INSTITUTO DE CIÊNCIAS BIOLÓGICAS

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DESENVOLVIMENTO DE UM *FRAMEWORK* PARA AVALIAÇÃO DA INTEGRIDADE ECOLÓGICA DE RIACHOS

CAMILA AIDA CAMPOS COUTO

BRASÍLIA – DF

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TESE DE DOUTORADO

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Tese de doutorado apresentada ao Programa de Pós-Graduação em Ecologia da Universidade de Brasília, como requisito para a obtenção do título de Doutor em Ecologia

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AGRADECIMENTOS

Apesar de eu costumar dizer que o Doutorado é uma jornada extremamente solitária, ela é, ao mesmo tempo, extremamente colaborativa. Ao longo de quatro anos e meio foram muitas pessoas e instituições que contribuíram, direta ou indiretamente, para a concretização deste projeto. Meus agradecimentos mais sinceros...

... aos meus dois "Júniors": meu orientador e meu marido. O primeiro, a pessoa que me instigou a fazer este doutorado, que me apoiou técnica e pessoalmente ao longo desta caminhada, que confia e admira meu trabalho, e pela qual eu tenho extremo respeito e apreço; e o segundo, meu parceiro de vida, aquele que confia mais em mim do que eu mesma, que me acompanhou no período do doutorado sanduíche na Austrália, que não me deixou desistir em vários momentos, que vibrou com minhas conquistas, que me acompanhou em campo quando ninguém mais podia ir, que está sempre ao meu lado. Sem meus dois "Júniors" talvez essa tese nem existisse.

... aos meus pais (Dirceu e Celeste) e irmãs (Carol e Érika) pelo amor e apoio incondicionais! Às pequenas Valentina e Catarina, para as quais dedico esta tese na esperança de dias melhores para o seu futuro. Vocês são minha base e referência, sempre.

... aos colegas do laboratório de Limnologia da UNB, pelo grande apoio nos trabalhos de campo e laboratório. Especialmente, agradeço a Flávio, Regina, Rafael e Gui, que além da ajuda técnica neste trabalho se tornaram grandes amigos. Ainda, um agradecimento mais que especial ao Alan Tonin que, além de amigo, acabou se tornando coautor de boa parte dos artigos que compõem esta tese, sendo sua ajuda crucial nos últimos meses de trabalho.

... à equipe, que hoje posso chamar também de amigos, do Laboratório de Citotaxonomia e Insetos Aquáticos do INPA: Prof. Neusa, Jeane, Gleison, Dani, Jeferson, Ana Pes e outros. Obrigada por tantos ensinamentos, por terem me feito conseguir distinguir famílias (e até gêneros!) de macroinvertebrados aquáticos em um mês intenso de imersão em Manaus! Vocês foram parte fundamental deste trabalho.

... à amiga Maíra e a estagiária Ana Maria pela ajuda na identificação das diatomáceas, e ao Cléber, não só por ter cedido a estrutura do seu laboratório na UFMG para estas análises, mas também pela amizade de longa data que é tão importante para mim. Obrigada pelos conselhos, incentivos e por sempre me fazer rir e acreditar.

... a todos os órgãos públicos e proprietários de áreas de preservação ambiental privadas que me concederam acesso para as coletas ao longo do ano de 2018. São eles: Instituto Brasília Ambiental (agradecimento especial à equipe da ESECAE), Instituto Chico Mendes de

Conservação da Biodiversidade (ICMBio), Marinha Brasileira, Exército Brasileiro, Jardim Botânico de Brasília, Instituto Brasileiro de Geografia e Estatística (Reserva Ecológica do IBGE), Fazenda Água Limpa (FAL-UNB), Chapada Imperial e Paraíso na Terra. Espero que este trabalho leve a vocês informações relevantes para suas belas ações de preservação da nossa natureza.

... à Agência Reguladora de Águas, Energia e Saneamento Básico do Distrito Federal (Adasa), minha "casa" desde 2009 e que, em 2017, me concedeu licença capacitação de três anos e meio para dedicação integral ao Doutorado, me remunerando por todo o período. Além do apoio financeiro, agradeço também pelo apoio logístico, com a disponibilização de veículos e motoristas para execução de parte do meu trabalho de campo. Espero poder retribuir todo apoio e confiança em mim depositados com um trabalho ainda mais qualificado.

... many thanks to the Australian Rivers Institute (Queensland, Australia), especially to professors Stuart Bunn and Mark Kennard for the welcoming and all necessary assistance in the one-year period of the Sandwich PhD in Australia. Many thanks also to the HDR colleagues, especially to my sweetie friends Naima and Rafaela, for their support and for so many special moments together.

… aos amigos e familiares do Brasil e Austrália. Sem família e amigos nada faz sentido. Especial agradecimento aos amigos brasileiros contemporâneos do Doutorado sanduíche em Brisbane. As semelhanças dos nossos sonhos e desafios nos uniram de uma forma única e inesquecível. Aline, Fernanda, Jéssica e Soraia, vocês foram essenciais.

... finalmente, agradeço à CAPES pelo financiamento de um ano de bolsa do programa CAPES-PrInt para execução das atividades no exterior e à FAP-DF pelos recursos disponibilizados ao Projeto Aquaripária, viabilizando as atividades de campo e laboratório.

muito obrigada.

Dedico esta tese aos meus pais, Dirceu e Celeste, e às minhas sobrinhas, Valentina e Catarina. Que a base que os primeiros me ofereceram sirva de alguma forma para ajudar a moldar um mundo melhor para os adultos do futuro. Que esta tese seja um incentivo para que vocês, crianças, acreditem e confiem na ciência, bem como lutem por nosso meio ambiente.

SUMÁRIO

APRESENTAÇÃO

Dos quatro anos e meio que me dediquei ao Doutorado, praticamente dois anos inteiros foram de trabalho de campo, explorando 52 trechos de rios do Distrito Federal (DF) e entorno, e em laboratório, analisando amostras. Eu pensava que, por já ter trabalhado por mais de dez anos na Agência Reguladora de Águas, Energia e Saneamento Básico do Distrito Federal (Adasa) nos setores de fiscalização e monitoramento de recursos hídricos, eu já conhecia muito bem os rios do DF. De fato, eu os conhecia um pouco, mas foi entrando em cada um deles, explorando seus arredores, seu leito, seus organismos, processos e águas que passei a entendê-los com muito mais profundidade.

Como servidora pública responsável pelo monitoramento da qualidade das águas do Distrito Federal durante tantos anos, pude perceber diversas inconsistências nos resultados de análises de qualidade de água em relação à realidade que eu presenciava nos locais de coleta. Comecei a me questionar se estávamos mesmo fazendo o melhor monitoramento que podíamos, se os objetivos estavam sendo alcançados, como estaria a qualidade do ecossistema aquático, e mais, o que eu poderia fazer para melhorar a gestão das nossas águas. Apesar da ampla rede de monitoramento operada pela Adasa, uma das mais robustas do Brasil, desde o ano de 2009, e uma série de parâmetros físicos, químicos e microbiológicos, eu sabia que o universo do monitoramento poderia ser muito mais abrangente e eficiente se olhássemos para outros elementos do ecossistema, além da água em si.

Meus questionamentos passaram a ser também levantados no âmbito da Câmara Técnica do Conselho de Recursos Hídricos do Distrito Federal (CRH/DF) e dali surgiu a ideia de realizar o Doutorado na UNB sob a supervisão do Prof. Dr. José Francisco Gonçalves Júnior, representante da Universidade no CRH. Com o interesse no assunto tomando forma, comecei a realizar pesquisa bibliográfica e encontrei uma série de estudos relacionados a indicadores biológicos, avaliação de integridade ecológica, saúde de rios, modelagem ecológica e modelos de monitoramento. Essa busca só alimentou em mim ainda mais o desejo de fazer a diferença e de, para isto, ter que me entregar a algo que até alguns anos atrás estava completamente fora de cogitação: um Doutorado. Foi assim que vontade e oportunidade se uniram, e foi assim que começou minha trajetória até aqui.

Após duas tentativas até ser aprovada no Programa de Pós-Graduação em Ecologia da UNB, vieram quatro anos e meio de uma verdadeira montanha-russa de emoções, repleta de desafios e conquistas, alegrias e tristezas, dificuldades e superações. Mas entre tantos altos e baixos, o que sempre me manteve firme foi a certeza de que eu precisava, e queria muito, cumprir minha missão. Sei que minha contribuição para a ciência e a gestão está longe do suficiente e da real atenção que nossas águas merecem, mas espero com esta tese ter contribuído para a gradual evolução dos programas de monitoramento. Sou muito grata por poder fazer parte da história da gestão das águas do meu país.

Foto: Adilon B. C. Júnior

RESUMO

O mundo vem passando por grandes transformações e, com elas, os impactos das atividades humanas sobre os mais diversos biomas e ambientes são notórios e preocupantes. Os ecossistemas aquáticos estão entre os mais ameaçados, levando a consequências que vão desde a perda da biodiversidade e alterações do equilíbrio ambiental até a eventos de escassez hídrica relacionados tanto a quantidade quanto a qualidade das águas. O monitoramento ambiental sempre teve como um dos principais objetivos avaliar as alterações que os agentes estressores exercem sobre os diversos elementos dos ecossistemas e, assim, direcionar ações de gestão. Ao longo dos anos, o monitoramento de ambientes aquáticos foi sofrendo modificações, transferindo o foco sobre a água como recurso para a água como parte de um ecossistema. As tradicionais análises de variáveis físicas e químicas foram sendo complementadas por indicadores biológicos por entender-se que estes são a ponta final da cadeia de processos na qual o ecossistema está inserido. Assim, indicadores biológicos são capazes de refletir o somatório de efeitos das pressões às quais o ecossistema está exposto, e de uma maneira menos oscilatória do que muitos parâmetros de qualidade de água. Apesar do avanço científico no tema e dos inúmeros exemplos de países e regiões do mundo onde esta abordagem integrativa já é aplicada até mesmo a nível de programas governamentais de monitoramento, muitas regiões, especialmente as tropicais, como o Brasil, ainda permanecem realizando o monitoramento tradicional. Considerando a relevância do Brasil no cenário hídrico e de biodiversidade mundial, é urgente que avanços nos métodos de monitoramento de ambientes aquáticos sejam implementados. Para tanto, é necessário o conhecimento sobre o comportamento de comunidades biológicas e processos ecossistêmicos diante de variações naturais e de impactos antrópicos. Assim, o objetivo deste trabalho foi traçar os caminhos necessários para o delineamento de um *framework* para avaliação da integridade ecológica de riachos, partindo da coleta de dados primários de campo, passando pela modelagem e desenvolvimento de índices, e chegando às aplicações na gestão. Primeiramente, fizemos uma caracterização da área de estudo, avaliando a influência de fatores como a sazonalidade e a distribuição espacial, por bacias hidrográficas e condições de proteção ambiental, sobre as características físicas, químicas e biológicas de riachos de cabeceira pertencentes a três grandes bacias hidrográficas brasileiras (Capítulo I). Demonstramos alterações na composição de duas comunidades biológicas (diatomáceas e macroinvertebrados) e de métricas estruturais e funcionais ao longo de gradientes naturais e de distúrbios antrópicos, possibilitando a identificação de potenciais táxons e métricas indicadoras, bem como limiares ecológicos, para os principais agentes estressores (Capítulos II e III). Por fim, desenvolvemos um índice de saúde de rios que, além de considerar os diversos indicadores de condição ambiental identificados nos capítulos anteriores, também levou em consideração as pressões humanas e as respostas da sociedade/governo, numa abordagem ainda mais integrativa (Capítulo IV). Apesar do grande esforço amostral, do avanço do conhecimento sobre os ecossistemas de riachos, e do *framework* proposto, sabe-se que o universo do monitoramento é ainda maior. Assim, este trabalho não esgota o tema da avaliação de integridade ecológica para a área de estudo e muito menos para outras regiões, mas soma-se ao conhecimento existente e abre inúmeras possibilidades para o futuro.

ABSTRACT

The world has experienced significant transformations and, with them, the impacts of human activities on different biomes and environments become increasingly notorious and worrisome. Aquatic ecosystems are among the most threatened ones, leading to consequences ranging from the loss of biodiversity and changes in the environmental balance to water scarcity scenarios which are related to both quantity and quality of water. Environmental monitoring has always had as one of its main objectives to access the changes that stressors exert on the various elements of ecosystems and, thus, to provide guidelines to management actions. Over the years, the monitoring of aquatic environments has undergone changes, with the focus being shifted from considering water rather as a resource to water being rather seen as part of an ecosystem. The traditional monitoring of physical and chemical variables has been complemented by biological indicators as it is understood that they represent the ending point of a long chain of processes in which the ecosystem is inserted. Thus, biological indicators may reflect the sum of pressures' effects to which the ecosystem is exposed, and in a less oscillatory way than many water quality variables. Despite scientific advances on the subject and the example of many countries and regions in the world where this integrative approach is already applied, even at the level of government monitoring programs, many regions, especially tropical countries such as Brazil still carrying out the traditional monitoring. Considering the relevance of Brazil in the global panorama of water resources and biodiversity, it is urgent that advances in methods for monitoring aquatic environments are implemented. Therefore, it is necessary the knowledge about the response of biological assemblages and ecosystem processes to natural variables and human impacts. Thus, the objective of this study was to trace the necessary paths for the elaboration of a stream ecological integrity assessment framework from the collection of primary field data, passing through the modelling and construction of indices, and, finally, heading to applications in water management. Firstly, we characterized the study area, accessing the influence of external factors such as seasonality and spatial distribution, by watersheds and environmental protection status, on abiotic characteristics of headwater streams belonging to three large Brazilian watersheds (Chapter I). Then, we demonstrated changes in the composition of two biological assemblages (diatoms and macroinvertebrates) and structural and functional metrics along natural and anthropogenic disturbances gradients, enabling the identification of potential taxa and indicator metrics, as well as ecological thresholds for the main stressors (Chapters II and III). Finally, we developed a river health index that, in addition to considering the bioindicators identified in the previous chapters, also took into account human pressures and the responses of society/government, in an even more integrative approach (Chapter IV). Despite the significant sampling effort, the advance of knowledge about stream ecosystems and the proposed framework, it is known that the universe of monitoring is even larger. With that in mind, this study does not exhaust the theme of ecological integrity assessment for the study area and much less for other regions, it rather adds itself to that and brings about countless possibilities for the future.

INTRODUÇÃO GERAL

1. Impactos humanos e a evolução do monitoramento de ecossistemas aquáticos

Ecossistemas aquáticos estão entre os mais ameaçados pelas atividades humanas (Gatti 2016). Além de ameaças persistentes, como fragmentação, degradação e perda de habitat, invasões de espécies e poluição, uma série de ameaças emergentes associadas às mudanças climáticas, novos contaminantes e outros estressores humanos estão afetando desproporcionalmente os ecossistemas de água doce em muitas partes do mundo (Vörösmarty et al. 2010; Reid et al. 2019). No Brasil, e em muitas outras economias em desenvolvimento na região tropical, as ameaças humanas à integridade dos ecossistemas de água doce estão aumentando rapidamente devido às altas densidades populacionais urbanas, deficiência no saneamento, mineração e remoção de vegetação nativa para pastagens e cultivos em larga escala (Ríos-Touma & Ramírez 2018).

Diante desta realidade, o monitoramento torna-se imprescindível, tanto para avaliar se um agente estressor afetou ou não o ambiente, quanto para determinar quais componentes são afetados e estimar a magnitude dos efeitos. No entanto, avaliar as mudanças nas condições ambientais é muitas vezes difícil, não ficando claro qual componente ambiental foi afetado pelo estressor e que tipo de mudança ocorreu (Smith 2014). Na tentativa de buscar entender e medir as consequências das atividades humanas, pesquisadores vêm desenvolvendo índices/modelos baseados tanto em aspectos estruturais quanto funcionais dos ecossistemas aquáticos (Schiller et al. 2017). Aspectos estruturais se referem à qualidade da água, habitat e composição das comunidades biológicas, enquanto aspectos funcionais estão relacionados aos processos que determinam os fluxos de energia e matéria nos ecossistemas (Tilman et al. 2014). Há mais informações sobre elementos estruturais do que funcionais e, apesar das muitas abordagens disponíveis para medir os processos ecossistêmicos fluviais, as abordagens estruturais são mais amplamente utilizadas, especialmente na gestão dos recursos hídricos (Young et al. 2008; Schiller et al. 2017).

Dentre os indicadores estruturais, destacam-se os parâmetros físicos e químicos da água, que compõem diversos índices amplamente utilizados em todo o mundo (Ewaid 2016; Pacheco et al. 2017; Wu et al. 2018), inclusive no Brasil. O Índice de Qualidade das Águas (IQA) foi criado em 1970, nos Estados Unidos, pela *National Sanitation Foundation*. A partir de 1975 começou a ser utilizado pela CETESB (Companhia Ambiental do Estado de São Paulo), e nas décadas seguintes outros Estados brasileiros adotaram o IQA, que hoje é o principal índice de qualidade da água bruta utilizado no país (ANA 2017).

A partir da década de 90, muitos programas de monitoramento mudaram seu foco de medidas principalmente físicas e químicas para a inclusão de mais medidas biológicas, considerando que a biota é geralmente o ponto final na sequência de efeitos da degradação/poluição sobre os ecossistemas aquáticos (Norris & Thoms 1999). Comunidades biológicas que vêm sendo amplamente estudadas são, por exemplo, os macroinvertebrados aquáticos (Ferreira et al. 2011; Baptista et al. 2013; Brito et al. 2020), os peixes (Karr 1981; Authman et al. 2015; Teresa & Casatti 2017) e as diatomáceas (Torrisi & Dell'Uomo 2006; Oeding & Taffs 2017; Pardo et al. 2018).

A respeito dos indicadores funcionais, são escassos para a maioria dos ecossistemas em países do mundo todo (Schiller et al. 2017), embora avaliar a resposta do funcionamento do ecossistema aos estressores seja fundamental para entender os efeitos sobre os serviços ecossistêmicos que produzem benefícios diretos para os seres humanos. Medições de processos ecossistêmicos não deveriam ser negligenciadas, uma vez que são sensíveis a fatores que são conhecidos por influenciar diretamente a saúde do rio, sendo possível, a partir deles, desenvolver modelos preditivos simples, mas poderosos (Bunn 1995). Indicadores funcionais podem ser baseados em processos como decomposição (Gulis et al. 2006; Chauvet et al. 2016), respiração, produtividade primária (Houser et al. 2005) e até na combinação de resposta dos diversos processos (Feio et al. 2010; Silva-Junior et al. 2014).

Quando são mínimas as atividades humanas em uma bacia hidrográfica, a biota é determinada pela interação da biogeografia e processos evolutivos no contexto climático e geológico local, se afastando das condições originais à medida que aumentam os impactos (Figura 1; Karr 1999). A expressão “saúde de rio” passou a ser utilizada neste contexto, embora haja divergências em relação à adequabilidade de seu uso (Karr 1999). Segundo Karr (1986) um ecossistema pode ser considerado saudável quando sua condição é estável, sua capacidade de resiliência preservada e o mínimo de apoio externo para seu manejo é necessário. Daí também surgiu a expressão “integridade ecológica” (IE) que, segundo Schallenberg et al. (2011) é o grau em que os componentes físicos, químicos e biológicos (incluindo composição, estrutura e processos) de um rio estão presentes, funcionando e sendo mantidos próximos à uma condição de referência que reflete impactos antropogênicos mínimos ou insignificantes.

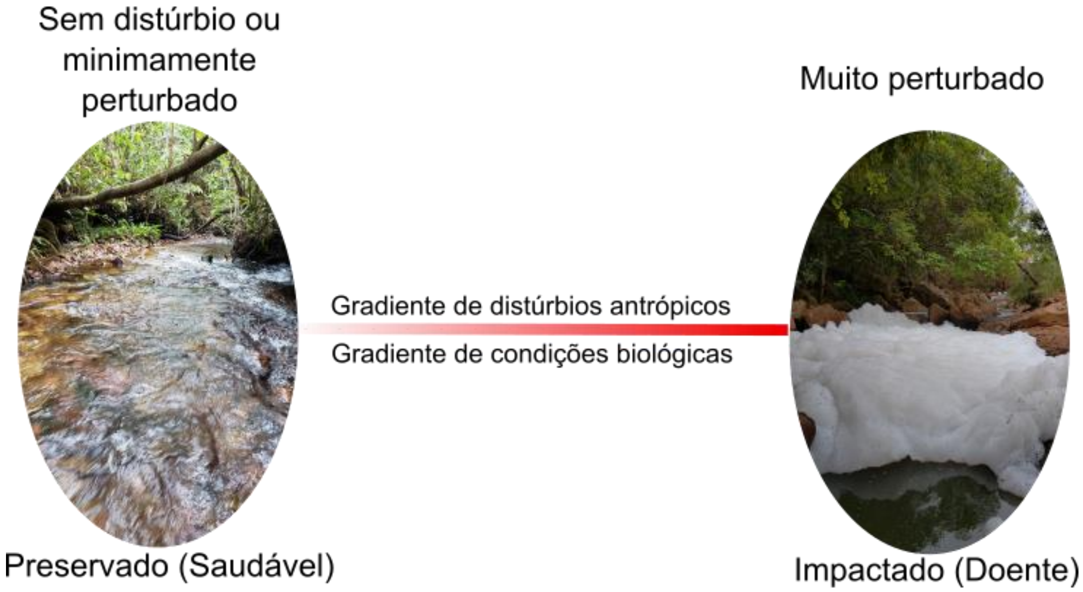

Figura 1: Gradiente de distúrbios antrópicos e de condições biológicas na avaliação da "saúde" ou integridade ecológica dos corpos hídricos. Adaptado de Karr (1999).

Duas metodologias vêm sendo utilizadas para acessar a saúde dos ecossistemas aquáticos: modelagem multimétrica e preditiva (Wang et al. 2019). O primeiro é baseado em índices bióticos incluindo diferentes aspectos (p. ex. diversidade, proporção de táxons, sensibilidade à poluição) (Luo et al. 2018). No segundo, a integridade ecológica de alguns rios é avaliada pelo desvio da composição de comunidades biológicas daquela que seria esperada na ausência de interferências humanas (Reynoldson et al. 1995). Seja pela abordagem multimétrica ou preditiva, muitos países e regiões do mundo têm aplicado sistematicamente o biomonitoramento para acessar as condições de saúde dos rios (Singhe & Saxena 2018). A abordagem multimétrica é usada, por exemplo, nos Estados Unidos (USEPA 2017). A modelagem preditiva é a base de alguns modelos como "*River Invertebrate Prediction and Assessment Classification System*" (RIVPACS, Reino Unido, Wright 1995) e "*Australian River Assessment System*" (AusRivAS, Austrália, Simpson & Norris 2000).

2. Histórico da gestão qualidade das águas no Brasil

Historicamente, a relação "sociedade x água" no Brasil passou por algumas mudanças. No período pré-colonial, os indígenas tinham a água como elemento primordial de vida. Algumas tribos até consideravam a água um ser vivo, e era comum haver rituais antes da pesca ou outro usos. Os impactos relatados nos ambientes aquáticos eram mínimos (ANA 2007). Com a colonização portuguesa, a pressão sobre os recursos naturais brasileiros foi acentuada. A organização política e social da colônia estruturava-se em torno da exploração da natureza e do trabalho escravo (Prado Júnior 1977). Segundo Benjamin (1999), em 500 anos de história pós-colonização, a natureza era vista como inimiga e o pensamento dominante era que para se desenvolver era preciso destruir. Da chegada dos portugueses em 1500 aos anos 2000, três fases podem ser identificadas na legislação ambiental brasileira: 1- Fase de exploração: de 1500 ao início da década de 1960. A questão ambiental, legalmente, não existia. A conquista de novas fronteiras (agrícola, pecuária e mineradora) era tudo o que importava na relação entre o homem e a natureza; 2- Fase fragmentária: dos anos 60 aos 80 do século XX. Algumas regulamentações começaram a aparecer, mas mais voltadas para a utilidade dos recursos ambientais do que para sua preservação. 3- Fase holística: a partir do início da década de 1980, com o lançamento da Política Nacional do Meio Ambiente, o meio ambiente passou a ser protegido integralmente, como um sistema ecológico integrado, com autonomia de valorização e garantias de implementação (Benjamin 1999).

A Constituição Federal Brasileira de 1988 estabeleceu em seu artigo 225 que "*todos têm direito a um meio ambiente ecologicamente equilibrado, ..., impondo ao Poder Público e à comunidade o dever de defendê-lo e preservá-lo para as gerações presentes e futuras*" (Brasil 1988). A Lei das Águas, promulgada em 1997 (Lei Federal n° 9433, Brasil 1997), introduziu os instrumentos necessários para a gestão integrada dos recursos hídricos no Brasil e, em relação ao monitoramento da qualidade da água, refere-se ao Enquadramento dos Corpos Hídricos por classes de uso.

Uma normativa do Conselho Nacional do Meio Ambiental (Resolução CONAMA nº 357, Brasil 2005) estabeleceu, para água doce, cinco classes de uso (especial, 1, 2, 3 e 4) e para cada uma os limites para mais de 200 variáveis físicas, químicas e microbiológicas da água. A qualidade da água aceitável para cada classe piora da classe especial até a classe 4. Na última, praticamente não há limites em relação às variáveis consideradas. A proposta de enquadramento dos corpos hídricos é feita pelos Comitês de Bacias Hidrográficas e deve ser baseada nos usos preponderantes pretendidos pela sociedade, tais como abastecimento humano, preservação da vida aquática, irrigação e navegação. Da classe especial até a classe 2, está previsto o uso para preservação/proteção das comunidades aquáticas. Na classe 4, os rios são designados apenas para navegação e composição paisagística. Embora a legislação estabeleça que *"a qualidade dos ambientes aquáticos pode ser avaliada por indicadores biológicos, quando for o caso, utilizando organismos aquáticos e / ou comunidades"*, não há orientação sobre como fazê-lo e quais parâmetros devem ser considerados.

Atualmente, o programa Qualiágua, coordenado pela Agência Nacional de Águas (ANA) promove uma tentativa de incentivar, nivelar e padronizar o monitoramento e a divulgação de dados da qualidade das águas em todo o país, definindo locais de amostragem estratégicos e variáveis mínimas de qualidade da água a serem monitoradas. Apesar das diversas variáveis e índices propostos no escopo do programa, a única abordagem relativa à vida aquática encontra-se no Índice de Proteção da Vida Aquática (IVA) que é baseado na concentração de substâncias que causam efeito tóxico sobre os organismos aquáticos, além do pH e do oxigênio dissolvido, e no estado trófico. Assim, não há avaliação das comunidades biológicas em si nem de qualquer processo ecossistêmico (ANA 2021).

Apesar da falta de referências oficiais, alguns poucos estados brasileiros vêm realizando o monitoramento biológico, principalmente com foco na comunidade de macroinvertebrados, como é o caso no estado de São Paulo (CETESB 2012) e o estado de Minas Gerais, em uma única bacia (Rio das Velhas; IGAM 2021). A escolha dos indicadores biológicos depende do conhecimento que se tem sobre a estrutura e funcionamento dos ecossistemas aquáticos das regiões, sendo fundamental uma etapa "básica" de estudos descritivos e exploratórios anteriormente à fase de aplicação (Buss et al. 2003). Diante da falta de recursos humanos e financeiros por parte de órgãos gestores no Brasil, o papel das universidades e centros de pesquisa é de grande relevância no desenvolvimento de estudos que estabeleçam critérios metodológicos para este tipo de monitoramento (Buss et al. 2003).

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OBJETIVO E ESTRUTURA DA TESE

1. Objetivo

O objetivo geral desta tese foi delinear um *framework* para avaliação da integridade ecológica de riachos, passando pela coleta de dados e modelagem das respostas de comunidades biológicas e processos ecológicos a gradientes naturais e de distúrbios antrópicos, até a proposta de índices e estratégias de gestão.

2. Estrutura da tese

Esta tese foi estruturada seguindo a ordem natural de ampliação do conhecimento e estabelecimento de um modelo para gestão de rios e bacias hidrográficas.

Cada capítulo da tese coincide com fases desejáveis em processos de delineamento de programas de monitoramento, sempre com a etapa seguinte englobando os resultados das etapas anteriores (Figura 2).

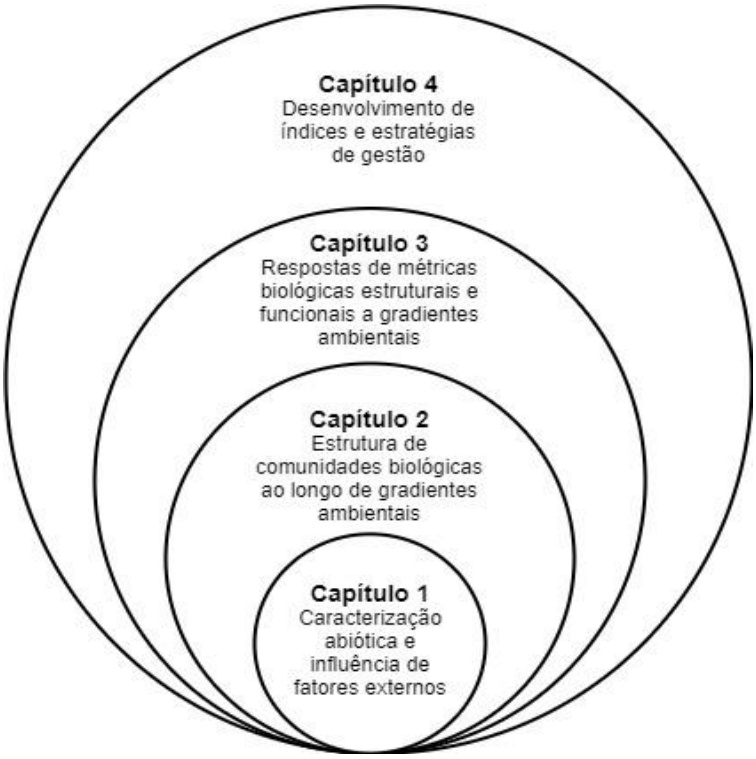

Figura 2. Estruturação dos capítulos da tese.

Capítulo 1: Influência de fatores temporais e espaciais sobre características abióticas de riachos do Cerrado.

Neste primeiro capítulo realizamos análises exploratórias do conjunto de dados de variáveis abióticas produzido ao longo do estudo. O objetivo foi avaliar a influência da distribuição espacial, por bacias hidrográficas e áreas de proteção, e da sazonalidade sobre fatores abióticos de riachos de cabeceira de três grandes bacias hidrográficas brasileiras, discutindo seus impactos para o monitoramento ambiental.

Capítulo 2: Uso das comunidades de Diatomáceas e Macroinvertebrados no apoio a gestão de bacias hidrográficas do Cerrado (título do artigo publicado na língua inglesa: Diatom and Macroinvertebrate assemblages to inform management of Brazilian savanna's watersheds).

Neste capítulo investigamos duas comunidades biológicas relevantes para o funcionamento dos ecossistemas aquáticos, as algas perifíticas do grupo das Bacillariophyta (Diatomáceas) e os macroinvertebrados aquáticos. O objetivo foi identificar alterações na composição das comunidades biológicas ao longo de gradientes naturais e de distúrbios antrópicos, identificando táxons e limiares ecológicos que possam ser aplicados em programas de biomonitoramento.

Capítulo 3: Definição de limiares relacionados a aspectos estruturais e funcionais dos ecossistemas para proteção dos riachos do Cerrado (título do artigo submetido na língua inglesa: Setting thresholds of ecosystem structure and function to protect streams of the Brazilian Savannah).

Neste capítulo modelamos as respostas de métricas biológicas estruturais (ex. índices de diversidade e de sensibilidade à poluição) e funcionais (ex. decomposição, e produção de biomassa algal) a gradientes naturais e de impactos humanos, utilizando a técnica de modelagem do tipo BRT (*Boosted Regression Tree*). Os objetivos foram identificar as métricas mais sensíveis e robustas, em termos de sensibilidade ao estressor, para serem utilizadas como indicadores de integridade ecológica, bem como acessar potenciais limiares nos gradientes ambientais.

Capítulo 4: Desenvolvendo um sistema de avaliação da saúde de rios para águas tropicais (título do artigo na língua inglesa: Progressing a river health assessment framework to tropical waters).

Neste último capítulo integramos os resultados dos capítulos anteriores, e finalizamos a tese com uma abordagem aplicada do conhecimento científico. Os objetivos foram delinear uma metodologia baseada na abordagem P-C-R (pressão, condição, resposta) para avaliar a saúde dos rios, e propor uma metodologia de avaliação da adequação das condições do rio às suas respectivas classes de uso, incluindo os aspectos biológicos atualmente negligenciados pela maioria dos programas de monitoramento no Brasil.

CAPÍTULO I

Influência de fatores temporais e espaciais sobre características abióticas de riachos do Cerrado

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Artigo a ser submetido no periódico *Environmental Monitoring and Assessment*

Resumo

O Cerrado é um bioma de grande relevância no contexto hidrológico e da biodiversidade brasileira. Entretanto, as atividades antrópicas têm colocado em risco o equilíbrio dos ecossistemas nele inseridos, incluindo os aquáticos. Dessa forma, conhecer e monitorar os riachos do Cerrado, especialmente os que ainda guardam muita proximidade com as condições naturais, é valioso tanto do ponto de vista da conservação quanto da gestão. Para se traçar estratégias de monitoramento eficientes, é necessário que haja conhecimento sobre as variações naturais que podem ocorrer em função de fatores como a distribuição espacial e a sazonalidade, além das variações em função do grau de exposição a impactos antrópicos. Neste estudo foram avaliados 52 trechos de riachos de cabeceira pertencentes a três grandes bacias hidrográficas brasileiras, localizados na porção central do Cerrado Brasileiro. Os locais de estudo foram classificados de acordo com o status de proteção ambiental (protegidos/não protegidos) e duas coletas foram realizadas nos períodos de chuva e estiagem de 2018. Os resultados demonstraram que o status de proteção ambiental foi o fator que mais contribuiu para as variações observadas nos locais de estudo, seguido pela distribuição por bacias hidrográficas. A sazonalidade teve influência apenas sobre a vazão. A bacia do Paranaíba apresentou o maior percentual de urbanização e todos os lançamentos pontuais de esgotos tratados. Isso foi refletido nos parâmetros de qualidade de água, que apresentaram as maiores diferenças entre locais protegidos e não protegidos nesta bacia. Na bacia do Preto, essencialmente agrícola, foram observados valores mais elevados de alguns parâmetros relacionados a degradação da qualidade da água (p.ex. fósforo e condutividade) nas áreas protegidas do que nas áreas agrícolas. A bacia do Maranhão apresentou poucas diferenças expressivas entre locais protegidos e não protegidos para qualidade de água. Os resultados apontam para a importância das áreas de proteção ambiental para a manutenção da qualidade da água, embora o papel das áreas protegidas seja mais evidente em bacias com impactos antrópicos mais intensos, como a bacia do Paranaíba. A pouca influência da sazonalidade sobre os fatores abióticos sugerem que apenas uma coleta anual seria suficiente para caracterização dos riachos da região, gerando economia de tempo e recursos na gestão.

