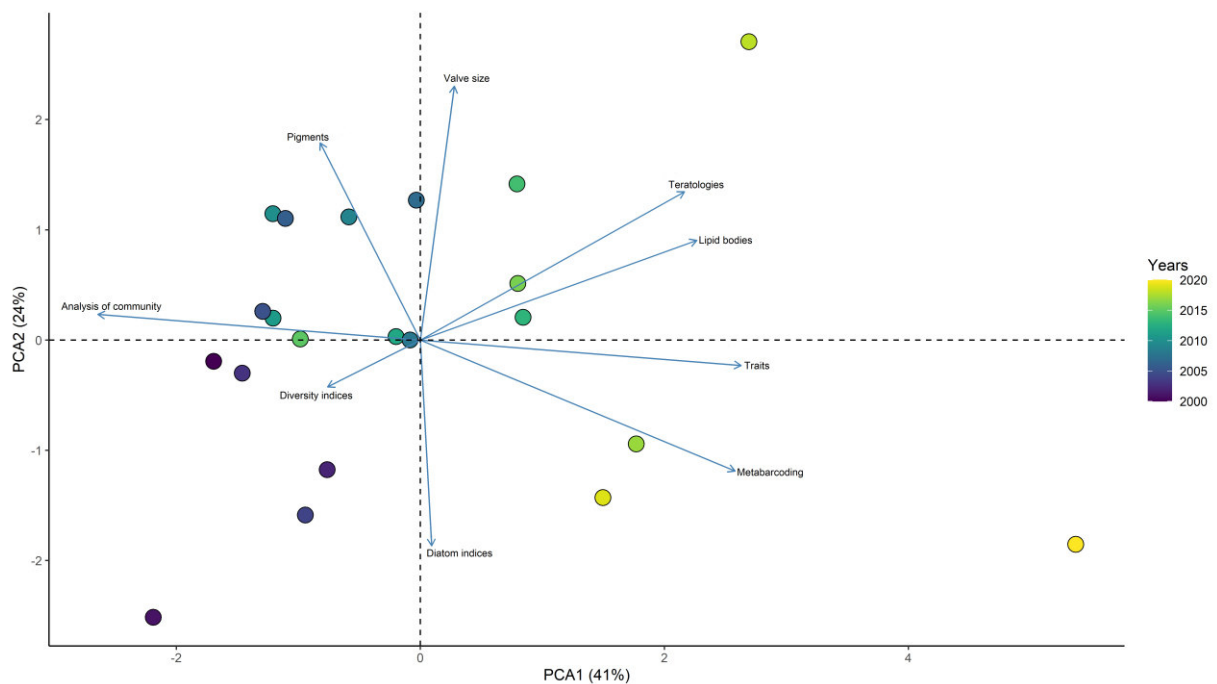


Figure 6. Principal component analysis (PCA) for diatom metrics used in studies of lotic environments in the last 20 years. The first principal component (PC1) explained 41% of the data variation. The second principal component (PC2) explained 24% of the data variation

Conclusion

Our overview has revealed that the number of studies seeking to assess the ecological status of lotic ecosystems has increased considerably in recent years, with more studies focusing on testing alternative non-taxonomic techniques that are more cost- and time-effective than their traditional counterparts. Nevertheless, studies need to advance in their choice of substrate for sampling, as there are still gaps to be filled concerning how the substrate influences indices and non-taxonomic metrics for biomonitoring. Moreover, only a few common indicator species have shown consistent responses as tolerant or sensitive to anthropogenic impacts. Finally, we expect that the information provided in this work instigates discussions and further studies that help the development of biomonitoring and assessment strategies of running waters in front of the Anthropocene.

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Declarations

Conflict of interest: The authors declare no competing interests.

References

- Alba Tercedor, J., Jáimez-Cuéllar, P., Álvarez, M., Avilés, J., Bonada-Caparrós, N., Casas, J., Mellado, A., Ortega, M., Pardo, I., Prat, N., Rieradevall, M., Robles, S., Sainz-Cantero, C. E., Sánchez-Ortega, A., Suárez, M. L., Vidal-Abarca, M. R., Vivas, S., & Zamora-Muñoz, C. (2002). Caracterización del estado ecológico de ríos 62ndice62riophy ibéricos mediante el índice IBMWP (antes BMWP'). *Limnetica*, 21, 175–185. <https://doi.org/10.23818/limn.21.24>
- Antoniades, D., Douglas, M. S. V., & Smol, J. P. (2009). Biogeographic distributions and environmental controls of stream diatoms in the Canadian Arctic Archipelago. *Botany*, 87(5), 443–454. <https://doi.org/10.1139/B09-001>
- Archibald, C. G. M., & Taylor, J. C. (2007). The assessment of diffuse pollution from acid-mine drainage using an update and revised diatom assessment procedure as an added-value biomonitoring tool. *Water Science and Technology*, 55(3), 151–160. <https://doi.org/10.2166/wst.2007.083>
- Baker, M. E., & King, R. S. (2010). A new method for detecting and interpreting biodiversity and ecological community thresholds. *Methods in Ecology and Evolution*, 1(1), 25–37. <https://doi.org/10.1111/j.2041-210X.2009.00007.x>
- Bartell, S. M. (2006). Biomarkers, bioindicators, and ecological risk assessment – a brief review and evaluation. *Environmental Bioindicators*, 1(1), 60–73. <https://doi.org/10.1080/15555270591004920>
- Beauger, A., Serieyssel, K. K., & Peiry, J. L. (2014). Diatom distribution in natural and impacted cut-off meanders of the Allier River, France. *Diatom Research*, 29(2), 119–145. <https://doi.org/10.1080/0269249X.2013.863224>

- Bere, T., & Tundisi, J. G. (2011a). The effects of substrate type on diatom-based multivariate water quality assessment in a tropical river (Monjolinho), São Carlos, SP, Brazil. *Water, Air & Soil Pollution*, 216, 391–409. <https://doi.org/10.1007/s11270-010-0540-8>
- Bere, T., & Tundisi, J. G. (2011b). Influence of ionic strength and conductivity on benthic diatoms communities in a tropical river (Monjolinho), São-Carlos-SP, Brazil. *Hydrobiologia*, 661(1), 261–276. <https://doi.org/10.1007/s10750-010-0532-0>
- Besse-Lototskaya, A., Verdonschot, P. F. M., Coste, M., & Vijver, B. V. (2011). Evaluation of European diatom trophic indices. *Ecological Indicators*, 11(2), 456–467. <https://doi.org/10.1016/j.ecolind.2010.06.017>
- Blindow, I. (1987). The composition and density of epiphyton on several species of submerged macrophytes – the neutral substrate hypothesis tested. *Aquatic Botany*, 29, 157–168. [https://doi.org/10.1016/0304-3770\(87\)90093-3](https://doi.org/10.1016/0304-3770(87)90093-3)
- Carayon, D., Eulin-Garrigue, A., Vigouroux, R., & Delmas, F. (2020). A new metric index for the evaluation of water ecological quality of French Guiana streams based on benthic diatoms. *Ecological Indicators*, 113, 106248. <https://doi.org/10.1016/j.ecolind.2020.106248>
- Cemagref. (1982). Etude des methodes biologiques d’appréciation quantitative de la qualité des eaux. Rapport Division Qualité des Eaux Lyon Agence de l’Eau Rhône-Méditerranéen-Corse, Pierre Bénite (pp. 2018).
- Cerisier, A., Vedrenne, J., Lavoie, I., & Morin, S. (2018). Assessing the severity of diatom deformities using geometric morphometric. *Botany Letters*, 166(1), 32–40. <https://doi.org/10.1080/23818107.2018.1474800>
- Chambers, P. A., McGoldrick, D. J., Brua, R. B., Vis, C., Culp, J. M., & Benoy, G. A. (2012). Development of environmental thresholds for nitrogen and phosphorus in streams. *Journal of Environmental Quality*, 41(1), 7–20. <https://doi.org/10.2134/jeq2010.0273>
- Chen, X., Zhou, W., Pickett, S. T. A., Li, W., Han, L., & Ren, Y. (2016). Diatoms are better indicators of urban streams conditions: A case study in Beijing, China. *Ecological Indicators*, 60, 265–274. <https://doi.org/10.1016/j.ecolind.2015.06.039>

- Chen, X., Stevenson, M. A., Zeng, L., & Qiao, Q. (2017). Diatom distribution in an alpine basin (central China) in relation to environmental factors and substrata. *Diatom Research*, 32(3), 251–262. <https://doi.org/10.1080/0269249X.2017.1371082>
- Chessman, B. C., Bate, N., Gell, P. A., & Newall, P. (2007). A diatom species index for bioassessment of Australian rivers. *Marine and Freshwater Research*, 58(6), 542–557. <https://doi.org/10.1071/MF06220>
- Clarke, K. R., & Ainsworth, M. (1993). A method of linking multivariate community structure to environmental variables. *Marine Ecology-Progress Series*, 92, 205–219. <https://doi.org/10.3354/meps092205>
- Coxon, A. P. M. (1982). *The user's guide to multidimensional scaling*. Heinemann.
- Debenest, T., Silvestre, J., Coste, M., Delmas, F., & Pinelli, E. (2008). Herbicide effects on freshwater diatoms: Introduction of nuclear alterations and silica cell wall abnormalities. *Aquatic Toxicology*, 88(1), 88–94. <https://doi.org/10.1016/j.aquatox.2008.03.011>
- Della Bella, V., Pace, G., Barile, M., Zedde, A., Puccinelli, C., Ciadamidaro, S., Danieli, P. P., Andreani, P., Aulicino, F. A., & Belfiore, C., & Mancini, L. (2012). Benthic diatom assemblages and their response to human stress in small-sized volcanic-siliceous streams of central Italy (Mediterranean eco-region). *Hydrobiologia*, 695(1), 207–222. <https://doi.org/10.1007/s10750-012-1195-9>
- Dickman, M. D., Peart, M. R., & Yin, W. W. S. (2005). Benthic diatoms as indicators of stream sediment concentration. *International Review of Hydrobiology*, 90(4), 412–421. <https://doi.org/10.1002/iroh.200410806>
- Dudgeon, D. (2019). Multiple threats imperil freshwater biodiversity in the Anthropocene. *Current Biology*, 29(19), R960–R967. <https://doi.org/10.1016/j.cub.2019.08.002>
- Dufrêne, M., & Legendre, P. (1997). Species assemblages and indicator species: the need for a flexible asymmetrical approach. *Ecological Monographs*, 67(3), 345–366. [https://doi.org/10.1890/0012-9615\(1997\)067\[0345:SAAIST\]2.0.CO;2](https://doi.org/10.1890/0012-9615(1997)067[0345:SAAIST]2.0.CO;2)
- Duong, T. T., Nguyen, H. Y., Le, T. P. Q., Nguyen, T. K., Tran, T. T. H., Le, N. D., Dang, D. K., Vu, T. N., Panizzo, V., & McGowan, S. (2019). Transitions in diatom assemblages and

pigments through dry and wet season conditions in the Red River, Hanoi (Vietnam). *Plant Ecology and Evolution*, 152(2), 163–177. <https://doi.org/10.5091/plecevo.2019.1627>

Dziengo-Czaja, M., Koss, J., & Matuszak, A. (2008). Teratological forms of diatoms (Bacillariophyceae) as indicators of water pollution in the western part of Puck Bay (southern Baltic Sea). *Oceanological and Hydrobiological Studies*, 50(1), 119–132. <https://doi.org/10.2478/v10009-007-0042-1>

Falasco, E., & Bona, F. (2011). Diatom community biodiversity in an Alpine protected area: a study in the Maritime Alps Natural Park. *Journal of Limnology*, 70(2), 157–167. <https://doi.org/10.4081/jlimnol.2011.157>

Feeley, H. B., Bradley, C., Free, G., Kennedy, B., Little, R., McDonnell, N., Plant, C., Trodd, W., Wynne, C., & Boyle, S. O. (2020). A national macroinvertebrate dataset collected for the biomonitoring of Ireland's river network, 2007–2018. *Scientific Data*, 7(208), 1–9. <https://doi.org/10.1038/s41597-020-00618-8>

Feio, M. J., Hughes, R. M., Casllisto, M., Nichols, S. J., Odume, O. N., Quintella, B. R., Kuemmerlen, M., Aguiar, F. C., Almeida, S. F. P., Alonso-EguíaLis, P., Arimoro, F. O., Dyer, F. J., Harding, J. S., Jang, S., Kaufmann, P. R., Lee, S., Li, J., Macedo, D.R., Mendes, A., Mercado-Silva, N., Monk, W., Nakamura, K., Ndiritu, G. G., Ogden, R., Peat, M., Reynoldson, T. B., Rios-Touma, B., Segurado, P., & Yates, A. G. (2021). The biological assessment and rehabilitation of the world's rivers: an overview. *Water*, 13(3), 371. <https://doi.org/10.3390/w13030371>

Fisher, J., & Dunbar, M. J. (2007). Towards a representative periphytic diatom sample. *Hydrology and Earth System Sciences*, 11(1), 399–407. <https://doi.org/10.5194/hess-11-399-2007>

Fliedner, A., Rüdél, H., Lohmann, N., Buchmeier, G., & Koschorreck, J. (2018). Biota monitoring under the Water Framework Directive: On tissue choice and fish species selection. *Environmental Pollution*, 235, 129–140. <https://doi.org/10.1016/j.envpol.2017.12.052>

Foley, J. A., DeFries, R., Asner, G. P., Barford, C., Bonan, G., Carpenter, S. R., Chapin, F. S., Coe, M. T., Daily, G. C., Gibbs, H. K., Helkowski, J. H., Holloway, T., Howard, E. A., Kucharik, C. J., Monfreda, C., Patz, J. A., Prentice, I. C., Ramankutty, N., & Snyder, P. K.

(2005). Global consequences of land use. *Science*, 309(5734), 570–574. <http://dx.doi.org/10.1126/science.1111772>

Garnier, S., Ross, N., Rudis, B., Sciaini, M., Camargo, A. P., & Scherer, C. (2021). Colorblind- Friendly color maps for R.

Glendell, M., Palarea-Albaladejo, J., Pohle, I., Marrero, S., McCreadie, B., Cameron, G., & Stutter, M. (2019). Modeling the ecological impact of phosphorus in catchments with multiple environmental stressors. *Journal of Environmental Quality*, 48(5), 1336–1346. <https://doi.org/10.2134/jeq2019.05.0195>.

Gomez, N. & Licursi, M. (2001). The Pampean Diatom Index (IDP) for assessment of rivers and streams in Argentina. *Aquatic Ecology*, 35(2), 173–181. <https://doi.org/10.1023/A:1011415209445>

Gray, N. F. (1998). Acid mine drainage composition and the implications for its impact on lotic systems. *Water Research*, 32(7), 2122–2134. [https://doi.org/10.1016/S0043-1354\(97\)00449-1](https://doi.org/10.1016/S0043-1354(97)00449-1)

Gray, J. B. V., & Vis, M. L. (2013). Reference diatom assemblage response to restauration of an acid mine drainage stream. *Ecological Indicators*, 29, 234–245. <https://doi.org/10.1016/j.ecolind.2013.01.002>

Grimmett, M. R., & Lebkuecher, J. G. (2017). Composition of algae assemblages in middle Tennessee streams and correlations of composition to trophic state. *Journal of Freshwater Ecology*, 32(1), 363–389. <https://doi.org/10.1080/02705060.2017.1314228>

Guimarães, P. S., & Garcia, M. (2016). Importância do habitat e do forófito para a composição da comunidade de diatomáceas perifíticas. *Iheringia Serie Botanica*, 71(1), 99–112.

Harding, W. R. & Taylor, J. C. (2014). Diatoms as indicators of historical water quality: A comparison of samples taken in the Wemmershoek catchment (Western Province, South Africa) in 1960 and 2008. *Water SA*, 40(4), 601–606. <https://doi.org/10.4314/wsa.v40i4.4>

Herrero, A., Guitiérrez-Cánovas, C., Vigiak, O., Lutz, S., Kumar, R., Gampe, D., Huber-García, V., Ludwig, R., Batalla, R., & Sabater, S. (2018). Multiple stressor effects on

biological quality elements in the Ebro River: present diagnosis and predicted responses. *Science of the Total Environment*, 630, 1608–1618. <https://doi.org/10.1016/j.scitotenv.2018.02.032>

Hill, M. O. (1979). TWINSpan – A FORTRAN Program for arranging multivariate data in an ordered two-way table by classifications of the individuals and attributes. Ithaca.

Hill, M. O., & Gauch, H. G. (1980). Detrended correspondence analysis: as improved ordination technique. *Vegetatio*, 42(13), 47–58. <https://doi.org/10.1007/BF00048870>

Ho, L., & Goethals, P. (2020). Research hotspots and current challenges of lakes and reservoirs: a bibliometric analysis. *Scientometrics*, 124, 603–631. <https://doi.org/10.1007/s11192-020-03453-1>

Kahlert, M., & Rašić, I. S. (2015). Similar small-scale variation of diatom assemblages of different substrates in a mesotrophic stream. *Acta Botanica Croatica*, 74(2), 363–376. <https://doi.org/10.1515/botcro-2015-0021>

Kassambara, A., & Mundt, F. (2020). Factoextra: extract and visualize the results of multivariate data analyses.

Kelly, M. G., & Whitton, B. A. (1995). The Trophic Diatom Index: a new index for biomonitoring eutrophication in rivers. *Journal of Applied Phycology*, 7(4), 433–444. <https://doi.org/10.1007/BF00003802>

Kelly, M. G., Juggins, S., Guthrie, R., Pritchard, S., Jamieson, J., Rippey, B., Hirst, H., Yallop, M. (2008). Assessment of ecological status in U. K. rivers using diatoms. *Freshwater Biology*, 53(2), 403–422. <https://doi.org/10.1111/j.1365-2427.2007.01903.x>

Kim, Y. S., Choi, J. S., Kim, J. H., Kim, S. C., Park, J. W., & Kim, H. S. (2008). The effects of effluent from a closed mine and treated sewage on epilithic diatom communities in a Korean stream. *Nova Hedwigia*, 86(3), 507–524. <https://doi.org/10.1127/0029-5035/2008/0086-0507>

Kókai, Z., Bácsi, I., Török, P., & Buczkó, K. (2015). Halophilic diatom taxa are sensitive indicators of even short term changes in lowland lotic systems. *Acta Botanica Croatica*, 74(2), 287–302. <https://doi.org/10.1515/botcro-2015-0025>

Kolkwitz, R., & Marsson, M. (1908). Ökologie der pflanzlichen saprobien. *Ver Deutsche Bot Ges*, 26, 505–5019.

Lavoie, I., Campeau, S., Fallu, M. A. & Dillon, P. J. (2006). Diatoms and biomonitoring: should cell size be accounted for? *Hydrobiologia*, 573(1), 1–16. <https://doi.org/10.1007/s10750-006-0223-z>

Lavoie, I., Lento, J., & Morin, A. (2010). Inadequacy of size distributions of stream benthic diatoms for environmental monitoring. *Journal of the North American Benthological Society*, 29(2), 586–601. <https://doi.org/10.1899/09-062.1>

Law, R. J., Elliott, A., & Thackeray, S. J. (2014). Do functional or morphological classifications explain stream phytobenthic community assemblages? *Diatom Research*, 29(4), 309–324. <https://doi.org/10.1080/0269249X.2014.889037>

Legendre, P., & Legendre, L. (1998). *Numerical Ecology*. Elsevier.

Lenoir, A., & Coste, M. (1996). Development of a practical diatom index of overall water quality applicable to the French National Water Board network. In B. A. Whitton, & E. Rott (Eds.), *Use of Algal for Monitoring Rivers* (pp. 29–45). Innsbruck University.

López-Barea, J. (1995). Biomarkers in ecotoxicology: an overview. In G. H. Degen, J. P. Seiler, & P. Bentley (Eds.), *Toxicology in Transition* (pp. 57–85). Springer.