Palavras-chave: Cerrado, sazonalidade, proteção ambiental, bacias hidrográficas, qualidade de água

Abstract

The Cerrado biome has great relevance in the hydrological and biodiversity context of Brazil. However, human activities have threatened the balance of ecosystems inserted into it, including the freshwater environments. Thus, knowledge and monitoring of Cerrado streams, especially those that are still very close to natural conditions, is valuable both from the point of view of conservation and management. To design efficient monitoring strategies, it is necessary to access the natural variations that can occur due to factors such as spatial distribution and seasonality, in addition to variations due to the degree of exposure to anthropogenic impacts. In this study, 52 sections of headwater streams belonging to three large Brazilian watersheds, located in the central portion of the Brazilian Cerrado, were evaluated. The study sites were classified according to their environmental protection status (protected/unprotected) and two samplings' campaigns were carried out in the rainy and dry periods of 2018. The results showed that the environmental protection status was the most relevant factor contributing to the variations observed in the study sites, followed by the distribution by watersheds. Seasonality only influenced the streamflow. Paranaíba watershed presented the highest percentage of urbanization and all point-sources of treated sewage release. This was reflected in the water quality parameters, which showed the greatest differences between protected and unprotected sites. In Preto watershed, essentially agricultural, some parameters related to water quality degradation (e.g., phosphorus and conductivity) presented higher values in protected areas than in agricultural areas. Maranhão watershed showed few significant differences between protected and unprotected sites for water quality. The results point to the importance of environmental protection areas for the maintenance of water quality, although the role of protected areas is more evident in areas with more intense anthropogenic impacts, such as the Paranaíba watershed. The little influence of seasonality on abiotic factors suggests that only one annual collection would be enough to characterize the region's streams, saving time and resources in management.

Key-words: Cerrado, seasonality, environmental protection, watershed, water quality

1. Introdução

O Cerrado é considerado a “caixa-d’água” do Brasil, recebendo este título por abrigar nascentes de rios de grande relevância no cenário nacional. Das doze regiões hidrográficas brasileiras, as águas do Cerrado drenam para oito, sendo fundamentais para as bacias do Paraguai, Parnaíba, São Francisco e Tocantins-Araguaia (Lima & Silva 2007). Além da relevância no contexto hidrológico, os rios do Cerrado formam importantes corredores de dispersão de espécies entre biomas vizinhos, tais como Amazônia e Mata Atlântica (Latrubesse et al. 2019). O Cerrado também é considerado um *hotspot* mundial de biodiversidade, abrigando cerca de 4.800 espécies de plantas e animais vertebrados (Strassburg et al. 2017).

Apesar da grande importância ecológica, o Cerrado é um bioma que vem sendo crescentemente afetado por atividades antrópicas, tendo cerca de 50% de sua vegetação natural removida (Beuchle et al. 2015) e possuindo apenas 19,8% de sua área original livre de perturbações e 7,5% protegida por alguma unidade de conservação (Strassburg et al. 2017). Especialistas alertam para a perda de biodiversidade antes mesmo de sabermos de sua existência (Azevedo-Santos et al. 2021). Nesse contexto, a monocultura de soja representa uma ameaça poderosa ao bioma Cerrado (Fearnside 2001), bem como as plantações de eucalipto, que cobrem mais de 5,5 milhões de hectares no Brasil, sendo a maior parte no Cerrado (IBA 2015). A construção de grandes barragens (Latrubesse et al. 2019) e os impactos advindos do processo de urbanização, assim como o lançamento de esgotos domésticos nos corpos hídricos (Pires et al. 2015) são outros exemplos de ameaças existentes. Além disso, a elevada quantidade de água captada para irrigação promove a redução das vazões dos riachos (Latrubesse et al. 2018), as quais já são naturalmente baixas. Projeções baseadas no modelo de desenvolvimento atual, com uma combinação de proteção limitada e grande pressão da expansão agrícola, preveem que 31 a 34% do Cerrado remanescente deixará de existir até 2050 (Strassburg et al. 2017).

No Cerrado da região central do Brasil, encontram-se riachos de pequena ordem que drenam para três grandes bacias hidrográficas brasileiras (Paranaíba, São Francisco e Tocantins-Araguaia), sendo, portanto, uma região estratégica em termos de conservação dos recursos hídricos e biodiversidade. Sabe-se que um bom monitoramento ambiental consegue embasar medidas de gestão apropriadas e efetivas (Bunn et al. 2010), visando a preservação e/ou recuperação dos ecossistemas aquáticos. Para tanto, é necessário que haja conhecimento sobre as variações naturais que podem ocorrer em função de fatores como a sazonalidade e a distribuição espacial (Rezende et al. 2014; Snell et al. 2018). Além disso, a avaliação das condições de referência, ou seja, condições naturais em locais livre de perturbação (ou com menor perturbação possível) geram informações relevantes para se entender o grau de distúrbio que outros locais se encontram (Hawkins et al. 2010).

A abordagem de condição de referência é amplamente utilizada em diversos lugares do planeta, tais como nos Estados Unidos da América (USEPA 2016), União Européia (União Européia 2000) e Austrália (Bunn et al. 2010), países e regiões que possuem um monitoramento das águas consolidado e contínuo há anos, servindo de exemplo para outros países. Apesar de no contexto da gestão nacional das águas no Brasil o Programa de Estímulo à Divulgação de Dados de Qualidade de Água (Qualiágua - ANA) prever amostragem em locais mais preservados, a resolução que estabelece os valores de referência para os diversos parâmetros de qualidade da água não faz distinção entre as diversas ecoregiões brasileiras e tampouco entre bacias hidrográficas (Resolução CONAMA nº 357/2005, Brasil 2005). Em um país de dimensões continentais, como é o caso do Brasil, isso pode comprometer a definição de limiares compatíveis com as características regionais (Feio et al. 2009).

Alguns estudos têm demonstrado as diferenças da qualidade da água em riachos de locais preservados e impactados (Silva et al. 2011; Muniz et al. 2012; Fonseca & Mendonça-Galvão 2014; Fonseca et al. 2014) na região central do Cerrado, relatando os baixos valores de nutrientes quando comparado a outras regiões (Fonseca et al. 2014). Apesar da relevância de tais estudos, existe uma lacuna de informações no que diz respeito ao efeito de fatores como a sazonalidade e da distribuição espacial por bacias hidrográficas e locais com diferentes status de preservação ambiental sobre as características físicas e químicas das águas dos riachos da região, bem como sobre características gerais de habitat, hidromorfologia e uso do solo. Informações de riachos de cabeceira de pequena ordem são de grande relevância para o contexto das águas do país, uma vez que os impactos ocorridos nestes locais são refletidos em trechos/rios de maior ordem (Morgan et al. 2013), muitos deles em outras unidades da federação e/ou biomas. Além disso, este conhecimento é fundamental no delineamento de programas de monitoramento eficientes e com custo-benefício otimizado.

Diante do exposto, o objetivo deste estudo foi avaliar a influência da distribuição espacial, por bacias hidrográficas e condição de proteção ambiental, e da sazonalidade sobre fatores abióticos de riachos de cabeceira de três grandes bacias hidrográficas brasileiras, discutindo seus impactos para o monitoramento ambiental.

2. Metodologia

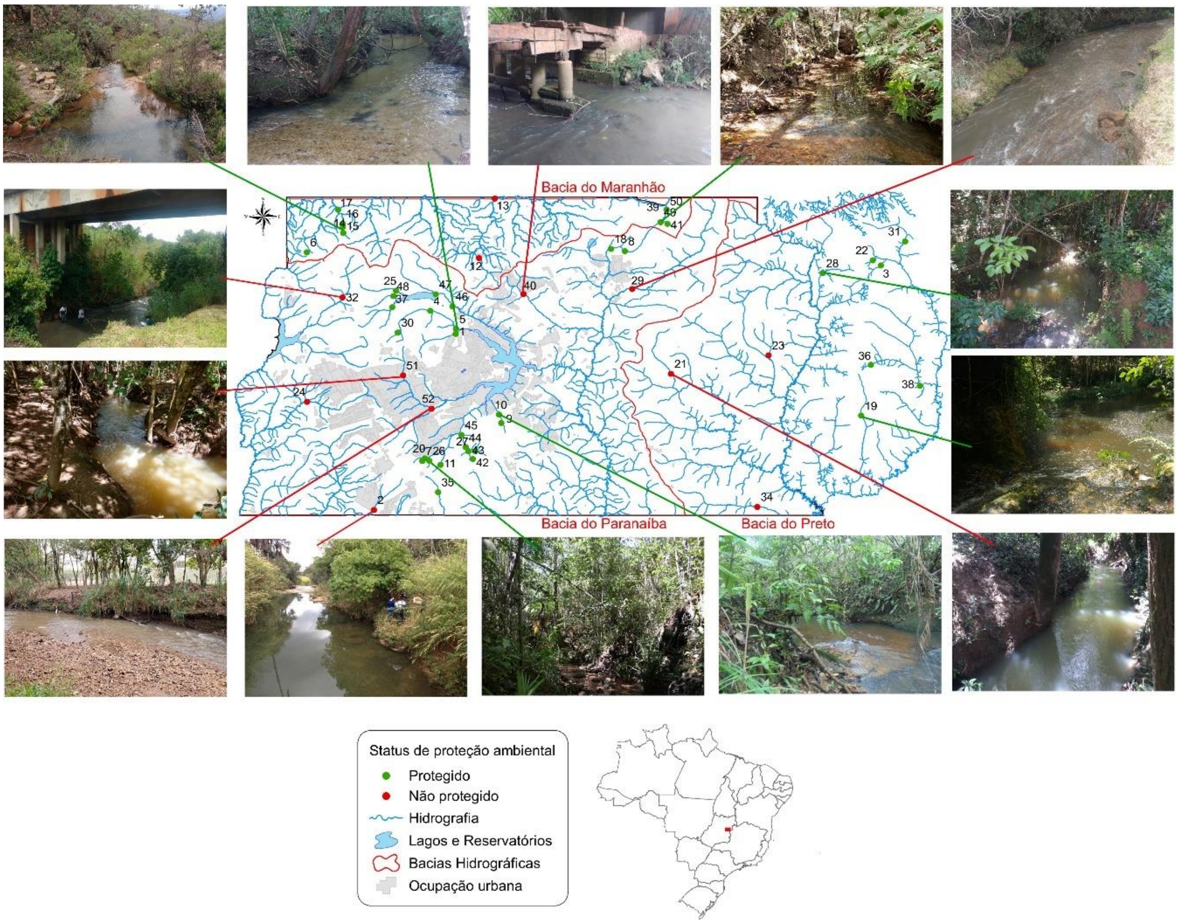
2.1. Área de estudo

A região de estudo foi o Distrito Federal e parte do entorno, totalizando uma área de cerca de 7000 km^2 no planalto central brasileiro. A região é caracterizada por uma malha hidrológica de muitas nascentes, córregos e pequenos rios e dá origem a cursos d'água de grandes bacias hidrográficas brasileiras e do continente sul-americano (Lima & Silva 2007).

Toda área de estudo pertence a uma única Unidade Ecológica - isto é, área com as mesmas características dominantes em termos de fisionomia e fenologia da vegetação, topografia e drenagem (Silva et al. 2006). A Unidade é caracterizada por savanas de planalto elevado (acima de 600 m a.s.l.), vegetação nativa dominante do tipo savana densa (cerrado), mas também encontradas savanas abertas (campo cerrado), matas de galeria e algumas matas decíduas. A paisagem é principalmente plana, mas muda para terreno ondulado em direção às bordas mais erodidas dos planaltos (Silva et al. 2006). O clima é caracterizado por períodos marcantes de chuva e de estiagem, com temperatura média e precipitação anual em torno de 20 °C e 1700 mm, respectivamente, mas com distribuição espacial desigual da precipitação na área (INMET 2021).

Foram selecionados para o estudo 52 trechos de riachos abrangendo três bacias hidrográficas: Maranhão (afluentes da bacia do Tocantins-Araguaia), Paranaíba e Preto (afluentes da bacia do São Francisco) (Figura 1), sendo 40 trechos localizados dentro de áreas protegidas (parques, estação ecológica, reservas) e 12 em áreas não protegidas. Apesar das diferenças de tamanho, todos os cursos d'água podem ser classificados como riachos (Chapman 1996) e são atravessáveis sem a necessidade de embarcação (fotos Figura 1).

As três bacias hidrográficas possuem diferentes usos do solo que variam desde áreas com predominância de vegetação natural (como é o caso da bacia do Maranhão), até áreas essencialmente agrícolas (bacia do Rio Preto) e com elevada ocupação urbana (bacia do Paranaíba). Além dos impactos provocados pela alteração na cobertura vegetal natural, os rios da região sofrem com interferências humanas diretas como a construção de barragens de diversos tamanhos, canalização de rios, mineração e, principalmente o lançamento de esgotos tratados e não tratados (GDF 2012; GDF 2020).



Código	Nome	Código	Nome	Código	Nome	Código	Nome
1	Acampamento	14	Dois Irmãos (I)	27	Onça (IBGE)	40	Sobradinho
2	Alagado	15	Dois Irmãos (II)	28	Pindaíba	41	Tabatinga
3	Areia	16	Dois Irmãos (III)	29	Pipiripau	42	Taquara (I)
4	Bananal (I)	17	Dois Irmãos (IV)	30	Poco D'água	43	Taquara (II)
5	Bananal (II)	18	Fumal	31	Poções	44	Taquara (III)
6	Barreiro	19	Fundo	32	Rodeador	45	Taquara (IV)
7	Bonito	20	Gama	33	Roncador	46	Torto
8	Brejinho	21	Jardim	34	São Bernardo	47	Três Barras
9	Cabeça de Veado (I)	22	João Pires	35	Saia Velha	48	Vargem Grande
10	Cabeça de Veado (II)	23	Lagoinha	36	Salobra	49	Vereda Grande (I)
11	Capetinga	24	Melquior	37	Santa Maria	50	Vereda Grande (II)
12	Contagem (I)	25	Milho Cozido	38	Santo Inácio	51	Vicente Pires (I)
13	Contagem (II)	26	Onça (FAL)	39	Serrinha	52	Vicente Pires (II)

Figura 1. Área de estudo e os 52 locais de coleta, com cores de acordo com o status de proteção ambiental (protegidos – verde, e não protegidos - vermelho). As três bacias hidrográficas (Paranaíba, Maranhão – afluentes do Tocantins-Araguaia, e Preto – afluentes do São Francisco) estão delimitadas pela linha vermelha. Fotos de alguns locais estão relacionadas aos pontos de coleta com setas verdes (locais protegidos) e vermelhas (locais não protegidos). Pontos de coleta em um mesmo curso d'água estão descritos por numeração sequencial de montante para jusante. FAL = Fazenda Águas Limpas; IBGE = Reserva Ecológica do IBGE (Instituto Brasileiro de Geografia e Estatística).

2.2. Desenho amostral de campo

Duas campanhas de campo foram realizadas para coleta de dados, a primeira nos meses de abril/maio de 2018 (final da estação chuvosa) e a segunda nos meses de agosto/setembro 2018 (final da estação de estiagem). Todos os locais foram amostrados em ambas as campanhas, com exceção do local 22 que se encontrava seco ao fina do período de estiagem.

Parâmetros físicos e químicos da água, e vazão, foram medidos nos quatro meses (dois meses de cada estação). Apenas em abril foram coletados sedimentos para análise granulométrica.

2.2.1. Coleta de água e medição de parâmetros físicos e químicos

Foram coletados cerca de 300mL amostras de água sub-superficiais de cada curso d'água. Além disto, foram filtrados, em campo, 30 mL de água, distribuídos em dois tubos Falcon de 15 mL. A filtração foi realizada com o auxílio de uma seringa de 20 mL, adaptador de filtração e filtros de celulose com porosidade de 0.22 µm. Os frascos e filtros foram mantidos resfriados e protegidos da luz até a chegada ao laboratório.

Os parâmetros oxigênio dissolvido e temperatura da água foram medidos *in loco*, com o auxílio sondas portáteis.

2.2.2. Caracterização hidromorfológica, granulometria dos sedimentos e sombreamento da mata ciliar

A vazão foi medida com auxílio de fluxômetro e trena. A seção escolhida para medição da vazão foi a mais reta e uniforme possível e a metodologia escolhida foi a da seção média, conforme Santos et al. (2001). As medições foram feitas em cada ocasião de amostragem, exceto quando as altas velocidades da água impediram. Nestes casos, os valores de vazão foram obtidos junto à Agência Reguladora de Águas, Energia e Saneamento Básico do Distrito Federal (Adasa). Os resultados para cada estação do ano foram gerados pela média dos valores encontrados nos dois meses de coleta.

Amostras de sedimento foram recolhidas, com auxílio de um tubo de PVC, em três pontos do segmento de rio (até 25 m acima ou abaixo da seção de medição da vazão). Os pontos foram selecionados de modo a representar os diferentes substratos existentes. A presença de grandes pedras ou lajes foi apenas descrita na ficha de campo.

Para o estudo foi considerada apenas a camada superficial de sedimentos (0 – 10 cm). O material coletado foi armazenado em sacos plásticos e encaminhado ao laboratório.

O sombreamento da vegetação ciliar sobre o leito dos corpos hídricos foi estimado visualmente e alocado em uma escala ordinal de 0 a 3 para uma das seguintes faixas de percentual de sombreamento: 0% - 0; 1-30% - 1; 30-60% - 2; > 60% - 3.

2.3. Análises laboratoriais

2.3.1. Análises físico-químicas da água

Imediatamente ao chegar no laboratório, os 300 mL de água bruta coletados foram utilizados para a análise de pH, turbidez e condutividade, por meio de sondas de bancada (equipamentos das marcas Jenway, Quimis e Metohm, respectivamente).

As amostras filtradas em campo foram analisadas em cromatógrafo Metrohm, utilizando colunas específicas para cátions (potássio), ânions (cloreto, nitrito, brometo, nitrato, fosfato, sulfato) e metais (ferro). A determinação do cátion foi feita na coluna MetroSep C4 250 / 4.0 mm utilizando ácido nítrico 1,7 mM / ácido dipololínico 0,7 nM como eluente com detecção de condutividade não suprimida. A determinação da amostra de ânions foi feita na coluna MetroSep A Supp 5 250 / 4,0 mm usando 3,2 mmol / L de carbonato de sódio e 1 mmol/L de bicarbonato de sódio como eluente, com detecção de condutividade suprimida. Os resultados para cada estação do ano foram gerados pela média dos valores encontrados nos dois meses de coleta.

2.3.2. Análise granulométrica dos sedimentos

A granulometria dos sedimentos do leito do rio foi analisada pelo método de peneiramento (Suguio 1973). As amostras foram secas ao ar por pelo menos 72h e, após a secagem, foram homogeneizadas e uma subamostra de aproximadamente 100 g foi incinerada em mufla a 550°C por 4 h. A diferença de peso entre a pré e pós-secagem foi utilizada para determinar a porcentagem de matéria orgânica em cada subamostra. Cada subamostra seca foi então peneirada por 15 min, passando por peneiras de diferentes diâmetros de malha (2 mm, 1 mm, 0,710 mm, 0,500 mm, 0,355 mm, 0,250 mm, 0,180 mm, 0,125 mm, 0,090 mm e 0,063 mm) com a ajuda de um agitador automático. Cada fração de amostra foi pesado e os resultados foram expressos como porcentagem do peso total da subamostra. As amostras de sedimentos foram agrupadas em 5 categorias: areia grossa (>2 - 0,710 mm), areia média (0,500 - 0,250 mm), areia fina (0,180 - 0,125 mm), areia muito fina (0,090 - 0,063 mm) e silte / argila (< 0,063 mm).

2.4. Análises geoespaciais – definição de características fisiográficas e de distúrbios humanos

As variáveis naturais foram definidas utilizando-se Sistema de Informações Geográficas (SIG). Um Modelo Digital de Elevação (resolução de 30 m) foi usado para delinear as bordas da área de drenagem a montante, usando as ferramentas de hidrologia no ArcMap 10.4 e dados topográficos do Banco de Dados Geomorfométrico do Brasil (TOPODATA; INPE 2016) em uma escala de 1: 50.000. Cada local de amostragem foi tratado como um ponto de saída. A partir desse modelo, extraímos a área de drenagem a montante, a declividade média do rio até o ponto de coleta, a elevação e a distância da nascente para cada local de amostragem.

Os dados do tipo de solo e do grupo geológico para o Distrito Federal e entorno foram junto à Agência Reguladora de Água, Energia e Saneamento Básico do Distrito Federal (Adasa). Ambos foram determinados em escala local, usando o ArcMap 10.4. Os três grupos geológicos presentes na área de estudo foram Bambuí, Paranoá e Canastra; e os três tipos de solos foram latossolo vermelho, plintossolo e cambissolo.

Os principais distúrbios antrópicos na área de estudo são a conversão da cobertura natural do solo para a agricultura, desenvolvimento urbano, mineração, lançamento de esgoto pontual (tratado ou não); e a presença de barragens (GDF 2012). Para avaliar o uso do solo, estabelecemos dois *buffers*: a área de drenagem e o corredor de mata ripária a montante do ponto de coleta (Figura 2).

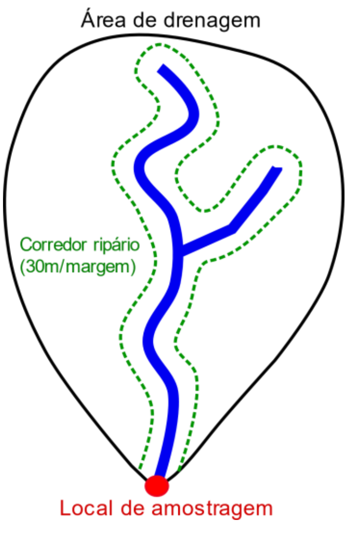


Figura 2. Representação gráfica dos *buffers* considerados neste estudo: corredor ripário (30 m de cada margem) e área de drenagem a montante do local de amostragem.

O Código Florestal Brasileiro (Lei Federal 12.651 / 2012, Brasil 2012) determina que a extensão da mata ripária depende da largura do rio. Rios com até 10 m de largura devem ter, no mínimo, 30 m de corredor ripário em cada margem. Rios com larguras maiores devem apresentar corredores proporcionalmente mais largos. Uma vez que a maioria dos rios na área de estudo apresentam larguras abaixo de 10 m, as análises de uso do solo na mata ripária cobriram 30 m de cada margem. Calculamos a proporção de cada tipo de uso do solo na área de drenagem a montante e no corredor ripário de cada local de amostragem usando um arquivo *shapefile* de usos do solo para o Distrito Federal (Reis & Lima 2015) e fotointerpretação da imagem de satélite RAPIDEYE com resolução de 5 metros para o estado de Goiás. Os usos do solo na área de drenagem e no corredor ripário foram agrupados em: urbano (ocupações urbanas consolidadas), agrícola (qualquer tipo de agricultura e pastagem) e modificado (assentamentos, plantações de eucalipto e solo exposto), além da cobertura vegetal natural.

O número de lançamentos pontuais de esgoto tratado provenientes de Estações de Tratamento de Esgoto (ETEs) e barragens presentes na área de drenagem a montante de cada local de coleta foram obtidos junto à Agência Reguladora de Águas, Energia e Saneamento Básico do Distrito Federal (Adasa).

2.5. Análises de dados

Neste trabalho utilizamos diversas ferramentas estatísticas, especialmente gráficas, tais como boxplots, para identificar e explorar os padrões existentes nos dados com relação às bacias hidrográficas (Paranaíba, Maranhão e Preto), estações do ano (chuva e estiagem) e status de proteção ambiental (protegido e não protegido). Também foram utilizados testes complementares para avaliar diferenças entre dois (*t* de Welch; Delacre et al. 2017) ou mais (ANOVA) grupos, e teste de correlação (Pearson) para analisar o grau de correlação entre métricas.

Análises de componentes principais (PCA) foram utilizadas para avaliar a distribuição dos locais de estudo em função das variáveis físicas e químicas da água, bem como a redundância entre variáveis, formação de gradientes e visualização de possíveis grupos. Por se tratar de variáveis com as mais diversas unidades de medida, os dados foram normalizados para a análise. A fim de facilitar a visualização dos resultados, variáveis redundantes ou com pouca contribuição na PCA foram excluídas.

Análises de variância permutacional multivariada (PERMANOVA, distância Bray-Curtis, 999 permutações) foram utilizadas para testar a significância ($p < 0{,}05$) das variações entre os grupos na PCA. Todas as análises foram conduzidas no R v.4.0.3 (R Core Team 2020), utilizando os pacotes psych (Revelle 2021), vegan (Oksanen et al. 2019), ggplot2 (Wickham 2016), FactoMineR (Lê et al. 2008), e factoextra (Kassambara & Mundt 2020).

3. Resultados

3.1. Características fisiográficas, hidromorfológicas e habitat

Para este grupo de características apenas a vazão poderia apresentar diferenças entre as duas estações do ano, considerando que as demais características são estáveis a curto e médio prazo. Em média, a vazão no período chuvoso (0,53 m^3 s^{-1}) foi cerca de três vezes maior do que no período de estiagem (0,17 $m^3.s^{-1}$). Poucos locais apresentaram vazão superior a 1 $m^3.s^{-1}$, sendo as maiores observadas em riachos localizados em áreas não protegidas na bacia do Paranaíba (Figura 3).

Os pontos amostrados apresentaram áreas de drenagem que variaram de 2 a 215 km^2 (média ± DP, 40 ± 49 km^2). A área de drenagem foi correlacionada positivamente com a distância da nascente ($r_{PEARSON}$ = 0,88), que variou de 0,5 a 34 km (8 ± 7 Km). Não foram observadas diferenças para estas métrica entre bacias, mas entre locais protegidos e não protegidos (teste-t_{15} = 2,7 p = 0,02) que apresentaram, em média, áreas de drenagem de 30 e 76 Km^2, respectivamente. A altitude variou entre 744 e 1220 m (1014 ± 97 m). Essa característica não apresentou diferenças significativas entre locais protegidos ou não protegidos, mas entre bacias (ANOVA F_2 = 15 $p < 0{,}001$). As menores altitudes foram registradas na bacia do Preto (895 m) e as maiores na bacia do Paranaíba (1055 m). Do mesmo modo, o declive dos riachos também apresentou diferença entre bacias (ANOVA F_2 = 11 $p < 0{,}001$), mas não entre o status de proteção ambiental. O declive médio na bacia do Maranhão (4,8°) foi superior aos das bacias do Paranaíba (2,7°) e do Preto (2°).

Em geral, os locais apresentaram solo do tipo latossolo (S1). Apenas 5 locais apresentam tipos de solo plintossolo (S2), todos pertencentes à bacia do Maranhão, e 1 local com solo do tipo cambissolo (S3), na bacia do Preto. Em relação ao grupo geológico, 81% dos locais pertencem ao grupo Paranoá (G1), nove ao grupo Bambuí (G3, todos localizados na bacia do Preto), e apenas uma estação encontra-se no domínio Canastra (G2, localizada na bacia do Maranhão).

Trinta e três locais de coleta apresentaram cobertura de mata ciliar superior a 60%. Apenas 3 locais na bacia do Paranaíba (dois não protegidos e um protegido) tiveram cobertura de 0% (isto é, ausência total de mata ciliar).

Os leitos dos riachos foram predominantemente compostos por areia grossa (> 2 a 0,71 mm; Figura 4). A proporção de matéria orgânica foi significativamente superior nos locais preservados do que nos não preservados (6% *versus* 3%; teste-t_{49} = -3,4 p = 0,001). Não houve diferenças significativas entre as bacias.

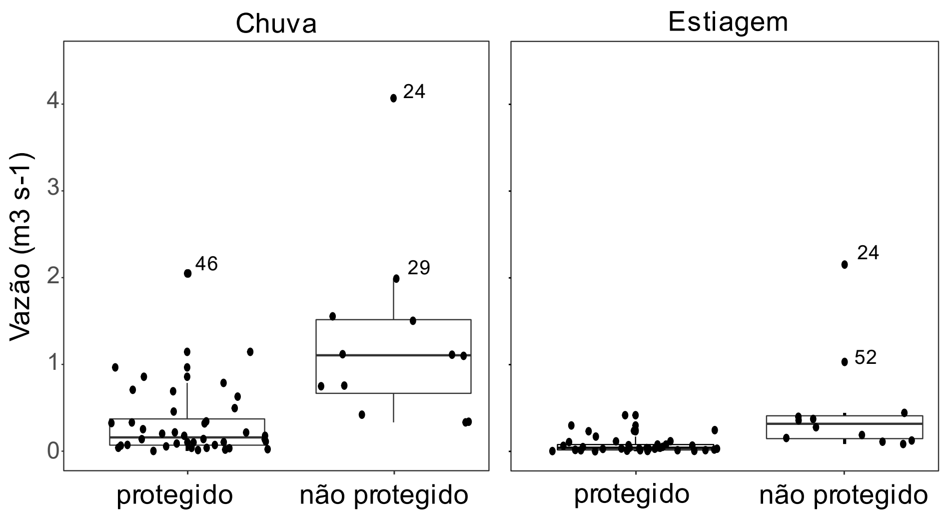


Figura 3. Vazões (m^{3}.s^{-1}) nos períodos de chuva e estiagem em locais protegidos e não protegidos. Em destaque os códigos dos locais que apresentaram as maiores vazões observadas em cada estação.

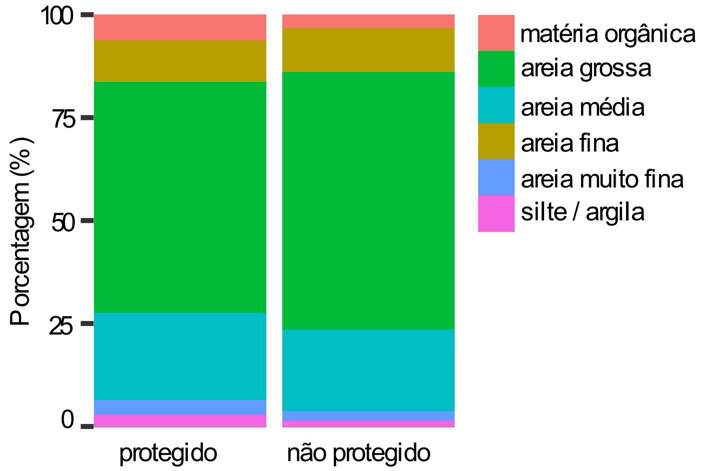


Figura 4. Granulometria dos sedimentos e percentual de matéria orgânica no leito dos riachos de locais protegidos e não protegidos.

3.2. Uso do solo e interferências humanas

O uso do solo e interferências humanas, assim como a maior parte das características fisiográficas e de habitat não apresentam variações em relação à estação do ano, uma vez que a coleta foi realizada em um único ano.

A vegetação natural foi predominante no corredor ripário, representando em média 90% da área total, enquanto nas áreas de drenagem a montante ela representou 70% (Figura 5A). A ocupação agrícola foi o principal uso antrópico tanto na área de drenagem (21%) quanto no corredor ripário (8%), seguida pela ocupação urbana, representando 8% da área de drenagem e 1% do corredor ripário. Os maiores percentuais de ocupação urbana (> 25%) na área de drenagem encontram-se nos pontos de coleta da bacia do Paranaíba, bem como os poucos locais que apresentaram algum grau de urbanização (> 10%) no corredor ripário (Figura 5B). Enquanto a bacia do Paranaíba englobou os maiores percentuais de ocupação urbana, a bacia do Preto apresentou quase 50% das áreas de drenagem ocupadas pela agricultura. A bacia do Maranhão foi a que apresentou maior percentual de áreas com vegetação natural (Figura 5A).

Foram detectados lançamentos regulares de esgoto tratado provenientes de estações de tratamento à montante de três dos 52 locais de estudo, todos na bacia do Paranaíba. Barragens foram identificadas nas três bacias (Figura 5B).

3.3. Variáveis físicas e químicas da água

A análise de componentes principais (PCA) utilizando dados dos 52 locais de estudo em duas estações do ano resumiu 62% da variabilidade dos parâmetros físicos e químicos da água (Dim 1 = 47% e Dim 2 = 15%; Figura 6). Condutividade, fosfato, turbidez, cloreto, nitrato e potássio determinaram o gradiente principal da PCA (Dim 1), enquanto oxigênio dissolvido, temperatura e pH foram mais correlacionados com a segunda dimensão da PCA (Dim 2).

O status de proteção ambiental (protegido *versus* não protegido) foi responsável pela maior separação dos locais de estudo (baseado no R^2 da PERMANOVA) (PERMANOVA: pseudo-F = 42,8 R^2= 0,21 p = 0,001), em comparação com as bacias (pseudo-F = 3,8 R^2= 0,04 p = 0,02) ou com as estações do ano (pseudo-F = 3,1 R^2= 0,02 p = 0,06). Quando analisadas separadamente cada bacia, houve diferenças significativas entre locais protegidos e não protegidos em todas elas (Figura 6).

A bacia do Paranaíba foi a única que apresentou diferenças significativas entre locais protegidos e não protegidos para os parâmetros fosfato (teste-t_{13} = -3,8 p = 0,002) e nitrato (teste-t_{13} = -3,5 p = 0,003). A condutividade foi significativamente distinta entre locais protegidos e não protegidos nas três bacias, entretanto, apenas na bacia do Preto ela foi maior nos locais protegidos do que nos não protegidos (Figura 7).

Potássio, oxigênio dissolvido e pH não apresentaram diferenças significativas entre bacias e status de proteção ambiental. Valores mais elevados de potássio foram observados em locais não protegidos na bacia do Paranaíba. Outliers identificados para oxigênio dissolvido (valores < 4 mg.L^{-1}) representam duas amostras de local protegido da bacia do Maranhão e duas amostras de local não protegido da bacia do Paranaíba. O pH foi ligeiramente mais próximo de neutro nos locais não protegidos. O cloreto foi diferente entre locais protegidos e não protegidos nas bacias do Maranhão e do Paranaíba, mas com as maiores diferenças sendo observadas na última (teste-t_{13} = -2,9 p = 0,01) (Figura 7).