Makarek, V., & Legendre, P. (2002). Nonlinear redundancy analysis and canonical correspondence analysis based on polynomial regression. *Ecology*, 83(4), 1146–1161. [https://doi.org/10.1890/0012-9658\(2002\)083\[1146:NRAACC\]2.0.CO;2](https://doi.org/10.1890/0012-9658(2002)083[1146:NRAACC]2.0.CO;2)

Martina, L. C., Principe, R., & Gari, N. (2013). Effect of a dam on epilithic algal communities of a mountain stream: before-after dam construction comparison. *Journal of Limnology*, 72(1), 79–94. <https://doi.org/10.4081/jlimnol.2013.e7>

Mbao, E. O., Gao, J., Wang, Y., Sitoki, L., Pan, Y., & Wang, B. (2020). Sensitivity and reliability of diatoms metrics and guilds in detecting the impact of urbanization on streams. *Ecological Indicators*, 116, 106506. <https://doi.org/10.1016/j.ecolind.2020.106506>

- Mendes, T., Almeida, S. F. P., & Feio, M. J. (2012). Assessment of rivers using diatoms: effect of substrate and evaluation method. *Fundamental and Applied Limnology*, 179(4), 267–279. <https://doi.org/10.1127/1863-9135/2012/0180>
- Merckx, T., Souffreau, C., Kaiser, A., Baardsen, L. F., Backeljau, T., Bonte, D., Brans, K. I., Cours, M., Dahirel, M., Debortoli, N., Wolf, K., Engelen, J. M. T., Fontaneto, D., Gianuca, A. T., Govaert, L., Hendrickx, F., Higuera, J., Lens, L., Martens, K., Matheve, H., Matthysen, E., Piano, E., Sablon, R., Schön, I., Doninck K. V., Meester, L. D., & Dyck, H. V. (2018). Body-size shifts in aquatic and terrestrial urban communities. *Nature*, 558(7708), 113–116. <https://doi.org/10.1038/s41586-018-0140-0>
- Moore, M. J. C., Langrehr, H.A., & Angradi, T. R. (2012). A submersed macrophyte index of condition for the Upper Mississippi River. *Ecological Indicators*, 13(1), 196–205. <https://doi.org/10.1016/j.ecolind.2011.06.003>
- Mora, D., Abarca, N., Proft, S., Grau, J. H., Enke, N., Carmona, J., Skibbe, O., Jahn, R., & Zimmermann, J. (2019). Morphology and metabarcoding: a test with stream diatoms from Mexico highlights the complementarity of identification methods. *Freshwater Science*, 38(3), 448–464. <https://doi.org/10.1086/704827>
- Moreno, J. L., Navarro, C., & De Las Heras, J. (2006). Proposal of an aquatic vegetation index (IVAM) for assessing the trophic status of the Castilla-La Mancha rivers: a comparison with other indexes. *Limnetica*, 25(3), 821–838.
- Morin, S., Bonet, B., Corcoll, N., Guasch, H., Bottin, M., & Coste, M. (2015). Cumulative stressors trigger increased vulnerability of diatom communities to additional disturbances. *Microbial Ecology*, 70, 585–595. <https://doi.org/10.1007/s00248-015-0602-y>
- Olenici, A., Blanco, S., Borrego-Ramos, M., Momeu, L., & Baci, C. (2017). Exploring the effects of acid mine drainage on diatom teratology using geometric morphometric. *Ecotoxicology*, 26(8), 1018–1033. <https://doi.org/10.1007/s10646-017-1830-3>
- Pandey, L. K., Kumar, D., Yadav, A., Rai, J., & Gaur, J. P. (2014). Morphological abnormalities in periphytic diatoms as a tool for biomonitoring of heavy metal pollution in a river. *Ecological Indicators*, 36, 272–279. <https://doi.org/10.1016/j.ecolind.2013.08.002>

- Pandey, L. K., & Bergey, E. A. (2016). Exploring the status of motility, lipid bodies, deformities and size reduction in periphytic diatom community from chronically metal (Cu, Zn) polluted waterbodies as a biomonitoring tool. *Science of the Total Environment*, 550, 372–381. <https://doi.org/10.1016/j.scitotenv.2015.11.151>
- Pandey, L. K., Bergey, E. A., Lyu, J., Park, J., Choi, S., Lee, H., Depuydt, S., Oh, Y. T., Lee, S. M., & Han, T. (2017). The use of diatoms in ecotoxicology and bioassessment: Insights, advances and challenges. *Water Research*, 118, 39–58. <https://doi.org/10.1016/j.watres.2017.01.062>
- Pandey, L. K., Sharma, Y. C., Park, J., Choi, S., Lee, H., Lyu, J., & Han, T. (2018). Evaluating features of periphytic diatoms communities as biomonitoring tools in fresh, brackish and marine waters. *Aquatic Toxicology*, 194, 67–77. <https://doi.org/10.1016/j.aquatox.2017.11.003>
- Pandey, L. K. (2020). In situ assessment of metal toxicity in riverine periphytic algae as a tool for biomonitoring of fluvial ecosystems. *Environmental Technology & Innovation*, 18, 100675. <https://doi.org/10.1016/j.eti.2020.100675>
- Park, J., Bergey, E. A., Han, T., & Pandey, L. K. (2020). Diatoms as indicators of environmental health on Korean islands. *Aquatic Toxicology*, 227, 105594. <https://doi.org/10.1016/j.aquatox.2020.105594>
- Passy, S. I., & Bode, R. W. (2004). Diatom model affinity (DMA), a new index for water quality assessment. *Hydrobiologia*, 524(1), 241–252. <https://doi.org/10.1023/B:HYDR.0000036143.60578.e0>
- Passy, S. I. (2007). Diatom ecological guilds display distinct and predictable behavior along nutrient and disturbance gradient in running waters. *Aquatic Botany*, 86(2), 171–178. <https://doi.org/10.1016/j.aquabot.2006.09.018>
- Pham, T. L. (2020). Using benthic diatoms as bioindicator to assess rural-urban river conditions in tropical area: a case study in the Sai Gon River, Vietnam. *Pollution*, 6(2), 387–398. <https://doi.org/10.22059/POLL.2020.292996.716>

- Piano, E., Falasco, E., & Bona, F. (2017). Mediterranean rivers: consequences of water scarcity on benthic algal chlorophyll a content. *Journal of Limnology*, 76(s1), 39–48. <https://doi.org/10.4081/jlimnol.2016.1503>
- Potapova, M., & Charles, D. F. (2007). Diatom metrics for biomonitoring eutrophication in rivers of the United States. *Ecological Indicators*, 7(1), 48–70. <https://doi.org/10.1016/j.ecolind.2005.10.001>
- Potapova, M., & Hamilton, P. B. (2007). Morphological and ecological variation within the *Achnanthes minutissimum* (Bacillariophyceae) species complex. *Journal of Phycology*, 43(3), 561–575. <https://doi.org/10.1111/j.1529-8817.2007.00332.x>
- Prygiel, J., Carpentier, P., Almeida, S., Coste, M., Druart, J. C., Ector, L., Guillard, D., Honoré, M. A., Iserentant, R., Ledeganck, P., Lalanne-Cassou, C., Lesniak, C., Mercier, I., Moncaut, P., Nazart, M., Nouchet, N., Peres, F., Peeters, V., Rimet, F., Rumeau, A., Sabater, S., Straub, F., Torrisi, M., Tudesque, L., Vijver, B. V., Vidal, H., Vizinet, J., & Zydek, N. (2002). Determination of the biological diatom index (IBD NF T 90-354): results of an intercomparison exercise. *Journal of Applied Phycology*, 14(1), 27–39. <https://doi.org/10.1023/A:1015277207328>
- R Core Team (2020). R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna. <http://www.R-project.org/>
- Richards, J., Tibby, J., Barr, C., & Goonan, P. (2020). Effect of substrate type on diatom-based water quality assessments in the Mount Lofty Ranges, South Australia. *Hydrobiologia*, 847(14), 3077–3090. <https://doi.org/10.1007/s10750-020-04316-9>
- Rimet, F. (2012). Recent views on river pollution and diatoms. *Hydrobiologia*, 683, 1-24. <https://doi.org/10.1007/s10750-011-0949-0>
- Rimet, F., & Bouchez, A. (2012). Life-forms, cell-sizes and ecological guilds of diatoms in European rivers. *Knowledge and Management of Aquatic Ecosystems*, 406, 1–12. <https://doi.org/10.1051/kmae/2012018>
- Rimet, F., Gusev, E., Kahlert, M., Kelly, M. G., Kulikovskiy, M., Maltsev, Y., Mann, D. G., Pfannkuchen, M., Trobajo, R., Vasselon, V., Zimmermann, J., & Bouchez, A. (2019).

Diat.barcode, na open-access curated barcode library for diatoms. *Scientific Reports*, 9, 15116. <https://doi.org/10.1038/s41598-019-51500-6>

Rivera, M. J., Luís, A. T., Grande, J. A., Sarmiento, A. M., Dávila, J. M., Fortes, J. C., Córdoba, F., Diaz-Curiel, J., & Santisteban, M. (2019). Physico-chemical influence of surface water contaminated by acid mine drainage on the populations of diatoms in dams (Iberian Pyrite Belt, SW Sapin). *International Journal of Environmental Research and Public Health*, 16(22), 4516. <https://doi.org/10.3390/ijerph16224516>

Rivera, M. J., Santisteban, M., Aroba, J., Grande, J. A., Dávila, J. M., Sarmiento, A., Fortes, J. C., Curiel, J., & Luís, A. T. (2020). Application of fuzzy logic techniques for biogeochemical characterization of dams affected by acid mine drainage (AMD) process in the Iberian Pyrite Belt (IPB), Spain. *Water Air and Soil Pollution*, 231(4), 142. <https://doi.org/10.1007/s11270-020-04501-5>

Rodríguez, P., Garraza, G. G., García, V., Granitto, M., & Escobar, J. (2020). Beaver dam effect of phytoplankton and periphyton composition and hydrology in streams from Tierra del Fuego (Argentina). *Hydrobiologia*, 847(6), 1461–1477. <https://doi.org/10.1007/s10750-020-04201-5>

Rumeau, A. & Coste, M. (1988). Initiation a la systématique des Diatomées d'eau douce pour l' utilisation pratique d'um 72ndice diatomique générique. *Bulletin Francais de la Pêche et la Pisciculture*, 309, 1–69.

Rusanov, A. G., Stanislavskaya, E. V., & Ács, E. (2012). Periphytic algal assemblages along environmental gradients in the rivers of the Lake Ladoga basin, Northwestern Russia: implication for the water quality assessment. *Hydrobiologia*, 695(1), 305–327. <https://doi.org/10.1007/s10750-012-1199-5>

Salomoni, S. E., Rocha, O., Callegaro, V. L., & Lobo, E. A. (2006). Epilithic diatoms as indicators of water quality in the Gravataí River, Rio Grande do Sul, Brazil. *Hydrobiologia*, 559, 233–246. <https://doi.org/10.1007/s10750-005-9012-3>

Schowe, K. A., & Harding, J. S. (2014). Development of two diatom-based indices: a biotic and multimetric index for assessing mine impacts in New Zealand streams. *New Zealand*

Journal of Marine and Freshwater Research, 48(2), 163–176.
<https://doi.org/10.1080/00288330.2013.852113>

Shen, R., Ren, H., Yu, P., You, Q., Pang, W., & Wang, Q. (2018). Benthic diatoms of the Ying River (Huaihe River Basin, China) and their application in water trophic status assessment. *Water*, 10(8), 1013. <https://doi.org/10.3390/w10081013>

Smith, V. H., & Schindler, D. W. (2009). Eutrophication science: where do we go from here? *Trends in Ecology and Evolution*, 24(4), 201–207. <https://doi.org/10.1016/j.tree.2008.11.009>

Souza, G. B. G., & Vianna, M. (2020). Fish-based indices for assessing ecological quality and biotic integrity in transitional waters: A systematic review. *Ecological Indicators*, 109, 105665. <https://doi.org/10.1016/j.ecolind.2019.105665>

Stevenson, R. J., Pan, Y., & Van Dam, H. (2010). Assessing environmental conditions in rivers and streams with diatoms. In J. Smol, & E. Stoermer (Eds.), *The diatoms: applications for the environmental and earth sciences* (2th ed., pp. 57–85). Cambridge University Press.

Stubbington, R., Paillex, A., England, J., Barthès, A., Bouchez, A., Rimet, F., Sánchez-Montoya, M. M., Westwood, C. G., & Datry, T. (2019). A comparison of biotic groups as di-phase indicators of ecological quality in intermittent rivers and ephemeral streams. *Ecological Indicators*, 97, 165–174. <https://doi.org/10.1016/j.ecolind.2018.09.061>

Tapolczai, K., Keck, F., Bouchez, A., Rimet, F., Kahlert, M., & Vasselon, V. (2019). Diatom DNA metabarcoding for biomonitoring: strategies to avoid major taxonomical and bioinformatical biases limiting molecular indices capacities. *Frontiers in Ecology and Evolution*, 7, 1–15. <https://doi.org/10.3389/fevo.2019.00409>

Taylor, J. C., Harding, W. R., Archibald, C. G. M., & Rensburg, L. V. (2005). Diatoms as indicators of water quality in the Jukskei-Crocodile river system in 1956 and 1957, a re-analysis of diatom count data generated by Bj Chohnoky. *Water SA*, 31(2), 237–246. <https://doi.org/10.4314/wsa.v31i2.5176>

ter Braak, C. J. F. (1986). Canonical correspondence analysis: a new eigenvector technique for multivariate direct gradient analysis. *Ecology*, 67(5), 1167–1179. <https://doi.org/10.2307/1938672>

- Townsend, S. A. & Gell, P. A. (2005). The role of substrate type on benthic diatom assemblages in the Daly and Roper Rivers of the Australian wet/dry tropics. *Hydrobiologia*, 548(1), 101–115. <https://doi.org/10.1007/s10750-005-0828-7>
- Tudesque, L., Grenouillet, G., Gevrey, M., Khazraie, K., & Brosse, S. (2012). Influence of small-scale gold mining on French Guiana streams: Are diatom assemblages valid disturbance sensors? *Ecological Indicators*, 14(1), 100–106. <https://doi.org/10.1016/j.ecolind.2011.07.018>
- Van Eck, N. J., & Waltman, L. (2010). Software survey: VOSviewer, a computer program for bibliometric mapping. *Scientometrics*, 84, 523–538. <https://doi.org/10.1007/s11192-009-0146-3>
- Vidaković, D. P., Radovanović, S. S., Predojević, D. D., Šovran, S. I., Živić, I. M., Stojanović, K. Z., & Krizmanić, J. Ž. (2018). Uncertainty of using habitat fidelity in biomonitoring based on benthic diatoms- the Raška River case study. *Biologia*, 73, 831–839. <https://doi.org/10.2478/s11756-018-0108-4>
- Vilmi, A., Karjalainen, S. M., & Heino, J. (2017). Ecological uniqueness of stream and lake diatom communities show different macroecological patterns. *Biodiversity Research*, 23(9), 1042–1053. <https://doi.org/10.1111/ddi.12594>
- Walsh, C. J., Roy, A. H., Feminella, J. W., Cottingham, P. D., Groffman, P. M., & Morgan, R. P. (2005). The urban stream syndrome: current knowledge and the search for a cure. *Journal of the North American Benthological Society*, 24(3), 706–723. <https://doi.org/10.1899/04-028.1>
- Wen, Y., Schoups, G., & Giesen, N. (2017). Organic pollution of rivers: combined threats of urbanization, livestock farming and global climate change. *Scientific Reports*, 7(1), 1–9. <https://doi.org/10.1038/srep43289>
- Wickham, H. (2016). *Ggplot2: elegant graphics for data analysis*. Springer.
- Wojtal, A. Z., & Sobczyk, Ł. (2012). The influence of substrates and physicochemical factors on the composition of diatom assemblages in karst springs and their applicability in water-quality assessment. *Hydrobiologia*, 695(1), 97–108. <https://doi.org/10.1007/s10750-012-1203-0>

Wu, N., Cai, Q., & Fohrer, N. (2012). Development and evaluation of a diatom-based index of biotic integrity (D-IBI) for rivers impacted by run-of-river dams. *Ecological Indicators*, 18, 108–117. <https://doi.org/10.1016/j.ecolind.2011.10.013>

Zalack, J. T., Smucker, N. J., & Vis, M. L. (2010). Development of a diatom index of biotic integrity for acid mine drainage impacted streams. *Ecological Indicators*, 10(2), 287–295. <https://doi.org/10.1016/j.ecolind.2009.06.003>

CAPÍTULO 2

The use of genus-level determinations and biovolume classes as surrogates to indicate environmental drivers of stream diatom communities in the Brazilian Pampa

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Abstract

The demand for low-cost and time-efficient biomonitoring and ecological assessment strategies has increased due to threats to biodiversity. We evaluated the ability of surrogates (genera and biovolume classes) to access information provided by diatom species. Moreover, we investigated whether the relative importance of spatial and environment on diatom communities identified at the genus or classified at biovolume classes reflects the ecological responses at the species. We used biotic, environmental, and spatial data from Pampa streams. We employed Procrustes analysis to detect whether the spatial variation of communities at the species level was represented by the surrogates and variation partitioning to evaluate the relative importance of spatial and environment. We found high congruence between the surrogates and diatom species communities. Furthermore, whilst both spatial and environment were important to explain species composition, only the environment was important for both surrogates. We suggest that the use of genus-level determinations can provide information about the environmental relationships of species, whereas biovolume classes may be useful to detect changes in diatom communities. These results are a first step to seek for strategies aiming to simplify biological monitoring and assessments at pampean streams, especially considering the demand for the identification of organisms at the species.

Key-words: congruence, grassland, lotic environment, metacommunity, Procrustes, variance partitioning

Introduction

Understanding freshwater biodiversity patterns is a challenge, especially in species-rich regions, such as the tropics and subtropics (e.g., Siqueira et al. 2012a; Trindade et al. 2018). This is because of the lack of reliable information about species identities (Hortal et al. 2015), in addition to the time spent on sample processing (Carneiro et al. 2010; de Oliveira et al. 2020). Such difficulties increase when anthropogenic impacts are part of the scenario as they may affect the composition of communities in different and unexpected ways, frequently resulting in biotic homogenisation (Siqueira et al. 2015; Petsch 2016) or differentiation (Vieira et al. 2022). As a consequence, biodiversity assessments and biomonitoring programmes of freshwater ecosystems tend to be expensive and time-consuming, which reduces the sampling effort in both space and time (Bevilacqua et al. 2015).

One way to overcome the absence of detailed information at the species level and to make biomonitoring programmes more cost- and time-effective is the use of surrogates (Margules & Pressey 2000). In general, a good surrogacy candidate needs to reflect the patterns observed at the species level (de Oliveira et al. 2020). However, a high congruence between information at the species level and the surrogate is not sufficient as it is necessary that the surrogates also respond to spatial and environmental predictors similarly to species, especially for biomonitoring and ecological assessment purposes. In freshwater ecosystems, coarser taxonomic resolutions are good surrogates of species (e.g., Godoy et al. 2019; Riato et al. 2022), a relationship usually referred to as the higher taxon approach (see de Oliveira et al. 2020 for a meta-analysis). Furthermore, functional classifications have been widely used as surrogates (Gallego et al. 2012; Mueller et al. 2013). However, it is still necessary to advance further and test not only the higher taxon approach in less studied ecosystems around the world but also the surrogacy efficiency of simple and easily obtainable morphometric characteristics of species. One alternative is the classification of organisms into size or biovolume classes, which could drastically reduce the effort to represent community structure without losing information provided at the species level (e.g., Wunsam et al. 2002; Ribeiro et al. 2020).

Diatoms are among the most common bioindicators in freshwaters due to their wide distribution and ease to sample (Stevenson et al. 2010). Moreover, diatom species are characterised by large differences in their tolerances to chemical and physical characteristics of freshwater ecosystems, such as nutrient concentrations and flow velocity (Virtanen & Soininen 2012). However, it is a highly diverse group for which species identification is based

on the analysis of small structures only distinguished by trained taxonomists, in addition to the need for a constant literature update since the nomenclature changes frequently (Rimet & Bouchez 2012a). In this sense, studies have evaluated the efficiency of using coarse taxonomic resolutions and easily obtainable traits as surrogates for biomonitoring and assessment programmes (e.g., Rimet & Bouchez 2012a; Algarte et al. 2014; Riato et al. 2022). Moreover, non-taxonomical metrics, such as the number and volume of lipid bodies, the frequency of valve deformities and size classes, have gained prominence in the literature in recent years (Costa & Schneck 2022). This is because biomonitoring and ecological assessments based on such metrics are more time- and cost-effective when compared to the obtention of information at the species level for all communities (e.g., Pandey et al. 2018a; Pandey et al. 2018b).

Here, we investigated the efficiency of a taxonomic and a morphometric approach as surrogates of information at the species level for stream diatom communities in the Brazilian Pampa region. Specifically, we tested whether the identification of genera and the use of biovolume classes are effective in representing the spatial variation in the structure of diatom communities. Moreover, we investigated the relative importance of environmental and spatial variables in determining this variation and whether the surrogates under evaluation respond similarly to diatom species.

Material and Methods

Study area

We sampled 25 low-order streams in the Ibicuí, Santa Maria and Vacacaí-Mirim hydrographic basins in southernmost Brazil (Figure 1) during austral summer (February 2020). The basins differ mostly in their land use and cover, with the Santa Maria and Vacacaí-Mirim basins dominated by agriculture, whereas the Ibicuí basin is mainly covered by natural grasslands. The climate is humid subtropical, with hot summers and without dry seasons ('Cfa' of Köppen classification) (Alvares et al. 2013). The sampling area is within the Pampa biome, also known as the Rio de la Plata Grasslands (Baeza et al. 2022). The region is characterised by the predominance of native grasslands (*campos*) (Overbeck et al. 2007), being the largest temperate grassland area in South America, covering approximately 75 million hectares in Uruguay, central-eastern Argentina and southernmost Brazil (Soriano 1991). The conversion of the natural landscape into agricultural areas, especially for the cultivation of soy, rice and silviculture, has been intensified in recent years (Overbeck et al. 2007; Oliveira et al. 2017).

threatening terrestrial and freshwater ecosystems. Moreover, conservation policies do not adequately contemplate the Pampa, which is considered the most neglected biome in Brazil (Overbeck et al. 2007) and among the most altered ecoregions of the world (Watson et al. 2016).

Sampled streams were dominated by grasslands within the riparian area, with sparse shrub formations and less commonly with riparian vegetation dominated by trees. At the Santa Maria and Vacacaí-Mirim hydrographic basins the streambeds are characterised by the predominance of sand and silt, whereas at the Ibicuí basin, it is common to observe pebbles, cobbles, and boulders, in addition to sand and silt.

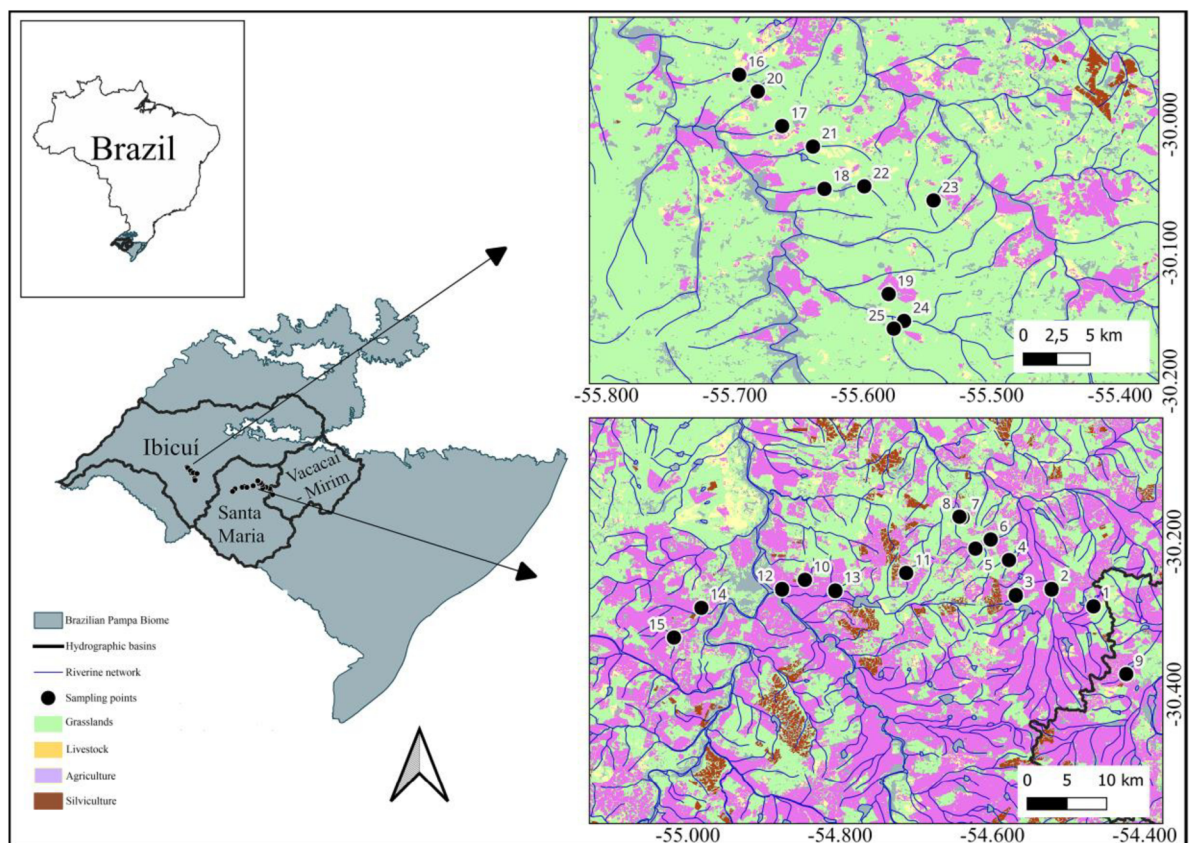


Figure 1. Twenty-five streams sampled in the Brazilian Pampa Biome distributed in Vacacaí-Mirim, Santa Maria and Ibicuí hydrographic basins, Rio Grande do Sul state.

Biological and environmental variables

At each stream site, we sampled biological and environmental variables from one stretch of 100 m. We sampled three subsamples of benthic diatoms from the surface of streambed sediment by using a *syringe*. The material from the three subsamples was pooled and

preserved in 4% formalin. In the laboratory, the material was acid-cleaned according to Metzeltin and García-Rodríguez (2012). We prepared permanent slides of diatoms using Naphrax[®] (Brunel Microscopes Ltd., Chippenham, UK) and counted 500 valves from each stream, using a light microscope with 1,000 $\times$ magnification. We identified diatoms at the lowest possible taxonomic level, mostly species, using specialised bibliography such as Metzeltin et al. (2005) and Metzeltin and García-Rodríguez (2012). After identification, we used published biovolume-based classes to classify the taxa into five biovolume classes: 1 ($\leq 99 \mu\text{m}^3$), 2 (100–299 μm^3), 3 (300–599 μm^3), 4 (600–1,499 μm^3), 5 ($> 1,500 \mu\text{m}^3$) (Rimet and Bouchez 2012b).

We measured pH, dissolved oxygen (mg L^{-1}), electrical conductivity ($\mu\text{S cm}^{-1}$) and total dissolved solids (g L^{-1}) using a Horiba U-50 multiparameter probe. At each stream, we made 10 measurements for each variable. We measured current velocity (cm s^{-1}), stream width (m) and depth (cm) at five random locations. We also sampled 500 mL of surface water at each stream for the analysis of total nitrogen (Allen et al. 1974) and total phosphorus (Valderrama 1981; Baumgarten et al. 1996). For further analyses, we used the mean of each variable per stream (Table I).

Data analyses

We performed principal components analysis (PCA; Legendre & Legendre 1998) to summarise the environmental variation among streams. For this, all variables were standardized to zero mean and unit variance. The interpretation of the results was based on the axes retained using the Broken-Stick criterion (Jackson 1993).

We used principal coordinates analysis (PCoA) to summarise the variation in diatom community composition for species, genus and biovolume classes, using presence-absence and abundance data. For this, we used the Sørensen (presence-absence) and the Bray-Curtis (logarithmised abundance + 1) dissimilarity coefficients (Legendre & Legendre 1998). To evaluate the congruence between the species dataset and the surrogates, we compared the ordination sample scores from PcoAs obtained with the species dataset with those obtained with each of the surrogates, using Procrustes analysis (Jackson 1995; Peres-Neto & Jackson 2001). The Procrustean superimposition is represented by the sum of squared differences (m^2) between matrices. In this sense, the lower the value of m^2 , the greater the similarity between ordination matrices. We transformed the values of m^2 to r (Procrustes correlation).

We used partial redundancy analysis (pRDA; Legendre & Legendre 1998) to evaluate the relative importance of spatial and environmental factors on the community composition of

diatoms for species, genus and biovolume class matrices. Before pRDA, we applied the Hellinger transformation on the biotic matrices (Legendre & Gallagher 2001). We generated the spatial predictor matrix using Moran eigenvector maps (MEM), with eigenvectors extracted from a Principal Coordinate of Neighbouring Matrices analysis (PCNM; Borcard & Legendre 2002). We used the minimum spanning tree criteria to truncate the distance matrix. In this analysis, the MEMs with the lowest values represent fine spatial scales, and those with higher values represent large spatial scales. The environmental matrix was determined after the exclusion of collinear variables with a variance inflation factor (VIF) higher than 3. The final environmental matrix contained eight variables: pH, dissolved oxygen, electrical conductivity, total phosphorus, total nitrogen, width, depth and current velocity. We then used redundancy analyses to test the significance of each global model (i.e., a model using the complete set of spatial predictors and another model using the complete set of environmental predictors, separately for each biological matrix).

We proceeded with the analysis only if the global model for a given set of predictors was significant ($P < 0.05$). In this case, we used a forward selection with two stopping criteria: the significance of each explanatory variable smaller than 0.05 and the adjusted coefficient of multiple determination (adjusted R^2) of the reduced model smaller than the adjusted R^2 of the global model, as recommended by Blanchet et al. (2008). The retained explanatory variables were then used in pRDA to partition biological variation into four fractions: [E] pure environmental component, [E-S] spatially structured environmental component, [S] pure spatial component and [UN] residual unexplained variation. Finally, we tested the significance of the pure environmental and pure spatial components using 999 permutations, and the explained variation was estimated using adjusted R^2 (Peres-Neto et al. 2006). Finally, to visualise the relationships between each biological dataset and the environment we constructed RDA plots for each approach using the selected environmental variables. We used the R environment for all analyses (R Core Team 2020), with the packages *car* (Fox & Weisberg 2011), *vegan* (Oksanen et al. 2017), *adespatial* (Dray et al. 2016) and *ggplot2* (Wickham 2016).

Results

The sampled streams varied in their environmental characteristics (Table I; Figure 2). According to the Broken-Stick criterion, the first two axes of the PCA were retained and explained 52% of the variation in environmental data. The first principal component

explained 32% of data variation, being positively related to current velocity and negatively to electrical conductivity, total dissolved solids, total nitrogen and stream width (Figure 2). The second principal component explained 20% of the variation and was positively related to dissolved oxygen and pH and negatively related to depth and total phosphorus (Figure 2).

Table I. Mean of environmental variables from 25 streams sampled in the Brazilian Pampa. Cond = electrical conductivity; DO = dissolved oxygen; TDS = total dissolved solids; TP = total phosphorus; TN = total nitrogen; Vel = current velocity.

Stream	pH	DO (mg L ⁻¹)	Cond (μS cm ⁻¹)	TDS (g L ⁻¹)	TP (μg L ⁻¹)	TN (mg L ⁻¹)	Vel (cm s ⁻¹)	Width (m)	Depth (cm)
1	8.2	8.4	0.24	0.23	85.0	1.2	<1.0	2.4	48.1
2	7.1	7.6	0.12	0.07	591.7	1.3	26.5	5.1	30.6
3	7.3	7.3	0.07	0.04	244.8	1.2	<1.0	2.2	19.3
4	7.8	11.1	0.22	0.14	72.6	0.7	<1.0	5.2	49.1
5	7.4	5.6	0.10	0.07	52.1	0.6	<1.0	5.9	49.8
6	6.9	4.3	0.06	0.04	105.6	1.3	<1.0	2.2	34.9
7	6.9	7.3	0.04	0.02	94.1	0.8	<1.0	2.5	23.9
8	7.2	7.9	0.06	0.03	38.8	0.6	<1.0	1.9	25.5
9	7.3	4.2	0.45	0.29	215.2	1.6	<1.0	3.9	30.3
10	8.0	8.7	0.30	0.19	102.3	1.5	<1.0	3.1	16.9
11	7.5	7.8	0.03	0.02	134.4	0.9	<1.0	1.2	20.2
12	7.7	8.6	0.04	0.02	108.1	0.8	<1.0	7.7	24.0
13	7.4	6.9	0.04	0.02	52.9	0.6	<1.0	5.4	56.0
14	6.6	5.0	0.03	0.02	57.0	0.6	<1.0	2.3	47.2
15	7.1	7.2	0.05	0.03	178.1	0.9	<1.0	2.2	45.0
16	7.3	6.6	0.15	0.09	43.0	0.4	21.0	1.1	17.5
17	7.9	7.9	0.07	0.04	47.1	0.6	23.9	3.0	14.6
18	7.1	8.0	0.04	0.02	30.6	0.5	66.4	2.2	21.9
19	7.5	6.2	0.08	0.06	49.6	0.7	<1	1.0	9.5
20	7.6	7.6	0.04	0.03	11.4	0.5	<1	5.5	5.4
21	7.4	7.0	0.06	0.04	25.7	0.6	9.7	4.1	16.8
22	8.4	7.8	0.12	0.08	29.0	0.8	<1	1.3	9.8
23	7.5	6.4	0.11	0.07	27.3	0.8	<1	1.9	39.8
24	7.5	8.0	0.07	0.05	33.1	0.7	30.5	4.1	7.3
25	7.3	6.7	0.07	0.04	96.5	0.6	38.5	4.7	11.1

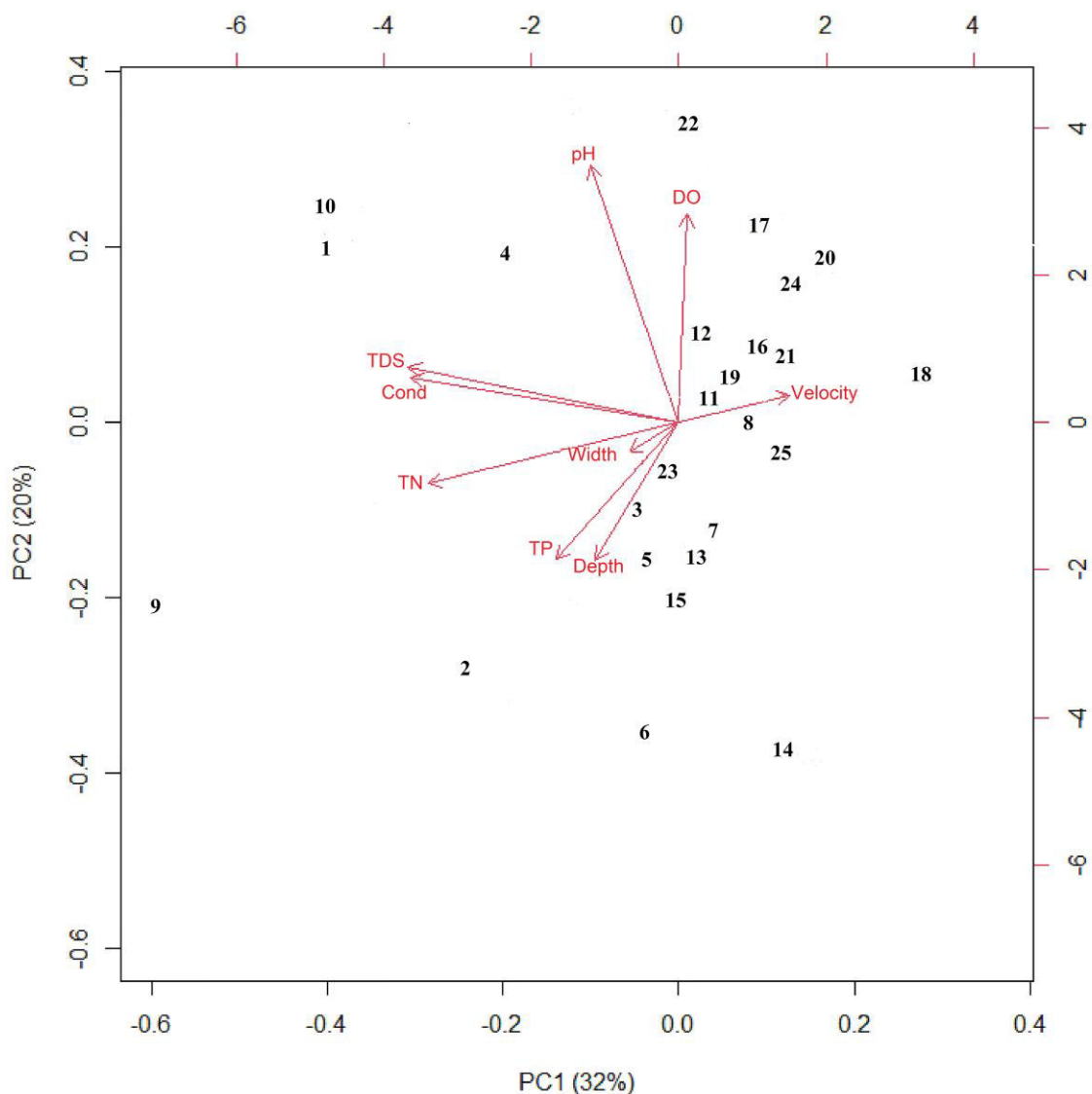


Figure 2. Principal Component Analysis (PCA) using environmental variables from 25 streams in the Brazilian Pampa. Numbers represent the streams sampled; TP = total phosphorus; TN = total nitrogen; Cond = electrical conductivity; TDS = total dissolved solids; DO = dissolved oxygen.

We found 245 diatom species from 50 genera (Table SI – Supplementary Material). Diatom species richness ranged from 37 to 81 per stream. The more abundant genera were *Navicula* (15%), *Nitzschia* (14%), *Encyonema* (8%) and *Achnantheidium* (6%). All other genera comprised less than 5% of abundance. Regarding diatom biovolume classes, the most abundant one was class 3 (300–599 μm^3). Only the smallest biovolume class (1) did not occur in all streams.

Both surrogates showed significant but low congruence with the dataset at the species level when presence-absence data were used, with identification at the genus-level and biovolume class data showing correlations of 0.57 and 0.56 ($P < 0.001$) with the species dataset, respectively. As expected, when using abundance data, congruence with the species-level data increased for both the genus-level ($r = 0.97$; $P < 0.001$) and the biovolume-class ($r = 0.84$; $P < 0.001$) datasets.

The variation in diatom species composition was partially explained by pure environmental, pure spatial and spatially structured environmental components, which accounted for similar variation for both presence-absence or abundance data. However, those three components together explained 14% of the variation for abundance data but only 5% for presence-absence data (Table 2; Figure 3). Furthermore, whilst only total nitrogen significantly explained the variation for presence-absence data, stream width, pH and total nitrogen explained the variation in composition based on abundance data. The selected spatial variables were PCNMs 1 and 3 for presence-absence data and 1 and 5 for abundance data.

Regarding both surrogates, variation partitioning showed similar patterns. In all cases (i.e., genus-level identification and biovolume-class approaches for both presence-absence and abundance data), the pure spatial fraction did not significantly explain the variation in diatom composition. However, both approaches presented similar results as those found for species in relation to the environmental component (Table II; Figure 3). The dataset based on biovolume classes better explained variation in composition, with the pure environmental and the spatially structured environmental components accounting together for 39% and 25% of the variation for presence-absence and abundance data, respectively (Table II; Figure 3). More important is that the selected environmental variables were mostly the same as those selected for the species dataset: total nitrogen for presence-absence data and stream width and total nitrogen for abundance data, for all three approaches (Table II). The exception was the relationship of species and genus-level datasets with pH, which was not selected when biovolume classes were used.

Table II. Results of the partial redundancy analysis (pRDA) of environmental and spatial models explaining the variation in stream diatom community structure for datasets identified at the species level, genus level, or by cell biovolume classes. Environmental and spatial variables (PCNM) selected in the final model. Values of significance ($P < 0.05$) for the global environmental (pG[E]) and global spatial (pG[S]) models. Adjusted R^2 values for pure environmental (Adj R^2 [E]), spatially-structured environmental (Adj R^2 [E–S]) and pure spatial (Adj R^2 [S]) fractions. Values of significance ($P < 0.05$) for the environmental (p[E]) and spatial (p[S]) fractions.

	Environmental	Spatial	pG[E]	pG[S]	Adj R^2 [E]	Adj R^2 [E–S]	Adj R^2 [S]	p[E]	p[S]
Presence-absence									
Species	TN	1,3	0.03	0.002	0.02	0.01	0.02	0.003	0.04
Genus	TN	1	0.02	0.040	0.06	0.01	0.02	0.001	0.05
Size class	TN	1	0.02	0.030	0.27	0.12	0.00	0.008	0.96
Abundance									
Species	Width, pH, TN	1, 5	0.001	0.006	0.06	0.05	0.03	0.007	0.04
Genus	Width, pH, TN	1	0.006	0.020	0.11	0.04	0.01	0.001	0.13
Size class	Width, TN	1	0.01	0.040	0.16	0.09	0.01	0.008	0.26

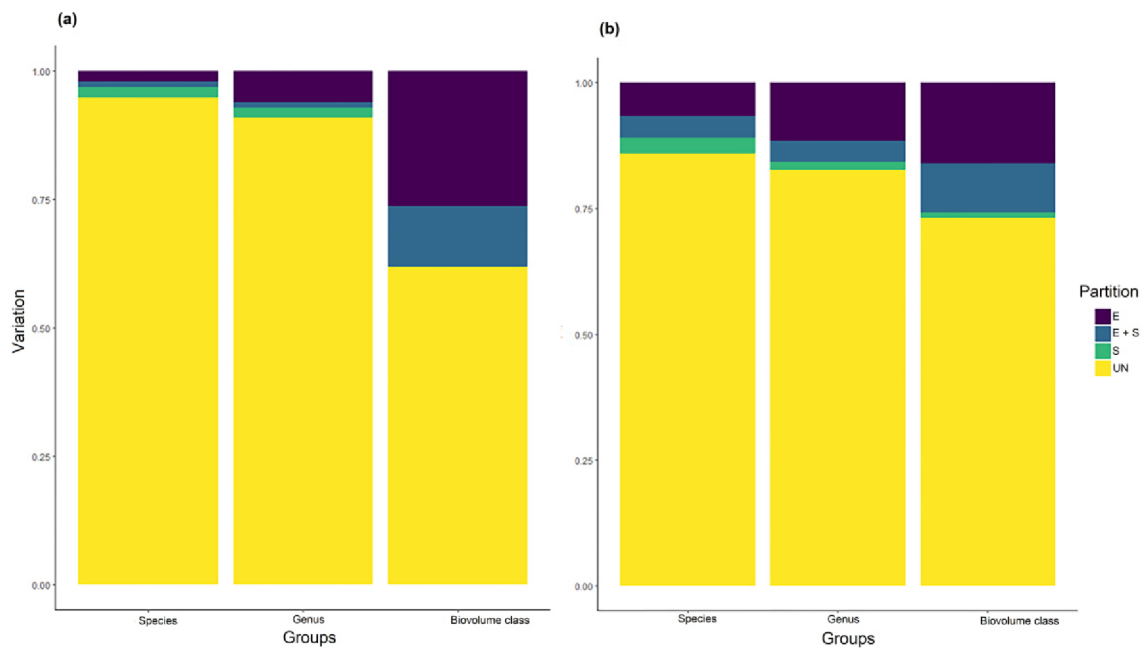


Figure 3. Partition of the variance of the diatom community composition (species and genus) and diatom biovolume classes for presence-absence (a) and abundance data (b). E = environmental; E+S = shared variation between space and environment; S= spatial; UN= Unexplained variation.

In fact, the directions of the relationships with environmental gradients were similar for all the three approaches (Figure 4). There was a strong gradient of total nitrogen at the first axis, in which species of *Cocconeis* (Figure 4a) and the genera *Cocconeis* and *Diadesmis* (Figure 4b) were positively associated with nitrogen-rich streams, mostly located at agricultural areas (see Figure 1), as was the case for biovolume class 5, the largest one (Figure 4c). The environmental gradient represented by the second axis of the RDAs showed that *Achnantheidium* and *Encyonema* were mainly related to narrow streams with high pH (Figure 4a and b), most of them occurring in grassland-dominated areas (see Figure 1), whereas *Navicula* was related to wide streams (Figure 4a and b) in agricultural areas. Moreover, the smallest biovolume classes (1, 2, and 3) were related to narrow streams with low total nitrogen concentrations (Figure 4c).

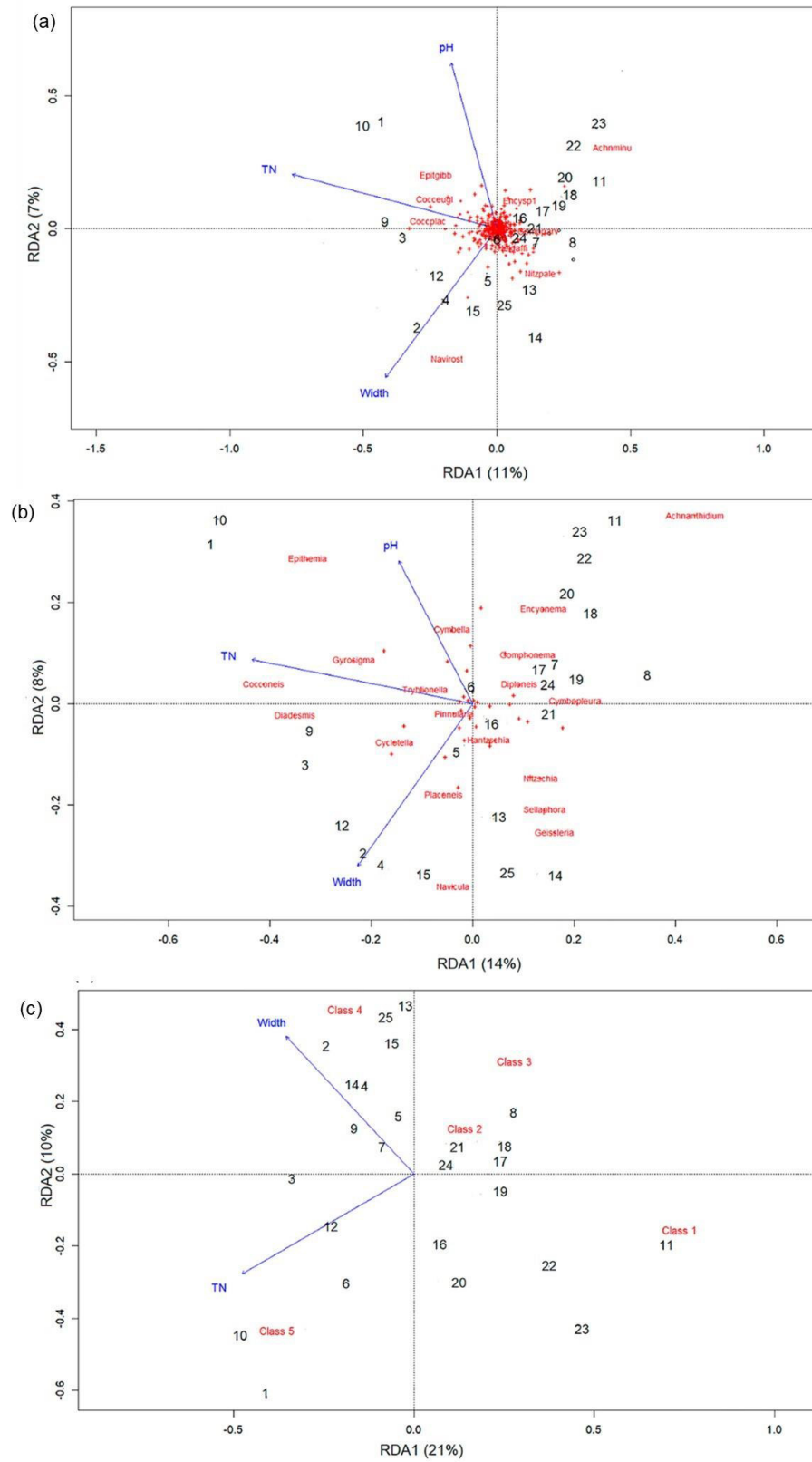


Figure 4 Redundancy Analysis (RDA) using diatom communities composition based on abundance data for species (a), genera (b) and biovolume classes (c). Species abbreviations: Achnminu: *Achnanthes minutissimum*; Coccegl: *Cocconeis placentula* var. *euglypta*; Coccpac: *Cocconeis placentula*; Cymbtumi: *Cymbella tumida*; Encysp1: *Encyonema* sp1; Naviros: *Navicula* cf. *rostellata*; Nitzpale: *Nitzschia palea*. Numbers represent the streams sampled; TN = total nitrogen.