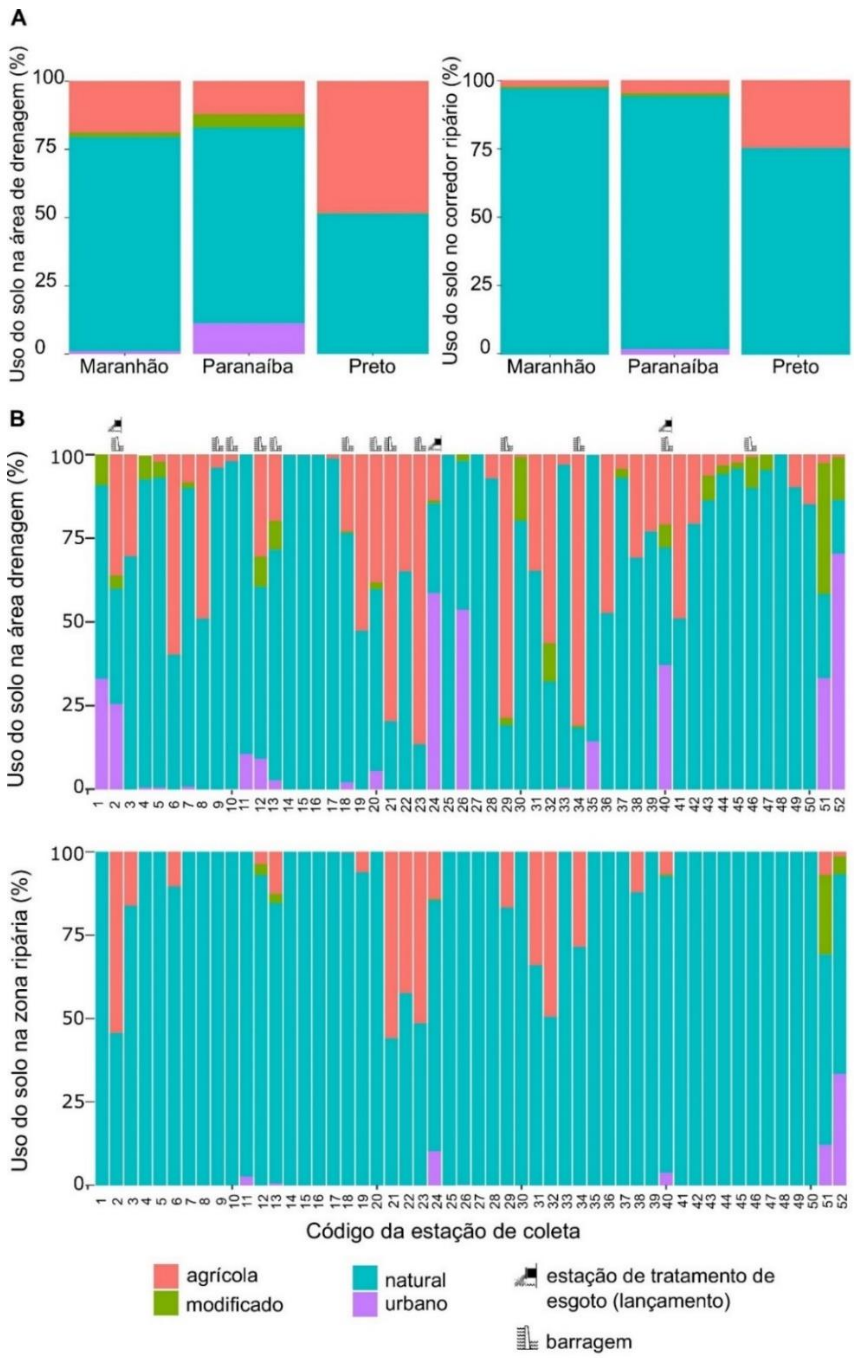


Figura 5. (A) Percentual de usos do solo na área drenagem e no corredor ripário por bacia hidrográfica, agrupados em quatro classes: natural, urbano, agrícola e modificado. (B) Percentual de usos do solo na área drenagem e no corredor ripário, e presença de lançamentos provenientes de estações de tratamento de esgoto e barragens por local de coleta.

Considerando que as maiores diferenças foram observadas entre locais protegidos e não protegidos, a Tabela 1 resume os valores medianos para cada parâmetro de acordo com o status de proteção ambiental.

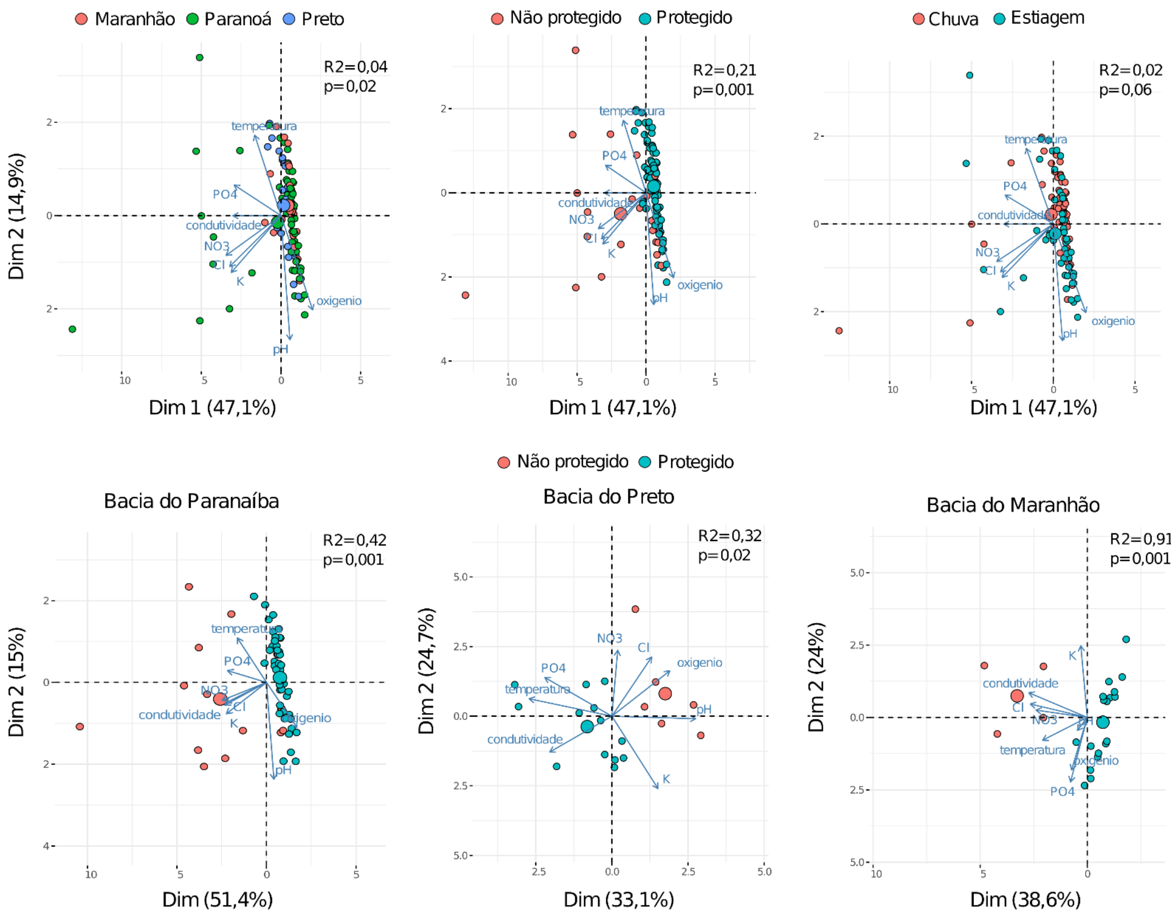


Figura 6. Distribuição dos pontos de amostragem em relação às variáveis físicas e químicas da água e agrupados por bacia (Paranoá, Preto e Maranhão), status de proteção ambiental (protegido e não protegido) e estação do ano (chuva e estiagem). Na segunda linha, locais protegidos e não protegidos por bacia hidrográfica.

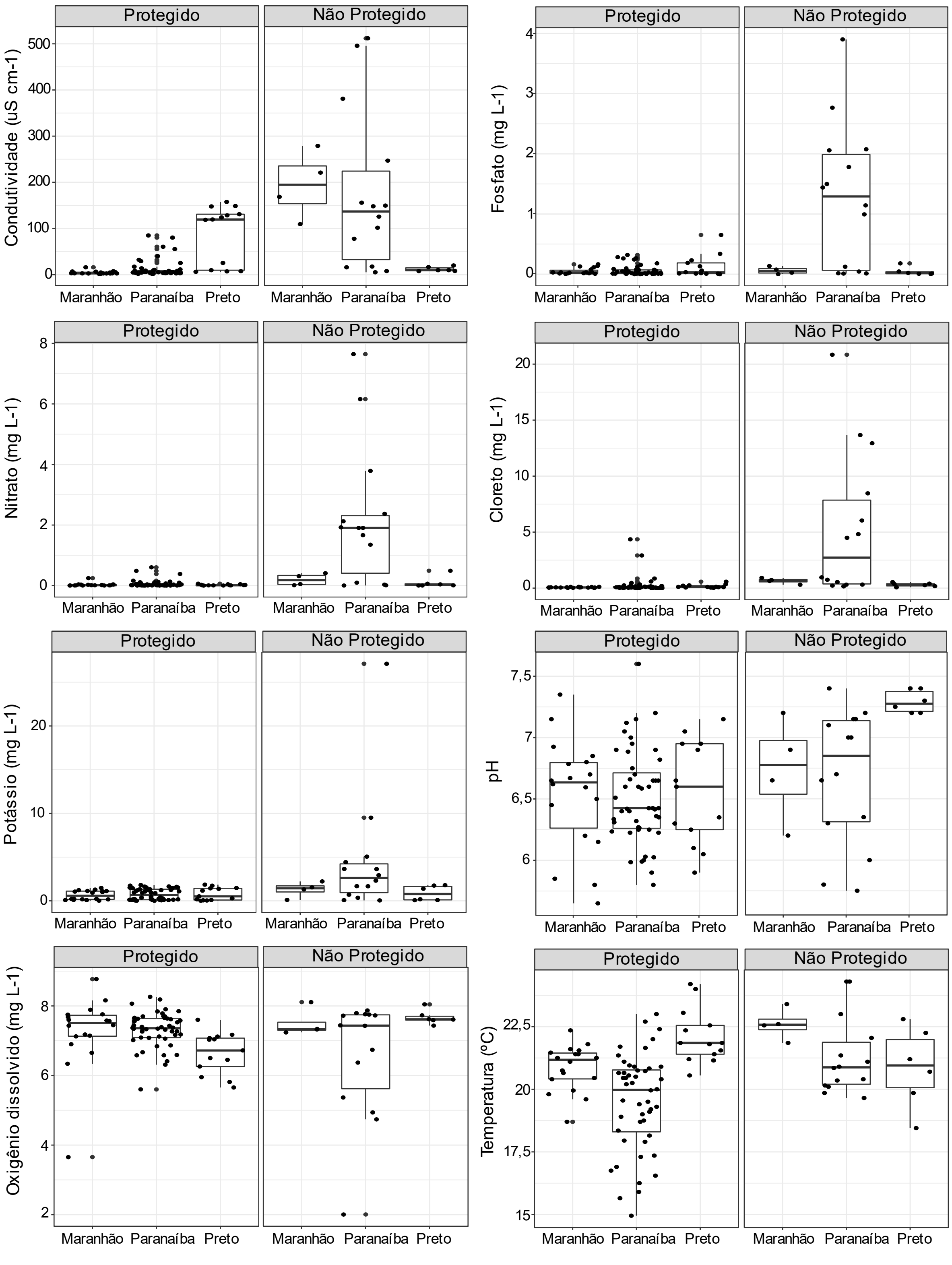


Figura 7. Boxplot das variáveis de qualidade da água para locais protegidos e não protegidos nas três bacias de estudo.

Tabela 1. Mediana de valores das principais variáveis físicas e químicas em locais protegidos e não protegidos considerando todos os locais de coleta e ambas as estações do ano (chuva e estiagem).

Variável	Locais protegidos	Locais não protegidos
Cloreto ($mg.L^{-1}$)	0,08	0,59
Condutividade ($\mu S.cm^{-1}$)	6	105
Fosfato ($mg.L^{-1}$)	0,03	0,1
Nitrato ($mg.L^{-1}$)	0,02	0,36
Oxigênio dissolvido ($mg.L^{-1}$)	7,3	7,5
pH	6,5	7,1
Potássio ($mg.L^{-1}$)	0,51	1,67
Temperatura (ºC)	20,7	21,2

4. Discussão

4.1. Influência do status de proteção ambiental, distribuição espacial e sazonalidade

Os resultados demonstraram variações naturais entre as bacias hidrográficas em relação a altitude, o declive, os tipos de solo e grupos geológicos para alguns locais. Embora essas características naturais tenham importância na determinação de fatores abióticos e bióticos de riachos (Li et al. 2012; Varanka & Luoto 2012), as variações observadas estiveram mais relacionadas ao uso do solo nas áreas de drenagem, que foi o grande diferencial entre as três bacias estudadas. Isto ficou claro quando comparados locais protegidos e não protegidos. As variáveis físicas e químicas apresentaram valores muito próximos entre locais protegidos das três bacias, enquanto as maiores diferenças foram observadas nos locais não protegidos.

A bacia do Paranaíba foi a que apresentou maiores percentuais de ocupação urbana e a que concentrou todos os lançamentos de esgoto. Apesar do percentual de ocupação urbana ter sido bem menor do que o de vegetação natural ou agricultura, os impactos provenientes dela são mais evidentes (Muniz et al. 2012), principalmente nos países em desenvolvimento (Capps et al. 2016). Além da impermeabilização do solo, poluição difusa, alteração do microclima, alterações da morfologia e estabilidade do canal, e acúmulo de lixo, o lançamento pontual de esgotos tratados e não tratados são grandes fontes de matéria orgânica, nutrientes e outros poluentes para os corpos hídricos, levando a alterações evidentes na qualidade da água (Silva et al. 2011) e na biota aquática (Murray et al. 2019). O fato de apenas a bacia do Paranaíba ter apresentado diferenças significativas entre locais preservados e não preservados para os parâmetros fosfato e nitrato, conhecidos indicadores de poluição por esgoto doméstico (Kaushal & Belt 2012; Mushtaq et al. 2019), ressaltam a influência da urbanização da bacia hidrográfica na qualidade das águas de seus riachos. Além disso, a bacia do Paranaíba foi a única que apresentou algum percentual de urbanização no corredor ripário. Apesar dos riachos terem apresentado elevado grau de preservação da vegetação ripária, sabe-se que pequenas modificações nessa vegetação podem resultar em grandes alterações na estrutura e no funcionamento dos ecossistemas aquáticos (Dala-Corte et al. 2019; Campos et al. 2021).

Quando os extremos de condições ambientais (preservados – impactados) são bem definidos, os resultados observados tendem a ser também mais evidentes (Stoddard et al. 2008). A bacia do Maranhão, mais preservada e que sofre impactos menos agressivos, e a bacia do Preto, com predominância de uso agrícola, apresentaram diferenças menos evidentes entre locais protegidos e não protegidos. Na bacia do Maranhão, os valores mais elevados de condutividade nos locais não protegidos podem estar relacionados ao grupo geológico (Canastra), que só foi registrado em um dos pontos não protegidos desta bacia (Rib. Contagem II – Cód. 13) (Netto et al. 2005). Já na bacia do Preto, a área considerada protegida é hoje um centro de treinamento do Exército, não fazendo parte do Sistema Nacional de Unidades de Conservação (SNUC 2000), o que nos leva ao entendimento de que não se trata de uma área livre de perturbações antrópicas, apesar de seu acesso restrito. A inversão dos resultados esperados para algumas variáveis na bacia do Preto, que apresentou valores de condutividade e fosfato mais elevados na área protegida, indica um possível estado intermediário de impacto, tanto na área protegida quanto na não protegida. O distúrbio intermediário, como o incremento não exagerado de nutrientes, pode muitas vezes favorecer algumas condições ecológicas (Budke et al. 2010; Capítulo 2), especialmente em uma região caracterizada por baixos valores dos mesmos (Fonseca & Mendonça-Galvão 2014).

Embora trabalhos tenham demonstrado diferenças significativas em fatores físicos, químicos e biológicos de riachos em função da sazonalidade (Ai et al. 2015; Rodrigues et al. 2018), outros também têm demonstrado ausência desse efeito (Couceiro et al. 2021). Neste trabalho o fator sazonalidade se mostrou influente apenas sobre a vazão dos riachos, que chegou a ser três vezes superior nos períodos de chuva. Os riachos, ou trechos deles, de maiores vazões foram os não protegidos, o que se explica pela tendência ao estabelecimento de áreas de proteção em regiões de nascentes ou bem próximas a elas (Mello et al. 2018). Assim, os trechos mais volumosos de rio tendem a ser os mais expostos aos impactos antrópicos. Riachos com vazões baixas são um desafio para a gestão das águas em termos quantitativos e qualitativos. No aspecto quantitativo, as demandas para abastecimento humano e irrigação podem gerar graves déficits que, quando combinados com estiagens prolongadas, provocam crises hídricas como a que ocorreu entre 2016 e 2018 na região de estudo (Lima et al. 2018).

No aspecto qualitativo, as elevadas vazões/velocidades e escoamento superficial durante as fortes chuvas somadas à reduzida vegetação natural que atuaria retendo o transporte de partículas de solo e poluentes (Taniwaki et al. 2017), podem causar alterações mais significativas na comunidade de macroinvertebrados nos locais não protegidos, tanto devido ao carreamento físico dos organismos quanto pela maior diluição de poluentes (Couceiro et al. 2021). Nos locais protegidos, em que as dimensões dos riachos são menores e onde há maior dependência de recursos externos, variações nas comunidades de macroinvertebrados poderiam estar associadas à disponibilidade de recursos (Couceiro et al. 2021), sendo beneficiadas nos períodos de estiagem em função da maior queda de folhas provocadas pelo estresse hídrico (Tonin et al. 2017).

Esta relação dos riachos de menores dimensões com a vegetação ripária fica evidente também pelos resultados de percentual de matéria orgânica no leito, o qual foi duas vezes maior em locais protegidos do que em locais não protegidos. Esse resultado suporta a relevância da preservação da mata ripária, uma vez que esta matéria orgânica alóctone é a principal fonte de energia em córregos de cabeceiras, sendo fundamental para organismos que dependem diretamente deste recurso, como muitos macroinvertebrados (Goncalves et al. 2006; Sánchez-Argüello et al. 2010), e também para toda cadeia alimentar, que depende direta ou indiretamente dos macroinvertebrados, tais como peixes e outros vertebrados e invertebrados.

4.2. Direcionamentos para gestão e programas de monitoramento ambiental

Conhecer valores de variáveis físicas e químicas regionais para locais com diferentes graus de impacto antrópico é muito importante do ponto de vista da gestão, uma vez que podem se tornar referência para programas de monitoramento. Neste trabalho conseguimos identificar e propor alguns valores de referência para diversos parâmetros físicos e químicos, que poderiam nortear programas de monitoramento.

Os valores observados neste estudo foram similares a outros estudos realizados na região para alguns parâmetros, mas bastante diferentes em relação ao outros parâmetros. Como exemplo, o fosfato em locais protegidos (0,03 $mg.L^{-1}$) foi mais elevado do que o observado por Fonseca et al. (2014) em região de estudo similar, provavelmente em decorrência dos valores mais elevados observados nas áreas protegidas da bacia do Preto. A condutividade para locais protegidos apresentou valores muito baixos (~6 $\mu S.cm^{-1}$) neste e em outros três estudos na região (Silva et al. 2011; Fonseca et al. 2014; Fonseca & Mendonça-Galvão 2014). Já os valores para locais não protegidos foram similares ao de Fonseca et al. (2014; ~100 $\mu S.cm^{-1}$) mas mais elevados do que o identificado por Silva et al. (2011; 25 $\mu S.cm^{-1}$). É possível que esta diferença tenha ocorrido pelo fato de Silva et al. (2011) terem considerado apenas 3 riachos localizados em áreas menos urbanizadas do que as consideradas neste estudo.

O oxigênio, apesar de ser amplamente utilizado como uma variável indicadora de qualidade da água, mostrou-se estável nas águas da região, com poucos locais apresentando valores abaixo de 5 $mg.L^{-1}$. As concentrações de oxigênio dissolvido são negativamente influenciadas pela decomposição da matéria orgânica, na qual a origem pode ser atribuída tanto à poluição orgânica quanto a fontes naturais, como material vegetal alóctone, aumentando a demanda bioquímica de oxigênio (Cunha et al. 2011). Os valores mais baixos de oxigênio (~2 $mg.L^{-1}$) foram observados para um ponto muito impactado (Rib. Sobradinho – Cód. 40) e um outro essencialmente preservado (Vereda Grande I – Cód. 49), em ambas as estações do ano. O primeiro recebe uma elevada carga de poluição orgânica proveniente de uma estação de tratamento de esgoto. O segundo apresenta um fluxo muito baixo de água e ao mesmo tempo conta com um elevado volume de matéria orgânica natural proveniente da vegetação ripária, característica típica de áreas de vereda.

Pelas diferenças observadas entre locais protegidos e não protegidos, fosfato, condutividade e nitrato demonstraram ser bons indicadores dos impactos antrópicos na região, mas o conjunto de parâmetros e as diferenças entre bacias indicam que a dicotomia protegido/não protegido não representa os gradientes de condições ambientais que são comumente observados na natureza. Quando o número de locais de coleta é reduzido, o uso de análises de gradiente pode ser mais vantajoso do que a categorização inicial (protegido/não protegido), para uma melhor classificação dos locais de estudo (Ligeiro et al. 2013). Aqui, além do número de locais não protegidos ter sido inferior ao de locais protegidos, o único critério para a classificação foi estar dentro ou fora dos limites de áreas consideradas protegidas, o que não é garantia de ausência de distúrbios antrópicos. Justamente devido ao alcance das atividades humanas, a existência de corpos d'água totalmente preservados (intactos) torna-se cada vez mais rara, o que tem levado pesquisadores a trabalharem com locais de minimamente ou o menos perturbado possível (Stoddard et al. 2006).

Ainda que nem sempre as áreas de proteção ambiental representem a qualidade necessária para a manutenção do equilíbrio ecológico dos riachos, uma vez que geralmente são criadas com o objetivo de preservação dos ecossistemas terrestres (Abell et al. 2007), em geral o estado do ecossistema com um todo é mantido em melhores condições do que nas áreas não protegidas. Nossos dados indicaram a importância das áreas de protegidas especialmente em bacias com algum grau de urbanização, como a bacia do Paranaíba.

Finalmente, outra informação relevante para o aspecto da gestão é o fato de a maioria das variáveis, com exceção da vazão, não ter sido afetada pela sazonalidade. Diante disso, podemos sugerir que apenas uma coleta anual seria suficiente para avaliação destes aspectos, reduzindo assim os custos de campo e o tempo de processamento das amostras, possibilitando o diagnóstico rápido e confiável das condições de qualidade de água, desde que se tenha um programa de monitoramento de longo prazo, assim como também sugerido por Couceiro et al. (2021). Entretanto, como as coletas foram realizadas a dois períodos em um único ano, novas coletas são recomendáveis para que haja confirmação dos padrões observados.

5. Conclusões

As maiores diferenças nos fatores abióticos dos riachos estudados foram entre locais protegidos e não protegidos, em detrimento das variações entre bacias e entre estações climáticas. Isso implica que estudos futuros ou o próprio monitoramento/gestão devem demandar mais esforços na definição de gradientes de perturbação antrópica (representativos e razoáveis) do que com variações naturais nas características de cada bacia e/ou nas condições ambientais devido a sazonalidade. Tais diferenças ficaram mais evidentes na bacia do Paranaíba, onde a urbanização é mais expressiva. Nessa bacia, fica clara a necessidade de redução da entrada de cargas orgânicas nos riachos e a importância das áreas de proteção ambiental, ainda que muitas delas não estejam completamente livres de impacto ou de pressão antrópica. As diferenças entre locais protegidos e não protegidos nem sempre é clara quando os impactos não são tão acentuados como no caso da urbanização, fazendo com que os estudos de gradientes sejam necessários e complementares. As informações geradas, tais como os valores de referência para variáveis de qualidade da água, são de grande relevância para o monitoramento das bacias hidrográficas, possibilitando assim melhorias e direcionamento nas ações de gestão ambiental.

Agradecimentos

Este trabalho contou com o apoio da Fundação de Amparo à Pesquisa do Distrito Federal (FAP-DF), Projeto Aquaripária (edital 05/2016-Pró-Águas; Proc. Nº: 193.000716 / 2016), que permitiu a execução de trabalhos de campo e análises laboratoriais; do Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) por meio de bolsa de pesquisa para José Francisco Gonçalves Jr (Proc. nº: 310641 / 2017-9); e da Agência Reguladora de Águas, Energia e Saneamento Básico do Distrito Federal (ADASA) que além do apoio financeiro à Campos C.A. também ofereceu apoio logístico de veículos para o trabalho de campo.

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CAPÍTULO II

Diatom and Macroinvertebrate assemblages to inform management of Brazilian savanna's watersheds

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Ecological Indicators https://doi.org/10.1016/j.ecolind.2021.107834

Abstract

Human activities are increasingly affecting freshwater ecosystems and biodiversity, especially in neotropical regions like the Brazilian savanna. Limited research and data availability have inhibited the development and implementation of systematic bioassessment programs and management guidelines. Identifying drivers of biological assemblages' composition and ecological thresholds along human disturbance gradients is an important step to protect and recover freshwater ecosystems while avoiding threats to biodiversity, goods and services of value to humans. The objectives of this study were to: 1) assess changes in the composition and density of periphytic diatom and macroinvertebrate assemblages relative to natural and human disturbance gradients in Brazilian savanna's streams, and 2) identify ecological thresholds for direct and indirect human disturbances and potential indicator taxa that could inform the development of biomonitoring programs in Brazil and other neotropical countries. Samplings were carried out in 52 stretches of streams located in central Brazil during two campaigns throughout 2018. Ordination analyses (NMDS) were applied to identify the main drivers of biological assemblages' composition and analyses of taxa distribution in disturbance gradients (TITAN) were carried out to detect possible thresholds and potential bioindicator taxa. Our results pointed out that the scale of land use in the catchment, treated sewage input, and water quality variables (nitrate, phosphate, and conductivity) were the main drivers of diatom and macroinvertebrate assemblages. Taxa such as *Eunotia* (diatoms) and some families of the orders Ephemeroptera, Plecoptera, and Trichoptera (EPT, macroinvertebrates) were associated with natural conditions, while *Nitzschia palea* and *Gomphonema* (diatoms) and Oligochaeta and Hirudinea (macroinvertebrates) were related to environments with a higher degree of disturbance. Although ecological thresholds along disturbance gradients varied among taxa and biotic groups, our results revealed that relatively minor increases in land use in the riparian zone (>0%) and in the upstream catchment (0–33%) were sufficient to trigger significant changes in macroinvertebrate and diatom assemblages. The limits of tolerance for conductivity, nitrate and phosphate were also low, varying between 7-163 µS.cm^{-1}, 0.3–1.0 mg.L^{-1} and 0.03–1.5 mg. L^{-1} , respectively. In general, the values we found were more restrictive than those provided by Brazilian government guidelines, suggesting that the latter would be insufficient to maintain the integrity of biological assemblages in Brazilian savanna's watersheds. We provided valuable knowledge about the sensitivities and tolerances of diatom and macroinvertebrate's taxa/assemblages that can be especially useful for the proper freshwater and watersheds management.

Key-words: diatoms, macroinvertebrates, gradients, human disturbances, ecological thresholds, environmental assessment

Resumo

As atividades humanas estão afetando cada vez mais os ecossistemas de água doce e a biodiversidade, especialmente em regiões neotropicais como a savana brasileira. A limitada disponibilidade de informações é um dos fatores que inibe o desenvolvimento e a implementação de programas de biomonitoramento sistemáticos e diretrizes de gestão. Identificar os preditores da composição das comunidades biológicas e limites ecológicos ao longo dos gradientes de perturbação humana é um passo importante para proteger e recuperar os ecossistemas de água doce, evitando ameaças à biodiversidade, bens e serviços de valor para os humanos. Os objetivos deste estudo foram: 1) avaliar as mudanças na composição e densidade de comunidades de diatomáceas perifíticas e macroinvertebrados em relação a gradientes naturais e de distúrbios humanos em riachos da savana brasileira, e 2) identificar limites ecológicos para distúrbios humanos diretos e indiretos e potenciais táxons indicadores que poderiam auxiliar no desenvolvimento de programas de biomonitoramento no Brasil e em outros países neotropicais. As amostragens foram realizadas em 52 trechos de riachos localizados na região central do Brasil durante duas campanhas ao longo de 2018. Análises de ordenação (NMDS) foram realizadas para identificar os principais preditores da composição das comunidades biológicas e análises de distribuição de táxons em gradientes de perturbação (TITAN) foram realizadas para detectar possíveis limiares e potenciais táxons bioindicadores. Nossos resultados apontaram que uso do solo na escala de bacia hidrográfica, a entrada de esgoto tratado e as variáveis de qualidade da água (nitrato, fosfato e condutividade) foram os principais fatores que influenciaram a composição das comunidades de diatomáceas e macroinvertebrados. Táxons como *Eunotia* (diatomáceas) e algumas famílias das ordens Ephemeroptera, Plecoptera e Trichoptera (EPT, macroinvertebrados) foram associados a condições naturais, enquanto *Nitzschia palea* e *Gomphonema* (diatomáceas) e Oligochaeta e Hirudinea (macroinvertebrados) foram relacionados a ambientes com um maior grau de perturbação. Embora os limiares ecológicos ao longo dos gradientes de perturbação variassem entre táxons e grupos bióticos, nossos resultados revelaram que aumentos relativamente pequenos no uso da terra na zona ribeirinha (> 0%) e na bacia hidrográfica a montante (0-33%) foram suficientes para desencadear mudanças significativas nas comunidades de macroinvertebrados e diatomáceas. Os limites de tolerância para condutividade, nitrato e fosfato também foram baixos, variando entre 7-163 $\mu S.cm^{-1}$, 0,3-1,0 $mg.L^{-1}$ e 0,03-1,5 $mg. L^{-1}$, respectivamente. Em geral, os valores encontrados foram mais restritivos do que aqueles fornecidos pelas diretrizes do governo brasileiro, sugerindo que este último seria insuficiente para manter a integridade das comunidades biológicas nas bacias hidrográficas do Cerrado brasileiro. Nós fornecemos conhecimento valioso sobre as sensibilidades e tolerâncias dos táxons / comunidades de diatomáceas e macroinvertebrados que podem ser especialmente úteis para o manejo adequado de água doce e bacias hidrográficas.

Palavras-chave: diatomáceas, macroinvertebrados, gradientes, distúrbios humanos, limiares ecológicos, avaliação ambiental

1. Introduction

Human activities are increasingly affecting freshwater ecosystems and biodiversity (Reid et al. 2019), which are sensitive to the cumulative impacts of multiple interacting stressors (Jackson et al. 2016). In addition to persistent threats such as habitat fragmentation, degradation and loss, species invasions and pollution, a range of emerging threats associated with climate change, new contaminants, and other human stressors are disproportionately impacting freshwater ecosystems in many parts of the world (Vorösmarty et al. 2010; Reid et al. 2019). In Brazil as in many other developing economies in the neotropics, human threats to the integrity of freshwater ecosystems are rapidly increasing due to high urban population densities, poor sewage and wastewater treatment, water resource development, mining, and native vegetation clearing for large scale pastures and cropping (Ríos-Touma & Ramírez 2018).

To understand the consequences of human activities on aquatic ecosystems and to prioritize remedial management actions, a range of monitoring indicators has been applied for assessment, diagnosis, and prognosis of ecosystem health and sustainability (Norris & Thoms 1999; Gergel et al. 2002; Rossberg et al. 2017). These include indicators of water quality (physical and chemical), ecosystem process (e.g., leaf litter decomposition, primary productivity) and biological assemblages' composition since they can be sensitive to a variety of anthropogenic disorders (Bunn et al. 2010). Although physical and chemical variables have been the most widely used in water quality monitoring programs around the world, indicators that incorporate biological assemblages are increasingly being used to evaluate river health as they can integrate processes over a range of spatial and temporal scales, providing a holistic view of the aquatic ecosystem (Feio et al. 2010; Buss et al. 2015; Bo et al. 2017).

Both large (e.g., watershed, regional) and local scale factors are important to determining variations in the composition and abundance of biological assemblages since they generate conditions that are appropriate or not for their survival and maintenance (Li et al. 2018; He et al. 2020). However, quantifying taxa and assemblages' responses to natural and anthropogenic changes remains an ongoing challenge for river health assessment and watershed management (Firmiano et al. 2017). Examining changes in the composition and abundance of taxa along environmental gradients allows not only to understand how individual taxa respond to different conditions but also to identify critical change points or thresholds in assemblages' responses (Baker & King 2010; Snyder & Young 2020). Although there are controversies regarding the applicability of thresholds to environmental impact assessment (Murray et al. 2018), the ability to identify such change points might be useful since it informs the limits of disturbance tolerated by biological assemblages and constituent taxa (Baker & King 2010), often indicating the existence of a gap between the limits acceptable by the assemblages and those applied by government guidelines. This scenario is well exemplified by the study developed by Rodrigues et al. (2016), which, by analysing damselfly assemblage, were able to verify the inefficiency of Brazilian legislation in preserving riparian vegetation and consequently the associated aquatic biodiversity in the Brazilian savanna. On the other side of the globe, Tibby et al. (2020) demonstrated that thresholds for diatoms in gradients of electrical conductivity and of phosphorous were considerably lower than the trigger values used in South Australia guidelines.

Many countries, such as Australia (Bunn et al. 2010), the European Union countries (European Union 2000), New Zealand (Schallenberg et al. 2011), and the United States of America (USEPA 2016), have incorporated biological assemblages as part of their river health assessment programs. In rapidly developing neotropical countries like Brazil, an increasing number of studies have demonstrated the potential of the analyses of biological assemblages in assessing and monitoring the health of aquatic environments (Buss et al. 2016; Macedo et al. 2016; Pereira et al. 2016). A recent, complete and comprehensive study in the Brazilian savanna region, developed a robust multimetric index (MMI) based on different aspects of macroinvertebrate assemblage characteristics to assess and monitor the ecological condition in neotropical savanna's streams (Silva et al. 2017). However, the development of systematic bioassessment programs and guidelines in national resolutions on environmental and water resources is in its infancy in this region, despite increasing concerns about the degradation of freshwater ecosystems (Sundar et al. 2020).

Macroinvertebrate assemblages are commonly used as bioindicators around the world (Buss et al. 2015; Sumudumali & Jayawardana 2021), not only because they are an essential part of the aquatic environment - since they transfer the energy to other trophic levels in the aquatic food web, but also because of their restricted mobility, relatively long life cycle if compared to other aquatic organisms, high taxa diversity with different levels of stress tolerance, pollution sensitivity, suitability of taxonomic keys and existence of well-defined sampling protocols (Sumudumali & Jayawardana 2021). Diatoms (Bacillariophyceae) are also widely used in monitoring programs as bioindicators (Pandey et al. 2018) due to their fundamental ecological role at the base of the food web and their sensitivity and rapid response to environmental fluctuations in comparison with organisms in higher trophic positions (Kelly et al. 2008). Changes in macroinvertebrate and diatom assemblages' composition and abundance are expected in the face of all pressures that aquatic ecosystems have been suffering (Silva et al. 2017; Gonzáles-Paz et al. 2020).

Most studies aimed at identifying ecological thresholds for aquatic biota typically focus only on a single biotic group (e.g., Smucker et al. 2013; Zhang et al. 2016; Brito et al. 2020) and very few consider two or more groups (e.g., Schröder et al. 2015). In Brazil, especially in neotropical savanna, fundamental knowledge about environmental controls of macroinvertebrates and diatoms biodiversity and responses to anthropogenic disturbances is critically scarce (Overbeck et al. 2015), posing a challenge for the implementation of biomonitoring to inform freshwater management.

Having these considerations in mind, the objectives of this study were (i) to assess changes in the composition and density of periphytic diatom and macroinvertebrate assemblages in relation to natural and human disturbance gradients in Brazilian savanna's streams, and (ii) to identify ecological thresholds and potential indicator taxa that could further inform the development of biomonitoring programs in Brazil and other neotropical countries.

2. Material and Methods

2.1. Study area

The study was carried out mostly in the Distrito Federal (DF; 5,802 km^2), located in the central Brazilian plateau (Figure 1). It is characterized by regions with distinct land uses: agriculture in the east; preservation and mining areas in the north; and urban densification in the central and south-western portion (GDF 2012). Eastern tributaries draining from the adjacent state of Goiás (GO), that present similar physical characteristics to those of the DF, were also included in the study area.

Fifty-two randomly selected sample sites were widely distributed within the study area to represent a broad range of natural conditions - relating to stream size, elevation, slope, geology, and other factors -, different land uses and anthropogenic disturbances in the drainage area (Table1). Sample sites were separated by at least 500 m when located in the same watercourse, and as long as different natural characteristics were observed. Site selection was constrained by road accessibility and all of them were located in wadeable streams of up to 5th order (Strahler 1957). The influence of the river size variation was considered in the study by some natural variables such as the drainage area, distance from the source and elevation.

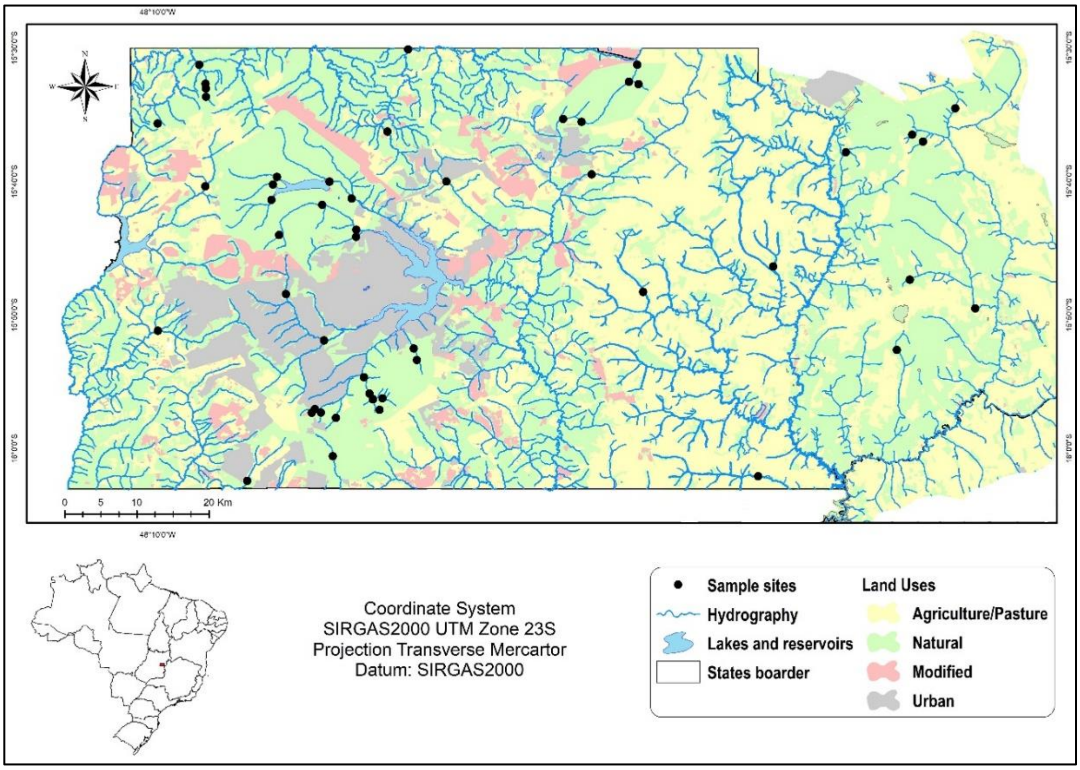

Figure 1. Study area, sample sites and land uses. Land uses were classified in agricultural (any kind of agriculture and pasture), natural (native vegetation), modified (new settlements, exposed soil and eucalyptus), or urban.

2.2. Natural variables and human disturbances

Natural variables (Table 1) minimally affected by human activities, were defined by Geographic Information System (GIS) processing. A Digital Elevation Model (DEM) (30 m resolution) was used to outline the upstream watershed borders, using the hydrology tools in ArcMap 10.4 and topographic data from Brazil’s Geomorphometric Database (TOPODATA; INPE 2018) on a scale of 1:50,000. Each sample site was treated as an outlet point. From this model, we extracted the upstream drainage area, the average slope of the river, elevation, and the distance from the source for each sample site.

Soil type and geological group data for Distrito Federal and its surroundings were sourced from the Federal District’s Water, Energy and Basic Sanitation Agency (ADASA). Both were determined on a local scale, i.e., at each sample site, using the ArcMap 10.4. The three geological groups present in the study area were the Bambui, Paranoa, and Canastra; and the three kinds of soils were red oxisol, plinthosol, and cambisol.

Natural variables that could potentially be affected by human activities (Table 1) included: local habitat (riverbed sediment granulometry and canopy cover shading), hydromorphological characteristics, and water quality (see description below).

The main anthropogenic disturbances in the study area are the removal of natural land cover for agriculture, urban development, mining, point-source sewage release (treated or not); and the presence of dams (GDF 2012). To evaluate the land use in the upstream catchment (CAT) we adapted the Land Use Index (LUI) proposed by Rawer-Jost et al. (2004). This index considers that the impact of urban areas is greater than agriculture and other kinds of land uses. Our adapted index considers agricultural and pasture areas with the same weight and includes modified areas (allotment, exposed soil, mining, and eucalyptus). The proposed Land Use Index (LUI) is calculated as (1):

LUI = 4x % urban areas (CAT_urb) + 2x % agricultural and pasture areas (CAT_agr) + % modified areas (CAT_mod) (1)

To evaluate the influence of alterations on the riparian corridor (RIP) in the biological assemblages we considered an upstream 30 m-buffer along each bank. The Brazilian Forest Code (Federal Law 12.651/2012) determines that the extent of riparian vegetation depends on the river width. Rivers up to 10 m in width should have, at least, 30 m of riparian vegetation on each bank. Rivers with 10 to 50 m width should have 50 m of riparian vegetation on each bank. Since most rivers in the study area have width below 10 m, the land use analyses in the riparian zone covered 30 m from each bank. The riparian corridor was also generated from the DEM model, considering the land uses within the 30 m along each bank. Similar to the catchments, land uses in the riparian corridor were also grouped into 3 categories: urban, agricultural and modified (Table 1). The percentage of occupation by native vegetation was not considered since it represents exactly the total area having subtracted the other uses. We calculated the proportion of each kind of land use in the upstream catchment and in the riparian corridor of each sample site using a land uses shapefile for Distrito Federal (Reis & Lima 2015) and by photointerpretation of RAPIDEYE satellite image with 5 m resolution for Goiás state (Table 1).

The number of point-source treated sewage releases and dams present in the upstream catchment of each sample site were obtained from the Federal District’s Water, Energy and Basic Sanitation Agency (ADASA). The number of upstream road crossings was calculated by Open Street Map data (OSM Foundation 2017). All geoprocessing analyses were carried out in ArcMap 10.4.

2.3. Field sampling and laboratory analyses

Two sampling campaigns were conducted in 2018, one at the end of the wet season (April/May) and another at the end of the dry season (September/October). Although rainfall data were not available for this study, since the regional hydrometeorological stations were out of operation throughout 2018, the historical rainfall in Distrito Federal is characterized by two well-marked periods, the wet season (October to March) and the dry season (April to September; INMET 2019). As it is an exploratory work, we consider it necessary to collect data that involves two sampling periods, thus guaranteeing a greater coverage on the conditions of our systems.

2.3.1. Water quality

A large number of water quality variables were measured at each sample site on each sampling occasion. Dissolved oxygen and water temperature were measured in situ using a YSI probe. A water sample (approximately 300 mL) was collected 15 cm under the surface – or between the surface and riverbed in case of the depth was conductivity and pH (Jenway, Quimis e Metrohm probes). The remainder was filtered (glass fibre 0.22 mm filter) and stored in 15 mL Falcon tubes at − 20 ◦C for analyses of ionic composition. Cation, anions, and metals were analysed on a Metrohm chromatograph using specific columns for cation (potassium), anions (fluoride, chloride, nitrite, bromide, nitrate, phosphate and sulphate) and metals (iron). Cation and metal determinations were performed on the MetroSep C4 250/4.0 mm column using 1.7 mM nitric acid / 0.7 nM dipicolinic acid as eluent with unsuppressed conductivity detection. Anion samples determinations were performed on the MetroSep A Supp 5 250/4.0 mm column using 3.2 mmol.L-1 sodium carbonate and 1 mmol.L-1 sodium bicarbonate as eluent, with suppressed conductivity detection.

2.3.2. Hydromorphology

Hydromorphological parameters were calculated in field, in a representative section of the stream stretch, and followed the middle section methodology (Santos et al. 2001). Measurements were taken on each sampling occasion, except when high water velocities prevented this. In these cases, gauged discharge values were obtained from the Federal District’s Water, Energy and Basic Sanitation Agency (ADASA). Riparian shading was estimated visually and allocated on an ordinal scale (ranging from 0 to 3) to one of the following classes: 0% − 0; 0–30% − 1; 30–60% − 2; >60% − 3.

The granulometry of the riverbed sediments was analysed only once (April) by the sieving method (Suguio 1973). Around 1 kg of sediments was sampled and air-dried for at least 72 h. After drying, the samples were homogenised, and a sub-sample (approximately 100 g) was incinerated in a muffle furnace at 550 ◦C for 4 h. The difference in weight between pre- and post-drying was used to determine the percentage of organic matter in each sub-sample. Each dried sub-sample was then sifted for 15 min passing through sieves of different mesh diameters (2 mm, 1 mm, 0.710 mm, 0.500 mm, 0.355 mm, 0.250 mm, 0.180 mm, 0.125 mm, 0.090 mm e 0.063 mm) with the help of an automatic shaker. Each size sample was weighed, and the results were expressed as a percentage of the total sub-sample weight. Prior to further analyses sediments samples were grouped into 5 categories: coarse sediments (>2–0.710 mm), medium sediments (0.500–0.250 mm), fine sediments (0.180–0.125 mm), very fine sediments (0.090–0.063 mm) and silt/clay (< 0.063).

2.3.3. Macroinvertebrates

Macroinvertebrates were collected in May and September 2018 with a *surber* sampler (0.09 m2 area and 0.250 mm mesh size). Five sub-samples distributed proportionally across the area covered by the most representative habitats were collected (e.g., macrophytes, stones, sand, and leaves), covering a length of 5 to 50 m, depending on access conditions along the riverbed. The sampling duration was 1 min in each sub-sample site. The five sub-samples were then combined to generate a single composite sample and it was preserved in 96% alcohol. Later, the macroinvertebrates were sorted and identified under a stereomicroscope. Identification was carried out mostly to family level following Hamada et al. (2018) and Hamada et al. (2019) and with the help of taxonomic specialists (see Acknowledgments). Few groups were classified only up to class or order. The results were expressed as the number of individuals/m^2 for each taxon.

2.3.4. Periphytic diatoms

Periphytic diatoms were sampled in May and September 2018 following the Water Framework Directive (WFD) protocol for Portuguese rivers (INAG I.P. 2008). Five 10 × 10 cm (total 500 cm^2 of surface area) pieces of slate stone tile were deployed in the riverbed and after approximately 30 days of incubation, they were retrieved. On average, a total surface area of about 250 cm^2 of tile at each site was scraped with a toothbrush to obtain the periphytic diatom sample. The scraped material was preserved in vials containing 0.33% Lugol solution and the identification and quantification of the periphytic diatoms were carried out under an inverted microscope using the Utermöhl method (Utermöhl 1931). We did not proceed the oxidation of organic matter previously to the identification as suggested by INAG I.P. (2008), because we were interested only in the active cells (which means cells with cellular content) at the time of sampling. Identification was carried out mostly to species level following specific taxonomic literature, with the help of taxonomic specialists (see Acknowledgments). The results were expressed as cells/cm^2/month for each taxon.

2.4. Data analyses

Since data distribution was not normal for many variables, Spearman correlation analysis was used to identify higher inter-correlated environmental and human disturbances variables. For pairs of variables with absolute correlation coefficients ≥ 0.6, we retained those correlated with the greatest number of other variables and also the most straightforward to obtain in the field or lab. These analyses resulted in the exclusion of 21 of the 40 initial variables considered (Table 1; Appendix B). All hydromorphological variables were excluded since they were highly correlated with the drainage area. Regarding habitat variables, the shading, the percentage of organic matter in the sediment (OM) and the percentage of coarse sediment (coa_sed) were retained. Among water quality variables, Cl, NO2 and SO4 were excluded. The percentage of modified area in the riparian corridor (RIP_mod) was also excluded since it was correlated with the percentage of urban area (RIP_urb). The only correlated variables we retained for subsequent analyses were LUI and RIP_agr since they refer to different scales of land use and occupation. Importantly, the retained human disturbance variables were minimally correlated with the natural environmental gradients (absolute Spearman's rho < 0.6).

We used ordination analyses (Nonmetric Multidimensional Scaling - NMDS) based on a Bray-Curtis dissimilarity matrix of the sample-by-taxon density data to summarise diatom and macroinvertebrate assemblages and evaluate relationships with natural and disturbance variables. Three dimensions (k = 3) was considered because of the reduced stress value compared with two dimensions. We used two-way Permutation Analyses of Variance (PERMANOVA) to test for differences in assemblages' composition between the dry/wet seasons. The p-values for pseudo-F were obtained from 999 data permutation. Different colours were used to distinguish seasonal samples in the ordination biplots. The R function "envfits" was used to relate the natural and human disturbances variables with sample position in ordination space. The projections of points onto vectors have a maximum correlation with corresponding gradients.

We used Threshold Indicator Taxa Analyses (TITAN; Baker & King 2010) to estimate thresholds for land uses and water quality gradients that were strongly related to variation in diatom and macroinvertebrate assemblages (as identified by ordination and envfit correlation analyses). Human disturbance variables included the Land Use Index (LUI) and its components – land use in the catchment scale (CAT_urb, CAT_agr, and CAT_mod), land use in the riparian scale (RIP_agr and RIP_urb), conductivity, phosphate and nitrate concentrations. TITAN is based on change point (King & Richardson 2003) and indicator value (z-value; Dufrêne & Legendre, 1997) analyses to detect significant changes in the frequency of occurrence and relative abundance of taxa along environmental gradients (Baker & King 2010). TITAN differentiates taxa with positive (Z+) and negative (Z−) responses to all gradients, with Z+ (tolerant) taxa increasing in frequency and abundance from the change point and Z− (sensitive) taxa decreasing. The response quality of each indicator taxon is measured by purity and reliability; both indices are obtained by resampling using the bootstrap method (500 resamples with replacement) to confirm the thresholds for each taxon and assemblage. Purity corresponds to the proportion of change points (if Z− or Z+) along with resampling that concurs with the observed value. Reliability corresponds to the proportion of the resampling that reports an indicator value with significant p-values (Baker & King 2010). We consider robust taxa those with purity and reliability above 90%. After taxon-specific change points have been identified, TITAN supplies an assemblage level threshold, reflecting the magnitude of assemblage changes as an indicator of coincident change point in the assemblage structure [sum (Z)], considering only the robust taxa (filtered results). Following recommendations (Baker & King 2010), we excluded taxa occurring at fewer than three sites. TITAN analyses were carried out with both seasons data together since we obtained much more consistent results (greater number of robust taxa) than considering wet and dry season separately, and also because it allowed us to have a broader view of the studied streams' system.

Prior to TITAN analyses, taxon density data were log (x + 1) transformed because of the wide ranges observed, although it is certainly acceptable to use untransformed data in this nonparametric analysis (King & Baker 2014). All statistical analyses were performed using the R program (R Development Core Team 2018) with specific packages for NMDS (Vegan; Oksanen et al. 2017), PERMANOVA (Adonis2; Anderson 2001; in Vegan), TITAN (TITAN2; Baker and King 2010) and graphs (Ggplot2; Wickham 2016).

Table 1. Description, average, standard deviation (SD), range (min = minimum and max = maximum) and number of data (N) of natural and human disturbances variables. (*) Data collected four times, but for analyses, we consider the average between April/May and August/September as representing dry and wet season, respectively. In one sample site, data were obtained only in the wet period. (**) For categorical variables, we indicated the number of samples in each category. (***) used only in TITAN analyses. Variables highlighted in bold were used in ordination analyses (the others were excluded by Spearman correlation analysis).

Natural Variables	**Description**	**Average (SD)**	**Min-Max**	**N**
GIS obtained variables				
drai_area	Total drainage area upstream of the sample site (Km^2)	40.52 (48,91)	2.21 - 215.42	52
Source_dist	Distance from the sample site to the river source (Km)	7.96 (7.37)	0.54 - 33.63	52
elevation	Altitude of the sample point relative to sea level (m)	1015.48 (96.63)	744 - 1220	52
slope	Average slope between two points along the river channel (m)	3.02 (1.72)	0.63 - 7.32	52
Soil**	Dominant soil type: red oxisol (S1) plinthosol (S2) cambisol (S3)	S1 - 46; S2 - 5; S3 - 1		52
Geol_group**	Geological group Paranoá (G1) Canastra (G2) Bambuí (G3)	G1 - 42; G2 - 1; G3 - 9		52
Habitat variables				
shading	% of riparian shading (0 = 0%; 1 = < 30%; 2 = between 30 and 60%; 2 = > 60%)	2.36 (0.96)	0 - 3	52
OM	% of organic matter in the riverbed sediment	6.15 (5.27)	0.61 - 26.66	52
coa_sed	% of coarse sediments (>2000 - 710mm) in the riverbed sediment	60.49 (25.86)	4.18 - 97.28	52
med_sed	% of medium sediments (500 - 250mm) in the riverbed sediment	21.99 (13.97)	1.06 - 52.16	52
fin_sed	% of fine sediments (180 - 125mm) in the riverbed sediment	10.94 (9.42)	0.42 - 45.39	52
v_fin_sed	% of very fine sediments (90 - 63mm) in the riverbed sediment	3.67 (3.75)	0.18 - 15.43	52
silt	%of silt and clay sediments (< 63mm) in the riverbed sediment	2.91 (4.51)	0.08 - 20.48	52
*Hydromorphological variables**				
av_depth	Average depht (m)	0.31 (0.18)	0.04 - 1	103
av_veloc	Average velocity (m)	0.27 (0.20)	0.01 - 1.5	103
av_width	Average width ($m\ s^{-1}$)	3.17 (2.72)	0.3 - 16.3	103
disch	Discharge ($m^3\ s^{-1}$)	0.35 (0.59)	0 - 4.77	103
*Water Quality (WQ) variables**				
temp	Temperature (°C)	20.52 (1.88)	13.8 - 26.6	103
DO	Dissolved Oxygen ($mg.L^{-1}$)	7.11 (0.92)	1.88 - 8.85	103
pH	Potential of Hydrogen	6.60 (0.45)	5 - 8.1	103
Cond	Electrical conductivity ($\mu S.cm^{-1}$)	56.07 (93.98)	1.3 - 584	103
turb	Turbidity (NTU)	7.92 (15.37)	0.04 - 197	103
K^+	Potassium ($mg.L^{-1}$)	1.27 (2.87)	0 - 46.55	103
Fe^{+2}	Iron ($mg.L^{-1}$)	1.47 (0.08)	1.3 - 1.85	103
F^-	Fluorine ($mg.L^{-1}$)	0.10 (0.64)	0 - 0.57	103
Cl^-	Chlorine ($mg.L^{-1}$)	0.92 (2.95)	0 - 35.4	103
NO_2^-	Nitrite ($mg.L^{-1}$)	0.04 (0.10)	0 - 1.23	103
Br^-	Bromide ($mg.L^{-1}$)	0.01 (0.01)	0 - 0.09	103
NO_3^-	Nitrate ($mg.L^{-1}$)	0.35 (1.11)	0 - 10.29	103
PO_4^{-3}	Phosphate ($mg.L^{-1}$)	0.23 (0.60)	0 - 6.26	103
SO_4^{-2}	Sulphate ($mg.L^{-1}$)	0.18 (0.51)	0 - 2.66	103
Human disturbances variables				
RIP_urb	% of urban area in riparian area	1.22 (5.08)	0 - 33.44	52
RIP_agr	% of agricultural and livestock areas in riparian area	7.82 (15.48)	0 - 55.93	52
RIP_mod	% of modified area in riparian area (allotment, exposed soil, eucalyptus)	0.70 (3.41)	0 - 23.91	52
CAT_urb***	% of urban area in upstream catchment	6.92 (16.23)	0 - 70.39	52
CAT_agr***	% of agricultural and livestock areas in upstream catchment	20.61 (25.03)	0 - 86.48	52
CAT_mod***	% of modified area in upstream catchment (allotment, exposed soil, eucalyptus)	3.28 (6.55)	2.21 - 215.42	52
LUI	Land Use Index for upstream catchment (see equation 1)	72.20 (75.25)	0 - 296.03	52
SR**	Presence/absence of point-source treated sewage release upstream	0 - 49; 1 - 3	0 - 1	52
Dam**	Presence/absence of upstream dams	0 - 39 ;11 - 13	0 - 1	52
N_dams	Number of upstream dams	0.47 (0.98)	0 - 4	52
Dist_dam	Distance between the sample point and the nearest dam	5.62 (3.33)	0.74 - 17.87	52
C_roads	Number of road crossings upstream	0.72 (1.65)	8 - 0	52

3. Results

3.1. Biological assemblages

We sampled 18 families, 26 genera, and 74 species of diatoms across the 52 study sites and two sampling seasons (wet and dry). The most frequent genus in both seasons was *Eunotia*, with *Eunotia* sp.1 presents in 78% of all samples. In the wet season, the densest species were *Eunotia* sp.1 (16%), *Gomphonema* cf. *parvulum* (10%) and *Nitzschia* sp.1 (9%). In the dry season, the densest species were *Nitzschia* cf. *palea* (31%), *Eunotia* sp.2 (12%) and *Gomphonema* cf. *lagenula* (11%; Appendix A).

We collected 50,880 macroinvertebrates belonging to 4 phyla, 10 classes, 11 orders and 54 families across all sites and sampling periods. The most common and widespread taxon was Chironomidae, occurring in all samples and forming 55% and 54% of the total density in the wet and dry seasons, respectively. After Chironomidae, the next most frequently occurring taxa, were Elmidae (90%), Ceratopogonidae (74%) and Perlidae (59%) in the wet season, and Elmidae (88%), Ceratopogonidae (82%) and Leptophlebiidae (82%) in the dry season. The densest taxa were Simulidae (36%), Oligochaeta (14%) and Elmidae (12%) in the wet season and Elmidae (17%), Simuliidae (17%) and Oligochaeta (14%) in the dry season (Appendix A).

3.2. Relationships of biological assemblages with environmental and human disturbances

The ordination of diatom and macroinvertebrate assemblages allowed the identification of a major gradient, predominantly characterized by direct and indirect human impacts (Figs. 2 and 3). The land use in the upstream catchment (LUI), the presence of point-source sewage release (SR), and water quality variables commonly related to pollution like phosphate, nitrate and conductivity, were the most relevant to taxa distribution. Among the natural variables, shading and elevation stand out as relevant factors for diatoms, while the percentage of organic matter in the sediment (OM) was more relevant for macroinvertebrates (Figs. 2 and 3, see "envfits" values in Appendix C).

Sites with a high density of genus *Eunotia* (diatom) and taxa Perlidae, Megapodagrionidae, Noteridae, Leptoceridae and Decapoda (macroinvertebrates) were characterised by low levels of human disturbance, more shading, higher elevation and higher percentage organic matter (OM). Sites with higher levels of human disturbance and greater drainage area, were characterised by several diatom taxa including *Nitzschia* cf. *palea*, *Gomphonema* cf. *lagenula*, *Diploneis* sp. and *Pinnularia* sp.1. Macroinvertebrates taxa displayed distinctive associations with different types of human disturbance. Simuliidae, Oligochaeta, Chironomidae and Hirudinea were related to sites with the presence of point-source sewage release and $PO4^{-2}$, while Physidae, Hydropsychidae and Dicteriadidae were more related to the land uses and higher conductivity values.

Diatom and macroinvertebrate assemblages' composition differed significantly between seasons (PERMANOVA; pseudo-F = 2.77, p = 0.003 for diatoms, and pseudo-F = 4.60, p = 0.001 for macroinvertebrates). But the contribution of the predictor variables (envfit) did not vary significantly when we performed the ordination (NMDS) analysis separated by season or with all data together (ANOVA F = 2.343, p = 0.107 for diatoms, and F = 0.347, p = 0.708 for macroinvertebrates). So here we only represent the results with both seasons data together.

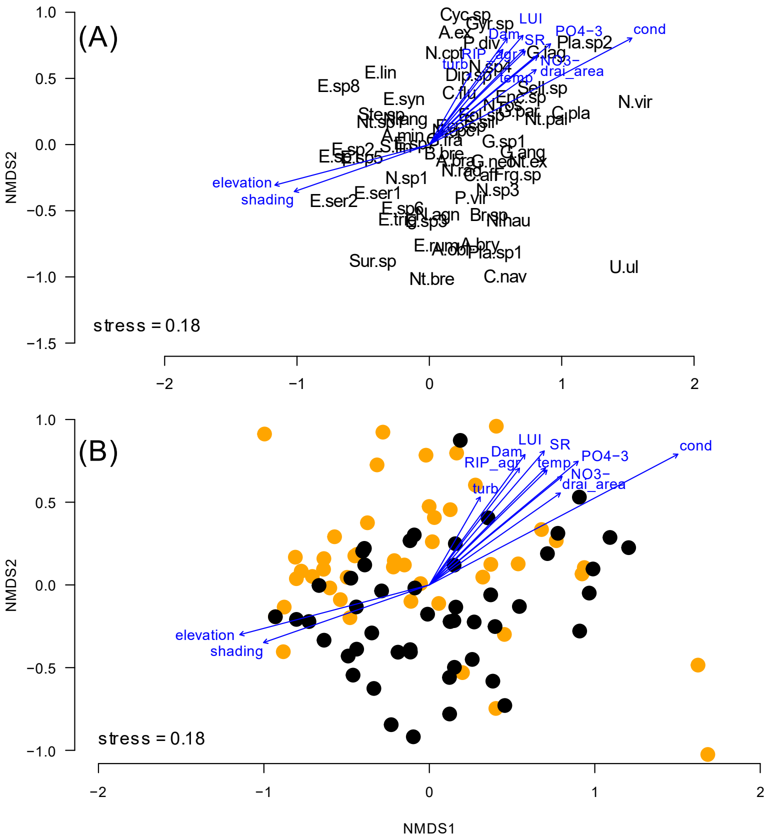

Figure 2. Distribution of diatom species (A) and sites (B), and the fitted vectors for significant ($p < 0.01$) environmental and human disturbance variables (blue arrows). Stronger relationships are represented by longer vectors. In B, sites are separated by season - wet (orange) and dry (black). Abbreviations: see Table 1. The complete list with taxa codes is in Appendix A.

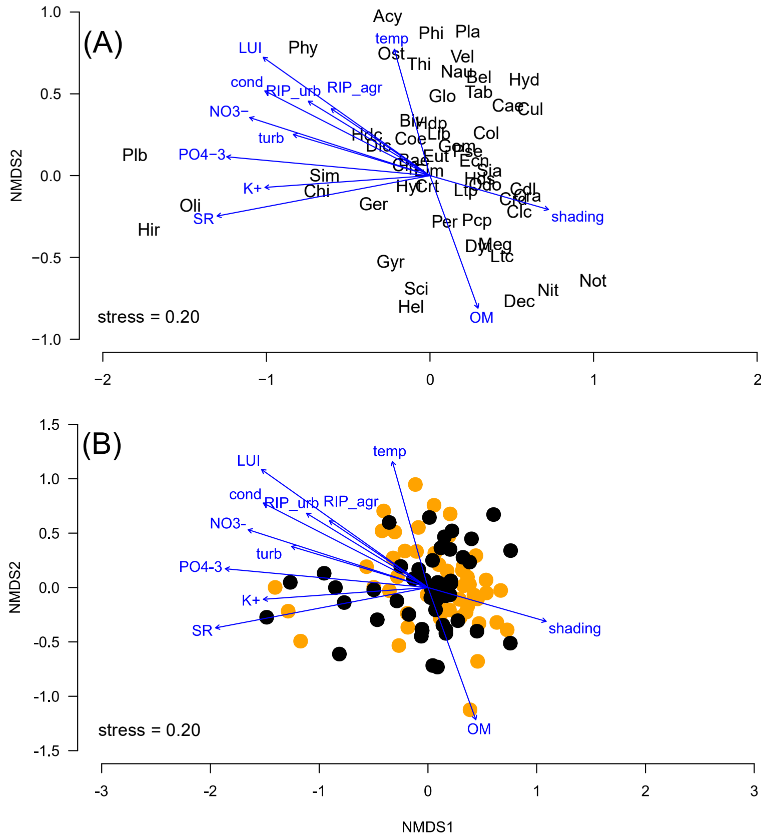

Figure 3. Distribution of macroinvertebrates taxa (A) and sites (B), and the fitted vectors for significant ($p < 0.01$) environmental and human disturbance variables (blue arrows). Stronger relationships are represented by longer vectors. In B, sites are separated by season - wet (orange) and dry (black). Abbreviations: see Table 1. The complete list with taxa codes is in Appendix A.

3.3. Environmental thresholds identified by TITAN analyses

TITAN analyses revealed substantial changes in diatom and macroinvertebrate assemblages along environmental disturbance gradients and different thresholds between the biotic groups. The assemblage threshold of the catchment land use index (LUI) was lower for sensitive (Z-) taxa of diatoms compared with macroinvertebrates (42 *versus* 173, Table 2). In contrast, the assemblage threshold of tolerant (Z+) taxa was higher for diatoms (159) than macroinvertebrates (42.5; Table 2). This suggests that sensitive diatoms were more responsive to minor changes in LUI than the sensitive macroinvertebrates, but the tolerant diatoms required greater changes in land uses than macroinvertebrates before they increased in density.

The Land Use Index in the upstream catchment (LUI) were the land use gradient that presented the largest number of robust taxa identified by TITAN analyses for both biological assemblages (Table 2, Figs. 4 and 5). We obtained different results in each LUI component. For diatoms, no sensitive (Z-) taxa were identified in CAT_urb. The thresholds observed in CAT_urb were higher for diatoms (Z+, 33.2%) than for macroinvertebrates (Z- 19.9%, Z+ 0.75%). The Z- thresholds in CAT_agr were lower for macroinvertebrates (0.18%) than diatoms (1.63%), but vice-versa for Z+ (macroinvertebrates 20.8%, diatoms 9.8%). The CAT_mod presented similar thresholds for both assemblages, near 0% for sensitive taxa and up to 5% for tolerant taxa. The thresholds were lower in the riparian land uses (RIP) than in the upstream catchment land use (LUI) components for both assemblages, with values around zero and no sensitive species detected for diatoms in RIP_urb gradient.

Among the physical and chemical parameters, conductivity gradient presented the largest number of robust sensitive and tolerant taxa for both assemblages (Table 2, Figs. 4 and 5). The assemblage thresholds for both sensitive (Z-) and tolerant (Z+) taxa were higher for diatoms (Z- 22.6 $\mu S.cm^{-1}$, Z+ 124.7 $\mu S.cm^{-1}$) than for macroinvertebrates (Z- 7.24 $\mu S.cm^{-1}$, Z + 163 $\mu S.cm^{-1}$). The change point in nitrate gradient was lower for both sensitive and tolerant diatoms (0.347 $mg.L^{-1}$, 0.486 $mg.L^{-1}$) than macroinvertebrates (0.976 $mg.L^{-1}$). The change point in phosphate gradient was around 0.04 $mg.L^{-1}$ (Z-) and 0.90 $mg.L^{-1}$ (Z+) for diatoms and 0.028 $mg.L^{-1}$ (Z-) and 1.47 $mg.L^{-1}$ (Z+) for macroinvertebrates (Table 2).

A wide range of change points were identified along disturbance gradients for taxa in each biotic group (Figs. 4 and 5). All sensitive (Z-) diatom species had LUI change points <50 and all the tolerant (Z+) species between 50 and 200 (Figure 4). For macroinvertebrates, most of the sensitive taxa had LUI change points >100 and most tolerant (Z+) taxa had change points <100 (Figure 5). For conductivity, most of the diatom and macroinvertebrate sensitive taxa (Z-) presented change points lower than 50 $\mu S.cm^{-1}$, but tolerant taxa (Z+) indicated a group increasing in low values and another group in higher than 100 $\mu S.cm^{-1}$ values (Figs. 4 and 5). In some land use gradients, taxa started to increase or decrease just after 0%, for example, the Megapodagrionidae (Z-) in CAT_agr gradient and all tolerant (Z+) diatom species in RIP_urb gradient.

The most common sensitive taxon among diatoms was *Eunotia* and the most common tolerant taxa were *Gomphonema*, *Nitzschia* and *Navicula* (Appendix E). The most sensitive macroinvertebrate taxa were Perlidae, Dytiscidae, Leptoceridae and Leptophlebiidae while the most common tolerant taxa were Hirudinea, Oligochaeta, Physidae and Planorbidae. Taxa belonging to EPT (Ephemeroptera/Plecoptera/Trichoptera) group represented 43.5% of the total sensitive (Z-) macroinvertebrate taxa (Appendix E).

Table 2. TITAN responses for diatom and macroinvertebrate assemblages among all gradients. For all gradients abbreviations, see Table 1. NI = not identified. For each group (diatoms and macroinvertebrates): number of robust sensitive (Z-) and tolerant (Z+) taxa, the assemblage threshold for filtered sensitive (FsumZ-) and tolerant (FsumZ+) taxa followed by 5th and 95th percentiles among bootstrap replicates (n=500). SumZ plots of all gradients for both assemblages can be found in Appendix D.