Discussion

We found that both surrogate approaches, identification to the genus level and classification of species in biovolume classes, showed high congruence with the dataset identified to the species level when abundance data were used, but not for presence-absence data. Moreover, surrogates partly reflected the environmental responses of species but not the effects of space. These results indicate that both approaches can be good candidates to represent the composition of diatoms in Brazilian Pampa streams.

The efficiency of freshwater surrogates has long been investigated as an alternative for describing biodiversity patterns and defining more cost- and time-efficient biomonitoring programmes (e.g., Bevilacqua et al. 2015; Godoy et al. 2019; Riato et al. 2022). Here, we found that the identification of diatoms at a coarse taxonomic level and the use of a morphometric non-taxonomical approach present high congruence with the original species matrix based on abundance data. Moreover, we found that the use of surrogates reduces the unexplained variation from partitioning analysis, which may be related to a reduction in the noise derived from multiple species in the analysis of community-environment relationships (e.g., Godoy et al. 2019). In this sense, our results are of great relevance, especially considering the demand of time and the effort required to assess communities at the species level for biomonitoring and ecological assessment programmes and considering the Linnean shortfall for microorganisms (e.g., Marrone et al. 2023), which may hinder the correct identification of species. This is true especially for less studied freshwater ecosystems, as is the case of Pampa streams in South America.

In addition to a high congruence, our results suggest that genus-level information can substitute species-level data to represent the main species-environment relationships of stream diatom communities. This has practical importance in the biomonitoring and ecological assessment context, for which useful surrogates or indicators need to detect the ecological status of the ecosystem of interest (Landeiro et al. 2012; Siqueira et al. 2012b). Moreover, we

suggest that biovolume classes may be good surrogacy candidates to detect changes in the composition of diatom communities, but it may be premature to consider this approach to represent species-environment relationships. This is because potential information may be lost due to the low number of environmental variables retained in the analysis in comparison with the species and genus-level approaches.

The environmental variables that best explained the variation in the composition of diatom communities clearly represent the ecological status of the studied streams. The importance of stream width in low-order Brazilian Pampa streams is probably related to habitat heterogeneity and differences in light incidence in the streambed (e.g., Pacheco et al. 2022). For example, despite the riparian zones of most of the sampled streams being dominated by natural grasslands, those streams less impacted by agriculture also have low-density arboreal riparian vegetation. In fact, the negative relationship of *Achnanthes* with stream width reinforces the suggestion of a relation with light incidence, as this genus is characterised by organisms living in the bottom layer of the periphytic biofilm and thus adapted to the reduced light availability (Passy 2007; Schneck et al. 2022). Furthermore, nitrogen is related to the variation of diatom species among freshwater ecosystems (e.g., B-Berés et al. 2014; Huttunen et al. 2020). In our study, streams with high nitrogen concentrations are those within agricultural landscapes, mainly at the Santa Maria and Vacacaí-Mirim hydrographic basins, in which nutrients are more easily leached to streams (Carpenter et al. 1998). Although nitrogen is often a limiting factor for algal growth (Kumar and Bera 2020), the excessive input of nitrogen of anthropogenic origin (i.e. agricultural runoff in the context of our study) degrades water quality, leading to eutrophication (Schindler 2006) and changes in the composition of freshwater diatom communities (e.g. Tan et al. 2014, Huttunen et al. 2020), as we found here. For instance, *A. minutissimum* and the genus *Encyonema*, which showed a negative relationship with nitrogen-rich streams, are commonly classified as sensitive to multiple stressors from agricultural runoff (e.g., Waite et al 2020). On the other hand, diatoms usually documented in polluted streams, such as *C. placentula* (e.g., Çelekli et al. 2019) and *Diadesmis* (e.g., Wood et al. 2019) were positively related with total nitrogen in our study. These results indicate that those species and genera more strongly related to nitrogen concentrations could be good indicators of agricultural impacts in Brazilian Pampa streams. Finally, regarding biovolume classes, the relation of large classes with agricultural streams can be explained by the limitation of nitrogen. This is because the increase in cell volume in algae communities can be driven by nitrogen availability

(Peter and Sommer 2014). Thus, we suggested that larger biovolume classes may be indicative of streams influenced by nitrogen input, while smaller biovolume classes may be indicative of grassland streams in Brazilian Pampa.

In our study, the spatial component was important only to explain the variation in the composition of species. The relevance of space for diatom communities may be associated with the differences in their dispersal capacity due to the auto-ecological characteristics of species within communities. For example, planktonic and motile species can benefit from dispersal through mass effects due to water flow in relation to attached diatoms (e.g., Jamoneau et al. 2018). Moreover, by grouping species into genera or biovolume classes, we simplified the data matrix in a way that most groups occur in all sites, reducing the pure spatial effect that may hinder the responses of organisms to major environmental factors of interest for biomonitoring and assessment programmes. As highlighted by Siqueira et al. (2012b), better indicator groups are those for which the metacommunity structure is determined mostly by environmental factors and not by spatial ones.

We found that the use of abundance data resulted in higher percentages of explained variance than presence-absence data, for both species and genera datasets. This effect of numerical resolution in analyses of communities is commonly observed (e.g., Mueller et al 2013; Szabó et al. 2018; Sgarbi et al. 2020) and related to the larger amount of ecological information provided by common species compared to rare species (Blanchet et al. 2016). Abundance data improve the detection of more subtle differences among communities, whereas incidence data are better in detecting large differences in species composition (Melo 2005, Blanchet et al. 2016), as may be the case in studies performed at larger spatial or environmental extents than ours (Wilson 2012). Thus, the choice between using incidence or abundance data should be a compromise between the effort needed to obtain abundance information, the spatial extent of the sampling, and the detail of ecological information needed for a specific purpose.

We recognise that the main caveat of our study is that our analyses were based on a single sampling in 25 streams. In this context, we cannot predict whether the surrogates would represent the variation in diatom species composition and the ecological responses of species at different seasons. Moreover, the snap-shot sampling may partly explain the high percentage of unexplained variation in the composition of diatom communities. This high percentage of unexplained variation is common in metacommunity studies with diatoms (e.g., Soininen et al. 2016; Szabó et al. 2018), and may be related to the stochasticity of community

organization or to processes involving unmeasured environmental variables (e.g., Szabó et al. 2018), such as silica content, heavy metals and pesticides or natural disturbance regimes resulting in uneven spate-producing rainfall. In this sense, to improve the knowledge of the validity of using surrogates for biomonitoring purposes in streams from the Pampa region, we suggest that a necessary further step is to test the surrogacy approach over time and, most importantly, over a larger spatial extent.

In conclusion, there is a high congruence between diatom communities at the levels of species and genus and between species and biovolume classes when abundance data are used. We also found that all datasets showed similar responses in relation to environmental factors and that the surrogates were not related to spatial factors, a desired characteristic for bioindicator candidates. We suggest that genus level is a good surrogate candidate to provide information about the species-environment relationships, whereas biovolume classes may be useful to detect changes in diatom communities. However, it is important to highlight that the use of such surrogates may affect our ability to detect species losses or more subtle changes in the composition of communities due to anthropogenic impacts, and should thus be used for specific purposes. Moreover, the use of biovolume classes may prevent the detection of disturbances based on the environmental tolerances of species. Finally, our results contribute to a deeper knowledge of surrogacy in freshwater ecosystems from the Brazilian Pampa biome and its importance to simplify aquatic biomonitoring and ecological assessments when using diatoms as bioindicators, especially in relation to taxonomic difficulties at the species level. Thus, the choice of surrogates to the detriment of species-level information may be an alternative to be evaluated for reducing the time and effort required for biomonitoring and ecological assessment studies.

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Author contributions Conceived and designed the study: APTC and FS. Collected the data: APTC. Analyzed and interpreted the data: APTC and FS. Drafted and revised the manuscript: APTC and FS.

References

- ALGARTE VM, RODRIGUES L, LANDEIRO VL, SIQUEIRA T & BINI LM. 2014. Variance partitioning of deconstructed periphyton communities: does the use of biological traits matter? *Hydrobiologia* 722(1): 279–790.
- ALLEN S, GRIMSHA WM, PARKINSON JA & Quarmby C. 1974. Chemical analysis of ecological materials. London: Blackwell Scientific Publishers, 565 p.
- ALVARES CA, STAPE JL, SENTELHAS PC, GONÇALVES JDM & SPAVOREK G. 2013. Köppen's climate classification map for Brazil. *Meteorol Z* 22(6): 711–726.
- BAEZA S, VÉLEZ-MARTIN E, DE ABELLEYRA D, BANCHERO S, GALLEGO F, SCHIRMBECK J, VERON S, VALLEJOS M, WEBER E, OYARZABAL M, BARBIERI A, PETEK M, GUERRA LARA M, SARRAILHÉ SS, BALDI G, BAGNATO C, BRUZZONE L, RAMOS S & HASENACK H. 2022. Two decades of land cover mapping in the Río de la Plata grassland regions: The MapBiomass Pampa initiative. *Remote Sens Appl: Soc Environ* 28: 100835.
- BAUMGARTEN MGZ, ROCHA JMB & NIENCHESKI LFH. 1996. Manual de análises em oceanografia química. Rio Grande: Editora da FURG, 132 p.
- B-BERÉS V, TÖRÖK P, KÓKAI E, KRASZNAI T, TÓTHMÉRÉSZ B & BÁCSI I. 2014. Ecological diatom guilds are useful but not sensitive enough as indicators of extremely changing water regimes. *Hydrobiologia* 738: 191–204.
- BEVILACQUA S, TERLIZZI A, MISTRI M & MUNARI C. 2015. New frameworks for species surrogacy in monitoring highly variable coastal ecosystems: Applying the BestAgg approach to Mediterranean coastal lagoons. *Ecol Indic* 52: 207–2018.
- BLANCHET FG, LEGENDRE P & BORCARD D. 2008. Forward selection of explanatory variables. *Ecography* 89(9): 2623–2632.

- BLANCHET FG, LEGENDRE P & He F. 2016. A new cost-effective approach to survey ecological communities. *Oikos* 125: 975–987.
- BORCARD D & LEGENDRE P. 2002. All-scale spatial analysis of ecological data by means of principal coordinates of neighbour matrices. *Ecol Modell* 153(1–2): 51–68.
- CARNEIRO FM, BINI LM & RODRIGUES LC. 2010. Influence of taxonomic and numerical resolution on the analysis of temporal changes in phytoplankton communities. *Ecol Indic* 10(2): 249–255.
- CARPENTER SR, CARACO NF, CORRELL DL, HOWARTH RW, SHARPLEY AN & SMITH VH. 1998. Nonpoint pollution of surface waters with phosphorus and nitrogen. *Ecol Appl* 8(3): 559–568.
- ÇELEKLI A, KAYHAN S, LEKESIZ O, TOUDJANI AA & ÇETIN T. 2019. Limno-ecological assessment of Aras River surface waters in Turkey: applications of diatom indices. *Environ Sci Pollut Res* 26: 8028–8038.
- COSTA APT & SCHNECK F. 2022. Diatoms as indicators in running waters: trends of studies on biological assessment and monitoring. *Environ Monit Assess* 194(10): 695.
- DE OLIVEIRA SS, ORTEGA JCG, RIBAS LGS, LOPES VG & BINI LM. 2020. Higher taxa are sufficient to represent biodiversity patterns. *Ecol Indic* 111: 105994.
- DRAY S, BLANCHET G, BORCARD D, GUENARD G, JOMBART T, LAROCQUE G, LEGENDRE P, MADI N & WAGNER HH. 2016. *adespatial*: Multivariate Multiscale Spatial Analysis. R Package version 0.0-7. [available on internet at [https:// CRAN.R-project.org/package=adespatial](https://CRAN.R-project.org/package=adespatial)]
- FOX J & WEISBERG S. 2011. *An R Companion to Applied Regression*. Sage, Thousand Oaks.
- GALLEGO I, DAVIDSON TA, JEPPESEN E, PÉREZ-MARTÍNEZ C, SÁNCHEZ-CASTILLO P, JUAN M, FUENTES-RODRÍGUEZ F, LEÓN D, PEÑALVER P, TOJA J & CASAS JJ. 2012. Taxonomic or ecological approaches? Searching for phytoplankton surrogates in the determination of richness and assemblage composition in ponds. *Ecol Indic* 18: 575–585.

- GODOY BS, FARIA APJ, JÜEN L, SARA L & OLIVEIRA LG. 2019. Taxonomic sufficiency and effects of environmental and spatial drives on aquatic insect community. *Ecol Indic* 107: 105624.
- HORTAL J, BELLO F, DINIZ-FILHO JAF, LEWINSOHN TM, LOBO JM & LADLE RJ. 2015. Seven shortfalls beset large-scale knowledge of biodiversity. *Annu Rev Ecol Evol Syst* 46(1): 523–549.
- HUTTUNEN KL, MUOTKA T, KARJALAINEN SM, LAAMANEN T & AROVIITA J. 2020. Excess of nitrogen reduces temporal variability of streams diatom assemblages. *Sci Total Environ* 713: 136630.
- JACKSON DA. 1993. Stopping rules in a principal component analysis: a comparison of heuristic and statistical approaches. *Ecology* 74(8): 2204–2214.
- JACKSON DA. 1995. PROTEST: a PROcrustean randomization TEST of community environment concordance. *Ecoscience* 2(3): 397–33.
- JAMONEAU A, PASSY SI, SOININEN J, LÉBOUCHER T & TISON-ROSEBERY J. 2018. Beta diversity of diatom species and ecological guilds: Response to environmental and spatial mechanisms along the stream watercourse. *Freshw Biol* 63(1): 62–73.
- KUMAR A & BERA S. 2020. Revisiting nitrogen utilization in algae: a review on the process of regulation and assimilation. *Bioresour Technol Rep* 12: 100584.
- LANDEIRO VL, BINI LM, COSTA FRC, FRANKLIN E, NOGUEIRA A, SOUZA JLP, MORAES J & MAGNUSSON WE. 2012. How far can go in simplifying biomonitoring assessment? An integrated analysis of taxonomic surrogacy, taxonomic sufficiency and numerical resolution in a megadiverse region. *Ecol Indic* 23: 366–373.
- LEGENDRE P & LEGENDRE L. 1998. *Numerical Ecology*. Amsterdam: Elsevier, 853 p.
- LEGENDRE P & GALLAGHER ED. 2001. Ecologically meaningful transformations for ordination of species data. *Oecologia* 129(2): 271–280.
- MARGULES CR & PRESSEY RL. 2000. Systematic conservation planning. *Nature* 405(6783): 243–253.

- MARRONE F, FONTANETO D & NASSELI-FLORES L. 2023. Cryptic diversity, niche displacement and our poor understanding of taxonomy and ecology of aquatic microorganisms. *Hydrobiologia* 850(6): 1221–1236.
- MELO AS. 2005. Effects of taxonomic and numeric resolution on the ability to detect ecological patterns at a local scale using stream macroinvertebrates. *Arch Hydrobiol* 164: 309–323.
- METZELTIN D, LANGE-BERTALOT H & GARCÍA-RODRÍGUEZ F. 2005. Diatoms of Uruguay – taxonomy, biogeography and diversity. Königstein: A.R.G. Gantner Verlag, 736 p.
- METZELTIN D & GARCÍA-RODRÍGUEZ F. 2012. Las diatomeas uruguayas. Montevideo: DIRAC, 208 p.
- MUELLER M, PANDER J & GEIST J. 2013. Taxonomic sufficiency in freshwater ecosystems: effects of taxonomic resolution, functional traits, and data transformation. *Aquat Sci* 32(3): 762–778.
- OKSANEN J, BLANCHET FG, KINDT R, LEGENDRE P, MINCHIN PR, O’HARA RB, SIMPSOM GL, SOLYMOS P, STEVENS MHH & WAGNER H. 2017. vegan: Community Ecology Package. R Package version 2.4-3 [available on internet at <http://CRAN.R-project.org/package=vegan>]
- OLIVEIRA TE, FREITAS DS, GIANEZINI M, RUVIARO CF, ZAGO D, MÉRCIO TZ, DIAS EA, LAMPERT VN & BARCELLOS JOJ. 2017. Agricultural land use change in the Brazilian Pampa biome: The reduction of natural grasslands. *Land Use Policy* 63: 394–400.
- OVERBECK GE, MULLER SC, FIDELIS A, PFADENHAUER J, PILLAR VD, BLANCO CC, BOLDRINI II, BOTH R & FORNECK ED. 2007. Brazil’s neglected biome: the southern Brazilian Campos. *Plant Ecol Evol Syst* 9(2): 101–116.
- PACHECO JP, CALVO C, AZNAREZ C, BARRIOS M, MEERHOFF M, JEPPESEN E, PEDERSEN AB. 2022. Periphyton biomass and life-form responses to a gradient of discharge in contrasting light and nutrient scenarios in experimental lowland streams. *Sci Total Environ* 806: 150505.

- PANDEY LK, SHARMA YC, PARK J, CHOI S, LEE H, LYU J & HAN T. 2018a. Evaluating features of periphytic diatom communities as biomonitoring tools in fresh, brackish and marine waters. *Aquat Toxicol* 194: 67–77.
- PANDEY LK, LAVOIE I, MORIN S, PARK J, LYU S, LEE H & HAN T. 2018b. River water quality assessment based on a multi-descriptor approach including chemistry, diatom assemblage structure, and non-taxonomical diatom metrics. *Ecol Indic* 84: 140–151.
- PASSY SI. 2007. Diatom ecological guilds display distinct and predictable behavior along nutrient and disturbance gradients in running waters. *Aquat Bot* 86(2): 171–178.
- PERES-NETO PR & JACKSON DA. 2001. How well do multivariate data sets match? The advantages of a Procrustean superimposition approach over the Mantel test. *Oecologia* 129: 169–178.
- PERES-NETO PR, LEGENDRE P, DRAY S & BORCARD D. 2006. Variation partitioning of species data matrices: estimation and comparison of fractions. *Ecology* 87(10): 2614–2625.
- PETER KH & SOMMER U. 2014. Interactive effect of warming, nitrogen and phosphorus limitation on phytoplankton cell size. *Ecol Evol* 5(5): 1011–1024.
- PETSCH DK. 2016. Causes and consequences of biotic homogenization in freshwater ecosystems. *Int Rev Hydrobiol* 101(3–4): 113–122.
- R CORE TEAM. 2020. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna. <http://www.R-project.org/>
- RIATO L, HILL RA, HERLIHY AT, PECK DV, KAUFMANN PR, STODDARD JL & PAULSEN SG. 2022. Genus-level, trait-based multimetric diatom indices for assessing the ecological condition rivers and streams across the conterminous United States. *Ecol Indic* 141: 109131.
- RIBEIRO L, BROTAS V, HERNÁNDEZ-FARIÑAS T, JESUS B & BARRILÉ L. 2020. Assessing alternative microscopy-based approaches to species abundance description of intertidal diatom communities. *Front Mar Sci* 7: 36.
- RIMET F & BOUCHEZ A. 2012a. Biomonitoring river diatoms: implications of taxonomic resolution. *Ecol Indic* 15(1): 92–99.

- RIMET F & BOUCHEZ A. 2012b. Life form, cell-sizes and ecological guilds of diatoms in European rivers. *Knowl Manag Aquat Ecosyst* 406: 1.
- SCHINDLER DW. 2006. Recent advances in the understanding and management of eutrophication. *Limnol Oceanogr* 51(1part2): 356–363.
- SCHNECK F, BINI LM, MELO AS, PETSCH DK, SAITO VS, WENGRAT S & SIQUEIRA T. 2022. Catchment scale deforestation increases the uniqueness of subtropical stream communities. *Oecologia* 199: 671–683.
- SGARBI LF, BINI LM, HEINO J, JYRKANKALLIO-MIKKOLA J, LANDEIRO VL, SANTOS EP, SCHNECK F, SIQUEIRA T, SOININEN J, TOLONEN KT & MELO AS. 2020. Sampling effort and information quality provided by rare and common species in estimating assemblage structure. *Ecol Indic* 110: 105937.
- SIQUEIRA T, BINI LM, ROQUE FO, COUCEIRO FRM, TRIVINHO-STRIXINO S & COTTENIE K. 2012a. Common and rare species respond to similar niche processes in macroinvertebrate metacommunities. *Ecography* 35(2): 183–192.
- SIQUEIRA T, BINI LM, ROQUE FO & COTTENIE K. 2012b. A metacommunity framework for enhancing the effectiveness of biological monitoring strategies. *PloS One* 7: e43626.
- SIQUEIRA T, LACERDA CGLT & SAITO VS. 2015. How does landscape modification induce biological homogenization in tropical stream communities? *Biotropica* 47(4): 509–516.
- SOININEN J, JAMONEAU A, ROSEBERY J & PASSY SI. 2016. Global patterns and drivers of species and trait composition in diatoms. *Global Ecol Biogeogr* 25: 940-950.
- SORIANO A. 1991. Rio de Plata Grasslands. In: Coupland RT. (Ed), *Ecosystems of the World: Natural Grasslands*, New York: Elsevier, p. 367–408.
- STEVENSON RJ, PAN Y & VAN DAM H. 2010. Assessing environmental conditions in rivers and streams with diatoms. In: SMOL J & STOERMER E. (Eds), *The Diatoms: Applications for the Environmental and Earth Sciences*, Cambridge: Cambridge University Press, pp. 57–85.