	LUI	**LUI components**			**RIP**		**Water Quality**		
		CAT_urb	**CAT_agr**	**CAT_mod**	**RIP_urb**	**RIP_agr**	**Cond**	**NO3**	**PO4**
Diatoms									
No. robust taxa (z−)	4	0	3	3	0	2	8	3	5
No. robust taxa (z+)	9	6	1	4	7	8	13	5	3
Fsum Z- (5th-95th percentiles)	42.48 (3.52 - 48.66)	NI	1.63 (0.58 - 9.83)	0 (0 - 2.610	NI	0 (0 - 0.12)	22.57 (2.72 - 50.27)	0.347 (0.001 - 0.545)	0.037 (0.005 - 0.310)
Fsum Z+ (5th-95th percentiles)	159.21 (105.16 - 206.85)	33.20 (2.10 - 53.77)	9.83 (8.37 - 79.60)	5.75 (0.63 - 11.38)	0 (0 - 0.06)	0.88 (0 - 11.32)	124.75 (13.58 - 139.47)	0.486 (0.082 - 2.122)	0.8975 (0.248 - 1.927)
Macroinvertebrates									
No. robust taxa (z−)	9	8	2	1	6	5	14	9	10
No. robust taxa (z+)	9	5	11	7	4	8	13	5	1
Fsum Z- (5th-95th percentiles)	172.95 (151.55 - 175.73)	19.94 (14.32 - 33.15)	0.18 (0.12 - 7.73)	0 (0 - 0.51)	3.20 (0 - 6.43)	0 (0 - 6.55)	7.24 (2.84 - 9.28)	0.976 (0.015 - 1.253)	0.028 (0.022 - 0.160)
Fsum Z+ (5th-95th percentiles)	42.49 (42.49 - 239.65)	0.75 (0.59 - 56.13)	20.83 (9.83 - 38.21)	7.91 (4.66 - 8.24)	0 (0 - 3.90)	0.12 (0 - 2.64)	163.00 (14.82 - 184.82)	0.976 (0.044 - 1.904)	1.470 (0.160 - 1.499)

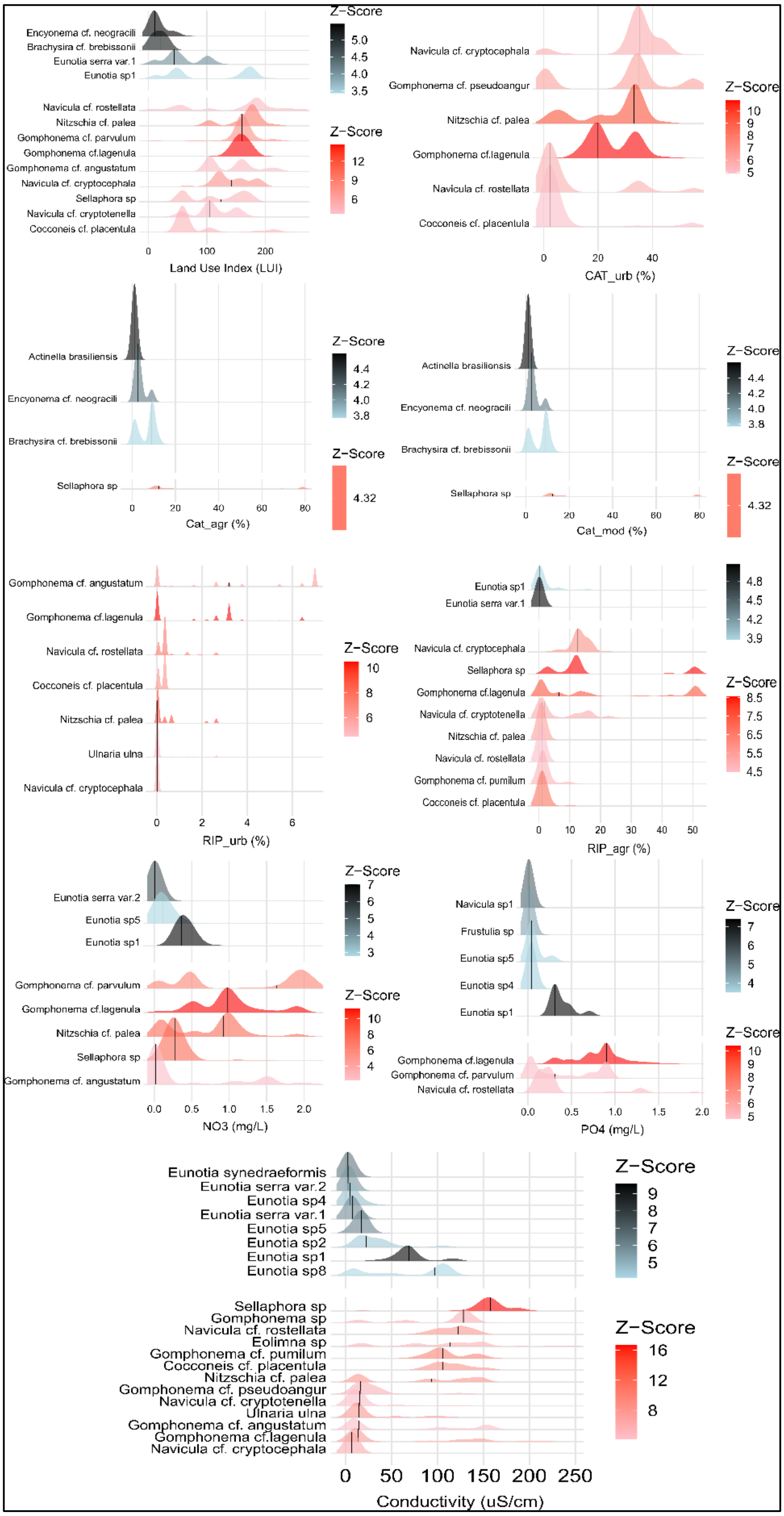

Figure 4. Results of TITAN showing taxon-specific maximum change points along all gradients for Diatoms. Robust sensitive taxa (Z-) and tolerant taxa (Z+) are represented in blue and red scales respectively. The blue/red colour intensity is proportional to the magnitude of the response. Black vertical bars = change point of each taxon. The area below the curve represent 5th and 95th percentiles among 500 bootstrap replicates.

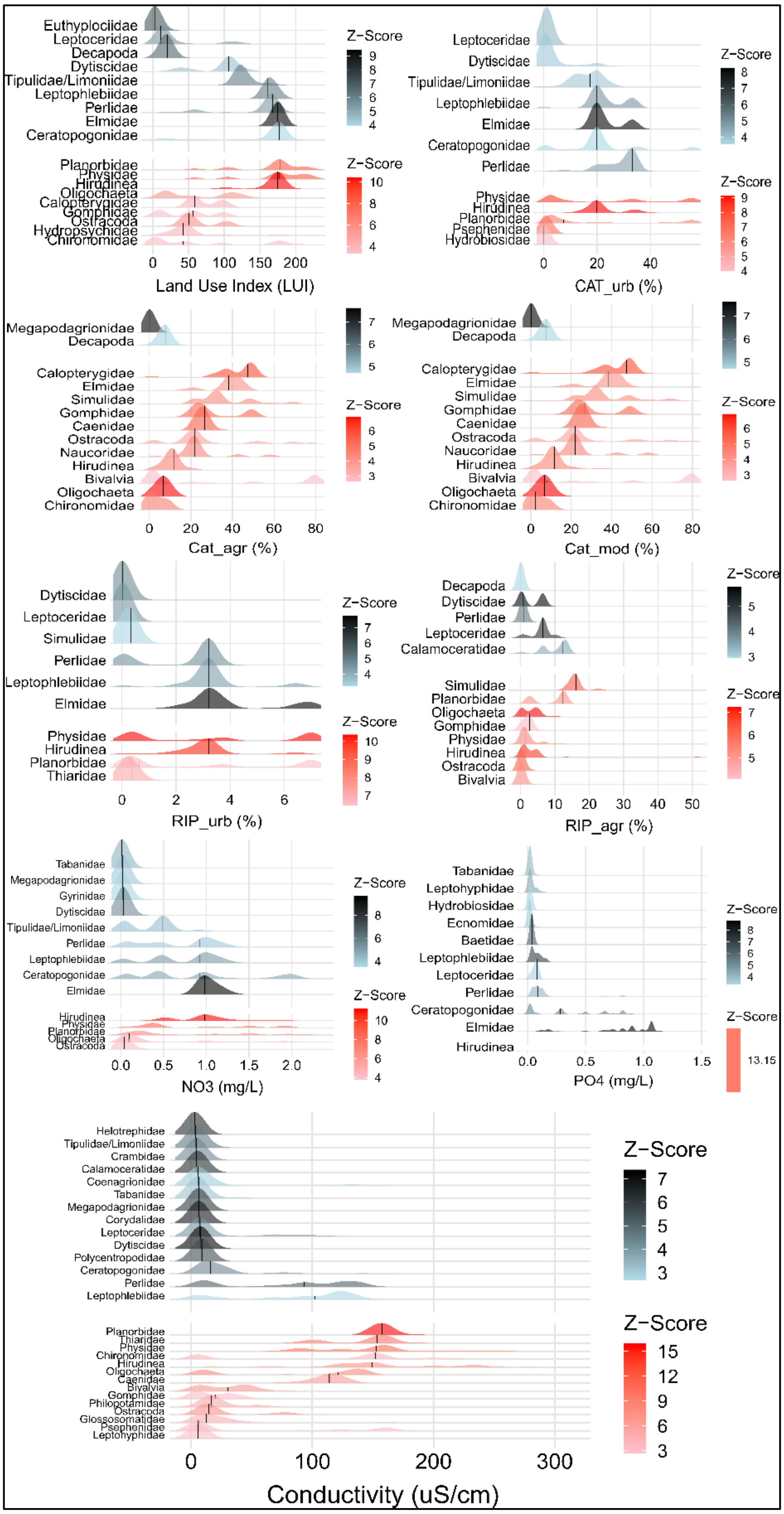

Figure 5. Results of TITAN showing taxon-specific maximum change points along all gradients for Macroinvertebrates. Robust sensitive taxa (Z-) and tolerant taxa (Z+) are represented in blue and red scales respectively. The blue/red colour intensity is proportional to the magnitude of the response. Black vertical bars = change point of each taxon. The area below the curve represent 5th and 95th percentiles among 500 bootstrap replicates.

4. Discussion

4.1. Influence of natural and human disturbance gradients on biological assemblages

Combined natural and human factors lead to changes in freshwater environment, that can ultimately impact biological assemblages. Considering both factors and their covariations allows a better understanding of the freshwater ecosystem (Tang et al. 2019). In this study, we observed that a large part of the natural factors considered did not have great relevance on the composition of both assemblages, with few exceptions. This may have occurred due to the relative homogeneity of the region since natural factors tend to be more relevant in explaining the variation in ecoregions and on national scales (Tang et al. 2019).

The natural variables that presented some correlation with the assemblages were elevation, shading and the percentage of organic matter (OM) in the river sediments. Jointly with local aspects, the elevation is reported as a determinant factor of the diatoms and macroinvertebrates' diversity (He et al. 2020). The last two factors - shading and OM - may be related to natural phenomena such as river enlargement from the source towards the mouth (Vannote et al. 1980; Hughes et al. 2011) but also to human disturbances, such as the removal of natural vegetation. Light availability has been reported as the most important factor controlling diatom assemblages in oligotrophic forested headwaters (Tornés & Sabater 2010). Besides this, the allochthonous OM from the riparian vegetation is the main source of energy in headwater streams and its input is fundamental for organisms that depend on this resource, like some macroinvertebrates (Gonçalves et al. 2006; Sánchez-Argüello et al. 2010).

Land uses on both scales (catchment and riparian) were important drivers in the composition of diatom and macroinvertebrate assemblages. It is known that human activities on different scales can lead to in-stream modifications, often through successive and complex processes that ultimately manifest in changes in the structure of biological assemblages (Allan 2004). Riparian vegetation plays a critical role in maintaining the structure and function of freshwater ecosystems, especially in headwaters or small rivers (Bunn & Davies 2000; Perona et al. 2009) as is the case of the streams in the study area. However, intact riparian zones can be insufficient to buffer the effects of altering catchment land uses, as demonstrated by Hlúbiková et al. (2014) and Giling et al. (2016). In this study, small changes in riparian vegetation were sufficient to trigger significant changes in biological assemblages. Therefore, this natural buffer is already broken under a small percentage of deforestation, leading to a greater effect of the catchment scale factors under the freshwater ecosystem.

We also detected strong relationships between biological assemblages' structures and the presence of point-source sewage release in the upstream catchment, particularly for macroinvertebrates. Organic pollution is a worldwide threat and the number of people affected by this is bound to increase from 1.1 billion in 2000 to 2.5 billion in 2050, with developing countries being disproportionately affected (Wen et al. 2017). The deficit in sewage collection and treatment in Brazilian cities has resulted in a significant input of pollutants reaching the streams and rivers, causing negative implications to the multiple uses of water resources (Agência Nacional de Águas – ANA 2017). Despite the self-depuration capacity of water bodies, the pollutant input causes changes in physical and chemical water characteristics, especially increasing turbidity, electrical conductivity, nutrient concentration and decreasing dissolved oxygen (Copetti et al. 2018). Nutrient pollution can have major and widespread impacts on biotic structure and function (Woodward et al. 2012) especially in headwater streams and small rivers that have relatively low discharge with minimal flushing or capacity for pollutant dilution, as is the case of the majority of watercourses in the study area (GDF 2012).

The water quality parameters considered in this study were not correlated with the direct human disturbances listed above, but in the ordination plots, some of them presented similar vectors (size and direction) to the main impacts, which may indicate that these parameters reflect unmeasured disturbances, such as the input of wastewater and diffuse pollutant into waterbodies. Electrical conductivity, nitrate, phosphate, turbidity, and dissolved oxygen are among the water quality variables most frequently altered by anthropogenic activities such as the release of domestic and industrial effluents, land use, and the use of fertilizers along with other agricultural inputs (Mello et al. 2020). Studies have already demonstrated the relationship of some of these parameters with the structure of biological assemblages, such as conductivity and phosphorus related to diatoms (Potapova & Charles 2003; Waite et al. 2019), and dissolved oxygen and turbidity related to macroinvertebrates (Okano et al. 2017; Corijmans et al. 2021).

4.2. Land use and water quality thresholds for diatom and macroinvertebrate assemblages

Catchment and riparian land use often result in changes in biological assemblages that are characterised by a decrease in the density and richness of taxa sensitive to environmental changes and an increase in the density of tolerant taxa (Feio et al. 2013; Martins et al. 2017). To reiterate: an ecological threshold refers to the point at which a small or abrupt change in a driver gradient may produce large responses in the ecosystem or particular components (e.g., species; Baker & King 2010). The ability to identify such thresholds is seen as an important aspect of managing ecological systems (Huggett 2005; Dodds et al. 2010) not only because they can influence ecosystem goods and services that people value (Martin et al. 2009) but also because they prevent the loss of biodiversity and ecosystem function (King & Richardson 2003).

Our results revealed that relatively minor increases in land uses in the riparian zone and in the upstream catchment were sufficient to trigger significant changes in macroinvertebrates and diatom assemblages. This suggests that the biotic assemblages in the small headwater streams we analysed are especially vulnerable due to the high connectivity of these ecosystems to adjacent landscapes (Taniwaki et al. 2018). In a study comprising several Brazilian biomes, Dala-Corte et al. (2020) demonstrated that the narrower the riparian-buffer the lower the threshold for removal of native vegetation. It was especially critical for macroinvertebrates considering that the reduction of only 6.5% of native vegetation cover within a 50-m riparian buffer was enough to cross thresholds for that biological assemblage. Similar low values were detected in Amazonian streams, where the thresholds for macroinvertebrates varied between 1 and 15% (~9%) and 0–35% (~1.4%) of forest-loss at the catchment and riparian levels, respectively (Brito et al. 2020). Even though the Brazilian Forest Code (Federal Law 12.651/ 2012) determines the maintenance of a riparian corridor between 30 and 100 m, depending on the river width, the preservation of the natural vegetation in private zones is limited to 35% in agricultural areas and unlimited in urban areas, which means that a much higher percentage of logging is permitted than it is tolerable according to the assemblages studied.

The limits of tolerance for conductivity were very low (7 – 163 $\mu S.cm^{-1}$) when compared to other studies. Sultana et al. (2019) demonstrated that for two catchments in Australia the conductivity threshold for macroinvertebrates ranged between 407 and 931 $\mu S.cm^{-1}$, furthermore Nguyen et al. (2017) found in western Ecuador change points between 930 and 1430 $\mu S.cm^{-1}$. Tibby et al. (2020) detected in South Australia significant declines in the relative abundance of sensitive species of diatoms at ~ 280 $\mu S.cm^{-1}$. A possible reason for this discrepancy may be the limited number of samples in the highest extremity of the gradient, which led to a low average conductivity (around 50 $\mu S.cm^{-1}$) and a few high values reaching a maximum of 500 $\mu S.cm^{-1}$. Nevertheless, our results indicate that some diatom and macroinvertebrate taxa responded robustly to even subtle changes in conductivity, indicating that it has an important correlation with biological assemblage’s composition.

Nitrogen and phosphorus are fundamental nutrients in triggering the eutrophication process of rivers and lakes (Figueredo et al. 2016; Zhang et al. 2017), and the export of phosphorus from agricultural land to water bodies is predicted to increase (Ockenden et al.

2016). Our results showed nitrate and phosphate thresholds values around 0.3–1.0 $mg.L^{-1}$ and 0.03–1.5 $mg.L^{-1}$, respectively, while the Brazilian legislation provides reference values of 10 and 0.1–0.15 $mg.L^{-1}$ for nitrate and total phosphorous, respectively. The threshold values of nitrate and phosphate were similar to those found in other regions of the world, both for diatoms (e.g., Hausmann et al. 2016; Tibby et al. 2020) and macroinvertebrates (e.g., Kail et al. 2012; Nguyen et al. 2017). The scientific literature indicates freshwater eutrophication as a major stressor (Brönmark & Hansson 2002) and cause of biodiversity loss in freshwater ecosystems (Taylor et al. 2014), but here we demonstrated that the loss of sensitive taxa occurs even before the risk of eutrophication.

That is very clear in the taxa-specific graphs where it is noticed that the change points of the sensitive taxa (Z-) are in general smaller and sharper than those of the tolerant taxa (Z+). The decline in sensitive taxa occurs due to increasing pressures such as physiological limitations, inaccessibility of resources, interruption of interspecific interactions and the strengthening of competition and predation pressure. In contrast, tolerant taxa are not directly affected by increasing environmental pressure, but abundance arises with increasing pressure as they fill ecological niches that become vague with the disappearance of competitors or when they are exposed to reductions in predation and subsidy of experimental resources. This makes the response of the tolerant taxa more gradual and less abrupt than that of the sensitive ones (King & Baker 2011; Sundermann et al. 2015).

Threshold analyses allowed for the identification of robust sensitive (ex: *Eunotia*, Coridalidae, Perlidae, Helotrephidae, Leptoceridae, Leptophlebiidae and others) and tolerant taxa (ex: *Gomphonema*, Hirudinea, Oligochaeta, Planorbidae, and others) and their individual responses to different disturbances. Factors such as taxa life history attributes, habitat diversity, and the influence of stochastic events appear to be implicated in producing these differential threshold responses (Huggett 2005), but also an important factor is the taxonomic resolution. In this study, we use the levels of genus/species for diatoms and family/order/ class for macroinvertebrates. More specific identifications can lead to new taxa-specific change points and must provide more informative description than higher taxonomic levels, however, studies have already shown that, in general, a coarse identification is sufficient for biomonitoring proposals (Bailey et al. 2001; Rimet & Bouchez, 2012; Vilmi et al. 2016).

Eunotia was related to good water quality conditions in studies carried out in Brazil (Salomoni et al. 2006) and England (Kelly, 1998), but Oeding & Taffs (2017) demonstrated that species within this genus exhibited a range of sensitivity values in Australian rivers. There is wide scientific consensus that *Nitzschia palea* is a specie tolerant to pollution (Kelly 1998;

Lobo et al. 2015; Oeding & Taffs 2017). For macroinvertebrates, the EPT (Ephemeroptera, Plecoptera, and Trichoptera) group represented 43.5% of the sensitive taxa identified by TITAN, and Oligochaeta, Hirudinea, and Physidae were the most common tolerant taxa, confirming the findings of other studies (Wagenhoff et al. 2012; Ferreira et al. 2014; Pardo et al. 2020). The robust response to a range of human disturbance gradients demonstrates the potential utility of these taxa as bioindicators for the study area. Ecological thresholds may support the development of numerical indicators of ecosystem health and also the identification of reference conditions to characterize assemblages' composition in the absence of disturbance (King & Richardson 2003; Baker & King 2010).

4.3. Implications for freshwater bioassessment

Despite its biological importance, the Brazilian savanna has been threatened by agribusiness and is one of the world's richest biodiversity hotspots with the lowest percentage of areas under full protection (MMA 2020). In addition to the low percentage of protected areas, the acceptable percentage of native vegetation removal and the water quality mandated by Brazilian legislation and management guidelines may be insufficient to maintain the integrity of biological assemblages (Martins et al. 2017). Our study has important implications and offers valuable information for the development of efficient management and policy tools on freshwater and watershed in the Brazilian savanna. Corroborating previous research in Brazil (Rodrigues et al. 2016; Dala- Corte et al. 2020; Brito et al. 2020) and elsewhere (e.g., Sultana et al. 2019; Tibby et al. 2020) we indicated that there is a gap between the needs of biological assemblages and current management guidelines.

Additionally, the study also demonstrated the importance of management considering more than one biological assemblage (Vilmi et al. 2016) and multiple stressors (Waite et al. 2019) since the responses are complementary. Changes in the biological composition attributed to a single variable do not necessarily represent a simple relationship to the environmental variable in focus but could include responses to other pressure variables linked with the corresponding variable (Sundermann et al. 2015). The study brings a simplification of a complex system, and although it considered multiple direct and indirect stressors, the change points cannot be used as a basis to derive causalities and the reduction of any stress factor below the change point of a sensitive taxon will be no guarantee for a recovery. The mapping of taxon-specific and assemblages change points can be used, in addition to the conservation of ecosystems and prevention of loss of biodiversity, in the indication of potentially polluted places and also in the prediction of future scenarios. (Sundermann et al. 2015).

The extent of temporal variability in biotic assemblages has important implications for bioassessment programs that aim to evaluate the health or integrity of aquatic ecosystems based on biological assemblages' structure and to assess the magnitude and likely sources of human disturbances (Tonkin et al. 2017). As it is an exploratory work, we consider it necessary to proceed sampling in two periods, thus guaranteeing greater coverage on the conditions of our systems. Although natural and human impact gradients were very similar, significant differences were detected in the composition of both biological assemblages between dry and wet seasons, as it has been shown in previous studies in the region (Bispo & Oliveira 2007) and elsewhere (e. g. Karaouzas et al. 2019; Snell et al. 2019). In this study, joint analysis of data from both seasons allows future monitoring programs to have reference taxa and thresholds, regardless of the period of sampling. In addition, with a single sampling in each season, we would not be able to affirm seasonality effects with certainty since this would require sampling in more than one year. Further research is required to understand the extent and drivers of temporal variation in biotic assemblages in Brazilian savanna's streams.

5. Conclusions

Working with different scales, stressors and responses is challenging but necessary to understand at least part of the environment's complexity (Allan 2004; Wagenhoff et al. 2012). Here we demonstrate that, despite being influenced by some natural characteristics, the structures of the diatom and macroinvertebrate assemblages were strongly affected by the gradients of anthropogenic disturbances, from local to the catchment scale. Our results may provide new perspectives on the management of freshwater in the study area and in other neotropical regions aided by the identification of specific taxa of diatoms and macroinvertebrates as potential bioindicators, and thresholds of important anthropogenic disorders (land use and water quality). In general, the values found were more restrictive than those provided by Brazilian government guidelines, which means they would be insufficient to maintain the integrity of biological assemblages in Brazilian savanna's watersheds. We offered valuable knowledge about the sensitivities and tolerances of diatom and macroinvertebrate's taxa/assemblages that can be especially useful for the proper freshwater and watersheds management.

Acknowledgements

The authors are thankful to the Laboratório de Citotaxonomia e Insetos Aquáticos (Instituto Nacional de Pesquisas da Amazônia - INPA) team, for their support in identifying macroinvertebrates. We also thank Maíra Campos, Ana Luiza Dornas and Cleber Figueredo, from Universidade Federal de Minas Gerais (UFMG), for their support in identifying diatoms. This work was funded by: the Institutional Internationalization Program of the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES-PrInt; Proc. no.: 88887.364699/2019-00) that financed the 1-year PhD sandwich of Campos C.A. in Brisbane, Australia; the Fundação de Amparo à Pesquisado do Distrito Federal (FAP-DF) for their financial support to Aquariparia Project (edital 05/ 2016; Proc. no.: 193.000716/2016) that allowed the execution of fieldwork and laboratory analyses; the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) through research fellowship to José Francisco Gonçalves JR (Proc. no.: 310641/2017-9); and the Agência Reguladora de Águas, Energia e Saneamento Básico do Distrito Federal (ADASA) that in addition to the financial support to Campos C. A. also offered logistical support of cars for the fieldwork. Thanks to the Australian River Institute (ARI) for hosting Campos C.A. for one year to develop the statistical and modelling analyses of the study. We greatly appreciate the collaboration of all students of the Laboratório de Limnologia (Universidade de Brasília - UNB) in fieldwork activities and laboratory analyses. Finally, we would like to thank all the institutions (Exército Brasileiro, Marinha Brasileira, IBRAM, ICMBio, Jardim Botânico, IBGE and UNB) and owners of environmental protected areas (Chapada Imperial and Paraíso na Terra), which allowed the collection of samples on the lands under their administration.

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Further reading

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SUPPLEMENTARY MATERIAL

APPENDIX A

Table A.1. Frequency and density of identified diatoms species in May (wet season) and September (dry season).

Species	Code	May		September	
		Freq (%)	Density (cell.cm^{-2})	Freq (%)	Density (cell.cm^{-2})
Achnantidium cf. exiguum	A.ex	4.35	29	3.92	42
Achnantidium cf. minutissimum	A.min	30.43	483	39.22	21826
Achnantidium cf. oblongella	A.obl	0.00	0	1.96	7
Actinella brasiliensis	A.bra	2.17	8	9.80	242
Adlafia cf. bryophila	A.bry	4.35	305	1.96	164
Brachysira sp	Br.sp	2.17	81	1.96	7
Brachysira cf. brebissonii	B.bre	4.35	163	11.76	594
Cocconeis cf. fluviatilis	C.flu	2.17	7	1.96	4
Cocconeis cf. placentula	C.pla	6.52	484	7.84	400
Cyclotella sp	Cyc.sp	2.17	55	1.96	55
Cymbella cf. affinis	C.aff	2.17	6	3.92	128
Cymbopleura cf. naviculiformis	C.nav	0.00	0	3.92	26
Diadesmis cf. confervacea	D.con	0.00	0	1.96	80
Diploneis sp	Dip.sp	6.52	98	3.92	70
Encyonema cf. neogracili	E.neo	8.70	465	13.73	524
Encyonema cf. silesiacum	E.sil	2.17	56	1.96	5765
Encyonema sp	Enc.sp	10.87	438	15.69	192
Encyonopsis cf. rumrichae	E.rum	2.17	51	3.92	444
Encyonopsis sp	Ecp.sp	0.00	0	5.88	445
Eolimna sp	Eol.sp	4.35	27	17.65	4362
Eunotia cf. lineolata	E.lin	4.35	853	0.00	0
Eunotia muscicola	E.mus	0.00	0	1.96	9
Eunotia serra var.1	E.ser1	15.22	529	27.45	1904
Eunotia serra var.2	E.ser2	6.52	205	9.80	611
Eunotia sp1	E.sp1	78.26	4289	78.43	14854
Eunotia sp2	E.sp2	28.26	1295	33.33	30338
Eunotia sp3	E.sp3	2.17	118	5.88	3095
Eunotia sp4	E.sp4	8.70	87	21.57	8118
Eunotia sp5	E.sp5	28.26	498	52.94	15406
Eunotia sp6	E.sp6	0.00	0	9.80	99
Eunotia sp7	E.sp7	13.04	240	27.45	3503
Eunotia sp8	E.sp8	60.87	2497	31.37	13464
Eunotia synedraeformis	E.syn	6.52	204	1.96	45
Eunotia trigibba	E.trig	0.00	0	1.96	23
Fragilaria sp	Frg.sp	0.00	0	5.88	105
Frustulia sp	Fru.sp	30.43	970	45.10	4371
Gomphonema sp	G.sp	8.70	202	9.80	183
Gomphonema cf. pseudoaugur	G.ps	6.52	61	9.80	277
Gomphonema sp1	G.sp1	0.00	0	7.84	108

Gomphonema cf. pumilum	G.pum	10.87	1458	11.76	904
Gomphonema cf. angustatum	G.ang	8.70	130	13.73	184
Gomphonema cf.lagenula	G.lag	6.52	331	19.61	26345
Gomphonema cf. neoapiculatum	G.neo	0.00	0	3.92	78
Gomphonema cf. gracile	G.fra	13.04	459	15.69	2345
Gomphonema cf. parvulum	G.par	17.39	2631	3.92	692
Gyrosigma sp	Gyr.sp	4.35	135	0.00	0
Navicula cf. cryptotenella	N.cpt	30.43	704	27.45	2373
Navicula sp4	N.sp4	0.00	0	1.96	4
Navicula cf. cryptocephala	N.cpc	19.57	321	29.41	1264
Navicula sp1	N.sp1	0.00	0	13.73	394
Navicula cf. rostellata	N.ros	15.22	167	11.76	118
Navicula sp2	N.sp2	0.00	0	1.96	5
Navicula cf. viridula	N.vir	0.00	0	1.96	5
Navicula sp3	N.sp3	0.00	0	1.96	4
Navicula cf. angusta	N.ang	4.35	72	11.76	483
Navicula cf. radiosa	N.rad	8.70	1104	7.84	114
Nitzschia cf. agnewii	N.agn	4.35	46	15.69	252
Nitzschia cf. haufleriana	N.hau	0.00	0	3.92	53
Nitzschia cf. palea	Nt.pal	10.87	317	25.49	76365
Nitzschia sp1	Nt.sp1	32.61	2516	11.76	71
Nitzschia cf. exilis	Nt.ex	0.00	0	3.92	64
Nitzschia cf. brevissima	Nt.bre	2.17	22	5.88	40
Pinnularia cf. gibba	P.gib	6.52	160	1.96	14
Pinnularia cf. mesolepta	P.mes.	6.52	100	9.80	66
Pinnularia cf. viridis	P.vir	4.35	96	17.65	185
Pinnularia sp1	P.sp1	4.35	245	1.96	46
Pinnularia cf. divergens	P.div	2.17	17	1.96	30
Planotidium sp1	Pla.sp1	0.00	0	1.96	4
Planotidium sp2	Pla.sp2	0.00	0	1.96	201
Sellaphora sp	Sell.sp	6.52	189	7.84	261
Stenopterobia sp	Ste.sp	2.17	11	1.96	15
Surirella linearis	S.lin	4.35	66	5.88	910
Surirella sp	Sur.sp	0.00	0	1.96	5
Ulnaria ulna	U.ul	13.04	680	21.57	4624

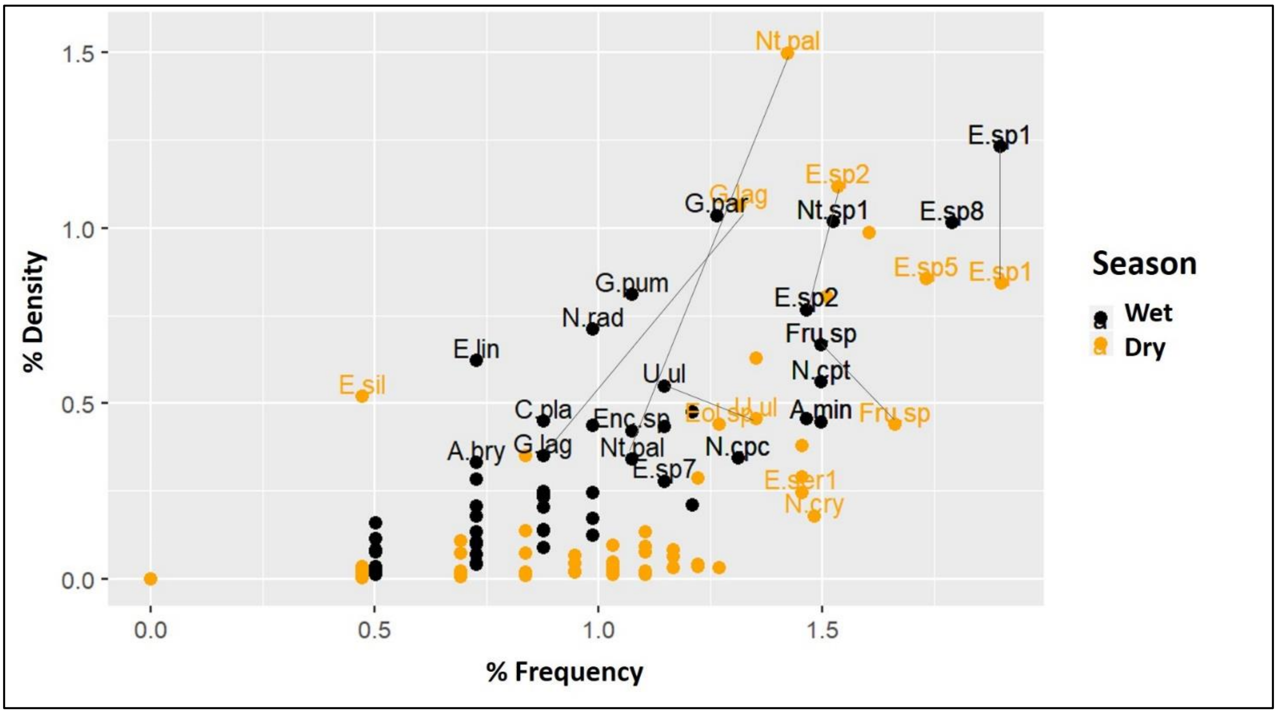


Figure A.1. Percentage of total frequency versus the percentage of total density of diatoms occurrence across the sampled sites in both campaigns, wet (May, black points) and dry (September, yellow points) seasons. Each point represents one species and the grey line links the same species across the two seasons. Data are plotted in log+1 scale and only the most frequent and abundant species in at least one month are labeled.

Table A.2. Frequency and density of identified macroinvertebrates taxa (low level) in May (wet season) and September (dry season).