- SZABÓ B, LENGYEL E, PADISÁK J & STENGER-KOVÁCS C. 2018. Benthic diatom metacommunity across small freshwater lakes: driving mechanisms, β -diversity and ecological uniqueness. *Hydrobiologia* 828: 183–198.
- TAN X, XIA X, ZHAO Q & ZHANG Q. 2014. Temporal variations of benthic diatom community and its main influencing factors in a subtropical river, China. *Environ Sci Pollut Res* 21: 434–444.
- TRINDADE CRT, LANDEIRO VL & SCHNECK F. 2018. Macrophyte functional groups elucidate the relative role of environmental and spatial factors on species richness and assemblage structure. *Hydrobiologia* 823: 217–230.
- VALDERRAMA JC. 1981. The simultaneous analysis of total nitrogen and total phosphorus in natural waters. *Mar Chem* 10(2): 109–112.
- VIEIRA MC, ORTEGA JCG, VIEIRA LCG, VELHO LFM & BINI LM (2022) Evidence that dams promote biotic differentiation of zooplankton communities in two Brazilian reservoirs. *Hydrobiologia* 849(3): 697–709.
- VIRTANEN L & SOININEN J. 2012. The roles of environment and space in shaping stream diatom communities. *Eur J Phycol* 47(2): 160–168.
- WAITE IR, PAN I & EDWARDS PM. 2020. Assessment of multi-stressors on compositional turnover of diatom, invertebrate and fish assemblages along an urban gradient in Pacific Northwest streams (USA). *Ecol Indic* 112: 106047.
- WATSON JEM, JONES KR, FULLER RA, DI MARCO M, SEGAN DB, BUTCHART SHM, ALLAN JR, MCDONALD-MADDEN E & VENTER O. 2016. Persistent disparities between recent rates of habitat conversion and protection and implications for future global conservation targets. *Conserv Lett* 9(6): 413–421.
- WICKHAM H. 2016. *Ggplot2: Elegant graphics for data analysis*. Houston: Springer.
- WILSON JB. 2012. Species presence/absence sometimes represents a plant community as well as species abundances do, or better. *J Veg Sci* 23: 1013–1023.

WOOD RJ, MITROVIC SM, LIM RP, WARNE MSJ, DUNLOP J & KEFFORD BJ. 2019. Benthic diatoms as indicators of herbicide toxicity in rivers – A new SPEcies at Risk (SPEARherbicides) index. *Ecol Indic* 99: 203–213.

WUNSAM S, CATTANEO A & BOURASSA N. 2022. Comparing diatom species, genera and size in biomonitoring: a case study from streams in the Laurentians (Québec, Canada). *Freshw Biol* 47(2): 325–340.

Table SI List of diatom taxa found in 25 streams on Brazilian Pampa, and the streams where each species occurred. Stream code according to Figure 1 presented in the main text.

Taxa	Stream code
<i>Achanthes coarctata</i> (Brébisson ex Smith) Grunow	20
<i>Achnanthes exigua</i> var. <i>constricta</i> (Grunow) Hust	4, 16, 17, 20, 25
<i>Achnanthes inflata</i> (Kützing) Grunow	1, 3, 12, 16,
<i>Achnantheidium minutissimum</i> (Kützing)	4, 5, 6, 7, 8, 9, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25
<i>Adlafia</i> sp. 1	25
<i>Adlafia</i> sp. 2	3, 11, 12
<i>Amphipleura lindheimeri</i> (Grunow)	1, 3, 4, 5, 9, 10, 16, 17, 19, 20, 24, 25
<i>Amphora</i> cf. <i>pediculus</i> (Kützing) Grunow	9, 16, 17
<i>Amphora veneta</i> (Kützing) Simonsen	24
<i>Aulacoseira ambigua</i> (Grunow) Simonsen	13, 14, 17
<i>Aulacoseira italica</i> (Ehrenberg) Simonsen	2, 4, 9, 12, 14, 15, 16
<i>Brachysira brebissonii</i> R. Ross	7, 11, 15
<i>Brachysira microcephala</i> (Grunow) Compère	11, 17, 22, 23, 24
<i>Brachysira</i> sp. 1	6, 7, 8
<i>Caloneis hyalina</i> Hustedt	6, 7, 12, 14, 17, 19, 22
<i>Caloneis</i> sp. 1	6, 7, 9
<i>Caloneis</i> sp. 2	21
<i>Capartogramma crucicola</i> (Grunow) R. Ross	2, 3, 4, 5, 6, 8, 12, 15, 16
<i>Cocconeis fluviatilis</i> J. H. Wallace	2, 4, 5, 9, 10, 12, 15, 16, 17, 21, 25
<i>Cocconeis placentula</i> Ehrenberg	1, 2, 3, 4, 5, 6, 9, 10, 12, 16, 18, 19, 23, 25
<i>Cocconeis placentula</i> var. <i>euglypta</i> Ehrenberg (Cleve)	1, 2, 3, 4, 5, 6, 9, 10, 12, 15, 16, 19, 22, 23, 24
<i>Craticula ambigua</i> (Ehrenberg) Mann	2, 3, 4, 7, 8, 9, 12, 13, 16, 21, 24
<i>Craticula cuspidata</i> (Kützing) Mann	17, 20, 23, 24, 25
<i>Craticula</i> cf. <i>pampeana</i> (Frenguelli) Lange-Bertalot	1
<i>Craticula subminuscule</i> (Manguin) Wetzel & Ector	3, 7, 12, 23, 25
<i>Craticula</i> sp. 1	17
<i>Craticula</i> sp. 2	13, 14, 15, 20
<i>Cyclotella cryptica</i> Reimann, Lewin & Guillard	1, 2, 4, 9, 16
<i>Cyclotella meneghiniana</i> Kützing	2, 3, 4, 5, 6, 9, 10, 12, 15, 16, 17, 23, 24, 25
<i>Cymbella</i> cf. <i>loescherae</i> Garcia & Dutra	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25
<i>Cymbella tumida</i> (Brébisson) Van Heurck	1, 3, 9, 10, 16, 17, 21, 22, 23, 24, 25
<i>Cymbella</i> sp. 1	3, 5, 9, 10, 12, 16, 17, 21, 22, 24
<i>Cymbella</i> sp. 2	7, 8, 11, 17, 24
<i>Cymbella</i> sp. 3	16, 23
<i>Cymbopleura acuta</i> (Schmidt) Krammer var. <i>acuta</i>	8
<i>Cymbopleura naviculiformis</i>	3, 4, 5, 7, 8, 9, 11, 14, 16, 17, 18, 20, 22, 23, 24
<i>Diadomesis confervaceae</i> Kützing	1, 2, 3, 4, 7, 9, 10, 12, 15,
<i>Diploneis</i> cf. <i>subovalis</i> Cleve	1, 2, 4, 5, 6, 7, 8, 9, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25
<i>Diploneis</i> sp. 1	12, 18
<i>Diploneis</i> sp. 2	17
<i>Diploneis</i> sp. 3	17
<i>Diploneis</i> sp. 4	21, 22, 25
<i>Diploneis</i> sp. 5	19

<i>Discostella stelligera</i> (Cleve & Grunow) Houk & Klee	3, 4, 16
<i>Encyonema</i> cf. <i>minutum</i> (Hilse) Mann	1, 2, 3, 4, 5, 8, 9, 10, 13, 15, 16, 17, 18, 19, 20, 21, 24, 25
<i>Eucyonema neogracile</i> Krammer	11
<i>Encyonema</i> sp. 1	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25
<i>Encyonema</i> sp. 2	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25
<i>Encyonema</i> sp. 3	15
<i>Encyonema</i> sp. 4	18, 25
<i>Encyonema</i> sp. 5	23
<i>Encyonopsis subminuta</i> Krammer & Reichardt	11, 22
<i>Encyonopsis</i> sp1	1, 3, 4, 6, 7, 8, 23
<i>Encyonopsis</i> sp2	7, 22
<i>Epithemia adnata</i> (Kützing) Brébisson	1, 5, 7
<i>Epithemia gibba</i> (Ehrenberg) Kützing	1, 3, 5, 10,
<i>Eunotia bidens</i> Ehrenberg	1, 2, 6, 14, 15, 22, 23
<i>Eunotia bilunaris</i> (Ehrenberg) Schaarschmidt	4, 6, 7, 8, 9, 12, 14, 18, 19, 20, 23
<i>Eunotia camelus</i> Ehrenberg	4, 5, 6, 7, 8, 9, 11, 15, 18, 19, 20, 21, 23
<i>Eunotia didyma</i> Grunow ex Zimmermann	3, 4, 6, 9, 12, 14, 19
<i>Eunotia indosinica</i> Glushchenko & Kulikovskiy	18, 19, 20, 21
<i>Eunotia juettnerae</i> Lange-Bertalot	11, 19
<i>Eunotia novaecaledonica</i> Gerd Moser	19, 22
<i>Eunotia paludosa</i> Grunow	7
<i>Eunotia</i> cf. <i>pirila</i> Carter & Flower	1, 2, 4, 5, 6, 7, 8, 9, 11, 12, 14, 15, 16, 18, 19, 20, 21, 22, 24, 25
<i>Eunotia rabenhorstii</i> var. <i>tridon</i> Cleve & Grunow	1, 5, 7, 10, 14, 19, 20, 22
<i>Eunotia</i> cf. <i>subarcuatoidea</i> Alles, Nörpel & Lange-Bertalot	4, 5, 7, 8, 11, 12, 14, 15, 17, 19, 20,
<i>Eunotia yberai</i> Frenguelli	1, 2, 3, 6, 7, 9, 10, 12, 14, 18, 19, 20, 22, 25
<i>Eunotia</i> sp. 1	2
<i>Eunotia</i> sp. 2	4, 6, 17, 22
<i>Eunotia</i> sp. 3	18
<i>Fallacia</i> sp. 1	4, 9
<i>Fallacia</i> sp. 2	4, 7, 8, 14, 18, 24
<i>Fallacia</i> sp3	16
<i>Fragilaria</i> sp. 1	1, 3, 4, 5, 8, 13, 16, 18, 19, 22, 25
<i>Fragilaria</i> sp. 2	11, 12, 13, 15, 17, 20
<i>Fragilaria</i> sp. 3	18, 21, 22, 23
<i>Fragilariforma</i> sp. 1	7
<i>Frustulia crassinervia</i> (Brébisson ex Smith) Lange-Bertalot & Krammer	2, 3, 4, 5, 6, 7, 8, 9, 11, 12, 14, 15, 17, 18, 19, 20, 21, 22, 23, 24
<i>Frustulia</i> cf. <i>vulgaris</i> (Thwaites) De Toni	3, 7, 16, 17, 18, 19
<i>Frustulia</i> sp. 1	11, 18, 19, 21
<i>Geissleria aikenensis</i> (Patrick) Torgan & Oliveira	4, 5, 13, 15, 16, 24, 25
<i>Geissleria</i> sp. 1	3, 4, 5, 7, 8, 11, 12, 14, 15, 18, 24, 25
<i>Gomphonema acuminatum</i> Ehrenberg	1, 3, 9, 20
<i>Gomphonema affinis</i> Metzeltin, Lange-Bertalot & Garcia-Rodriguez	1, 2, 3
<i>Gomphonema augur</i> Ehrenberg	2, 15
<i>Gomphonema brasiliense</i> Grunow	7, 13, 17, 21, 23
<i>Gomphonema capitatum</i> Ehrenberg	1, 16
<i>Gomphonema gracile</i> Ehrenberg	9, 10, 11, 12, 13, 14, 17, 18, 19

<i>Gomphonema</i> _cf. <i>kobayasii</i> Kociolek & Kingston	1, 16, 19, 24, 25
<i>Gomphonema lagenula</i> Kützing	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 17, 18, 19, 20, 21, 22, 24, 25
<i>Gomphonema</i> cf. <i>lujanense</i> Reichardt & Maidana	15
<i>Gomphonema laticollum</i> Reichardt	15
<i>Gomphonema parvulum</i> (Kützing) Kützing	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25
<i>Gomphonema pumilum</i> (Grunow) Reichardt & Lange-Bertalot	1, 4, 5, 7, 8, 9, 11, 18, 20, 21, 22, 23, 24
<i>Gomphonema turris</i> Ehrenberg	1, 5, 6, 10, 15
<i>Gomphonema</i> sp. 1	1, 6, 7, 8, 9, 11, 13, 16, 17, 18, 19, 22, 23, 25
<i>Gomphonema</i> sp. 2	1, 7, 11, 15, 16, 18, 19, 20, 23
<i>Gomphonema</i> sp. 3	1, 9, 10, 15,
<i>Gomphonema</i> sp. 4	2, 9, 18
<i>Gomphonema</i> sp. 5	10
<i>Gomphonema</i> sp. 6	15, 18, 20
<i>Gomphonema</i> sp. 7	15
<i>Gomphonema</i> sp. 8	17, 24
<i>Gomphonema</i> sp. 9	17, 24
<i>Gomphonema</i> sp. 10	18
<i>Gomphonema</i> sp. 11	18
<i>Gomphonema</i> sp. 12	22, 23
<i>Gomphonema</i> sp. 13	19
<i>Gomphonema</i> sp. 14	20, 22
<i>Gomphonema</i> sp. 15	25
<i>Gomphonema</i> sp. 16	25
<i>Gyrosigma acuminatum</i> (Kützing) Rabenhorst	1, 2, 3, 4, 9, 10, 12, 15, 17, 18, 20, 22
<i>Gyrosigma scalproides</i> (Rebenhorst) Cleve	3, 16, 19
<i>Gyrosigma nodiferum</i> (Grunow) Reimer	3, 5, 9, 10, 16, 17, 19, 20, 22
<i>Hantzschia amphioxys</i> (Ehrenberg) Grunow	2, 3, 4, 5, 6, 8, 9, 12, 13, 14, 15, 16, 17, 19, 20, 21, 22, 23, 24, 25
<i>Hantzschia amphioxys</i> var. <i>vivax</i> (Grunow)	18
<i>Hantzschia</i> sp. 1	22, 23
<i>Hippodonta capitata</i> (Ehrenberg) Lange-Bertalot, Metzeltin & Witkowski	3, 4, 5, 12, 14, 15, 16
<i>Humidophila contenta</i> (Grunow) Lowe	4, 5, 7, 9, 12, 14, 16, 17, 18, 20, 21, 22, 25
<i>Iconella</i> cf. <i>alata</i> (Hustedt) Kapustin & Kulikovskiy	5
<i>Iconella</i> cf. <i>engleri</i> (Müller) Cocquyt & Jahn	14, 15, 22
<i>Iconella guatemalensis</i> (Ehrenberg) Ruck & Nakov	1, 12, 15, 23, 24
<i>Iconella splendida</i> (Ehrenberg) Ruck & Nakov	16
<i>Iconella tenera</i> (Gregory) Ruck & Nakov	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 14, 15, 16, 19, 20, 21, 22, 23, 24, 25
<i>Iconella tenera</i> f. <i>minor</i> (Gregory) Ruck & Nakov	11, 13, 16, 19, 22, 24, 25
<i>Luticola dapalis</i> (Frenguelli) Mann	4, 16, 25
<i>Luticola goeppertiana</i> (Bleisch) Mann ex Rarick, Wu, Lee & Edlund	1, 2, 4, 5, 10, 12, 17, 19, 21, 25
<i>Luticola</i> cf. <i>saxophila</i> (Bock ex Hustedt) Mann	20, 21
<i>Luticola</i> sp. 1	1, 2, 3, 6, 15, 24, 25
<i>Luticola</i> sp. 2	3, 6, 7
<i>Luticola</i> sp. 3	4, 5, 6, 7, 12, 14, 16, 17, 18, 19, 20, 21, 22
<i>Luticola</i> sp. 4	23
<i>Luticola</i> sp. 5	1, 4, 5, 9, 10, 16, 18, 19, 20
<i>Luticola</i> sp. 6	1, 2, 9

<i>Melosira varians</i> Agardh	1, 2, 3, 4, 5, 6, 12, 15, 25
<i>Navicula angusta</i> Grunow	11
<i>Navicula cryptocephala</i> Kützing	1, 2, 3, 4, 5, 6, 7, 8, 9, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25
<i>Navicula</i> cf. <i>cryptotenella</i> Lange-Bertalot	8, 11, 13, 14
<i>Navicula neomundana</i> (Lange-Bertalot & Rumrich) Lange-Bertalot, Jarlman & Vijver	5, 7, 21
<i>Navicula</i> cf. <i>radiosa</i> Kützing	1, 2, 3, 4, 6, 7, 9, 11, 12, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 25
<i>Navicula</i> cf. <i>rostellata</i> Kützing	2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 13, 14, 15, 16, 17, 18, 19, 21, 22, 23, 24, 25
<i>Navicula</i> cf. <i>schroeteri</i> Meister	1, 2, 3, 4, 5, 9, 10, 15, 16, 17, 18, 21, 25
<i>Navicula</i> sp. 1	1, 2, 3, 6, 7, 11, 19, 24, 25
<i>Navicula</i> sp. 2	3, 4, 6, 21, 22, 23, 24, 25
<i>Navicula</i> sp. 3	2, 3, 10, 14, 16
<i>Navicula</i> sp. 4	11
<i>Navicula</i> sp. 5	17, 18
<i>Navicula</i> sp. 6	17, 25
<i>Navicula</i> sp. 7	22
<i>Neidium</i> cf. <i>affine</i> (Ehrenberg) Pfitzer	2, 3, 4, 5, 7, 8, 9, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 44, 25
<i>Neidium affine</i> var. <i>longiceps</i> (Gregory) Cleve	21
<i>Neidium</i> cf. <i>amphigomphus</i> (Ehrenberg) Pfitzer	19, 21, 24
<i>Neidium</i> sp. 1	3, 4, 5, 6, 7, 8, 13, 14, 17, 18, 19, 22, 23, 24, 25
<i>Neidium</i> sp. 2	6, 8, 14, 18, 21
<i>Neidium</i> sp. 3	14, 17, 21
<i>Neidium</i> sp. 4	24
<i>Nitzschia</i> cf. <i>acicularis</i> (Kützing) Smith	3, 4, 13, 14, 16, 19, 21
<i>Nitzschia</i> cf. <i>amphibia</i> (Grunow)	19, 22, 23
<i>Nitzschia clausii</i> Hantzsch	3, 4, 5, 6, 7, 9, 12, 14, 15, 16, 18, 19
<i>Nitzschia dissipata</i> (Kützing) Rabenhorst	2, 3, 8, 9, 10, 16, 19, 21,
<i>Nitzschia</i> cf. <i>filiformis</i> (Smith) Van Heurck	2, 3, 6, 9, 14, 15, 16, 17, 21, 24
<i>Nitzschia</i> cf. <i>frustulum</i> (Kützing) Grunow	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25
<i>Nitzschia</i> cf. <i>gracilis</i> Hantzsch	1, 2, 3, 4, 5, 7, 9, 10, 12, 14, 15, 16, 17, 19, 21, 22, 24, 25
<i>Nitzschia inconspicua</i> (Grunow)	17, 21
<i>Nitzschia palea</i> (Kützing) Smith	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25
<i>Nitzschia terrestris</i> (Petersen) Hustedt	6, 7, 9, 12, 14, 18, 20
<i>Nitzschia</i> sp. 1	1, 6, 9, 10, 12, 15, 16, 17, 19, 21, 22, 23, 24
<i>Nitzschia</i> sp. 2	1, 3, 4, 5, 13, 14, 15, 17, 18, 19, 20, 21, 22, 23
<i>Nitzschia</i> sp. 3	15
<i>Nitzschia</i> sp. 4	1, 3
<i>Nitzschia</i> sp. 5	1, 9
<i>Nitzschia</i> sp. 6	2, 3, 4, 13, 14, 15, 16, 18, 19, 21, 22, 23, 24, 25
<i>Nitzschia</i> sp. 7	3, 13, 14, 15, 17, 18, 19, 21, 23
<i>Nitzschia</i> sp. 8	15, 16, 17, 18, 23
<i>Nitzschia</i> sp. 9	16, 22, 23
<i>Nitzschia</i> sp. 10	16, 19, 25
<i>Nitzschia</i> sp. 11	19, 21
<i>Orthoseira roeseana</i> (Rabenhorst) Pfitzer	1, 6
<i>Pinnularia</i> cf. <i>borealis</i> Ehrenberg	10, 12, 14, 18, 19, 20, 21
<i>Pinnularia meridiana</i> Metzeltin & Krammer	13, 22

<i>Pinnularia microstauron</i> (Ehrenberg) Cleve	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25
<i>Pinnularia</i> cf. <i>viridiformis</i>	1, 2, 6, 9, 13, 14, 17, 20, 21, 24
<i>Pinnularia</i> sp. 1	1, 2, 5, 9, 19,
<i>Pinnularia</i> sp. 2	1, 3, 5, 7, 8, 11, 12, 16, 17, 21, 22
<i>Pinnularia</i> sp. 3	1, 2, 3, 4, 5, 6, 7, 12, 13, 14, 15, 17, 19, 20, 25
<i>Pinnularia</i> sp. 4	2, 6
<i>Pinnularia</i> sp. 5	2, 3, 4, 5, 6, 7, 10, 12, 13, 14, 15, 19, 20, 24
<i>Pinnularia</i> sp. 6	2, 3
<i>Pinnularia</i> sp. 7	3, 6, 9
<i>Pinnularia</i> sp. 8	7, 10, 12, 14
<i>Pinnularia</i> sp. 9	6, 7, 9, 11, 17, 19, 20, 21, 22, 23, 25
<i>Pinnularia</i> sp. 10	8, 9, 15, 16
<i>Pinnularia</i> sp. 11	16
<i>Pinnularia</i> sp. 12	21, 22
<i>Pinnularia</i> sp. 13	25
<i>Pinnularia</i> sp. 14	24
<i>Placoneis clementis</i> (Grunow) Cox	4, 13, 15, 16, 24
<i>Placoneis</i> cf. <i>constans</i>	1, 3, 4, 5, 6, 7, 12, 13, 14, 15, 16, 17, 18, 19, 21, 22, 24, 25
<i>Placoneis rhombelliptica</i> Metzeltin, Lange-Bertalot & García-Rodríguez	5, 14, 19
<i>Placoneis symmetrica</i> (Hustedt) Lange-Bertalot	5, 6, 8, 9, 10, 14
<i>Placoneis uruguayensis</i> Metzeltin, Lange-Bertalot & García-Rodríguez	25
<i>Placoneis</i> cf. <i>zula</i> Kulikovskiy, Lange-Bertalot & Metzeltin	25
<i>Placoneis</i> sp. 1	1, 2, 3, 4, 5, 6, 15, 18, 19, 22
<i>Placoneis</i> sp. 2	1, 3
<i>Placoneis</i> sp. 3	3, 5, 12, 25
<i>Placoneis</i> sp. 4	4, 16
<i>Placoneis</i> sp. 5	24
<i>Planothidium</i> cf. <i>frequentissimum</i> (Lange-Bertalot) Lange-Bertalot	2, 4, 15,
<i>Planothidium</i> cf. <i>lanceolatum</i> (Brébisson ex Kützing) Lange-Bertalot	2, 3, 4, 5, 6, 9, 12, 15, 16,
<i>Planothidium</i> sp. 1	25
<i>Planothidium</i> sp. 2	25
<i>Psammothidium</i> sp. 1	8, 13, 15, 16, 17, 22, 25
<i>Pseudostaurosira</i> sp. 1	1, 2
<i>Rhopalodia</i> cf. <i>brebissonii</i> Krammer	6, 7, 8, 9, 10, 17, 18, 21, 23, 24
<i>Rhopalodia gibberula</i> (Ehrenberg) Müller	17, 18
<i>Rhopalodia</i> sp. 1	3, 5, 9, 10, 18, 23
<i>Sellaphora</i> cf. <i>atomoides</i> (Grunow) Wetzel & Van de Vijver	3, 4, 6, 7, 8, 15, 16, 17, 22, 23, 24, 25
<i>Sellaphora</i> cf. <i>laevisima</i> (Kützing) Mann	2, 3, 4, 5, 6, 7, 8, 9, 12, 13, 14, 15, 16, 17, 19, 21, 22, 24, 25
<i>Sellaphora multiconfusa</i> (Lange-Bertalot) Wetzel, Ector, Van de Vijver, Compère & Mann	15, 16, 17, 24
<i>Sellaphora pupula</i> (Kützing) Mereschkovsky	1, 2, 4, 6, 7, 8, 9, 12, 13, 14, 15, 16, 17, 19, 20, 21, 22, 23, 24, 25
<i>Sellaphora</i> cf. <i>triundulata</i> Metzeltin & Lange-Bertalot	2
<i>Sellaphora ventraloconfusa</i> (Lange-Bertalot) Metzeltin & Lange-Bertalot	14
<i>Sellaphora</i> sp. 1	1, 3, 4, 5, 6, 7
<i>Sellaphora</i> sp. 2	2, 3, 4, 5, 12, 14, 15, 17, 19

<i>Stauroneis</i> cf. <i>anceps</i> Ehrenberg	17, 19, 21
<i>Stauroneis</i> cf. <i>gracilis</i> Ehrenberg	20
<i>Stauroneis kriegeri</i> Patrick	14, 15, 16, 17, 19, 25
<i>Stauroneis</i> sp. 1	2, 6, 7, 8, 12, 14
<i>Stauroneis</i> sp. 2	19, 22, 24
<i>Stauroneis</i> sp. 3	24
<i>Surirella angusta</i> Kützing	2, 5, 6, 9, 12, 14, 24
<i>Surirella</i> aff. <i>engleri</i> f. <i>angustior</i> Müller	5
<i>Surirella minuta</i> var. <i>peduliformis</i> Frenguelli	1, 3, 4, 7, 9, 18, 25
<i>Surirella</i> sp. 1	7, 16
<i>Surirella</i> sp. 2	18, 22, 24, 25
<i>Surirella</i> sp. 3	22, 24, 25
<i>Surirella</i> sp. 4	24
<i>Surirella</i> sp. 5	24, 25
<i>Surirella</i> sp. 6	24, 25
<i>Synedra gouldarii</i> Brébisson, ex Cleve & Grunow	3
<i>Tryblionella coarctata</i> (Grunow) Mann	16, 21
<i>Tryblionella levidensis</i> Smith	1, 3, 5, 9, 10, 12,
<i>Ulnaria acus</i> (Kützing) Aboal	1, 2, 3, 4, 6, 8, 9, 10, 11, 12, 13, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25
<i>Ulnaria ulna</i> (Nitzsch) Compère	1, 2, 3, 5, 6, 10, 12, 15, 16, 17, 18, 19, 20, 21, 22, 24

CAPÍTULO 3

The use of biological indices and species morphology of diatoms for stream environmental assessment

O uso de índices biológicos e da morfologia de diatomáceas na avaliação ambiental de riachos

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Abstract: Aim: Our aim was to verify whether non-taxonomic (morphological) diatom metrics performed as well as taxonomic (diversity and diatom indices) metrics for ecological assessments and biomonitoring in streams in the Brazilian Pampa. **Methods:** We sampled physical, chemical, and biological variables from 15 streams (classified into two groups according to total nitrogen concentrations). We sampled diatoms from stream sediment surface using a syringe. We calculated common diatom indices used for stream ecological assessment, as well as the percentage of deformed valves in the communities, and the average valve width and shape of *Achnantheidium minutissimum*. **Results:** We found no significant differences between the two stream groups based on diversity or diatom indices. Among the morphological metrics, the average valve width of *A. minutissimum* was significantly higher in streams with lower nitrogen concentrations, and its valve shape differed between the two stream groups. **Conclusion:** Our study is one of the first to investigate morphological biomarkers in diatoms for ecological assessments in the Brazilian Pampa biome. However, we highlight the importance of replicating this study involving a larger sample size with lotic environments influenced by different impacts to improve understanding of the use of non-taxonomic metrics for ecological assessments and biomonitoring.

Key words: diversity index, diatom index, teratology, *Achnantheidium minutissimum*

Resumo: Objetivo: Nosso objetivo foi verificar se métricas não taxonômicas (morfológicas) de diatomáceas apresentam o mesmo desempenho que métricas taxonômicas (índices de diversidade e de diatomáceas) para avaliações ecológicas e biomonitoramento em riachos do Pampa brasileiro. **Métodos:** Amostramos variáveis físicas, químicas e biológicas de 15 riachos (classificados em dois grupos de acordo com as concentrações de nitrogênio total). Amostramos diatomáceas da superfície do sedimento utilizando uma seringa. Calculamos os índices de diatomáceas comumente utilizados na avaliação ecológica de riachos, bem como a percentagem de valvas deformadas nas comunidades e a largura e forma médias das valvas de *Achnantheidium minutissimum*. **Resultados:** Não encontramos diferenças significativas entre os dois grupos de riachos com base na diversidade ou nos índices de diatomáceas. Entre as métricas morfológicas, a largura média da valva de *A. minutissimum* foi significativamente maior nos cursos de água com concentrações de nitrogênio mais baixas, e a forma das suas valvas diferiu entre os dois grupos de riachos. **Conclusões:** Nosso estudo é um dos primeiros a investigar biomarcadores morfológicos em diatomáceas para avaliações ecológicas no bioma Pampa brasileiro. No entanto, destacamos a importância de replicar este estudo

envolvendo um tamanho amostral maior e com ambientes lóticos influenciados por diferentes impactos para melhorar a compreensão acerca do uso de métricas não taxonômicas para avaliações ecológicas e biomonitoramento.

Palavras-chave: índice de diversidade, índice de diatomáceas, teratologia, *Achnantheidium minutissimum*

Introduction

All ecosystems are subjected to some extent of human-induced degradation (Dudgeon, 2019; Bologna & Aquino, 2020). Freshwater environments support high biodiversity and provide essential ecosystem services to humans (e.g., Newton et al., 2018). However, these ecosystems have been destroyed by human activities at a rapid pace through impacts such as hydrological changes, water pollution, habitat degradation, eutrophication, and land use (Dudgeon, 2019; He et al., 2019).

Artificial eutrophication is caused by excessive input of nutrients through sewage or agricultural runoff, and has a high potential to negatively affect the health of freshwater ecosystems (Dodds, 2006; Atwell & Bouldin, 2022). It is widely known that an exacerbated increase in nutrients, especially in low-order streams, causes a decrease in species richness of diatom communities (Torres-Franco et al., 2019; Costa et al., 2022), and leads to changes in the structure of these communities (e.g., Soininen et al., 2004). In addition, morphological alterations, such as changes in the valve size of *Achnantheidium minutissimum* (Vilmi et al., 2015) and an increased frequency of deformed valves (Dziengo-Czaja et al., 2008) were evidenced in response to eutrophication.

The detection of such impacts depends on ecological assessments and further biomonitoring of ecosystems (e.g., Friberg et al., 2011). However, due to the accelerated process of environmental degradation, strategies for efficient ecological assessment and biomonitoring need to consider the cost and time necessary to process the sampled biological material in laboratory (Bennett et al., 2017). Studies that seek for such approaches commonly evaluate the use of taxonomic surrogates (Landeiro et al., 2012), reduced taxonomic resolution (Oliveira et al., 2020) or reduced counting effort (Castro et al., 2023). However, the use of non-taxonomic approaches is less evaluated, despite their potential to effectively reduce the cost and time of sample processing (Costa & Schneck, 2022). Among these approaches, we highlight the use of biomarkers such as lipid bodies measurement in diatom

cells or the presence of deformed valves, which have been shown to be efficient for metrics for assessing water quality (Pandey et al., 2018)

Diatoms commonly stand out in biomonitoring and ecological assessment studies due to specific characteristics such as a fast life cycle, wide distribution, and fast responses to environmental changes (Stevenson et al., 2010). Assessment of alpha diversity (e.g., species richness or Shannon and Simpson indices) (e.g., Gokce & Gulbenk, 2019) or variation in community structure, as well as diatom indices (e.g., Pillsbury et al., 2019), are used frequently to evaluate anthropogenic impacts (Costa & Schneck, 2022). However, these metrics tend to be time-consuming and costly due to the need for a specialist in diatom taxonomy to identify all organisms at the species level. On the other hand, alternative metrics like morphological assessments, such as average width valve (Vilmi et al., 2015), percentage of teratological valves (Pandey et al., 2018) or valve shape alterations (Olenici et al., 2017) have been highlighted in the literature as potentially good pollution biomarkers.

Here, we investigated how efficient different taxonomic and morphological metrics are at differentiating streams with high total nitrogen concentrations from streams less impacted by nitrogen input in the Brazilian Pampa. The tested metrics were (i) alpha diversity, specifically species richness and the Shannon index; (ii) three diatom indices, *l'Indice de Polluosensibilité Spécifique* (IPS; Cemagref, 1982), Trophic Diatom Index (TDI; Kelly & Whitton, 1995; Kelly, 1998) and Pampean Diatom Index (IDP; Gómez & Licursi, 2001); (iii) the percentage of deformed frustules; (iv) valve width of *Achnanthes minutissimum*; and (v) valve shape of *A. minutissimum*. We expected that morphological metrics (percentage of deformed frustules, cell size and shape of *A. minutissimum*) would be as efficient as taxonomical metrics (diversity and diatom indices) in detecting environmental differences among the two groups of streams. Specifically, we expected that the percentage of deformed frustules and the valve width of *A. minutissimum* would be higher in streams influenced by higher nitrogen concentration. Moreover, we expected that the valve shape of *A. minutissimum* would differ between the two groups of streams.

Materials and Methods

Study area

We sampled 15 low-order streams in the municipalities of São Gabriel, Rosário do Sul, and Alegrete (Figure 1) in January 2020. The climate of the region is subtropical Cfa according to

Köppen classification (Alvares et al., 2013). Sites are in the Brazilian Pampa biome which is characterized by the predominance of grasslands (*campos*) (Overbeck et al., 2007). The Brazilian Pampa suffers from the accelerated conversion of its natural landscape into agriculture and silviculture (Overbeck et al., 2007; Oliveira et al., 2017).

We classified the streams into two groups based on total nitrogen concentration because it was already shown that N was one of the most important variables in explaining the diatom community structure (Costa & Schneck, *paper accepted*). We used hierarchical cluster analysis with Euclidean distance (Kassambara, 2017) to determine the stream groups according to total nitrogen streams concentration. In this sense, group 1 (N = 7) is represented by the streams with the highest concentrations of total nitrogen and group 2 (N = 8) is represented by the streams with the lowest concentrations of total nitrogen (Figure 1).

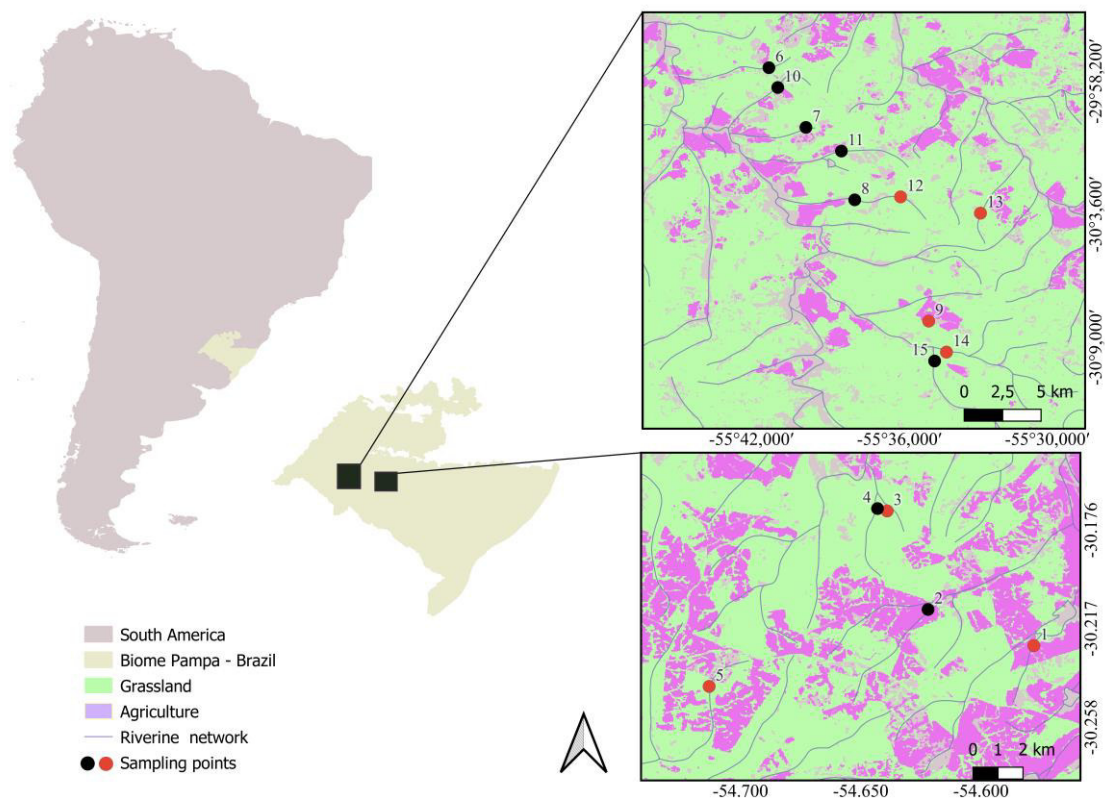


Figure 1. Fifteen streams sampled in the Brazilian Pampa biome. Red points refer to group 1 with higher nitrogen concentrations, and black points refer to group 2 with lower concentrations of nitrogen.

All 15 pampean streams studied here are found with mesotrophic characteristics according to the Trophic State Index of Laparelli (2004) based on total phosphorus

concentration. In relation to total nitrogen concentrations the group 1 ranged from 0.74 to 0.92 mg L⁻¹ and group 2 ranged from 0.44 to 0.63 mg L⁻¹ (Table 1).

Environmental variables

We used a multiparameter probe to measure pH, dissolved oxygen (DO; mg L⁻¹), electrical conductivity (EC; $\mu\text{S cm}^{-1}$), and total dissolved solids (TDS; g L⁻¹) (Table 1). We made 10 measurements for each variable. We used a measure tape to obtain the width and depth at five points in each stream (Table 1). We also measured the flow water at five points using a flowmeter. Moreover, we sampled 500 ml of surface water at each stream for analyses of total nitrogen (TN; Allen et al., 1974), and total phosphorus (TP; Valderrama, 1981; Baumgarten et al., 1996) (Table 1).

Table 1. Mean of environmental variables from 15 streams sampled on Brazilian Pampa. Cond = electrical conductivity; DO = dissolved oxygen; TDS = total dissolved solids; TP = total phosphorus; TN = total nitrogen; Vel = current velocity; Group 1 is represented by the streams with the highest concentrations of total nitrogen and group 2 is represented by the streams with the lowest concentrations of total nitrogen.

Stream	Group	pH	DO (mg L ⁻¹)	Cond ($\mu\text{S cm}^{-1}$)	TDS (g L ⁻¹)	TP ($\mu\text{g L}^{-1}$)	TN (mg L ⁻¹)	Vel (cm s ⁻¹)	Width (m)	Depth (cm)
1	1	7.8	11.1	0.22	0.14	72.6	0.7	<1.0	5.2	49.1
2	2	7.4	5.6	0.10	0.07	52.1	0.6	<1.0	5.9	49.8
3	1	6.9	7.3	0.04	0.02	94.1	0.8	<1.0	2.5	23.9
4	2	7.2	7.9	0.06	0.03	38.8	0.6	<1.0	1.9	25.5
5	1	7.5	7.8	0.03	0.02	134.4	0.9	<1.0	1.2	20.2
6	2	7.3	6.6	0.15	0.09	43.0	0.4	21.0	1.1	17.5
7	2	7.9	7.9	0.07	0.04	47.1	0.6	23.9	3.0	14.6
8	2	7.1	8.0	0.04	0.02	30.6	0.5	66.4	2.2	21.9
9	1	7.5	6.2	0.08	0.06	49.6	0.7	<1.0	1.0	9.5
10	2	7.6	7.6	0.04	0.03	11.4	0.5	<1.0	5.5	5.4
11	2	7.4	7.0	0.06	0.04	25.7	0.6	9.7	4.1	16.8
12	1	8.4	7.8	0.12	0.08	29.0	0.8	<1.0	1.3	9.8
13	1	7.5	6.4	0.11	0.07	27.3	0.8	<1.0	1.9	39.8
14	1	7.5	8.0	0.07	0.05	33.1	0.7	30.5	4.1	7.3
15	2	7.3	6.7	0.07	0.04	96.5	0.6	38.5	4.7	11.1

Diatom sampling

We sampled benthic diatoms from the surface of streambed soft sediment using a syringe and from stones using a brush. After sampling we preserved the material with 4% formalin. In the laboratory, the biological material was oxidized according to Simonsen (1974) adapted by Moreira-Filho & Valente-Moreira (1981). Finally, we prepared permanent slides using Naphrax® (Brunel Microscopes Ltd., Chippenham, UK). For the quantitative analyses (500 valves counted for each stream) we only used the communities from sediment because it was the substrate that occurred in all the 15 streams. The percentage of deformed valves was obtained simultaneously with the quantification of the community. For morphometric analysis (width and shape), we used populations of *A. minutissimum* (represented by 50 specimens from each stream) (Figure 2) from sediment and stones. We chose this species because it occurred abundantly in the streams. For diatoms identification and counting we used a Zeiss optical microscope with a magnification of 1000×. We identified taxa at the lowest possible taxonomic level using specialized bibliography.

Morphometric analyses

Each of the 750 specimens of *Achnantheidium minutissimum* (50 per stream) were photographed using an optical microscope with camera at a magnification of 1000×. For all specimens, we obtained valve width measurements. Next, we identified the images and converted them into.tps files using tpsUtil software version 1.78 (Rohlf, 2013). For each specimen, two landmarks were recorded, and valve morphology was modeled using two configurations of curves, each containing 25 semi-landmarks - totaling 50 semi-landmarks (Figure 2). The landmarks and semi-landmarks were recorded using the tpsDIG version 2.31 (Rohlf, 2015). To determine *A. minutissimum* valve shape through geometric morphometrics we used coordinates from generalized Procrustes analyses (GPA; Rohlf & Slice, 1990). This allows to obtain shape information with the effects of translation, scaling, and rotation minimized. We ran Principal Component Analysis (PCA) to summarize the information about *A. minutissimum* shape and identify the axes of maximal and minimal shape variance among all specimens. These analyses were performed with the symmetrical component.

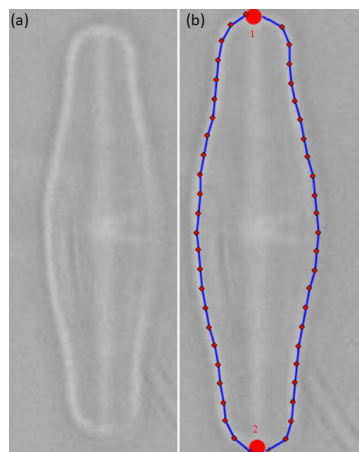


Figure 2. *Achnantheidium minutissimum*; the species of diatom used for morphometric analysis (valve width and shape) (a), and position of landmarks (1 and 2) and 50 semi-landmarks forming two curves along the valve outline (b).

Data analysis

For each stream, we obtained the species richness, the Shannon diversity index, and calculated three diatom indices: the *l'Indice de Polluosensibilité Spécifique* (IPS; Cemagref, 1982), the Trophic Diatom Index (TDI; Kelly & Whitton, 1995; Kelly, 1998), and the Pampean Diatom Index (IDP; Gómez & Licursi, 2001). Non-taxonomical metrics were represented by the percentage of deformed frustules within 500 valves, valve width and shape of *A. minutissimum*.

We used t-tests to evaluate whether species richness, Shannon index, each of the three diatom indices, percentage of deformed frustules and average of *A. minutissimum* valve width would successfully differentiate the two categories of streams. The assumptions of normality and homoscedasticity were satisfied. Finally, we used the first principal component of PCA to run Discriminant Analysis with a cross-validation test to detect differences in *A. minutissimum* valve shape between stream categories.

We used the R environment (R Core Team, 2020) to perform all analyses. For diatom indices we used the *DiaThor* package (Gelís et al., 2022), for geometric morphometrics analysis we used *Geomorph* (Adams & Otárola-Castillo, 2013) and *MASS* (Venables & Ripley, 2002), and for graphic illustrations we used *ggplot2* (Whickham, 2016).