Taxa	Code	May		September	
		Freq (%)	Density (ind m2)	Freq (%)	Density (ind m2)
Ancylidae	Acy	4.08	4	2.00	9
Baetidae	Bae	40.82	264	80.00	2818
Belostomatidae	Bel	6.12	9	2.00	2
Bivalvia	Biv	28.57	302	38.00	1331
Caenidae	Cae	4.08	9	8.00	18
Calamoceratidae	Clc	22.45	78	24.00	80
Calopterygidae	Clp	14.29	29	24.00	69
Ceratopogonidae	Crt	73.47	431	82.00	964
Chironomidae	Chi	100.00	11473	100.00	36156
Coenagrionidae	Coe	51.02	104	58.00	220
Collembola	Col	0.00	0	12.00	18
Corduliidae	Cdl	2.04	2	2.00	7
Corydalidae	Crd	22.45	36	14.00	27
Crambidae	Cra	20.41	38	18.00	29
Culicidae	Cul	0.00	0	2.00	2
Curculionidae	Cur	0.00	0	2.00	2
Decapoda	Dec	6.12	7	12.00	16
Dicteriadidae	Dic	2.04	2	4.00	7
Dytiscidae	Dyt	28.57	80	42.00	267
Ecnomidae	Ecn	4.08	4	26.00	47
Elmidae	Elm	89.80	2527	88.00	5249
Empididae	Emp	26.53	42	30.00	127
Euthyplociidae	Eut	12.24	80	8.00	29
Gerridae	Ger	0.00	0	8.00	11
Glossosomatidae	Glo	16.33	42	22.00	131
Gomphidae	Gom	30.61	167	28.00	78
Gripopterygidae	Gri	4.08	7	4.00	7
Gyrinidae	Gyr	12.24	20	26.00	44
Helotrephidae	Hel	2.04	16	10.00	40
Hidracarina	Hyd	18.37	49	32.00	91
Hirudinea	Hir	18.37	2569	10.00	1227
Hydrobiidae	Hdb	4.08	22	6.00	22
Hydrobiosidae	Hds	10.20	16	18.00	53
Hydrophilidae	Hdp	4.08	4	12.00	33
Hydropsychidae	Hdc	51.02	647	48.00	462
Hydroptilidae	Hyt	20.41	42	48.00	173
Leptoceridae	Ltc	34.69	240	40.00	122
Leptohyphidae	Lth	32.65	680	58.00	2036
Leptophlebiidae	Ltp	46.94	267	82.00	1924
Libellulidae	Lib	48.98	122	48.00	182
Megapodagrionidae	Meg	6.12	13	18.00	31
Naucoridae	Nau	30.61	60	28.00	76
Nitidulidae	Nit	0.00	0	2.00	2
Noteridae	Not	4.08	13	2.00	2
Odontoceridae	Odo	30.61	131	22.00	84

Oligochaeta	Oli	44.90	2998	72.00	4229
Ostracoda	Ost	18.37	216	24.00	1780
Perlidae	Per	59.18	322	64.00	453
Philopotamidae	Phi	10.20	20	10.00	18
Physidae	Phy	4.08	9	8.00	109
Planariidae	Pla	2.04	13	6.00	13
Planorbidae	Plb	6.12	84	4.00	47
Polycentropodidae	Pcp	18.37	33	20.00	78
Psephenidae	Pse	12.24	47	16.00	78
Scirtidae	Sci	0.00	0	2.00	2
Sialidae	Sia	8.16	11	8.00	18
Simulidae	Sim	57.14	7542	68.00	5236
Tabanidae	Tab	12.24	16	24.00	76
Thiaridae	Thi	4.08	264	4.00	33
Tipulidae/Limoniidae	Tip	53.06	122	42.00	180
Veliidae	Vel	2.04	2	8.00	22

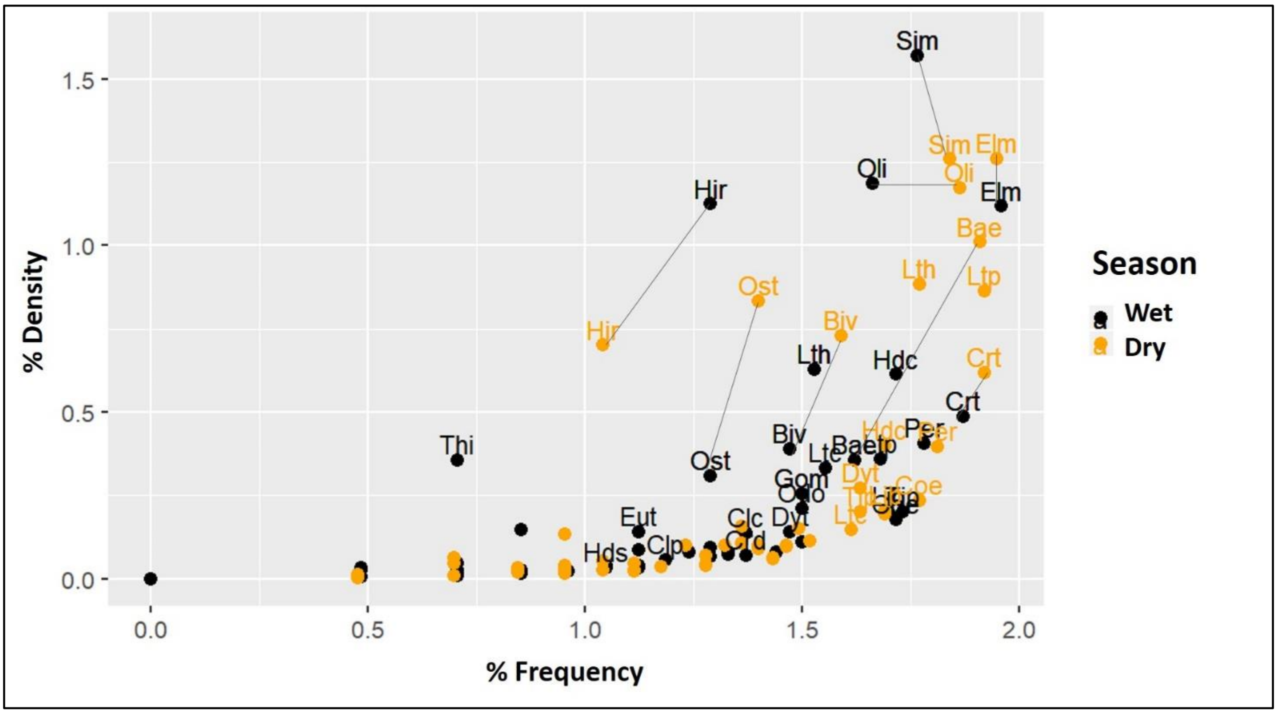


Figure A.2. Percentage of total frequency versus the percentage of total density of macroinvertebrates taxa occurrence across the sampled sites in both campaigns, wet (May, black points) and dry (September, yellow points) seasons. Each point represents one taxon and the grey line links the same taxon in two months. Data are plotted in log+1 scale and only the most frequent and abundant taxa in at least one month are labeled.

APPENDIX B

Table B.1. Spearman's rho correlation coefficients for relationships between natural and human disturbances variables.

Variables	*GIS obtained variables*									
	drai_area	source_dist	elevation	slope	S1	S2	S3	G1	G2	G3
drai_area	1.00									
source_dist	0.92	1.00								
elevation	-0.55	-0.50	1.00							
slope	-0.59	-0.44	0.36	1.00						
S1	0.05	-0.08	0.10	-0.36	1.00					
S2	-0.15	0.01	-0.01	0.40	-0.90	1.00				
S3	0.20	0.18	-0.21	-0.02	-0.39	-0.05	1.00			
G1	-0.31	-0.23	0.60	0.19	0.14	-0.01	-0.30	1.00		
G2	0.21	0.24	-0.24	0.12	-0.39	0.43	-0.02	-0.30	1.00	
G3	0.25	0.15	-0.54	-0.24	0.00	-0.15	0.32	-0.93	-0.06	1.00
OM	-0.27	-0.38	0.07	-0.19	0.21	-0.19	-0.08	-0.07	-0.16	0.13
coa_sed	-0.18	-0.06	0.08	0.37	-0.23	0.16	0.19	-0.08	-0.03	0.09
med_sed	0.15	0.04	-0.04	-0.31	0.28	-0.22	-0.17	0.10	0.00	-0.10
fin_sed	0.17	0.08	-0.07	-0.40	0.14	-0.04	-0.23	0.13	0.04	-0.15
v_fin_sed	0.11	-0.01	-0.10	-0.31	0.00	0.09	-0.18	-0.05	0.16	-0.01
silt	0.19	0.07	-0.16	-0.36	0.03	0.03	-0.13	-0.17	0.15	0.12
shading	-0.29	-0.38	0.24	0.00	0.11	-0.16	0.10	0.05	-0.16	0.01
av_depth	0.41	0.41	-0.05	-0.15	-0.07	-0.01	0.19	-0.02	0.01	0.02
av_veloc	0.37	0.36	-0.03	-0.24	0.00	-0.01	0.00	0.04	0.00	-0.04
av_width	0.64	0.68	-0.28	-0.16	-0.16	0.14	0.07	0.03	0.23	-0.12
disch	0.64	0.67	-0.16	-0.26	-0.08	0.04	0.11	0.02	0.17	-0.08
temp	0.11	0.10	-0.44	-0.08	-0.13	0.18	-0.09	-0.32	0.17	0.27
DO	-0.08	0.07	0.26	0.29	-0.22	0.17	0.15	0.12	-0.04	-0.11
pH	0.14	0.17	-0.13	-0.01	-0.01	-0.08	0.20	-0.20	-0.03	0.22
cond	0.51	0.43	-0.51	-0.18	0.05	-0.06	0.03	-0.34	0.22	0.27
turb	0.46	0.45	-0.26	-0.24	0.19	-0.27	0.14	-0.35	0.00	0.36

K+	0.15	0.15	-0.05	0.04	0.08	-0.09	0.00	0.03	-0.01	-0.03
Fe+2	0.11	0.10	-0.04	0.01	0.04	0.00	-0.08	-0.04	-0.08	0.07
F-	0.09	0.09	-0.05	0.10	0.05	-0.05	0.00	-0.04	0.01	0.04
Cl-	0.39	0.41	-0.22	-0.15	0.07	-0.08	0.01	-0.19	0.19	0.13
NO2-	0.13	0.13	-0.10	-0.01	0.00	0.00	-0.01	0.02	0.00	-0.02
Br-	-0.10	-0.07	-0.07	0.18	0.00	0.05	-0.11	-0.12	0.08	0.09
NO3-	0.13	0.12	-0.06	-0.03	0.05	0.01	-0.15	0.11	0.18	-0.18
PO4-3	0.11	0.14	-0.07	-0.08	0.06	-0.03	-0.07	0.04	0.05	-0.06
SO4-2	0.21	0.20	-0.24	0.00	0.00	0.00	-0.01	-0.11	0.23	0.03
RIP_urb	0.22	0.28	-0.11	0.02	0.01	0.02	-0.06	0.09	0.29	-0.20
RIP_agr	0.46	0.44	-0.43	-0.19	0.01	-0.11	0.22	-0.59	0.17	0.55
RIP_mod	0.36	0.40	-0.13	-0.05	-0.03	0.06	-0.06	0.04	0.35	-0.18
CAT_urb	0.24	0.25	0.09	-0.01	0.20	-0.16	-0.12	0.32	0.15	-0.39
CAT_agr	0.23	0.09	-0.43	-0.12	0.11	-0.23	0.23	-0.46	0.05	0.47
CAT_mod	0.41	0.43	0.13	-0.12	0.13	-0.16	0.03	0.27	0.18	-0.35
LUI	0.32	0.23	-0.27	-0.20	0.25	-0.35	0.17	-0.26	0.03	0.26
SR	0.24	0.25	-0.05	0.05	0.09	-0.08	-0.04	0.12	-0.04	-0.11
Dam	0.36	0.34	-0.29	0.04	-0.07	-0.04	0.24	-0.18	0.24	0.10
N_dams	0.34	0.33	-0.29	0.07	-0.12	-0.02	0.31	-0.20	0.29	0.10
Dist_dam	0.39	0.39	-0.32	0.07	-0.10	0.00	0.24	-0.21	0.31	0.10
C_Roads	0.52	0.55	-0.31	-0.17	-0.10	0.00	0.23	-0.20	0.30	0.10

	Habitat characteristics						
	OM	coa_sed	med_sed	fin_sed	v_fin_sed	silt	shading
OM	1.00						
coa_sed	-0.44	1.00					
med_sed	0.41	-0.90	1.00				
fin_sed	0.37	-0.93	0.75	1.00			
v_fin_sed	0.46	-0.77	0.50	0.80	1.00		
silt	0.50	-0.71	0.53	0.64	0.89	1.00	
shading	0.03	0.13	-0.12	-0.12	-0.16	-0.23	1.00
av_depth	-0.17	-0.09	0.07	0.09	0.09	0.11	-0.20

av_veloc	-0.05	0.13	-0.10	-0.07	-0.14	-0.09	-0.16
av_width	-0.47	0.05	-0.12	0.05	-0.01	-0.05	-0.40
disch	-0.34	0.03	-0.04	0.04	-0.05	-0.03	-0.35
temp	0.15	-0.06	0.01	0.10	0.19	0.19	-0.33
DO	-0.37	0.20	-0.22	-0.19	-0.17	-0.15	-0.05
pH	-0.20	0.19	-0.25	-0.14	-0.13	-0.15	-0.02
cond	-0.23	-0.01	0.05	0.00	-0.09	-0.04	-0.34
turb	-0.18	-0.01	0.05	-0.04	-0.09	0.05	-0.30
K+	-0.14	0.03	-0.01	-0.03	-0.07	-0.05	-0.23
Fe+2	-0.16	0.15	-0.05	-0.18	-0.22	-0.15	-0.09
F-	-0.11	0.05	0.04	-0.10	-0.15	-0.05	-0.07
Cl-	-0.19	0.05	0.01	-0.03	-0.16	-0.09	-0.23
NO2-	-0.02	0.05	-0.05	-0.03	-0.05	-0.02	-0.18
Br-	0.03	-0.05	0.09	0.03	-0.04	0.02	-0.01
NO3-	-0.05	-0.02	0.04	0.07	0.05	0.06	-0.25
PO4-3	-0.08	-0.06	0.08	0.07	-0.02	-0.01	-0.12
SO4-2	-0.14	-0.03	0.01	0.10	-0.01	-0.05	-0.16
RIP_urb	-0.31	0.14	-0.12	-0.10	-0.10	-0.04	-0.29
RIP_agr	-0.09	0.08	-0.05	-0.11	-0.07	0.05	-0.36
RIP_mod	-0.19	0.05	0.01	-0.08	-0.08	0.01	-0.42
CAT_urb	-0.31	0.05	-0.01	0.00	-0.15	-0.18	-0.14
CAT_agr	0.16	-0.04	0.06	-0.01	0.02	0.06	-0.10
CAT_mod	-0.29	-0.20	0.26	0.15	-0.01	0.06	-0.22
LUI	-0.04	0.06	0.00	-0.08	-0.10	-0.02	-0.14
SR	-0.09	-0.03	-0.03	0.09	0.02	-0.02	-0.38
Dam	-0.29	0.13	-0.12	-0.11	-0.15	-0.11	-0.09
N_dams	-0.30	0.18	-0.17	-0.16	-0.18	-0.14	-0.08
Dist_dam	-0.31	0.11	-0.10	-0.09	-0.13	-0.10	-0.14
C_Roads	-0.22	0.01	0.00	0.00	-0.05	0.05	-0.55

Hydromorphological characteristics

	av_depth	av_veloc	av_width	disch
av_depth	1.00			
av_veloc	0.33	1.00		
av_width	0.34	0.26	1.00	
disch	0.72	0.70	0.74	1.00
temp	0.22	0.20	0.19	0.26
DO	0.11	-0.08	0.23	0.12
pH	-0.08	-0.14	0.10	-0.03
cond	0.24	0.33	0.37	0.43
turb	0.38	0.36	0.36	0.51
K+	-0.14	-0.20	0.06	-0.13
Fe+2	0.14	0.21	0.09	0.24
F-	-0.03	-0.16	-0.04	-0.12
Cl-	0.16	0.31	0.38	0.44
NO2-	0.27	0.34	0.35	0.46
Br-	-0.07	-0.25	-0.17	-0.23
NO3-	0.18	0.33	0.33	0.43
PO4-3	0.30	0.31	0.21	0.41
SO4-2	0.15	0.18	0.34	0.33
RIP_urb	0.08	0.23	0.31	0.34
RIP_agr	0.18	0.15	0.31	0.32
RIP_mod	0.16	0.29	0.39	0.43
CAT_urb	0.12	0.28	0.41	0.42
CAT_agr	-0.12	-0.05	0.08	-0.05
CAT_mod	0.31	0.29	0.45	0.52
LUI	0.02	0.18	0.26	0.24
SR	0.20	0.15	0.38	0.34
Dam	0.20	0.15	0.34	0.31
N_dams	0.20	0.13	0.33	0.29
Dist_dam	0.22	0.13	0.38	0.34

C_Roads	0.23	0.33	0.47	0.51										
	Water quality													
	temp	DO	pH	cond	turb	K+	Fe+2	F-	Cl-	NO2-	Br-	NO3-	PO4-3	SO4-2
temp	1.00													
DO	-0.18	1.00												
pH	-0.16	0.32	1.00											
cond	0.32	-0.29	0.02	1.00										
turb	0.23	0.05	0.07	0.32	1.00									
K+	-0.25	-0.10	0.31	0.31	-0.09	1.00								
Fe+2	0.05	0.05	-0.07	0.16	0.28	-0.02	1.00							
F-	-0.11	0.01	0.19	0.16	-0.11	0.46	0.07	1.00						
Cl-	0.30	-0.14	0.09	0.64	0.35	0.15	0.22	0.09	1.00					
NO2-	0.34	0.07	-0.15	0.13	0.35	-0.33	0.21	-0.25	0.21	1.00				
Br-	0.02	-0.01	0.01	-0.01	-0.12	0.12	-0.01	0.14	0.04	-0.12	1.00			
NO3-	0.33	-0.12	-0.08	0.40	0.26	-0.02	0.23	0.00	0.48	0.41	-0.16	1.00		
PO4-3	0.32	-0.01	-0.19	0.17	0.47	-0.25	0.30	-0.23	0.23	0.63	-0.11	0.40	1.00	
SO4-2	0.25	-0.15	-0.03	0.51	0.33	0.03	0.18	-0.13	0.57	0.39	-0.02	0.42	0.42	1.00
RIP_urb	0.10	-0.05	0.03	0.44	0.33	0.25	0.19	0.06	0.51	0.29	-0.03	0.51	0.38	0.44
RIP_agr	0.39	-0.03	0.28	0.52	0.53	0.20	0.14	0.13	0.59	0.16	0.05	0.23	0.17	0.33
RIP_mod	0.22	-0.08	0.00	0.46	0.33	0.29	0.23	0.09	0.52	0.23	-0.05	0.39	0.29	0.39
CAT_urb	-0.07	-0.08	-0.07	0.49	0.13	0.23	0.11	0.06	0.50	0.22	-0.08	0.63	0.30	0.41
CAT_agr	0.32	-0.13	0.29	0.19	0.23	0.07	0.01	0.09	0.23	0.05	0.06	-0.06	-0.10	0.13
CAT_mod	-0.16	0.13	-0.04	0.33	0.28	0.19	0.11	0.09	0.34	0.05	-0.06	0.30	0.14	0.21
LUI	0.30	-0.11	0.25	0.47	0.48	0.26	0.09	0.10	0.61	0.19	0.00	0.39	0.23	0.32
SR	0.17	-0.22	0.05	0.39	0.32	0.40	0.18	0.11	0.37	0.32	0.00	0.38	0.39	0.37
Dam	0.08	0.07	0.28	0.38	0.13	0.13	0.06	0.25	0.43	0.11	0.14	0.20	-0.01	0.35
N_dams	0.06	0.07	0.27	0.37	0.10	0.12	0.03	0.24	0.40	0.09	0.14	0.17	-0.04	0.34
Dist_dam	0.10	0.07	0.28	0.41	0.16	0.16	0.08	0.24	0.45	0.12	0.16	0.23	0.02	0.38
C_Roads	0.27	0.05	0.25	0.50	0.48	0.30	0.16	0.12	0.61	0.24	0.00	0.41	0.29	0.40

Human disturbances

	RIP_urb	RIP_agr	RIP_mod	CAT_urb	CATagr	CAT_mod	LUI	SR	Dam	N_dams	Dist_dam	C_Roads
RIP_urb	1.00											
RIP_agr	0.31	1.00										
RIP_mod	0.75	0.42	1.00									
CAT_urb	0.64	0.05	0.48	1.00								
CAT_agr	-0.08	0.65	0.09	-0.28	1.00							
CAT_mod	0.41	0.11	0.53	0.64	-0.17	1.00						
LUI	0.51	0.68	0.46	0.37	0.59	0.29	1.00					
SR	0.35	0.34	0.36	0.40	0.13	0.19	0.38	1.00				
Dam	0.17	0.42	0.16	0.21	0.39	0.18	0.29	0.24	1.00			
N_dams	0.17	0.39	0.17	0.20	0.37	0.17	0.26	0.19	0.99	1.00		
Dist_dam	0.23	0.46	0.23	0.24	0.39	0.23	0.32	0.28	0.99	0.97	1.00	
C_Roads	0.61	0.73	0.70	0.35	0.38	0.42	0.62	0.47	0.43	0.42	0.48	1.00

APPENDIX C

Table C.1. Results of "envfit" (R package "vegan") tests for correlations between variables and the diatom assemblage ordination.

	NMDS1	NMDS2	NMDS3	r	p-values
drai_area	0.819	0.566	0.098	0.142	0.004
elevation	-0.962	-0.248	-0.112	0.214	0.001
S1	-0.541	-0.226	0.810	0.066	0.109
S3	-0.070	0.992	0.107	0.032	0.377
G1	-0.815	-0.077	-0.575	0.085	0.038
G2	0.707	0.408	-0.578	0.052	0.148
OM	-0.898	-0.326	-0.297	0.091	0.034
coa_sed	-0.743	-0.275	0.610	0.000	1.000
shading	-0.901	-0.315	0.300	0.187	0.001
temp	0.677	0.684	-0.272	0.162	0.003
DO	-0.509	0.254	-0.822	0.042	0.238
pH	0.257	0.687	0.680	0.034	0.354
cond	0.883	0.460	0.093	0.437	0.001
turb	0.349	0.576	0.739	0.118	0.009
K+	0.974	-0.016	0.228	0.107	0.018
Fe+2	0.494	0.856	-0.150	0.023	0.534
F-	0.258	-0.360	0.897	0.128	0.011
Br-	0.173	-0.874	-0.455	0.008	0.863
NO3-	0.774	0.633	-0.012	0.162	0.001
PO4-3	0.704	0.573	0.419	0.247	0.001
RIP_urb	0.938	0.345	0.007	0.088	0.033
RIP_agr	0.478	0.602	0.639	0.197	0.001
LUI	0.582	0.666	0.468	0.216	0.002
SR	0.674	0.649	0.352	0.169	0.003
Dam	0.569	0.770	0.290	0.156	0.005

Table C.2. Results of “envfit” (R package “vegan”) tests for correlations between variables and the macroinvertebrates assemblage ordination.

	NMDS1	NMDS2	NMDS3	r	p-values
drai_area	-0.608	-0.774	0.174	0.087	0.038
elevation	0.332	0.935	-0.127	0.082	0.047
S1	0.237	0.575	0.783	0.078	0.040
S3	-0.096	0.181	-0.979	0.054	0.157
G1	0.497	0.868	0.025	0.038	0.307
G2	0.067	-0.729	0.682	0.050	0.173
OM	0.299	0.836	-0.460	0.236	0.001
coa_sed	-0.143	-0.804	0.578	0.098	0.030
shading	0.935	0.274	0.228	0.150	0.001
temp	-0.241	-0.867	-0.437	0.201	0.001
DO	0.897	-0.285	0.339	0.088	0.028
pH	-0.724	0.030	0.689	0.050	0.172
cond	-0.872	-0.448	0.197	0.334	0.001
turb	-0.916	-0.289	-0.280	0.212	0.001
K+	-0.967	0.070	0.246	0.273	0.001
Fe+2	-0.850	-0.203	-0.485	0.050	0.205
F-	-0.125	-0.008	0.992	0.028	0.411
Br-	-0.578	-0.722	0.380	0.011	0.787
NO3-	-0.892	-0.287	0.351	0.382	0.001
PO4-3	-0.976	-0.091	0.196	0.406	0.001
RIP_urb	-0.744	-0.455	0.490	0.249	0.001
RIP_agr	-0.804	-0.554	0.217	0.140	0.004
LUI	-0.796	-0.570	0.204	0.411	0.001
SR	-0.980	0.187	0.076	0.444	0.001
Dam	-0.489	-0.422	0.763	0.094	0.029

APPENDIX D

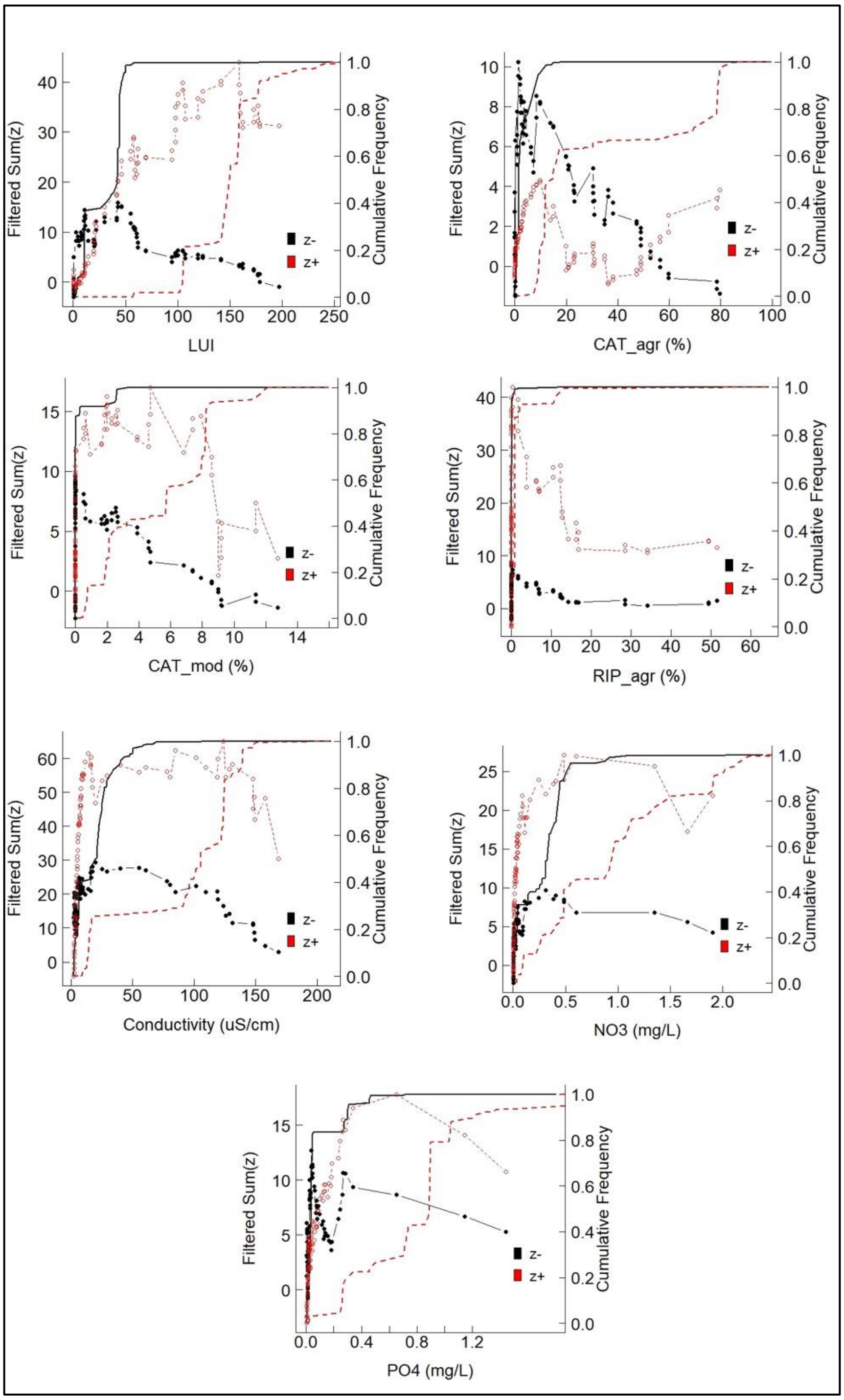


Figure D.1. TITAN sum(Z−) and sum(Z+) values corresponding to all candidates change points along the analyzed environmental gradient. Black and red vertical lines represent the cumulative frequency distribution of change points for 500 bootstrap replicates for sum(Z−) and sum(Z+), respectively.

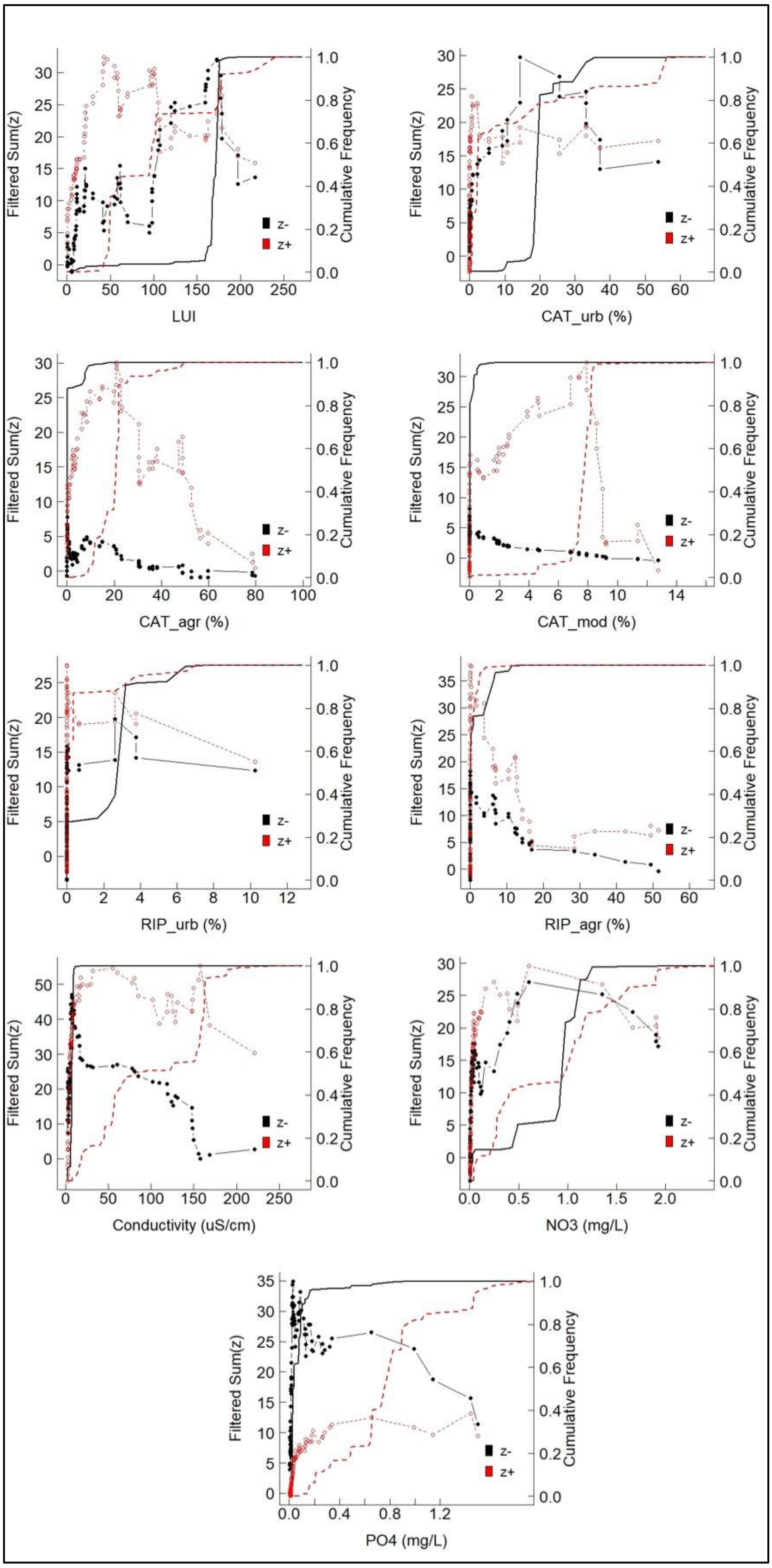
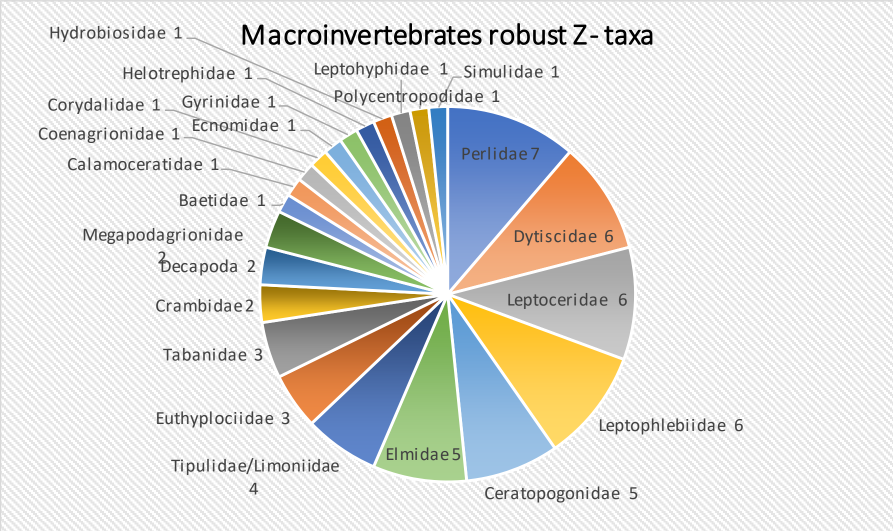


Figure D.2. TITAN sum(Z−) and sum(Z+) values corresponding to all candidate change points along the analyzed environmental gradient. Black and red vertical lines represent the cumulative frequency distribution of change points for 500 bootstrap replicates for sum(Z−) and sum(Z+), respectively.

APPENDIX E



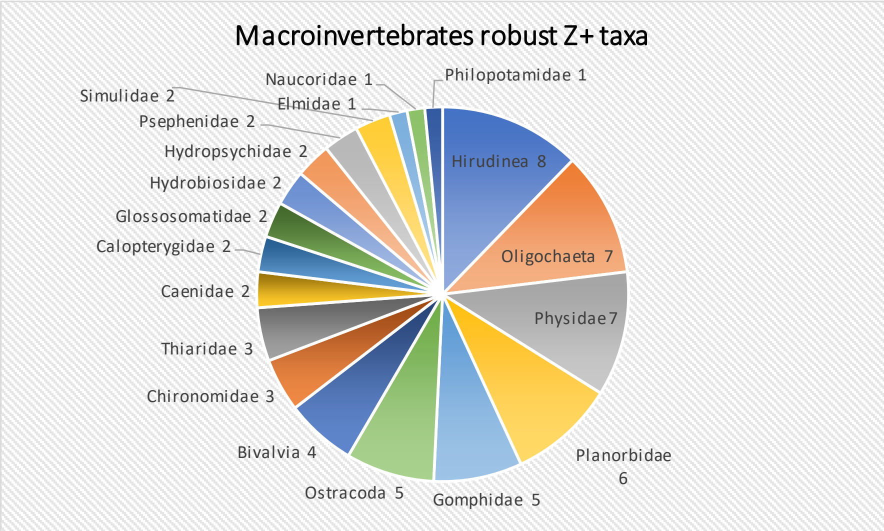
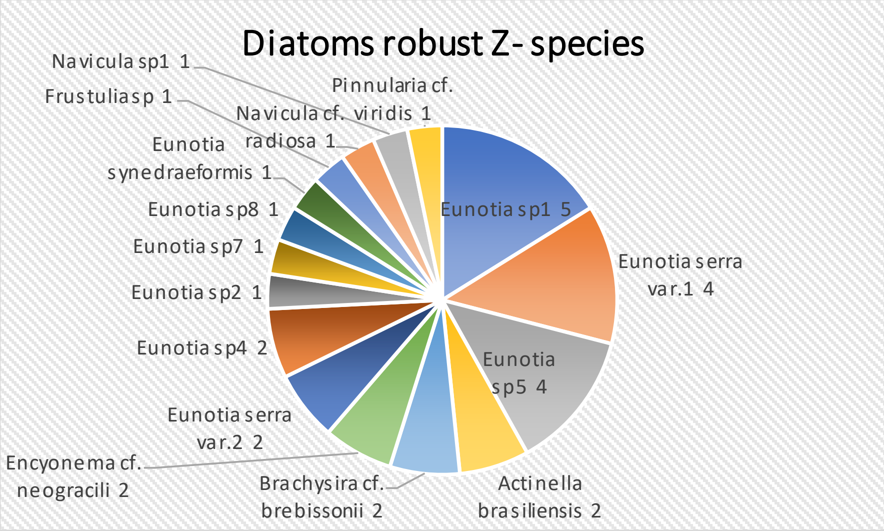


Figure E.1. Number of times each macroinvertebrate taxon was detected as robust sensitive (Z-) or tolerant (Z+) by TITAN analyses among all the disturbances gradients.



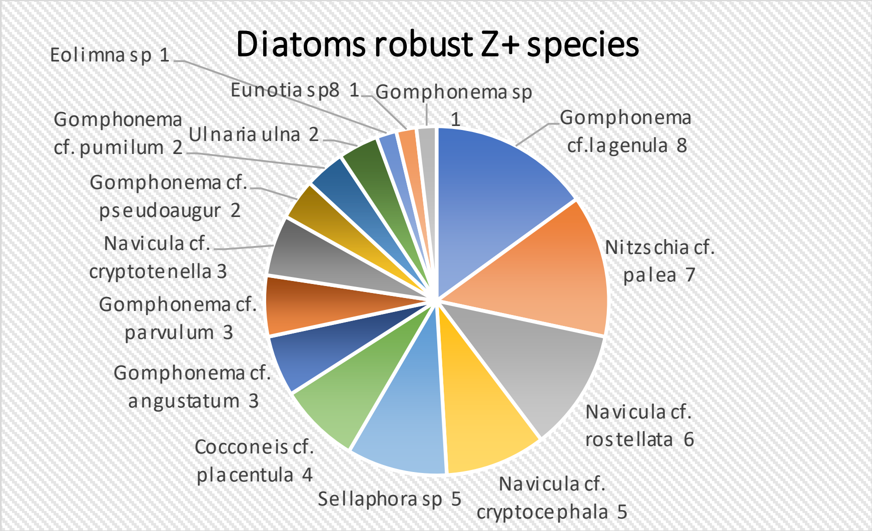


Figure E.2. Number of times each diatom taxon was detected as robust sensitive (Z-) or tolerant (Z+) by TITAN analyses among all the disturbances gradients.