Results

We found 229 diatom taxa distributed among the 15 studied streams. The mean species richness was 64.85 ± 14.36 in group 1, and 67 ± 11.33 in group 2. The mean of Shannon

index was 3.31 ± 0.42 in group 1, and 3.43 ± 0.18 in group 2. There were no differences between stream groups in relation to diatom species richness ($t = -0.33$; $P = 0.74$) (Figure 3a) and the Shannon index ($t = -0.70$; $P = 0.50$) (Figure 3b). In addition, none of the diatom indices differed between the groups of streams (IPS: $t = 1.34$, $P = 0.20$; IDP: $t = 1.39$, $P = 0.18$; TDI: $t = 0.86$, $P = 0.40$; Figure 4).

Table 2. Values of the metrics per stream. S = diatom species richness; H' = Shannon diversity index; IDP = Pampean Diatom Index; IPS = *l'Indice de Polluosensibilité Spécifique*; TDI = Trophic Diatom Index; DVD = Diatom valve deformation; MVW = Mean valve width of *A. minutissimum*; Group 1 is represented by the streams with the highest concentrations of total nitrogen and group 2 by the streams with the lowest concentrations of total nitrogen.

Stream	Group	S	H'	IDP	IPS	TDI	DVD (%)	MVW (μm)
1	1	76	3.67	2.16	12.63	63.18	2.2	3.81
2	2	69	3.67	1.99	14.42	48.99	2.8	3.73
3	1	67	3.65	2.52	14.10	32.49	1.0	3.43
4	2	48	3.14	2.03	14.13	37.24	2.6	3.57
5	1	37	2.54	1.48	17.40	31.08	2.0	3.47
6	2	81	3.62	2.58	11.76	49.86	0.6	3.80
7	2	79	3.40	2.55	11.13	67.30	1.4	3.74
8	2	66	3.42	2.29	14.60	46.01	0.6	3.57
9	1	78	3.61	2.22	12.10	51.30	1.6	3.64
10	2	54	3.44	2.07	15.63	44.34	0.2	3.65
11	2	67	3.40	2.41	10.77	64.88	0.2	3.71
12	1	71	3.27	1.97	14.63	51.16	0	3.63
13	1	55	2.87	1.69	16.44	44.23	0.2	3.73
14	1	70	3.38	2.59	13.57	59.87	0	3.74
15	2	72	3.18	2.99	12.37	65.13	0	3.80

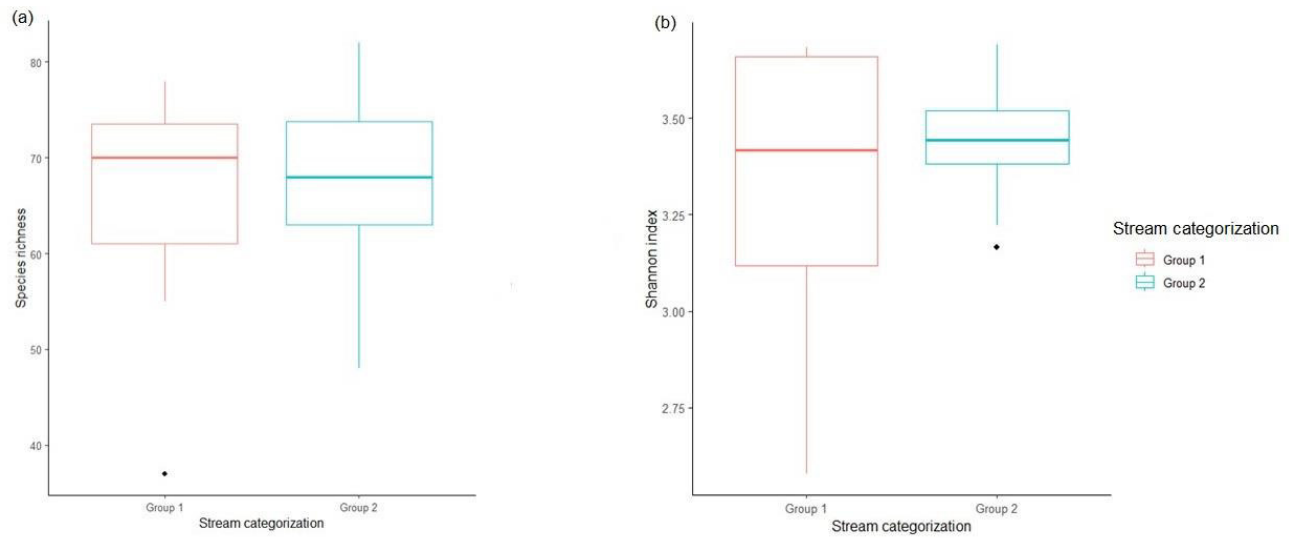


Figure 3. Species richness (a), and Shannon index (b) of stream diatoms in group 1 (higher total nitrogen concentration) and group 2 (lower total nitrogen concentration).

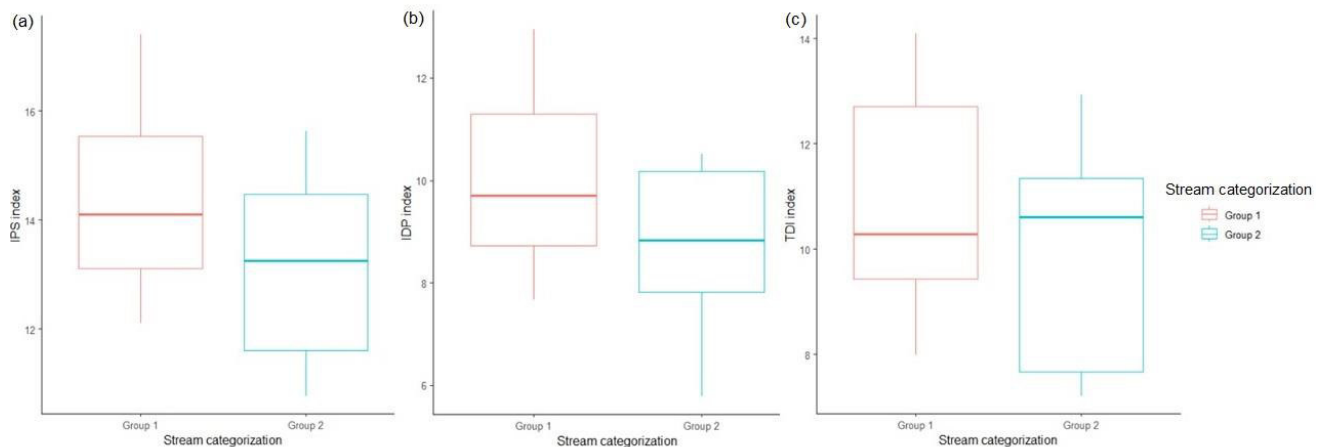


Figure 4. Values of *l'Indice de Polluosensibilité Spécifique* (a), Pampean Diatom Index (b), and Trophic Diatom Index for streams in group 1 (higher total nitrogen concentration) and group 2 (lower total nitrogen concentration).

Regarding to non-taxonomic metrics, the percentage of deformed valves did not differ between the two stream categories ($t = -0.09$; $P = 0.92$; Figure 5a). On the other hand, the

valve width of *A. minutissimum* was greater in streams from group 2, that is, with less nitrogen concentrations ($t = -2.52$; $P = 0.01$; Figure 5b).

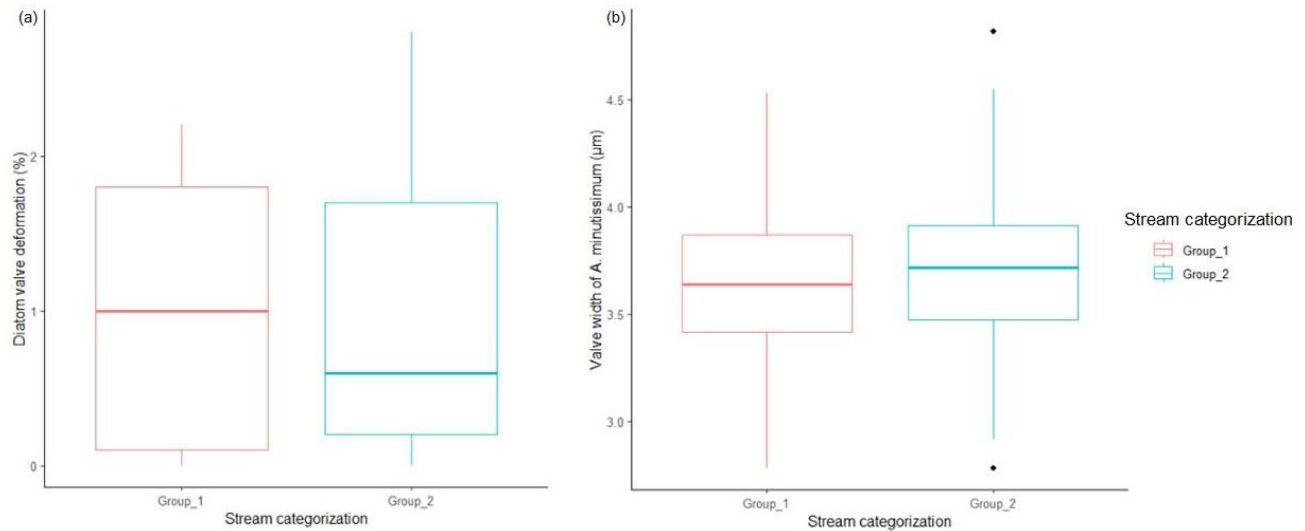


Figure 5. Percentage valve deformation (in 500 valves counted) and valve width of *Achnantheidium minutissimum* (b) for streams in in group 1 (higher total nitrogen concentration) and group 2 (lower total nitrogen concentration).

Regarding valve shape, the first principal component (85.15% of total variability) mostly described changes at the apices of the *A. minutissimum* (6a), so that apices valve outline changed from elliptic (predominant shape in group 2) (Figure 6a) (negative position in morphospace along the first principal component) to capitate (predominant shape in group 1) (Figure 6c) (positive position in morphospace along the first principal component). The valve shape differed between stream groups ($P = 0.005$). The cross-validation test identified correctly 47% of individuals in group 1 and 53% for group 2.

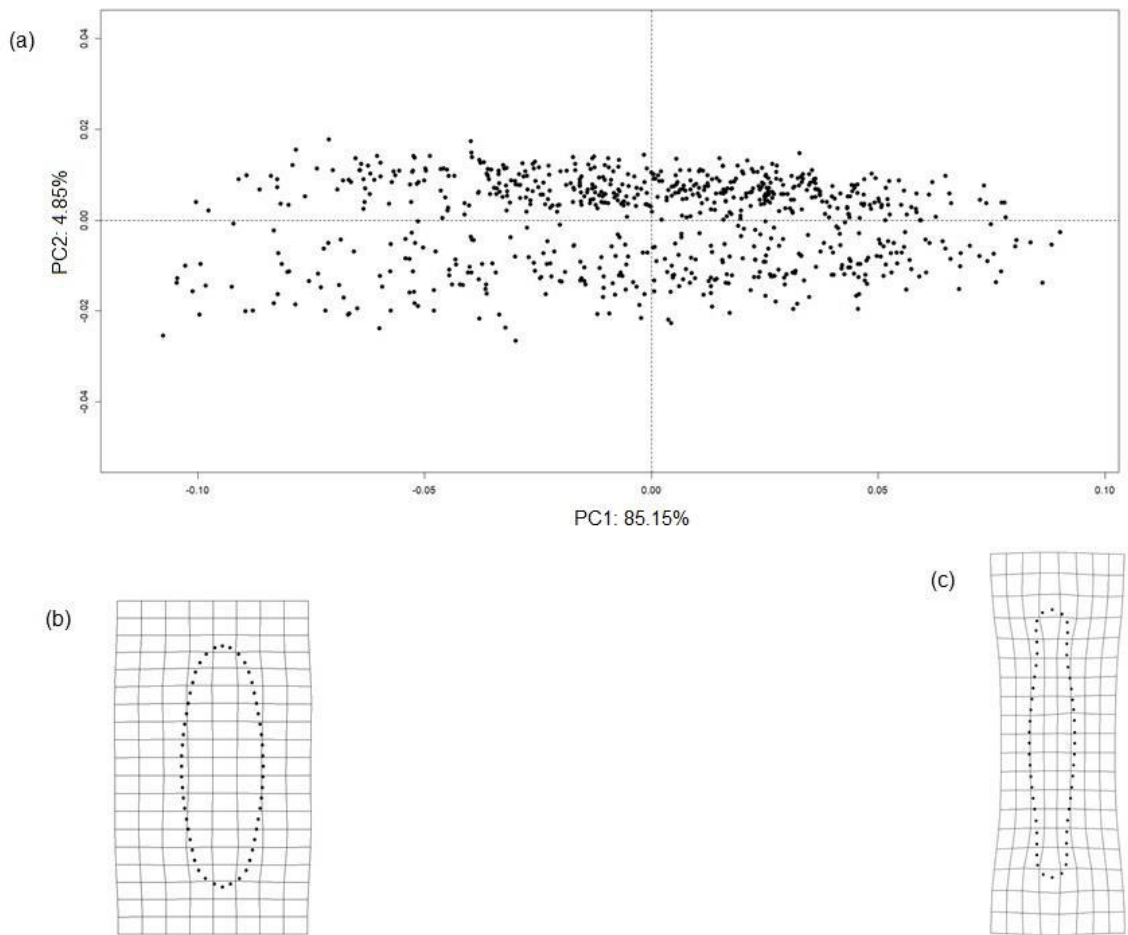


Figure 6. Principal Component Analysis (PCA) of total valve shape variation in *A. minutissimum* populations (a); negative extreme position in morphospace in first principal component (b), and positive position in morphospace in first principal component (c).

Discussion

We found that taxonomical metrics (both alpha diversity and diatom indices) were not able to distinguish streams categorized according to total nitrogen concentrations. On the other hand, despite the similar percentage to deformed valves between stream groups, the valve width of *A. minutissimum* was greater in the group of streams with lower concentrations of total nitrogen. In addition, the valve shape of *A. minutissimum* differed between stream groups.

We expected that species richness and Shannon index would be lower in streams with higher total nitrogen concentrations than in streams with reduced concentration of nitrogen. In the last years, diatom diversity indices have been commonly used in biomonitoring or ecological assessment of lotic environments (Costa & Schneck, 2022). However, different diversity metrics may have different sensitivity in relation to the input of nutrients in the

aquatic environment (e.g., Blanco et al., 2012). For instance, while total phosphorus concentration can be related positively to the Shannon index (Lavoie et al., 2018), in relation to species richness this relation is commonly negative (Licursi et al., 2016; Costa et al., 2022). In fact, algal diversity can be influenced not only by local factors, such as physical and chemical characteristics, but also by factors acting at regional scales, such as climate or landscape (Chase & Leibold, 2002). In this sense, using only diatom diversity indices for stream ecological assessments is commonly discouraged (Blanco et al., 2012).

In general, diatom indices are based on values assigned to species depending on their sensitivity and frequency (e.g., Stoermer & Smol, 1999; de la Rey et al., 2008), and are widely used to assess the ecological status of freshwater ecosystems (Costa & Schneck, 2022). Here, none of the diatom indices used (IDP, IPS and TDI) showed significant differences between stream groups. This lack of effective response of traditional diatom indices is not a new finding, as they can be influenced by factors such as taxonomic uncertainty or regional differentiation such as ecoregion parameters (Besse-Lototskaya et al., 2011). Moreover, the initial effects of eutrophication in lentic environments were also not detected by traditional diatom indices, but by morphological biomarker metrics (Vilmi et al., 2015).

Non-taxonomical metrics performed better in distinguishing the two groups of streams. However, the greater average width of *A. minutissimum* in streams with lower nitrogen concentrations than in those with more nitrogen is opposite to previous empirical evidence. For example, a positive relationship between valve width and nutrient concentrations was found in European temperate lakes (Vilmi et al., 2015). This positive relationship can be expected because algae cell growth is commonly limited by nutrients such as nitrogen (Peter & Sommer, 2014). The opposite relationship we found needs to be better investigated, as it could be related to the effect of other pollutants or to the concentration of nutrients in the sediment. Despite this, we highlight the potential of using the valve width of diatoms as a biomarker due to the easiness to measure this metric in comparison to obtaining data on community composition.

We carried out morphometric analyses with *A. minutissimum* because was a species that occurred abundantly in several streams and previous studies had already shown changes in the valve shape in response to heavy metals (Cerisier et al., 2018) and water acidification (Olenici et al., 2017). Our result on valve shape of *A. minutissimum* indicate that geometric morphometry is a tool that could also be potentially used to identify freshwater ecosystems

differing in their environmental characteristics (e.g., Olenici et al., 2017). Despite being more complex than using linear morphometrics, such as valve width, measuring the valve shape of diatom populations can be a less time-consuming and cheaper alternative than identifying communities at the species level to apply traditional taxonomic metrics.

Different than we expected, the percentage of deformed valves in diatom communities did not differ between stream groups. The percentage of diatom-deformed valves is easy to measure when compared to community assessment approaches at the species level and has been used to assess anthropogenic impacts such as heavy metals (Pandey et al., 2014), pesticides (Lavoie et al., 2017), and eutrophication (Falasco et al., 2009). Here, the highest percentage of deformed frustules was 2.6%, within the expected for preserved environments (<5%, according to Lavoie et al., 2017). In this sense, we can suggest that the higher nitrogen concentrations in group 1 were not considered important in increasing the frequency of occurrence of deformed valves in the communities.

In conclusion, our study is a first step to investigate the use of biomarkers for ecological assessments in streams of the Brazilian Pampa biome. The non-taxonomical metrics responded better to different total nitrogen concentrations. In this sense, we emphasize that studies that include greater replication in space and time are necessary to determine the most effective metrics to ecological assessments of streams from the Brazilian Pampa.

References

- Adams, D.C., & Otárola-Castillo, E., 2013. Geomorph: an R package for the collection and analysis of geometric morphometric shape data. *Methods Ecol. Evol.* 4, 393–399. <https://doi.org/10.1111/2041-210X.12035>
- Allen, S., Grimsha, W.N., Parkinson, J.A., & Quarmby, C., 1974. Chemical analysis of ecological materials. London: Blackwell Scientific Publishers.
- Alvares, C.A., Stape, J.L., Sentelhas, P.C., Gonçalves, J.D.M., & Sparovek, G., 2013. Köppen's climate classification map for Brazil. *Meteorol. Z.* 22(6), 711–728. <https://doi.org/10.1127/0941-2948/2013/0507>

- Atwell, A.K., & Bouldin, J.L., 2022. Effects of agricultural intensity on nutrients and sediment contributions within the Cache River Watershed, Arkansas. *Water*. 14(16), 2528. <https://doi.org/10.3390/w14162528>
- Baumgarten, M.G.Z., Rocha, J.M.B., & Niencheski, L.F.H., 1996. Manual de análises em oceanografia química. Rio Grande: Editora da FURG.
- Bennett, J.R., Rühland, K.M., & Smol, J.P., 2017. No magic number: determining cost-effective sample size and enumeration effort for diatom-based environmental assessment analyses. *Can. J. Fish. Aquat. Sci.* 74(2), 208–215. <https://doi.org/10.1139/cjfas-2016-0066>
- Bere, T., & Tundisi, J.G., 2010. Biological monitoring of lotic ecosystems: the role of diatoms. *Braz. J. Biol.* 70(3), 493–502. <https://doi.org/10.1590/S1519-69842010005000009>
- Besse-Lototskaya, A., Verdonschot, P.F.M., Coste, M., & Vijver, B.V., 2011. Evaluation of European diatom trophic indices. *Ecol. Indic.* 11, 456–467. <https://doi.org/10.1016/j.ecolind.2010.06.017>
- Blanco, S., Cejudo-Figueiras, C., Tudesque, L., Bécares, E., Hoffmann, L., & Ector, L., 2012. Are diatom diversity indices reliable monitoring metrics? *Hydrobiologia* 695, 199–206. <https://doi.org/10.1007/s10750-012-1113-1>
- Bologna, M., & Aquino, G., 2020. Deforestation and world population sustainability: a quantitative analysis. *Sci. Rep.* 10(1), 1–9. <https://doi.org/10.1038/s41598-020-63657-6>
- Castro, E., Siqueira, T., Melo, A.S., Bini, L.M., Landeiro, V.L., & Schneck, F., 2023. Reduced enumeration effort, but not coarse taxonomic resolution, is sufficient to represent beta diversity patterns of stream benthic diatoms. *Limnologia* 102, 126107. <https://doi.org/10.1016/j.limno.2023.126107>
- Cemagref, A., 1983. Etude des methodes biologiques d’appréciation quantitative de la qualité des eaux. Rapport Division Qualité des Eaux Lyon Agence de l’Eua Rhône-Méditerranéen-Corse, Pierre Bénite, p. 218.
- Chase, J.M., & Leibold, M.A., 2002. Spatial scale dictates the productivity-biodiversity relationship. *Nature* 416, 427–430. <https://doi.org/10.1038/416427a>

- Costa, A.P.T. & Schneck, F., 2022. Diatom as indicators in running waters: trends of studies on biological assessment and monitoring. *Environ. Monit. Assess.* 194(10), 695. <https://doi.org/10.1007/s10661-022-10383-3>
- Costa, A.P.T., Castro, E., da Silva, C.F.M., & Schneck, F., Eutrophication changes community composition and drives nestedness of benthic diatoms from coastal streams. *Acta Limnol. Bras.* 34, e14. <https://doi.org/10.1590/S2179-975X0122>
- De la Rey, P.A., van Rensburg, L., & Vooslo, A., 2008. On the use of diatom-based biological monitoring Part 1: A comparison of the response of diversity and aut-ecological diatom indices to water quality variables in the Marico-Molopo River catchment. *Water* 34, 53–60.
- Doods, W.K., 2006. Eutrophication and trophic state in rivers and streams. *Limnol. Oceanogr.* 51(1), 671–680. https://doi.org/10.4319/lo.2006.51.1_part_2.0671
- Dudgeon, D., 2019. Multiple threats imperil freshwater biodiversity in the Anthropocene. *Curr. Biol.* 29(19), R960–R967. <https://doi.org/10.1016/j.cub.2019.08.002>
- Dziengo-Czaja, M., Koss, J., Matuszak, A., 2008. Teratological forms of diatoms (Bacillariophyceae) as indicators of water pollution in the western part of Puck Bay (southern Baltic Sea). *Oceanol. Hydrobiol. Stud.* 37, 119–132. <https://doi.org/10.2478/v10009-007-0042-1>
- Falasco, E., Bona, F., Badino, G., Hoffmann, L., & Ector, L., 2009. Diatom teratological forms and environmental alterations: a review. *Hydrobiologia* 623, 1–35. <https://doi.org/10.1007/s10750-008-9687-3>
- Friberg, N., Bonada, N., Bradlay, D.C., Dunbar, M.J., Edwards, F.K., Grey, J., Hayes, R.B., Hildrew, A.G., Lamouroux, N., Trimmer, M., & Woodward, G., 2011. Biomonitoring of human impacts in freshwater ecosystems: the good, the bad and the ugly. *Adv. Ecol. Res.* 44, 1–68. <https://doi.org/10.1016/B978-0-12-374794-5.00001-8>
- Gelis, M.M.N., Sathcq, M.B., Jupke, J., & Cochero, J., 2022. DiaThor: R package for computing diatom metrics and biotic indices. *Ecol. Modell.* 465, 109859. <https://doi.org/10.1016/j.ecolmodel.2021.109859>

- Gokce, D., & Gulbenk, H., 2019. Assessment of water quality and epilithic algae community structure of Tohma River, Turkey. *Fresenius. Environ. Bull.* 28(2A), 1203–1217. <https://doi.org/10.13140/RG.2.2.33469.18408>
- Gómez, N., & Licursi, M., 2001. The Pampean Diatom Index (IDP) for assessment of rivers and streams in Argentina. *Aquat. Ecol.* 35, 173–181. <https://doi.org/10.1023/A:1011415209445>
- He, F., Zarfl, C., Bremerich, V., David, J.N.W., Hogan, Z., Kalinkat, G., Tockner, K., & Jähnig, S.C., 2019. The global decline of freshwater megafauna. *Glob. Change Biol.* 25(11), 3883–3892. <https://doi.org/10.1111/gcb.14753>
- Kassambara, A., 2017. Practical Guide to Cluster Analysis in R. USA: STHDA.
- Kelly, M.G., & Whitton, B.A., 1995. The Trophic Diatom Index: a new index for monitoring eutrophication rivers. *J. Appl. Phycol.* 7, 433–444. <https://doi.org/10.1007/BF00003802>
- Kelly, M.G., 1998. Use of the Trophic Diatom Index to monitor eutrophication in rivers. *Water Res.* 32(1), 236–242. [https://doi.org/10.1016/S0043-1354\(97\)00157-7](https://doi.org/10.1016/S0043-1354(97)00157-7)
- Lamparelli, M.C., 2004. Graus de trofia em corpos d'água do Estado de São Paulo: avaliação dos métodos de monitoramento. [Tese de doutorado em Ciências]. São Paulo: Departamento de Ecologia, Universidade de São Paulo.
- Landeiro, V.L., Bini, L.M., Costa, F.R.C., Franklin, E., Nogueira, A., Souza, J.L.P., Moraes, J., & Magnusson, W.E., 2012. How far can we go in simplifying biomonitoring assessments? An integrated analysis of taxonomic surrogacy, taxonomic sufficiency and numerical resolution in a megadiverse region. *Ecol. Indic.* 23, 366–373. <https://doi.org/10.1016/j.ecolind.2012.04.023>
- Lavoie, I., Campeau, S., Darchambeau, F., Cabana, G., & Dillon, P.J., 2008. Are diatoms good integrators of temporal variability in stream water quality? *Freshw. Biol.* 53, 827–841. <https://doi.org/10.1111/j.1365-2427.2007.01935.x>
- Lavoie, I., Hamilton, P.B., Morin, S., Tiam, S.K., Kahlert, M., Gonçalves, S., Falasco, E., Foritn, C., Gontero, B., Heudre, D., Kojadinovic-Sirinelli, M., Manoylov, K., Pandey, L.K., & Taylor, J.C., 2017. Diatom teratologies as biomarkers of contamination: Are all deformities

ecologically meaningful? Ecol. Indic. 82, 539–550.
<http://dx.doi.org/10.1016/j.ecolind.2017.06.048>

Licursi, M., Gómez, N., & Sabater, S., 2016. Effects of nutrient enrichment on epipellic diatom assemblages in a nutrient-rich lowland stream, Pampa region, Argentina. *Hydrobiologia*, 766, 135–150. <https://doi.org/10.1007/s10750-015-2450-7>

Moreira-Filho, H., & Valente-Moreira, I.M., 1981. Avaliação taxonômica e ecológica das diatomáceas (Bacillariophyceae) epífitas em algas pluricelulares obtidas nos litorais nos estados do Paraná, Santa Catarina e São Paulo. *Bol. Mus. Mun.* 47, 1–17.

Newton, A., Brito, A.C., Icely, J.D., Derolez, V., Clara, I., Angus, S., Schernewski, G., Inácio, M., Lillebø, A.I., Sousa, A.I., Bájaoui, B., Solidoro, C., Tosic, M., Canedo-Arguelles, M., Yamamuro, M., Reizopoulou, S., Tseng, H.C., Canu, D., Roselli, L., Maanan, M., Cristina, S., Ruiz-Fernández, A.C., Lima, R.F., Kjerfve, B., Rubio-Cisneros, N., Perez-Ruzafa, A., Marcos, C., Pastres, R., Pranovi, F., Snoussi, M., Turpie, J., Tuchkovenko, Y., Dyack, B., Brookes, J., Povilanskas, R., & Khokhlov, V., 2018. Assessing, quantifying, and valuing the ecosystem services of coastal lagoons. *J. Nat. Conserv.* 44, 50–65. <https://doi.org/10.1016/j.jnc.2018.02.009>

Olenici, A., Blanco, S., Borrego-Ramos, M., Momeu, M., & Baciú, C., 2017. Exploring the effects of acid mine drainage on diatom teratology using geometric morphometric. *Ecotoxicology* 26(4), 1018–1030. <https://doi.org/10.1007/s10646-017-1830-3>

Oliveira, T.E., Freitas, D.S., Gianezini, M., Ruviaro, C.F., Zago, D., Mércio, T.Z., Dias, E.A., Lampert, V.N., & Barcellos, J.O.J., 2017. Agricultural land use change in the Brazilian Pampa Biome: The reduction of natural grasslands. *Land use Policy* 63, 394–400. <https://doi.org/10.1016/j.landusepol.2017.02.010>

Oliveira, S.S., Ortega, J.C.G., Ribas, L.G.S., Lopes, V.G., & Bini, L.M., 2020. Higher taxa are sufficient to represent biodiversity patterns. *Ecol. Indic.* 111, 105994. <https://doi.org/10.1016/j.ecolind.2019.105994>

Overbeck, G.E., Müller, S.C., Fidelis, A., Pfadenhauer, J., Pillar, V.D., Blanco, C.C., Boldrini, I.I., Both, R., & Forneck, E.D., 2007. Brazil's neglected biome: the southern

Brazilian *Campos*. *Perspect. Plant Ecol. Evol. Syst.* 9(2), 101–116.
<https://doi.org/10.1016/j.ppees.2007.07.005>

Pandey, L.K., Kumar, D., Yadav, A., Rai, J., & Gaur, J.P., 2014. Morphological abnormalities in periphytic diatoms as a tool for biomonitoring of heavy metal pollution in a river. *Ecol. Indic.* 36, 272–279. <https://doi.org/10.1016/j.ecolind.2013.08.002>

Pandey, L.K., Lavoie, I., Morin, S., Park, J., Lyu, J., Choi, S., Lee, H., & Han, T., 2018. River water quality assessment based on a multi-descriptor approach including chemistry, diatom assemblage structure, and non-taxonomical diatom metrics. *Ecol. Indic.* 84, 140–151. <https://doi.org/10.1016/j.ecolind.2017.07.043>

Peter, K.H., & Sommer, U., 2014. Interactive effect of warming, nitrogen and phosphorus limitation on phytoplankton cell size. *Ecol. Evol.* 5(5), 1011–1024. <https://doi.org/10.1002/ece3.1241>

Pillsbury, R., Stevenson, R.J., Munn, M.D., & Waite, I., 2019. Relationships between diatom metrics based on species nutrient traits and agricultural land use. *Environ. Monit. Assess.* 191(4), 1–26. <https://doi.org/10.1007/s10661-019-7357-8>

Potapova, M., & Hamilton, P.B., 2007. Morphological and ecological variation within the *Achnantheidium minutissimum* (Bacillariophyceae) species complex. *J. Phycol.* 43(3), 561–575. <https://doi.org/10.1111/j.1529-8817.2007.00332.x>

R Core Team, 2020. R: A language and environment for statistical computing [online]. Vienna: R Foundation for Statistical Computing.

Rohlf, F.J., 2013. tps Utility program SUNY at stony Brook

Rohlf, F.J., 2015. The tps series of software. *Hystrix. It. J. Mamm.* 26, 9–12. <https://doi.org/10.4404/hystrix-26.1-11264>

Simonsen, R., 1974. The diatom plankton of the Indian Ocean Expedition of RV Meteor. *Forsch. Ergeb.* 19, 1–107.

Soininen, J., Paavola, R., & Muotka, T., 2004. Benthic diatom communities in boreal streams: community structure in relation to environmental and spatial gradients. *Ecography* 27, 330–342. <https://doi.org/10.1111/j.0906-7590.2004.03749.x>

- Stevenson, R.J., Pan, Y., & Van Dam, H., 2010. Assessing environmental conditions in rivers and streams with diatoms. In: Smol, J., & Stoermer, E., eds. *The diatoms: Applications for the environmental and earth sciences*. Cambridge: Cambridge University Press, 57–85.
- Suárez, B., Barrios, M., & Mello, F.T., 2022. macroinvertebrate's response to different land use in lowland streams from Uruguay: use of artificial substrates for biomonitoring. *Neotrop. Biodivers.* 8(1), 136–146. <https://doi.org/10.1080/23766808.2022.2049178>
- Torres-Franco, A.F., Alatrasta-Góngora, G.R., Guzmán-Rodríguez, N.P., Calizaya-Anco, J.A., Mota, C.R., Figueredo, C.C., 2019. Physicochemical and diatom trophic state indexes: A complementary approach for improving water sustainability in a high Andean urban stream. *Ecohydrol. Hydrobiol.* 19, 577–587. <https://doi.org/10.1016/j.ecohyd.2019.01.007>
- Umetsu, C.A., Aguiar, F.C., Ferreira, M.T., Cancian, L.F., & Camargo, A.F., 2018. Addressing bioassessment of tropical rivers using macrophytes: The case of Itanhaém Basin, São Paulo, Brazil. *Aquat. Bot.* 150, 53–63. <https://doi.org/10.1016/j.aquabot.2018.07.004>
- Valderrama, J. C., 1981. The simultaneous analysis of total nitrogen and total phosphorus in natural waters. *Mar. Chem.* 10(2), 109–112. [https://doi.org/10.1016/0304-4203\(81\)90027-X](https://doi.org/10.1016/0304-4203(81)90027-X)
- Venables, W.N., & Ripley, B.D., 2002. *Modern Applied Statistics with S (Statistics and Computing)*. New York: Springer.
- Vilmi, A., Karjalainen, S.M., Landeiro, V.L., & Heino, J., 2015. Freshwater diatoms as environmental indicators: evaluating the effects of eutrophication using species morphology and biological indices. *Environ. Monit. Assess.* 187(5), 1–10. <https://doi.org/10.1007/s10661-015-4485-7>
- Wickham, H., 2016. *ggplot2: Elegant graphics for data analysis*. Springer.

CONSIDERAÇÕES FINAIS E PERSPECTIVAS

A principal proposta da tese aqui apresentada foi trazer informações e avançar no conhecimento científico acerca de abordagens de biomonitoramento e avaliações ecológicas de ambientes lóticos, utilizando diatomáceas como modelo biológico de estudo e riachos de baixa ordem inseridos no bioma Pampa como a principal área de estudo. Desenvolvi os trabalhos ao longo do meu doutoramento buscando testar métricas diatomológicas que visassem otimizar o tempo de análises, especialmente para identificação das comunidades em nível de espécie. Isso porque, como exposto ao longo desse documento, estratégias de biomonitoramento e avaliações ecológicas de ambientes aquáticos continentais com melhor custo-benefício são cada vez mais necessárias frente aos crescentes impactos de origem antrópica (e.g. BEVILACQUA *et al.*, 2015).

No primeiro capítulo conduzi uma revisão sistemática e cienciométrica global com o intuito de avaliar as principais tendências sobre o uso de diatomáceas no biomonitoramento e avaliações ecológicas de ambientes lóticos com base em 764 artigos. Dentre os principais achados estão:

- (1) Pesquisas sobre essa temática estão bem consolidadas na América do Norte e na Europa. Esse era um resultado esperado, pois tanto nos Estados Unidos como em países da União Europeia existem políticas governamentais (e consequentemente forte investimento nas ciências ambientais) que visam monitorar os ambientes aquáticos com base em avaliações de dados abióticos e diversas comunidades biológicas, dentre essas as diatomáceas.
- (2) Dentre as comunidades, a epilítica foi amostrada com maior frequência nos estudos. Apesar de, o efeito de diferentes substratos sobre a colonização, diversidade e composição de comunidades perifíticas estar descrito na literatura, verificamos que menos que 5% dos artigos buscaram avaliar o efeito de diferentes substratos sobre as métricas diatomológicas de biomonitoramento e avaliações ecológicas.
- (3) A eutrofização artificial está entre os principais impactos avaliados nos ecossistemas lóticos através do uso de métricas diatomológicas. Ainda, 641 espécies foram classificadas em algum momento como tolerante ou sensível a determinado impacto antrópico. *Nitzschia palea* e *Gomphonema parvulum* foram espécies que se destacaram em relação à frequência com que aparecem nos artigos, comumente indicadas como tolerantes a diversos fatores estressores (e.g. SALOMONI *et al.*, 2006; ARCHIBALD;

TAYLOR, 2007; PHAM, 2020). Ainda, detectei algumas inconsistências em relação a faixas de tolerâncias para algumas espécies, especialmente para *Achnantheidium minutissimum* e *Cocconeis placentula*.

- (4) Por fim, verifiquei que, ao longo do tempo, métricas taxonômicas tradicionais (como avaliação da diversidade, composição da comunidade e índices de diatomáceas) são amplamente utilizados com o intuito de avaliar a integridade dos ambientes lóticos. Por outro lado, métricas alternativas vêm ganhando destaque na literatura, como o uso do DNA *metabarcoding*, avaliações de teratologias, corpos lipídicos e tamanho das valvas.

No segundo capítulo busquei verificar se grupos substitutos de espécies, como gêneros e classes de biovolume de diatomáceas são eficientes em representar a variação espacial na estrutura das comunidades de diatomáceas inseridas em riachos do bioma Pampa. Além disso, testei se as comunidades baseadas nos grupos substitutos responderiam aos padrões ambientais e espaciais da maneira similar às comunidades identificadas em nível de espécie. Dentre os principais achados desse capítulo estão:

- (1) A congruência entre grupos substitutos e a matriz original de espécies é consideravelmente maior quando são utilizados dados de abundância comparado aos dados de presença e ausência.
- (2) O componente espacial foi importante para explicar a composição de comunidades em nível de espécie, mas ao simplificar a matriz em gêneros e classes de biovolume o componente espacial não foi significativo.
- (3) Por fim, o componente ambiental foi importante para explicar a composição de comunidades em nível de espécie e substitutos. Essa informação tem aplicabilidade em contextos de avaliações ambientais, pois aqui os substitutos refletiram o estado ecológico dos riachos estudados de forma similar às espécies.

Esse último resultado é interessante, especialmente se levarmos em consideração que os grupos substitutos foram usados aqui com o intuito de testar a redução dos esforços de identificação de comunidades em nível de espécie. Vale ressaltar que, além do custo elevado e o tempo demandado para a identificação de espécies, pesquisadores com especialidade em taxonomia estão cada vez mais escassos em todas as áreas (WÄGELE *et al.*, 2011).

No terceiro capítulo verifiquei a eficiência de métricas taxonômicas comumente utilizadas nas avaliações ecológicas, como índices de diversidade e diatomológicos, bem como métricas

não-taxonômicas, como a presença de teratologias em valvas e morfometria linear e geométrica de *Achnanthes minutissimum*, para diferenciar riachos classificados de acordo com as concentrações de nitrogênio total na água. Dentre os principais achados desse capítulo estão:

- (1) Não houve diferença significativa para os índices de diversidade (riqueza e Shannon) e diatomológicos utilizados ao comparar as duas categorias de riachos.
- (2) Em oposição às expectativas, a porcentagem de valvas deformadas nas comunidades não diferiu entre as categorias de riachos.
- (3) A morfometria linear de *A. minutissimum* diferiu entre as categorias de riachos. No entanto, era esperado valvas maiores em riachos com maiores concentrações de nitrogênio, pois já foi evidenciado que o aumento gradativo de nitrogênio e fósforo acarreta em aumento da valva dessa espécie (VILMI *et al.*, 2015). No entanto, destaco que esse resultado foi encontrado para ambientes lênticos e temperados, de forma que para ambientes lóticos e subtropicais esse padrão não foi corroborado.
- (4) Por fim, houve diferenças na forma da valva avaliada através de morfometria geométrica de *A. minutissimum* entre as categorias de riachos. Essa espécie foi escolhida para essa análise por dois motivos: (i) ocorreram em grande parte dos riachos e, (ii) já existem evidências de que ela apresenta variação na forma da valva em função de estressores (OLENICI *et al.*, 2017; CERISIER *et al.*, 2018).

Para finalizar esta tese deixo aqui algumas perspectivas para futuros estudos com base nos achados descritos ao longo desse documento. Primeiramente destaco que uma das maiores críticas relacionadas aos capítulos 2 e 3 é o número de riachos amostrados, considerado baixo para estudos de biomonitoramento e avaliações ecológicas. Dessa forma, penso que um delineamento amostral que contemple um maior número de riachos distribuídos pelo bioma Pampa, bem como uma replicação temporal, poderia ser mais adequado e compor informações mais robustas para testar com maior confiabilidade as abordagens de grupos substitutos, porcentagem de valvas deformadas nas comunidades e morfometrias linear e geométrica. Além disso, a replicação temporal permitiria monitorar esses ambientes com periodicidade. Por fim, levando em consideração que a morfometria geométrica é uma ferramenta capaz de detectar variações da forma de corpo de diferentes organismos, outras espécies comumente abundantes (e.g., *Gomphonema parvulum* e *Nitzschia palea*) podem ter

potencial para serem testadas frente a diversos impactos antrópicos, como alterações hidrológicas, urbanização e mineração.

REFERÊNCIAS

ARCHIBALD, C. G. M.; TAYLOR, J. C. The assessment of diffuse pollution from acid-mine drainage using an update and revised diatom assessment procedure as an added-value biomonitoring tool. **Water Science and Technology**, v. 55, n. 3, p. 151–160, 2007.

BEVILACQUA, S.; TERLIZZI, A.; MISTRI, M.; MUNARI, C. New frameworks species surrogacy in monitoring highly variable coastal ecosystems: Applying the BestAgg approach to Mediterranean coastal lagoons. **Ecological Indicators**, v. 52, p. 207–218, 2015.

CERISIER, A.; VEDRENNE, J.; LAVOIE, I.; MORIN, S. Assessing the severity of diatom deformities using geometric morphometric. **Botany Letters**, v. 166, n. 1, p. 32–40, 2018.

OLENICI, A.; BLANCO, S.; BORREGO-RAMOS, M.; MOMEU, L.; BACIU, C. Exploring the effects of acid mine drainage on diatom teratology using geometric morphometric. **Ecotoxicology**, v. 26, p. 1018–1030, 2017.

PHAM, T. L. Using benthic diatoms as bioindicator to assess rural-urban river conditions in tropical area: A case study in the Sai Gon River, Vietnam. **Pollution**, v. 6, n. 2, p. 387–398, 2020.

SALOMONI, S. E.; ROCHA, O.; CALLEGARO, V. L.; LOBO, E. A. Epilithic diatoms as indicators of water quality in the Gravataí River, Rio Grande do Sul, Brazil. **Hydrobiologia**, v. 559, p. 233–246, 2006.

VILMI, A.; KARJALAINEN, S. M.; LANDEIRO, V. L.; HEINO, J. Freshwater diatoms as environmental indicators: evaluating the effects of eutrophication using species morphology and biological indices. **Environmental Monitoring and Assessment**, v. 187, p. 1–10, 2015.

WÄGELE, H.; KLUSSMANN-KOLB, A.; KUHLMANN, M.; HASZPRUNAR, G.; LINDBERG, D.; KOCH, A.; WÄGELE, J. W. The taxonomist – an endangered race. A practical proposal for its survival. **Frontiers in Zoology**, v. 8, p. 25, 2011.

ANEXOS

Anexo 1: Publicações extra doutoramento entre 03/2019 e 10/2023.

1. **COSTA, A. P. T.**; CROSSETTI, L. O.; HARTZ, S. M.; BECKER, F. G.; HEPP, L. U.; BOHNENBERGER, J. E.; LIMA, M. S.; GUIMARÃES, T.; SCHNECK, F. Land cover is the main correlate of phytoplankton beta diversity in subtropical coastal shallow lakes. **Aquatic Ecology**, v. 54, n.4, p. 1015–1028, 2020. <https://doi.org/10.1007/s10452-020-09790-w>
2. TOURINHO, M. P.; **COSTA, A. P. T.**; MARTINS, K. P.; BANDEIRA, M. G. S.; BARBOSA, F. G. Scientific knowledge on threatened species of the Brazilian Red List: freshwater fish as a case study. **Environmental Biology of Fishes**, v. 103, p. 719–731, 2020. <https://doi.org/10.1007/s10641-020-00978-5>
3. **COSTA, A. P. T.**; CASTRO, E.; DA SILVA, C. F. M.; SCHNECK, F. Eutrophication changes community and drives nestedness of benthic diatoms from coastal streams. **Acta Limnologica Brasiliensia**, v. 34, p. e14, 2022. <https://doi.org/10.1590/S2179-975X0122>
4. GARCÍA-RODRÍGUEZ, F.; **COSTA, A. P. T.**; FRANTZ, A. M.; DA SILVA, C. F. M.; MARTINS, K. P.; MORAES, A. L. M.; ALVES, F. L.; BUGONI, L.; PALMA-SILVA, C.; ALBERTONI, E. A.; MATTOS, P. H.; PINHO, G. L. L.; AGOSTINI, V. O. The combined use of paleolimnological and long-term limnological information to identify natural and anthropogenic environmental changes. **Acta Limnologica Brasiliensia**, v. 34, p. e28, 2022. <https://doi.org/10.1590/S2179-975X3322>