CAPÍTULO III

Setting thresholds of ecosystem structure and function to protect streams of the Brazilian Savannah

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Artigo submetido em 13 de julho de 2021 no periódico *Science of the Total Environment.*
Status em 09 de agosto de 2021: em revisão.

Abstract

Ecological metrics has been proven to be effective in monitoring programs aimed at assessing freshwater ecosystem integrity. Structural and functional aspects of the ecosystem may allow for a comprehensive view of the multiple human impacts that occur at different scales. However, a gap in the effective use of such ecological tools lies in the identification of the relative importance of different mechanisms that cause impacts and the interactions between them. Using Boosted Regression Tree (BRT) models, we evaluated the relative importance of natural and human impact factors, from local to catchment scales, on metrics related to diatom and macroinvertebrate assemblages and ecosystem processes (litter decomposition, algal and microbial biomass production and sediment respiration). The study was carried out in 52 stream reaches of the Brazilian Savannah in central Brazil. At least three metrics of each ecological group presented models with relatively good performance. Conductivity was the most relevant factor to explain the variation of ecological metrics, followed by other water quality variables and catchment-scale land uses. In general, macroinvertebrate metrics and algal biomass production responded to both water quality and land use factors, while metrics of diatoms and microbial biomass responded more strongly to water quality variables. Litter decomposition metrics were influenced by seasonality. The nonlinear responses allowed the detection of gradual or abrupt-changes curves, indicating potential thresholds of important drivers, like conductivity (100-200 $\mu S.cm^{-1}$), phosphate (0.5 $mg.L^{-1}$) and catchment-scale urbanization (10-20%). Considering the best performance models and the ability to respond rather to stress than to natural factors, the potential bioindicators identified in the study area were the macroinvertebrates abundance, the percentage of Ephemeroptera/Plecoptera/Trichoptera, the percentage of Oligochaeta/Hirudinea, the percentage of genus *Eunotia*, the Trophic Diatom Index and the algal biomass production. The results reinforced the importance of integrative monitoring programs, which include metrics from different ecological groups. We also consider BRT models and the multi-metric approach powerful tools that can enhance and give better direction to freshwater management.

***Key-words*:** ecosystem integrity, boosted regression tree, ecological metrics, freshwater management

Resumo

Métricas ecológicas têm se mostrado eficazes indicadoras em programas de monitoramento que visam avaliar a integridade de ecossistemas de água doce. Os aspectos estruturais e funcionais do ecossistema podem permitir uma visão abrangente dos múltiplos impactos humanos que ocorrem em diferentes escalas. No entanto, uma lacuna para uso efetivo de tais ferramentas ecológicas reside na identificação da importância relativa dos diferentes mecanismos que causam impactos e as interações entre eles. Usando modelos de árvore de regressão com *boosting* (*Boosted Regression Tree* - BRT), avaliamos a importância relativa dos fatores naturais e de impactos humanos, da escala local a bacias hidrográficas, nas métricas relacionadas a comunidades de diatomáceas e macroinvertebrados e processos ecossistêmicos (decomposição, produção de biomassa algal e microbiana, e respiração de sedimentos) O estudo foi realizado em 52 trechos de riachos do Cerrado no Brasil central. Pelo menos três métricas de cada grupo ecológico apresentaram modelos com bom desempenho. A condutividade foi o fator mais relevante para explicar a variação das métricas ecológicas, seguida por outras variáveis de qualidade da água e usos do solo na escala da bacia. Em geral, as métricas de macroinvertebrados e a produção de biomassa algal responderam tanto à qualidade da água quanto aos fatores de uso da terra, enquanto as métricas de diatomáceas e biomassa microbiana responderam mais fortemente às variáveis de qualidade da água. As métricas de decomposição foram influenciadas pela sazonalidade. As respostas não lineares permitiram a detecção de curvas de mudanças graduais ou abruptas, indicando potenciais limiares de fatores importantes, como condutividade (100-200 $\mu S.cm^{-1}$), fosfato (0,5 $mg.L^{-1}$) e urbanização em escala de bacias (10 - 20%). Considerando os modelos com melhor desempenho e a capacidade de responder mais ao estresse do que aos fatores naturais, os potenciais bioindicadores identificados na área de estudo foram a abundância de macroinvertebrados, a porcentagem de Ephemeroptera / Plecoptera / Trichoptera, a porcentagem de Oligochaeta / Hirudinea, a porcentagem de gênero *Eunotia*, o Índice de Diatomáceas Tróficas e a produção de biomassa algal. Os resultados reforçaram a importância de programas de monitoramento integrativo, que incluem métricas de diferentes grupos ecológicos. Também consideramos os modelos de BRT e a abordagem multimétrica como ferramentas poderosas que podem aprimorar e dar melhores direcionamentos ao gerenciamento dos ecossistemas de água doce.

Palavras-chave: integridade ecossistêmica, modelagem ecológica, métricas ecológicas, manejo de água doce

1. Introduction

Freshwater ecosystems are among the most threatened by human activities (Gatti 2016). The knowledge of the various components is these ecosystems is of paramount importance to the elaboration of public policies on conservation or recovery (Bunn et al. 2010). Biomonitoring data has been increasingly used in determining the ecological conditions of aquatic environments (Norris & Thoms 1999; Poquet et al. 2009; Pardo et al. 2018), although physical, chemical and microbiological analyses remain the most widespread (Ewaid 2016; Pacheco et al. 2017; Wu et al. 2018). Ecosystem responses are integrative since they reflect both physical/chemical (e.g., habitat and water quality) and biological changes (e.g., the introduction of exotic species) characterizing the ecosystem integrity or the "river health" (Karr 1991, 1999).

A comprehensive ecosystem integrity assessment should consider both structural and functional characteristics (Bunn & Davies 2000). While the structure of an ecosystem comprises physical and chemical attributes related to water quality, composition of biological assemblages and habitat conditions, its functioning is related to the processes regulating energy and matter fluxes (Tilman et al. 2014). Moreover, structural and functional attributes are strongly influenced by the surrounding landscape (Allan 2004). Human activities in the upstream drainage area are considered the main threats to ecosystem integrity, impacting habitat, water quality, and biota through complex pathways acting on different temporal and spatial scales (Townsend et al. 2003; Allan 2004).

Effective biomonitoring programs focus on metrics that respond to human impacts and are easy to understand and communicate (Karr 2006). Furthermore, low sensitivity to natural and spatial variations is desirable as these may be confounded with the effects of anthropogenic stressors (Norris & Hawkins 2000). The most commonly used metrics to assess freshwater ecosystem integrity are those related to biological assemblages, such as species richness and diversity, abundance, proportion of tolerant/sensitive taxa and organismal traits (e.g., feeding habits, body size, mobility), and indices of sensitivity to pollution (Hering et al. 2006b). Macroinvertebrates, diatoms, macrophytes and fish are groups usually chosen for that propose (Son et al. 2018; Waite et al. 2019) since they provide robust parameters to the identification of several human disturbances and present particular features that facilitate such application (e.g., life cycle, habitat, size; Merritt & Cummins 1996; Kelly et al. 2008).

Much less explored in the context of biomonitoring are aquatic fungi and bacteria, which are key decomposers of organic matter in streams. The composition and biomass of both fungal and bacterial assemblages might be affected by the limitation of nutrients in streams (Medeiros et al. 2015). Thus, an increase in nutrients caused by the point-source sewage release or fertilisers, for example, would lead to changes in the microbial community and, consequently, in rates of organic matter decomposition (Gessner & Chauvet 2002). The responses of some ecosystem processes to stressors are fundamental for understanding the effects on ecosystem services that produce direct benefits to people. But despite this, there is still a lot of reluctance among managers and little use of functional indicators (e.g., litter decomposition) in monitoring programs (Schiller et al. 2017).

Although many studies have pointed out to the applicability of several ecological metrics for assessing freshwater ecosystem integrity, the main gap lies in the relative importance of different mechanisms that cause impacts and the interactions between them (Wenger et al. 2009). According to Sutherland et al. (2013), one solution to this is the use of modelling as a tool for measuring and monitoring systems. In the context of environmental management, most models used in monitoring programs consider biological assemblages, especially benthic invertebrates (AUSRIVAS, Smith et al. 1999; RIVPACS, Wright et al. 1984; USEPA, 2016), as indicators. Some studies suggest the use of ecosystem processes for this purpose (Gessner & Chauvet 2002; Feio et al. 2010; Woodward et al. 2012), and rare are those that present a multi-metric approach using structural and functional aspects (Castela et al. 2008; Clapcott et al. 2014).

In this context, Boosted Regression Tree (BRT) models have been used as a robust tool to identify the influence of environmental variables, natural or those related to human activities, on ecological metrics, making it possible to evaluate the shape of the responses and to make forecasts by using new data (Clapcott et al. 2012; Waite et al. 2019). This approach allows the identification of gradients, from which is possible to detect non-linear responses, change points, and interactions among predictors.

In this study, we evaluate how ecological metrics respond to natural environmental gradients and human related stressors at different spatial scales (local and catchment) in streams of the Brazilian Savannah. By using BRT models and metrics of the structure (e.g., assemblages of diatoms and macroinvertebrates) and functioning (e.g., litter decomposition, sediment respiration, and, algal and microbial biomass production) of stream ecosystems we aim to (1) identify the most suitable metrics, in terms of sensitivity to stressors, to be used as indicators of stream integrity, and (2) assess the response and potential thresholds of ecological metrics along environmental gradients to inform environmental management.

2. Material and Methods

2.1. Study area

The study was conducted in the central Brazilian plateau (*ca.* 1000 m a.s.l.) in an area of approximately 6700 km^2 dominated by Cerrado (Brazilian Savannah) vegetation. This region is characterized by headwaters belonging to three of the major Brazilian watersheds (Tocantins-Araguaia, São Francisco, and Paranaíba; Figure 1). Fifty-two stream reaches were selected to represent a broad range of natural environmental conditions, land uses and anthropogenic disturbances in the upstream catchment. When more than one reach was sampled in the same stream, they were at least 500m apart from each other (to reduce their spatial dependence) and comprised different natural characteristics. All streams are wadeable and perennial of up to 5th order (Strahler 1957). Despite some samplings at different spots in the same river can generate understanding of a pseudo-sample, the assemblages studied do not move over long distances and their composition is closely related to local factors such as habitat and water quality. Thus, spots on the same river can be considered independent samples in this case. Also, Mantel test (Supplementary Material) carried out previously showed that dissimilarities in taxa distribution is more related with natural/impacts characteristics than with geographic distance in our study area. In addition, as our focus is on the gradient analysis of natural conditions and impacts, the presence of sites with similar characteristics, if happen, would represent only an overlap in the gradient, not interfering in the results of the analysis.

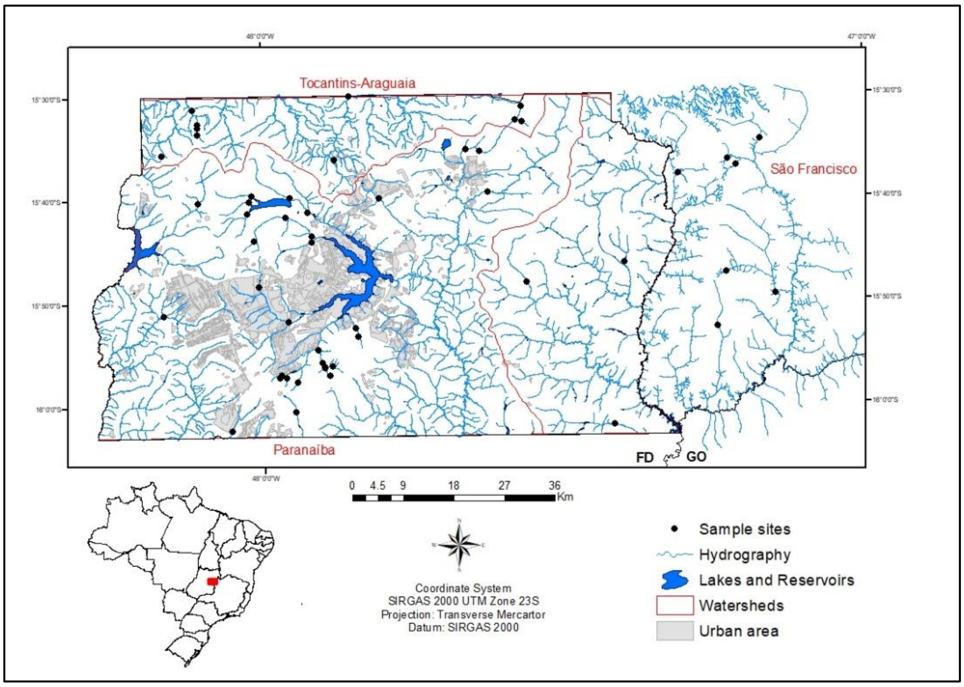

Figure 1. Spatial distribution of the 52 stream reaches in the regional river network within the three watersheds (Tocantins-Araguaia, São Francisco and Paranaíba) in central Brazil.

2.2. Sampling, analysis and metrics

Two sampling campaigns were conducted in 2018, one in the end of the wet season (April/May) and another in the end of the dry season (August/September).

2.2.1. Predictor variables

A large number of variables related to natural conditions and human stressors were previously measured at each stream reach (Campos et al. 2021). From this dataset, we retained only uncorrelated variables (absolute Pearson's $r < 0.6$) which include natural characteristics not affected by human activities (drainage area and elevation), natural characteristics that could be affected by human activities (% of organic matter in streambed sediment, % of coarse sediments, riparian shading), water quality variables commonly used in monitoring programs and considered as indirect indicators of human disturbances (conductivity, turbidity, dissolved oxygen, pH, nitrate and phosphate) and primary sources of human disturbances (land use in riparian and catchment scales and the presence/absence of point-source treated sewage release and dams). All of them will be considered hereinafter as predictors (Table 1). The season (wet and dry) was also considered as a predictor since it may affect some of our biological response metrics.

2.2.2. Response metrics

A large number of ecological metrics were considered in this study (Table 2). The structural metrics are related to the diatom and macroinvertebrate assemblages' composition. The functional metrics include relevant ecosystem processes such as leaf litter decomposition (microbial and total), sediment respiration and algal and microbial biomass production.

2.2.3. Biological assemblages sampling

Macroinvertebrates were sampled using a *surber* (0.09 m^2 area and 0.25 mm mesh size) to collect five sub-samples per site covering the proportional diversity of habitats. The sub-samples were then integrated and preserved in 96% alcohol to be sorted and identified under a stereomicroscope to the lower taxonomic level possible (until family). Diatoms were sampled from five 10 x 10 cm pieces of artificial substrates (slate stones) that were incubated in the riverbed for approximately 30 days. Nearly 250 cm^2 were scraped and the shaved material was preserved in vials containing 0.33% Lugol solution. The identification and quantification of the organisms were carried out under an inverted microscope and using the Utermöhl method (Utermöhl 1931). Identification of macroinvertebrates and diatoms was carried out mostly to family and species level, respectively, with the assistance of taxonomic specialists (see Acknowledgments).

2.2.4. Biological assemblage metrics

For diatoms and macroinvertebrates were included metrics relative to the assemblages' structure and sensitivity to pollution. The structure was composed of richness, abundance, diversity (Shannon-Wiener, Simpson) and evenness (Pielou) indices. The percentage abundance of pollution-sensitive taxa was calculated for the diatom genus *Eunotia*, for diatoms, and for the macroinvertebrate orders Ephemeroptera/Plecoptera/Trichoptera (EPT) and the Plecoptera order alone. The percentage abundance of pollution-tolerant taxa was calculated for the diatom species *Nitzschia palea*, and for the macroinvertebrate classes Oligochaeta/Hirudinea.

Some pollution sensitivity indices were adapted for diatoms and macroinvertebrates. The TDI (Trophic Diatom Index) was adapted from Kelly (1998). Although this index has been developed in Europe, it has the most complete species list. Only 8 of the 74 species identified were not described in the TDI list, hence we attributed the lowest value (1) to them. The Biological Monitoring Working Party (BMWP) was adapted from four BMWP indices developed in different regions. The main reference was Monteiro et al. (2008) since their BMWP was developed for Goiás state (Brazil). Taxa not registered by Monteiro et al (2018) received the score suggested by Junqueira & Campos (1998) who studied macroinvertebrates in Minas Gerais state (Brazil). In the case of taxa that were not registered by the first two authors, we used the scores proposed by Uherek & Gouveia (2014), who studied Amazon rivers (Brazil). In the last case, the scores from Alba-Tercedor & Sánchez-Ortega (1988; Iberian Peninsula) were applied. Taxa without published sensitivity grades were attributed with the lowest score (1). The Average Score per Taxon (ASPT) index Armitage et al. (1983) was calculated by dividing the score of each taxon by the total number of scoring taxa.

2.2.5. Ecosystem processes

The respiration rates on river sediments were measured following Feio et al. (2010), with some adaptations, as an indication of river metabolism. Three PVC chambers (30 cm long, ø 4.4 cm) were half-filled with riverbed sediment (<1 cm diameter; collected up to 15 cm depth) and then filled in with stream water and sealed with rubber stoppers. To control, one PVC chamber was filled in only with river water. Respiration rates were measured as the depletion of dissolved oxygen in the chambers after approximately 30 min. The volume of water in each chamber was measured using a beaker.

The respiration rate for each site was given by the expression (1):

$$Rr = \Sigma\ s[V \times (Of - Oi) \times t] - c[V \times (Of - Oi) \times t] \quad (1)$$

where Rs (mg O_2 L^{-1} h^{-1}) is the respiration rate, "s" is each chamber, V is the volume (L) of water in each chamber, Of is the final O_2 concentration (mg.L^{-1}), measured with a YSI probe), Oi is the initial O_2 concentration (mg.L^{-1}), "t" is the incubation period (hours) and "c" is the control chamber. Respiration was measured only in September (dry season).

The microbial (fine mesh bag-FMB) and total (coarse mesh bag-CMB) leaf litter decomposition rates were calculated by the decrease in leaves weight after 30 days of incubation on riverbeds. Freshly fallen leaves litter of *Hyeronimia alchorneoides*, a common tree species that occurs in the riparian vegetation of Brazilian Savannah streams, were collected during the dry season previously to this study fieldwork. Portions with approximately 3 ±0.5 g of dry air leaves were placed in fine- (0.05 mm mesh; 13 x 20 cm size) and coarse-mesh litter bags (10 mm mesh; 18 x 23 cm size). The use of FMB (only microbial effects) and CMB (microbial and invertebrates assemblages' effects) allows distinguishing the contribution of microorganisms and macroinvertebrates to the loss of leaf litter mass. Moreover, CMB may also add the physical water abrasion effect (Tonin et al. 2018).

In the laboratory, six leaf discs (10 mm diameter) were cut from each sample. A set of a three-leaves disc was used to determine ergosterol content (as an indirect measure of fungal biomass on decomposing leaves; see the detailed description below) and another similar set was used to determine the total ATP content (as an indirect measure of the total microbial biomass; see the detailed description below). The results were expressed in % of decomposed biomass standardized for 30 days.

A similar piece of artificial substrate area scraped for diatoms (approx. 250 cm^2) was scraped off for Chlorophyll *a* determination (with pheophytin *a* correction), an indirect measure of periphytic algal biomass. The material was filtered (glass fibre 0.45 mm filters) and frozen until analysis. Chlorophyll *a* concentration (µg m^{-2}) was determined spectrophotometrically after acetone extraction (Wetzel & Likens 1991).

Aquatic fungal biomass was indirectly measured by the ergosterol concentration, according to Gessner (2005). Lipids were extracted and saponified at 80 °C. The extracted lipids were partitioned into a non-polar phase and ergosterol was purified by solid-phase extraction. A final purification and quantification of ergosterol were achieved by HPLC (DIONEX Summit P580, Sunnyvale, CA, U.S.A.) where the ergosterol peak is detected at 8 min. The results were expressed in µg erg g^{-1}.

The total microbial biomass was estimated by ATP concentration according to Abelho (2005). Sets of three-leaves discs from each litter bag were homogenized with HEPES solution and oxalic acid for ATP extraction, and the centrifugation supernatant was neutralized with NaOH solution. A set of ATP-free (48-hour ultraviolet) leaf discs went through the same procedure to measure extraction efficiency. ATP reading was conducted in a luminometer. The results were expressed in nmol ATP g^{-1}.

2.3. Data Analysis

In order to quantify the relationships between selected predictors and response metrics we used Boosted Regression Tree (BRT) analysis. BRTs provide a means to fit nonlinear relationships between predictors to response metrics, including interaction effects by using a boosting strategy to combine results from a large number (often thousands) of simple regression tree models (Friedman 2001). Three elements are fundamental in the execution of the BRT models: (i) tree complexity (*tc*), which controls whether the interactions are fitted; (ii) the learning rate (*lr*), which determines the contribution of each tree to the growing model; and (iii) the number of trees (*nt*) necessary for the optimization of the model, which is determined based on the two previous parameters (Elith et al. 2008). We adopted the tree complexity (*tc*) equal to 5, and the learning rate varying between 0.01 and 0.0001, guaranteeing that at least 1000 trees were generated for each metric (see all settings in Supplementary Material A). The bag fraction (*bf*) represents the proportion of training data to be selected, without replacement, at each interaction, thus controlling the stochasticity of randomization. We applied *bf* equal to 0.75. Within the BRT, the cross-validation (CV) technique provides a means for testing the model using part of the training data, while still using all data at some stage to fit the model. It is useful especially in cases of relatively low sample sizes (Elith et al. 2008), as is the case of this study.

The response variables included count data (e.g., Inv_ and Diat_Richness), proportional data (e.g., %EPT and %Plecoptera) and indices bounded between 0 and some maximum (e.g., BMWP, TDI). For the count data, we tested two models, the first fitted assuming a Poisson-error (discrete probability) distribution and the second using log (x+1) values and assuming a Gaussian-error (continuous probability) distribution. Both models were examined, and results were reported for the best model based on goodness of fit and examination of residual errors. Left- and right-bounded data (e.g., %Eunotia) were log (x+1) transformed and models fitted assuming Gaussian errors (Clapcott et al. 2012).

BRT outputs included the performance of training data (% variation explained) and test data (CV correlation), enabling us to evaluate the potential to explain training data and the predictive power for new data, respectively. Furthermore, the relative influence (contribution) of each predictor to explain the training data was scaled so that the sum adds up to 100% and a higher number indicated a stronger influence on the response. Lastly, partial dependence plots indicated the shapes of relationships between predictors and the response variable (e.g., linear, curvilinear, and sigmoidal) taking into account the average effect of all other predictors (Elith et al. 2008).

In a second step, the models were reduced with the exclusion of predictor variables that contributed less than 2% to explain each response variable, since the reduction of variables is desirable considering that BRT models tend to overfit models (Elith et al. 2008; Brown et al. 2012). The results presented refer to the reduced final models. Sewage release (SR) was excluded from the reduced models in all response metrics (less than 2% of relative contribution). All statistical analyses were performed using the gbm package (Greenwel 2020) from R v.4.0.3 (R Core Team 2020) and specific code for BRT provided by Elith et al. (2008).

Table 1. Description, average, range (minimum and maximum) and the number of samples (N) of natural and human disturbances variables. (*) Data collected four times, but for analysis, we consider the average between April/May and August/September. (**) For categorical variables, we indicated the number of samples in each category.

Variables	**Description**	**Average (min-max)**	**N**
drai_area	Drainage area upstream of the sample site (Km^2)	40.52 (2.21 – 215.42)	52
elevation	Altitude of the sample site relative to the sea level (m)	1015 (744 – 1220)	52
shading	% of riparian shading (0 = 0%; 1 = < 30%; 2 = between 30 and 60%; 2 = > 60%)	0 – 3; 1 – 9; 2 – 7; 3 - 33	52
OM	% of organic matter in the riverbed sediment	6.15 (0.61 – 26.66)	52
coa_sed	% of coarse sediments (>2000 - 710 mm) in the riverbed sediment	60.49 (4.19 – 97.28)	52
DO*	Dissolved Oxygen ($mg.L^{-1}$)	7.11 (1.88 – 8.85)	103
cond*	Electrical conductivity ($\mu S.cm^{-1}$)	56 (1 – 584)	103
turb*	Turbidity (NTU)	8 (0.04 – 197)	103
NO_3^-*	Nitrate ($mg.L^{-1}$)	0.35 (0 – 10.29)	103
PO_4^{-3}*	Phosphate ($mg.L^{-1}$)	0.23 (0 – 6.26)	103
RIP_urb	% of urban area in the riparian corridor	1 (0 – 33)	52
RIP_agr	% of agricultural and livestock areas in the riparian corridor	8 (0 – 56)	52
CAT_urb	% of urban area in upstream catchment	7 (0 – 70)	52
CAT_agr	% of agricultural and livestock areas in upstream catchment	21 (0 – 86)	52
CAT_mod	% of modified area in upstream catchment (allotment, exposed soil, eucalyptus)	3 (0 – 39)	52
SR**	Presence (1)/absence (0) of point-source treated sewage release upstream	0 - 49; 1 - 3	52
Dam**	Presence (1)/absence (0) of dams upstream	0 - 39; 1 - 13	52

Table 2. Description, range, average (minimum and maximum) and the number of samples (N) of ecological response metrics.

Ecological group	**Response metrics**	**Description**	**Average**	**N**
Diatoms	Diat_Rich	Diatom species richness	7.52 (1 – 17)	97
	Diat_Abund	Diatom species abundance	2857.61 (6.12 – $9x10^4$)	97
	Diat_Shannon	Shannon-Wiener index	1.40 (0 – 2.52)	97
	Diat_Simpson	Simpson index	0.64 (0 – 0.9)	97
	Diat_Pielou	Pielou index	0.74 (0 – 1)	97
	%Eunotia	% abundance of *Eunotia*	56.81 (0 – 100)	97
	%Nitz_palea	% abundance of *Nitzchia palea*	2.28 (0 – 76.76)	97
	TDI	Trophic Diatom Index (Kelly 1998, adapted)	15.44 (0 – 92.01)	97
Macroinvertebrates	Inv_Rich	Macroinvertebrate taxa richness	14.66 (3 – 28)	99
	Inv_Abund	Macroinvertebrate taxa abundance	513.94 (6 – $6.4x10^3$)	99
	Inv_Shannon	Shannon-Wiener index	1.49 (0.43 – 2.23)	99
	Inv_Simpson	Simpson index	0.62 (0.19 – 0.86)	99
	Inv_Pielou	Pielou index	0.58 (0.19 – 0.96)	99
	%EPT	% abundance of Ephemeroptera, Plecoptera and Trichoptera	17.72 (0 – 76.47)	99
	%Plecoptera	% abundance of Plecoptera	2.53 (0 – 31.82)	99
	%OLI_HIR	% abundance of Oligochaeta and Hirudinea	3.74 (0 – 70.29)	99
	BMWP	Biological Monitoring Work Party (Monteiro et al. 2008; Junqueira & Campos 1998; Uherek & Gouveia 2014; and Alba-Tercedor & Sanches-Ortega 1988, adapted)	87.00 (15 – 170)	99
	ASPT	Average Score per Taxon (Armitage 1983)	5.90 (4.83 – 6.93)	99
Ecosystem Processes	Mic_dec	% of decomposed biomass in fine mesh litter bags	65.2 (32.79 – 119.70)	95
	Tot_dec	% of decomposed biomass in coarse mesh litter bags (microbial + invertebrates)	61.69 (15.28 – 118.05)	95
	Resp	Sediment respiration rate (mg O_2 h^{-1})	0.13 (0 – 1.31)	50
	Chl	Algal biomass (Chlorophyll *a* concentration ug m^{-2})	0.69 (0 – 11.63)	94
	Erg	Fungal biomass (Ergosterol concentration mg Erg /g AFDM)	0.05 (0 – 0.27)	101
	ATP	Microbial biomass (ATP concentration nmol ATP / g AFDM)	0.01 (0 – 0.07)	101

3. Results

3.1. Performance of BRT models

For macroinvertebate metrics, the highest percentages of variance explained were observed for % Oligochaeta/Hirudinea (91%), Macroinvertebrate abundance (84%), % Plecoptera (82%) and % EPT (70%). For diatom metrics, BRT models explained the highest percentage of variation for: %Eunotia (87%), Trophic Diatom Index (TDI, 84%) and Diatom richness (77%). Metrics of ecosystem processes were best predicted for algal biomass production (Chl, 96.47%), microbial decomposition (Mic_dec, 82%) and total decomposition (Tot_dec, 73.45%) (Figure 2). The medians of the structural and functional metrics were very similar, around 60% (Figure 2).

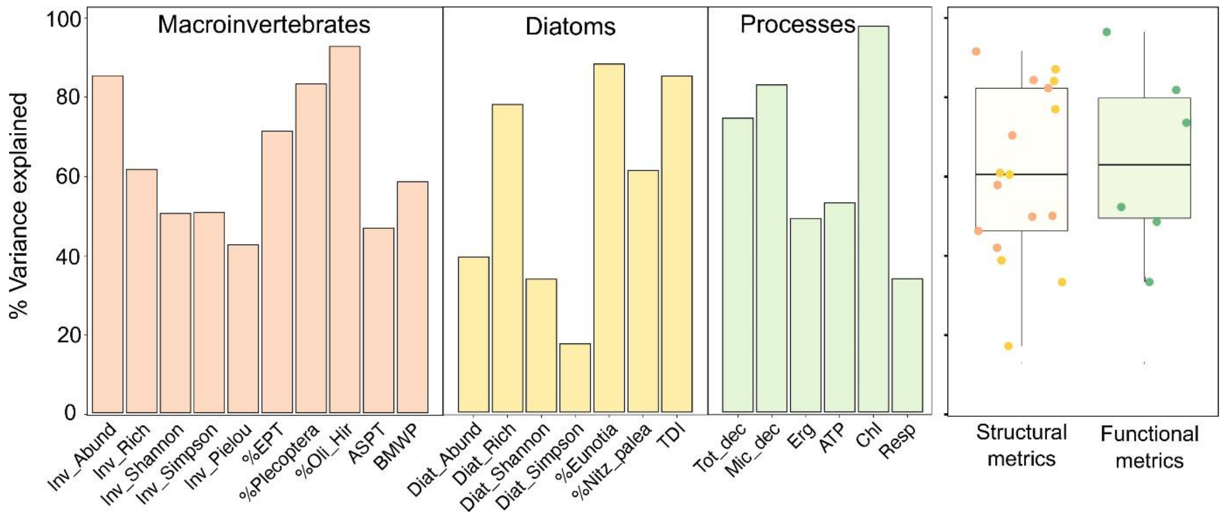

Figure 2. Percentage of variance explained for Macroinvertebrate, Diatom, and Ecosystem Process metrics models. Diat_Pielou is not shown because it was not possible to run the model. Boxplot of structural (Diatoms and Macroinvertebrates) and functional (Ecosystem Processes) metrics results. See all settings and statistics in the Supplementary Material.

3.2. Relative contributions of predictor variables

Seasonality was a relevant predictor only for decomposition. Predictors related to the river size (drainage area and elevation) were important to explain some metrics, but especially %Plecoptera, for which the two predictors combined explained 29% of its variation. Habitat variables were relevant to explain few metrics but, among them, the percentage of organic matter in river sediment (OM) was the most relevant, particularly for macroinvertebrates' metrics, ergosterol and ATP (Figure 3).

Water quality variables were relevant in explaining almost all metrics. Conductivity presented higher contributions for most metrics (macroinvertebrates, diatoms and ecosystem processes). Turbidity, dissolved oxygen, nitrate, and phosphate were also relevant for certain response metrics (Figure 3).

Among the land use predictors, agricultural and urban occupation in the upstream catchment (CAT_agr and CAT_urb) presented the highest contribution in explaining most of the response metrics (Figure 3). Macroinvertebrate metrics were the most influenced by them, but also the abundance of diatoms and sediment respiration. For macroinvertebrates, some metrics were rather explained by urban occupation in the upstream catchment (e.g., %EPT, 13%), other by agricultural (e.g., Inv_Simpson, 13%) and other by both, like the macroinvertebrates richness (CAT_agr 14%, CAT_urb 10%) and the BMWP (CAT_agr 14%, CAT_urb 13%). Generally, catchment-scale metrics were more relevant to ecological variables than riparian-scale metrics, except for the abundance of diatoms and respiration rate, which were mostly influenced by urbanization (RIP_urb) and agricultural activities in the riparian corridor (RIP_agr), respectively. The influence of the presence of dams was minimal in all models.

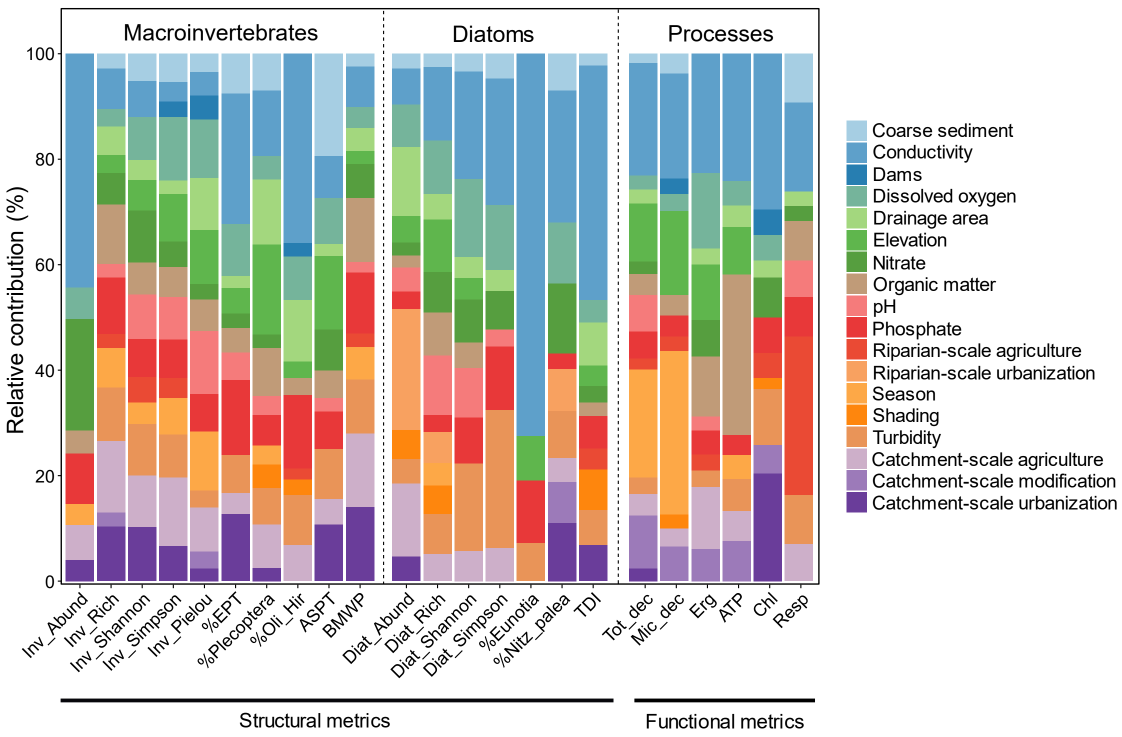

Figure 3. Relative contribution (0-100%) of predictor variables on the variance explained of each response ecological metrics (structural and functional). SR was excluded because its contribution was 0% in all models.

3.3. Ecological response relationships with environmental gradients

The relationships between predictors and response metrics presented some features in common: 1- the majority of response shapes were non-linear; 2- some of the response metrics presented an early increase or decrease followed by the continuity of the curve in the opposite direction; 3- for some of them, it is possible to identify common values from which the curves abruptly changed, which points out to the existence of potential thresholds. For example, change points of most conductivity curves were around 100 $\mu S.cm^{-1}$. For phosphorous, change points were around 0.5 $mg.L^{-1}$, and CAT_urb between 10 and 20% (Figs. 4, 5 and 6).

Conductivity, phosphate, nitrate and land use in the catchment had a positive influence in metrics commonly associated with increased human disorders, like Macroinvertebrates abundance, %Oligochaeta/Hirudinea, TDI, Diatom richness and algal biomass; while the opposite behaviour was observed for metrics related to a good ecological status like %EPT and %Eunotia. The increase in the drainage area and the reduction in elevation, i.e. the widening of the river from the source towards the mouth, were negatively related to the %Plecoptera, %Eunotia and Diatom richness, and positively related to the increase in %Oligochaeta/Hirudinea, TDI, total and microbial decomposition (Figs. 4, 5 and 6).

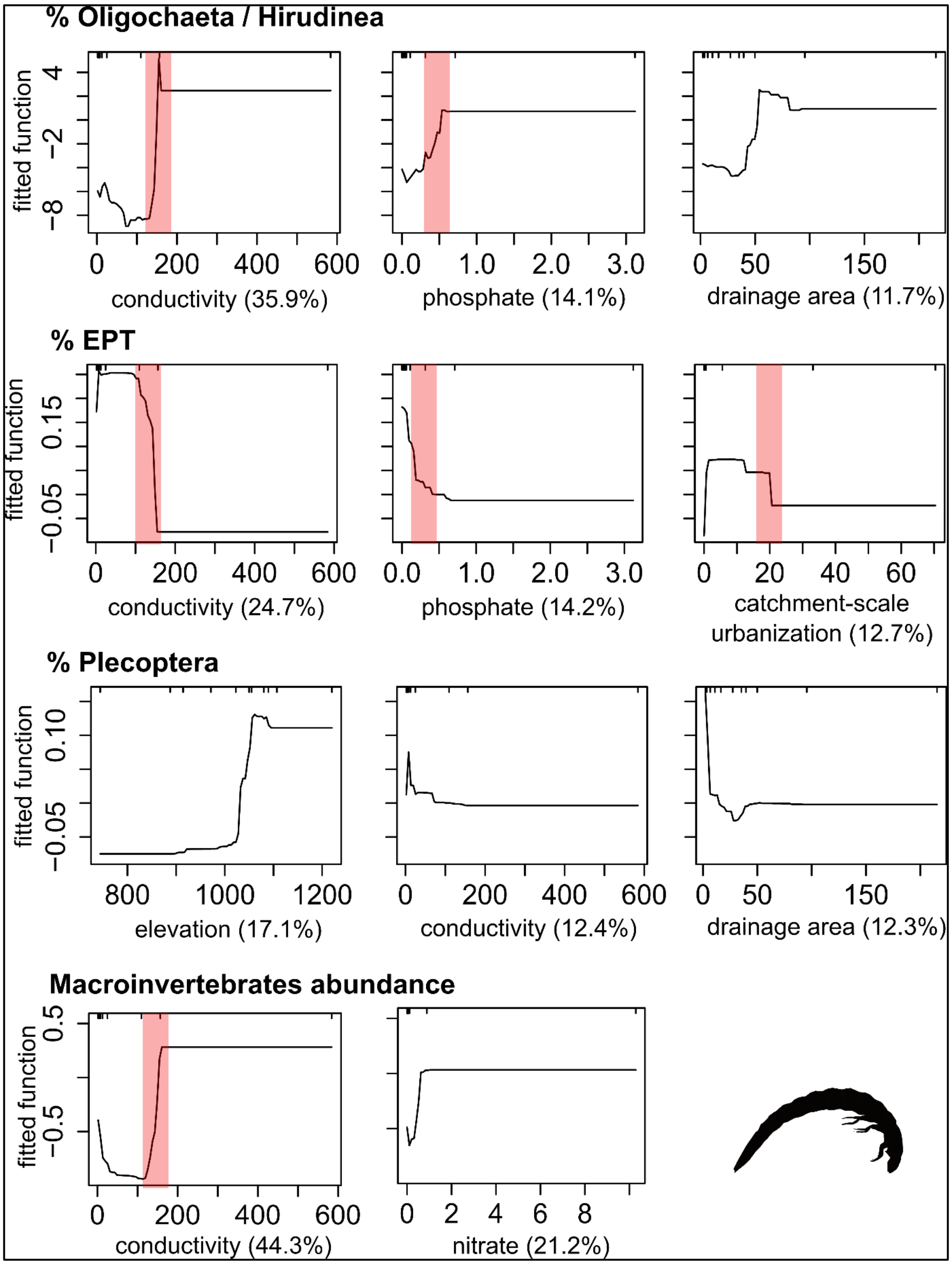

Figure 4. Boosted regression tree (BRT) fitted functions for the best performance models of Macroinvertebrate metrics. Partial plots representing predictors which contributed more than 10% for explaining deviance in the ecological metrics. Rug plots show the distribution of data, in deciles, of the variable on the X-axis. Red box represent the potential threshold zone.

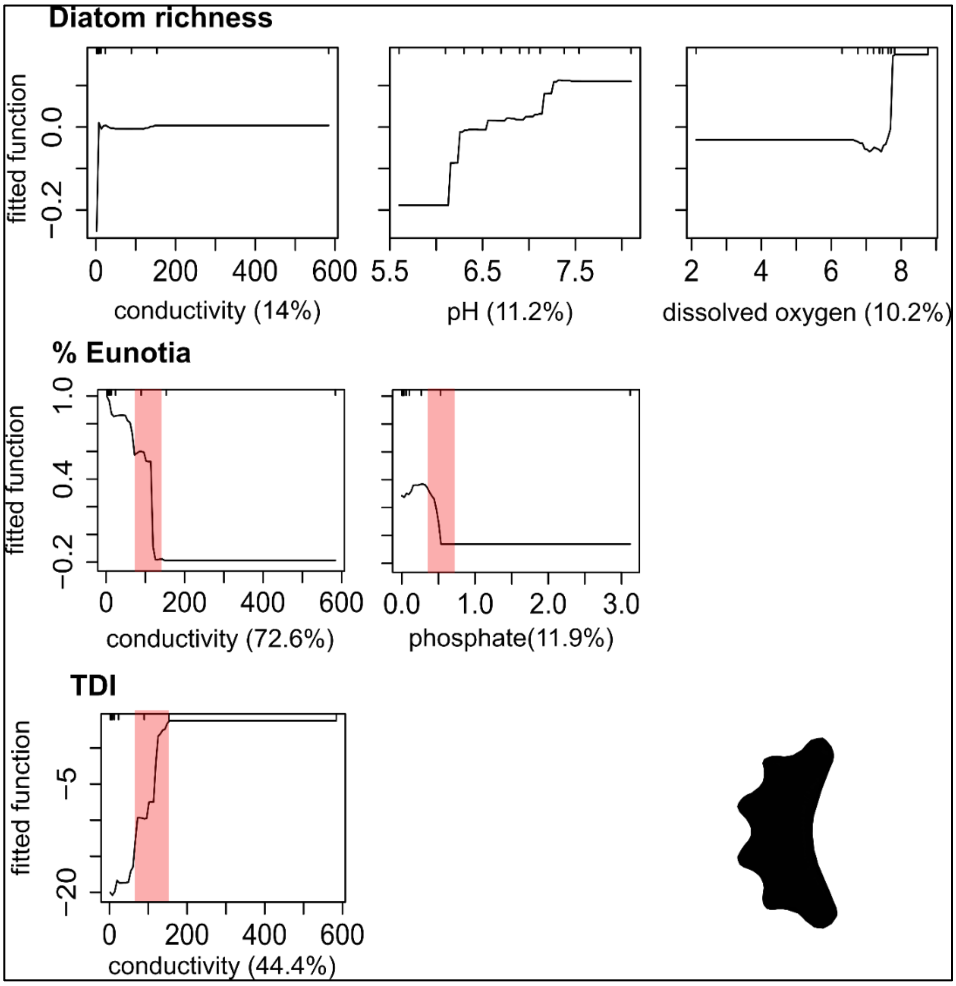

Figure 5. Boosted regression tree (BRT) fitted functions for the best performance models of Diatom metrics. Partial plots representing predictors which contributed more than 10% for explaining deviance in the ecological metrics. Rug plots show the distribution of data, in deciles, of the variable on the X-axis. Red box represent the potential threshold zone.

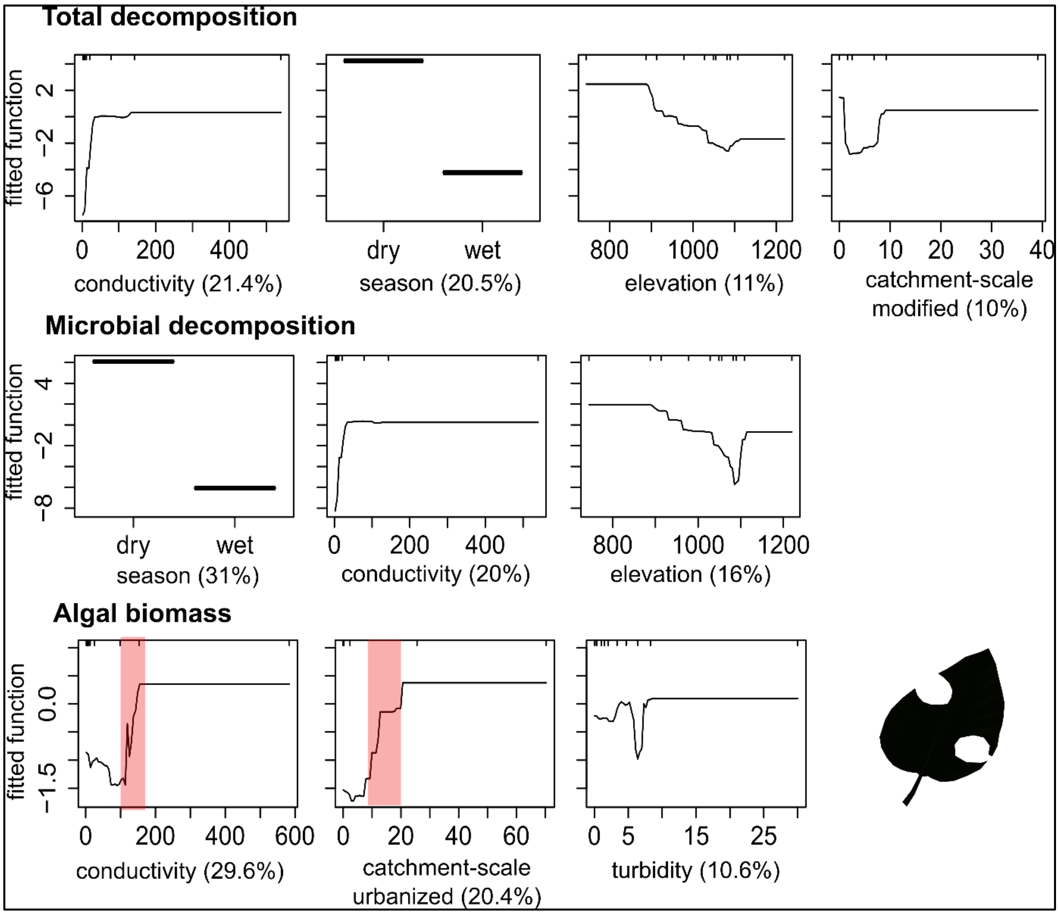

Figure 6. Boosted regression tree (BRT) fitted functions for the best performance models of functional metrics. Partial plots representing predictors which contributed more than 10% for explaining deviance in the ecological metrics. Rug plots show the distribution of data, in deciles, of the variable on the X-axis. Red box represent the potential threshold zone.

4. Discussion

4.1. Effects of water quality variables on response metrics

In general, water quality variables are considered response metrics, because they may respond to direct human disturbances, such as land use changes (Fierro et al. 2017) and wastewater input (Barrenha et al. 2018). However, our results clearly demonstrate that they are extremely relevant in indicating the behavior of ecological metrics, then being considered as predictors. Studies involving large areas or places with limited available information are unlikely to have access to all types of direct human impacts affecting sampling sites. It may include diffuse pollution and even global phenomena that are often ignored in such studies (Zimmerman et al. 2008). Thus, water quality variables represent predictors at the local scale, which may result from the interaction of various activities at other scales (Allan 2004). In this study, the most important predictors to explain the ecological metrics were physical and chemical variables often used to indicate human disturbances (Álvarez-Cabria et al. 2016;

Heathwaite 2010; Uriarte et al. 2011), such as phosphorous and nitrate concentrations, but especially conductivity. For instance, we reported major changes in ecological metrics when conductivity stood between 100 and 200 $\mu S.cm^{-1}$, suggesting a potential threshold. It is often reported that values above this threshold indicate loss of water quality, but when high conductivity is due to the natural context (Fravet & Cruz 2007, FUNASA 2014, CETESB 2016). In terms of relative importance, conductivity was the main predictor for the studied metrics.

Phosphorus is a fundamental nutrient triggering eutrophication of freshwaters (Figueredo et al. 2016; Zhang et al. 2017), and the export of phosphorus from agricultural fields to waterbodies is predicted to increase (Ockenden et al. 2016). Our results showed potential thresholds for phosphate around 0.5 $mg.L^{-1}$ and its contribution was especially relevant for metrics sensitive to pollution, such as %EPT, %Oli_Hir and %Eunotia, as shown elsewhere (Kelly et al. 1998; Salomoni et al. 2006, Ferreira et al. 2014; Pardo et al. 2020).

4.2. Relationships of land use variables with the response metrics

Urban and agricultural occupations in the upstream catchment were the most important land use factors to explain the response metrics. It is well reported that the logging of natural vegetation in the upstream catchment affects stream ecosystem via complex pathways (Allan 2004), including changes in temperature, habitat diversity, hydromorphology, sunlight, nutrient cycling and nutrient availability (Einheuser et al. 2013). These changes are translated into alterations in the structure and functioning of streams ecosystem (Clapcott et al. 2012).

It was observed that values of urban occupation in the upstream catchments between 10 and 20% led to a decrease in the abundance of EPT group and an increase of algal biomass. Brito et al. (2020) reported abrupt changes in the composition of macroinvertebrates with the removal of 57% to 79% of native vegetation in the Amazon forest, while Dala-Corte et al. (2020) reported values between 3% and 40% across biomes in Brazil. This indicates more restrictive values for our study region, suggesting that parts of the Brazilian Savannah are more susceptible to the conversion of native areas. Additionally, the increase of algal biomass related to urbanization process confirms a recent study that shows 32% greater effect on stream functioning than in its structure in the tropics (Wiederkehr et al. 2020).

Changes in the riparian vegetation are commonly associated with alterations in biological assemblages and ecosystem processes (Encalada 2010; Fierro et al. 2017), especially in headwaters or small rivers that are light-limited systems and rely on plant litter inputs from surrounding vegetation (Bunn & Davies, 2000; Perona et al. 2009). Although in our study we did not observe a robust relationship to macroinvertebrates, we showed a consistent negative relationship between diatoms and urbanization or diatoms and agriculture in the riparian corridor, indicating greater local than catchment-scale effect. This finding suggests reliable benefits of forested riparian buffers for stream biodiversity in urban environments, also supported by previous studies (e.g., Mutinova et al. 2020).

4.3. Shapes of response curves

Most of our models presented a unidirectional response for direct (land use) and indirect (water quality) human disturbances. Overall, increasing human disturbance (e.g., conductivity and changes in land use) led to a decrease of pollution-sensitive taxa (e.g., percentage of EPT group and *Eunotia*) and an increase of pollution-tolerant taxa (e.g., percentage of Oligochaeta and Hirudinea, the Trophic Diatom Index and the algal biomass production).

The nonlinear responses promote insights about the subsidy-stress theory (Odum et al. 1979), which predicts that the increase of limiting resources (e.g., nutrients, light) in an environment may have an initial positive effect on biological communities and ecosystem functions but up to a certain threshold; with further increases causing adverse effects. In other words, this would be the “too much of a good thing” syndrome (Odum et al. 1979). In this context, considering that Brazilian Savannah streams are poor in nutrients (Markewitz et al. 2006), nutrient inputs possibly promote the maintenance of more species/individuals. But at the other extreme of the gradient, intense disturbances are expected to reduce the number of species that are able to colonize or tolerate high impact levels (Odum et al. 1979). The behavior of EPT curve face to the increase in the conductivity and catchment urbanization is an example of this. It presented an initial low value followed by a sharp increase, but the further decrease indicates their sensitivity to disturbed environments (Ligeiro et al. 2013; Siegloch et al. 2017).

The evaluation of the response curves from BRT models are also a good starting point for a discussion on thresholds for the considered predictors, although it requires some attention, once certain variables do not show abrupt changes, but a gradient. Furthermore, the response curves take into account the effect of the other predictors (interaction), for they are not the perfect representation of the effect of each variable (Elith et al. 2008). Notable change points could be observed, for example, for conductivity, phosphate, and the percentage of urbanization in the upstream catchment, as already discussed.

4.4. Implications for management

BRT models provided robust information on the main predictors driving each ecological metric and how metrics responded to natural and human related predictors, allowing the identification of potential indicators of stream integrity. While the percentage of EPT group abundance and the algal biomass would be good indicators of urbanization in the upstream catchment, the percentage of *Eunotia* would indicate changes in water quality. In contrast, other metrics were poorly explained by the predictors or mostly influenced by natural predictors, which make them inappropriate indicators of environmental disturbances for management purposes (Norris & Hawkins 2000). This was the case of the Simpson Index for Diatoms which showed a low variance explained (17%), the percentage of Plecoptera which was mostly influenced by natural characteristics (elevation and drainage area) and decomposition primarily influenced by seasonality.

The different responses to the set of predictors, including structural and functional ecosystem metrics, can lead to a comprehensive interpretation of river conditions (Feio et al. 2010). The prediction of ecological conditions is relevant from the point of view of management since these are more complex data to be acquired, but of extreme relevance for understanding the health of water bodies (Karr 2006). Knowledge about the importance of each predictor for the response metrics allows, for example, to predict some ecological conditions in places with limited availability of biological data.

Also, the diversity of ecological metrics explored in this study can be used to design a multi-metric index, one that should encompass different types of metrics (structural and functional) and aspects of biodiversity (Hering et al. 2006a). However, the advantage of using multi-metric approach rather than a multi-metric index is the opportunity to understand differences among ecological indicators and develop recovery/conservation initiatives taking into account local particularities and needs. As compared by Karr (2006), the ecological metric response curve is like the “dose-response curves” for toxicologists, which allow understanding the effects of a chemical on individual organisms. On the other hand, a multi-metric index has the advantage of providing a precise picture of a site’s overall condition, besides of being easier to communicate and be understood by managers and society (Hering et al. 2006b), although important information may be lost in the simplification process (Baker & King 2010).

Finally, the potential thresholds identified in the present study are important signs of major changes in ecological responses and should be employed in eventual review processes of guidelines to public policies for river health preservation and recovery (Huggett 2005). Brazilian national environmental guidelines do not consider, for example, the conductivity

(CONAMA nº 357, Brasil 2005) notwithstanding the importance of this variable as an ecosystem driver, as demonstrated above. In addition, further attention should be paid to the context of land use, especially to the urbanization processes in the upstream catchment. Currently, in Brazil, greater attention has been given to the riparian vegetation (Federal Law nº 12651, Brasil 2012), but, as we have demonstrated, for purposes of biodiversity conservation and maintenance of ecosystem processes, it is necessary to consider the entire context of the catchment in which the stream is located.

5. Conclusions

Our results demonstrated the importance of considering a set of ecological response metrics (structural and functional) and environmental factors (natural and disturbances), allowing a complete view of the freshwater ecosystem condition. The percentage of variance explained enabled us to identify the best models. The relative importance of predictors on ecological metrics pointed to metrics that are most affected by factors on a local (e.g., percentage of *Eunotia* abundance) and catchment (e.g., algal biomass) scales. Also, the nonlinear responses permitted the detection of gradual or abrupt change curves, pointing out to the existence of potential thresholds of important drivers, like the conductivity (100-200 $\mu S.cm^{-1}$), phosphate (0.5 $mg.L^{-1}$) and catchment-scale urbanization (10-20%). Considering the best performance models and the ability to respond more strongly to the human disturbances than to natural factors, the potential bioindicators identified for the study area were macroinvertebrates abundance, percentage of Ephemeroptera/Plecoptera/Trichoptera, percentage of Oligochaeta/Hirudinea, percentage of *Eunotia*, Trophic Diatom Index and algal biomass. Although we have worked with many biotic and abiotic variables and the BRT model considered the interaction between them, models are simplified representations of a complex system, therefore presenting limitations. Nevertheless, the consistency and reasonableness of influential metrics within a given set of ecological metrics provide a weight of evidence in support of the models' results (Waite et al. 2019). The BRT models and the multi-metric approach proved to be powerful tools that can be effectively employed to enhance and give better direction to freshwater management, not only to the streams of the Brazilian Savannah but also to water bodies in other regions.

Acknowledgments

This work was supported by the Institutional Internationalization Program of the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES-PrInt; Proc. no.: 88887.364699/2019-00) that financed the 1-year PhD sandwich of Campos C.A. in Brisbane, Australia; the Fundação de Amparo à Pesquisa do Distrito Federal (FAP-DF) for their financial support to Aquariparia Project (edital 05/2016-Águas; Proc. no.: 193.000716/2016) that allowed the execution of fieldwork and laboratory analyses; the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) through research fellowship to José Francisco Gonçalves Jr (Proc. no.: 310641/2017-9); and the Agência Reguladora de Águas, Energia e Saneamento Básico do Distrito Federal (ADASA) that in addition to the financial support to Campos C.A. also offered logistical support of vehicles for the fieldwork.

The authors are thankful to the Laboratório de Citotaxonomia e Insetos Aquáticos (Instituto Nacional de Pesquisas da Amazônia - INPA) team, for their support in identifying macroinvertebrates. We also thank Maíra Campos, Ana Luiza Dornas and Cleber Figueredo, from Universidade Federal de Minas Gerais (UFMG), for their support in identifying diatoms. Our sincere gratitude to the Australian River Institute (ARI) for hosting Campos C.A. for one year to develop the statistical and modelling analyses of the study. We greatly appreciate the collaboration of all students of the Laboratório de Limnologia (Universidade de Brasília - UNB) in fieldwork activities and laboratory analyses. Many thanks to Erika Helena Campos, who carried out the final English review. Finally, we would like to thank all institutions (Exército Brasileiro, Marinha Brasileira, IBRAM, ICMBio, Jardim Botânico, IBGE and UNB) and owners of environmental protected areas (Chapada Imperial and Paraíso na Terra), which allowed the collection of samples on the lands under their administration.

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SUPPLEMENTARY MATERIAL

Table S1. Settings and statistics for all reduced BRT models. tc = tree complexity; lr = learning rate; # of trees = number of trees. na = not applicable. Check abbreviations in Table 2.

Biological Groups	**Metrics**									
Macroinvertebrates										
	Inv_Rich	**Inv_Abund**	**Inv_Shannon**	**Inv_Simpson**	**Inv_Pielou**	**%EPT**	**%Plecoptera**	**%Oli_Hir**	**BMWP**	**ASPT**
Data transformation	na	na	na	na	na	log(x+1)	log(x+1)	na	na	na
Model error	Poisson	Poisson	Gaussian	Gaussian	Gaussian	Gaussian	Gaussian	Guassian	Poisson	Gaussian
tc	5	5	5	5	5	5	5	5	5	5
lr	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.01	0.001	0.001
# of trees	2000	4050	1350	1450	1100	2450	4150	2000	1700	1250
Total Deviance	2.46	927.60	0.21	0.032	0.031	0.21	0.15	89.59	15.28	0.17
Residual Deviance	0.97	146.39	0.10	0.016	0.018	0.06	0.03	7.62	6.46	0.09
Variance Explained (%)	60.80	84.22	49.76	50.00	41.94	70.33	82.19	91.49	57.72	46.15
Training data correlation	0.88	0.88	0.87	0.86	0.84	0.89	0.93	0.96	0.86	0.78
CV Deviance (mean)	2.21	525.95	0.20	0.032	0.031	0.16	0.12	54.41	13.42	0.16
CV Deviance (se)	0.34	161.59	0.03	0.005	0.004	0.03	0.02	17.78	2.12	0.01
CV Deviance Explained (%)	10.32	43.30	1.45	0.00	0.000	22.49	19.18	39.27	12.18	8.28
CV correlation	0.35	0.46	0.19	0.239	0.177	0.45	0.53	0.62	0.38	0.36

Diatoms

	Diat_Rich	Diat_Abund	Diat_Shannon	Diat_Simpson	Diat_Pielou	%Eunotia	%Nitz_palea	TDI
Data transformation	na	log (x+1)	na	na		log (x+1)	log(x+1)	na
Model error	Poisson	Gaussian	Gaussian	Gaussian		Gaussian	Gaussian	Gaussian
tc	5	5	5	5		5	5	5
lr	0.001	0.001	0.0001	0.0001		0.001	0.001	0.001
# of trees	3650	1050	7900	3850		4350	3450	3950
Total Deviance	2.30	0.50	0.307	0.041		0.44	0.14	430.48
Residual Deviance	0.53	0.31	0.205	0.034		0.06	0.06	68.74
Variance Explained (%)	76.91	38.76	33.22	17.07		86.99	60.42	84.03
Training data correlation	0.92	0.77	0.81	0.73		0.94	0.82	0.93
CV Deviance (mean)	1.99	0.48	0.299	0.04		0.16	0.12	235.97
CV Deviance (se)	0.19	0.09	0.038	0.007		0.02	0.04	39.98
CV Deviance Explained (%)	13.33	3.41	2.61	2.44		63.01	19.44	45.18
CV correlation	0.44	0.25	0.282	0.215		0.68	0.40	0.61

Ecosystem Processes

	Tot_dec	Mic_dec	Chl	Resp	Erg	ATP
Data transformation	na	na	na	na	log	log
Model error	Gaussian	Gaussian	Gaussian	Gaussian	Gaussian	Gaussian
tc	5	5	5	5	5	5
lr	0.001	0.001	0.01	0.0005	0.001	0.001
# of trees	2900	4300	2300	4150	1800	1600
Total Deviance	191.329	164.82	3.20	0.04	0.128	0.088
Residual Deviance	50.804	30.05	0.11	0.03	0.066	0.042
Variance Explained (%)	73.45	81.77	96.47	33.33	48.44	52.27
Training data correlation	0.90	0.92	0.99	0.68	0.82	0.82
CV Deviance (mean)	130.112	91.86	1.78	0.04	0.127	0.075
CV Deviance (se)	22.615	30.24	0.92	0.03	0.026	0.007
CV Deviance Explained (%)	32.00	44.27	44.38	4.76	0.78	14.77
CV correlation	0.54	0.75	0.48	0.21	0.388	0.419

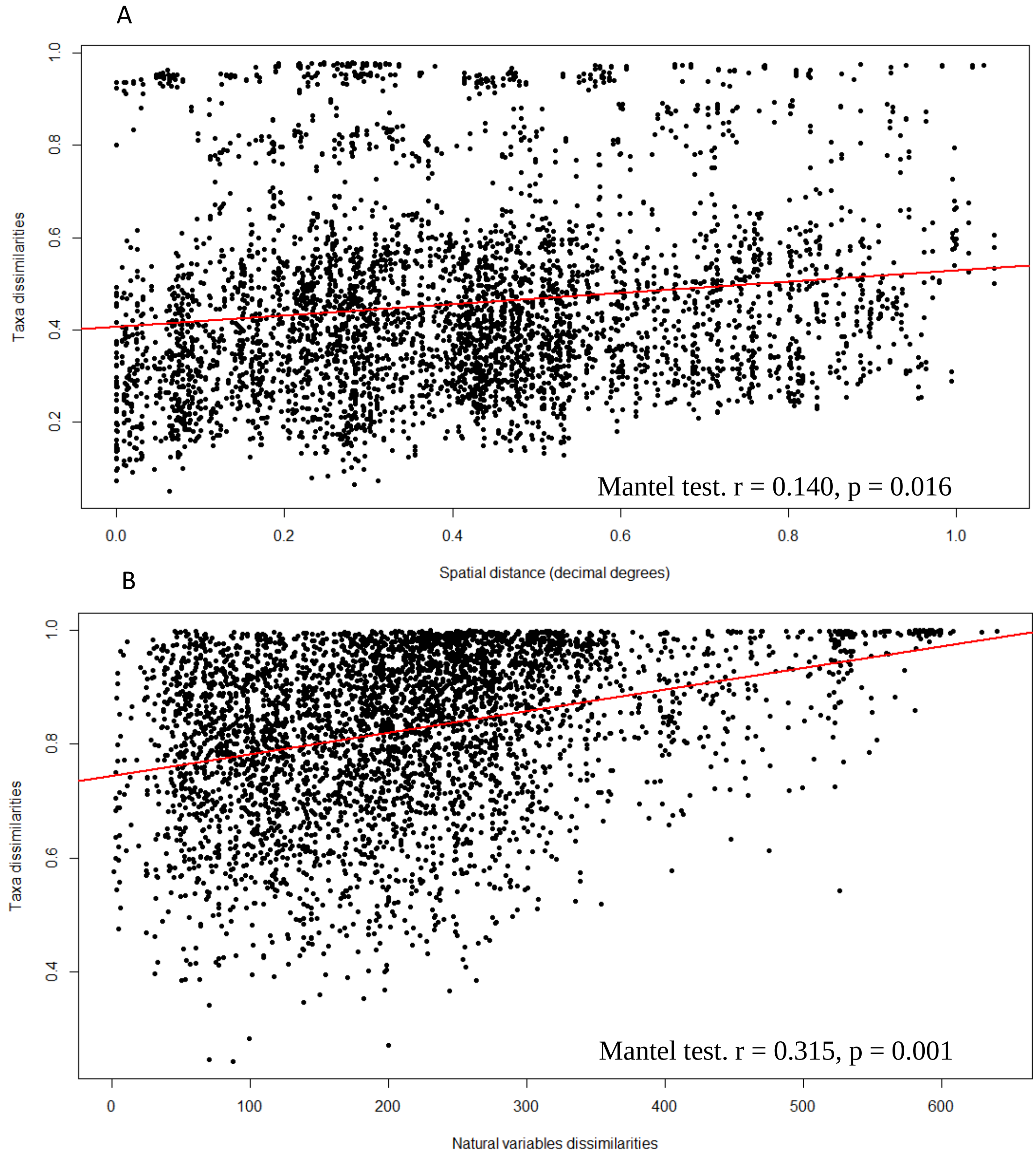

Figure S1. Correlation between taxa dissimilarities (diatoms and macroinvertebrates) and geographic distance (A) and natural variables dissimilarities (B). For both, Mantel test result are described in each graph.

CAPÍTULO IV

Progressing a river health assessment framework to tropical waters

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Artigo a ser submetido no periódico *Ambio*

Abstract

Brazil is extremely important in the world's context of biodiversity and freshwater reserves, but its natural resources has been threatened by diverse anthropogenic activities. In the context of water quality management, the Brazilian legislation foresees the classification of its rivers (or stretches of them) into classes according to designated best uses. From the special class to class 4, there is a gradient of decreasing water quality. Although the Federal legislation mentions biomonitoring, there are no clear guidelines for its implementation, nor is there any concern to assess the ecological integrity (river health) of aquatic ecosystems, focusing purely on physical, chemical, and microbiological factors related to water quality itself. In this study, we outlined a framework that encompasses two management tools: (i) a river health index considering the P-C-R (Pressure-Condition-Response) approach and (ii) an index of suitability to the class of use (SCU). The PCR approach integrates pressures on aquatic ecosystems (anthropogenic activities), their conditions in terms of hydrology, water quality and biological elements and societal/governmental response (designated best use using the classes of use). The SCU measures whether rivers' conditions are in accordance with their classes of use. We used the Harmony Degree (HD), method based on the fuzzy membership function, to calculate both indices. The results showed that most sites had a good river health condition, with the worst conditions occurring in areas with high urban density or under intense agricultural use. SCU indicated that few streams were poorly or not compatible with their classes of use, suggesting their classifications were based on current conditions but not on future intentions. With both tools in hands, the managers have the opportunity to assess the real situation of aquatic ecosystems and define the best management strategy.

Key-words: ecological integrity, river health framework, freshwater management

Resumo

O Brasil é extremamente importante no contexto mundial de biodiversidade e reservas de água doce, mas seus recursos naturais têm sido ameaçados por diversas atividades antrópicas. No contexto da gestão da qualidade da água, a legislação brasileira prevê a classificação de seus rios (ou trechos) em classes de acordo com os usos pretendidos mais exigentes em termos de qualidade de água. Da classe especial para a classe 4, há um gradiente de diminuição da qualidade da água. Embora a legislação federal mencione o biomonitoramento, não há diretrizes claras para sua implementação, nem há qualquer preocupação em avaliar a integridade ecológica (saúde) dos ecossistemas aquáticos, com foco puramente em fatores físicos, químicos e microbiológicos relacionados à qualidade da água em si. Neste estudo, delineamos um sistema de avaliação que engloba duas ferramentas de gestão: (i) um índice de saúde de rio considerando a abordagem P-C-R (Pressão-Condição-Resposta) e (ii) um índice de adequação à classe de uso (SCU). A abordagem P-C-R integra pressões sobre os ecossistemas aquáticos (atividades antropogênicas), suas condições em termos de hidrologia, qualidade da água e elementos biológicos, e a resposta social / governamental (classes de uso). A SCU mede se as condições dos rios estão de acordo com suas classes de uso. Utilizamos o Grau de Harmonia (HD), método baseado na lógica difusa (*fuzzy*), para calcular os dois índices. Os resultados mostraram que a maioria dos locais apresentou um bom estado de saúde ecossistêmica, com as piores condições ocorrendo em áreas com alta densidade urbana ou de uso agrícola intenso. O SCU indicou que poucos riachos foram pouco compatíveis ou incompatíveis com suas classes de uso, sugerindo que suas classificações foram baseadas nas condições atuais, e não em intenções de usos futuros. Com as duas ferramentas em mãos, os gestores têm a oportunidade de avaliar a real situação dos ecossistemas aquáticos e definir a melhor estratégia de manejo.

Palavras-chave: integridade ecológica, avaliação de saúde do rio, gestão de água doce

1. Introduction

Brazil shelters in its territory a substantial part of the world's biodiversity (about 14%; Brazil 2003), freshwater resources (12%; Rebouças 2006) and remaining tropical forests that play a significant role in the regional and global climate system (Fernandes et al. 2017). All this biological wealth attributed to different Brazilian biomes has been historically threatened by pressures such as deforestation (Crouzeilles et al. 2017) and organic pollution (Wen et al. 2017). The discharge of untreated domestic sewage is the main factor degrading water bodies in Brazil, since only 55% of the country's municipalities have a sewage collection system, covering only 44% of dwellings. Furthermore, of the total volume of sewage collected, only 69% was treated in 2008, the remainder being released directly into water bodies without any treatment (IBGE 2012). In central Brazil, another major threat is the transformation of extensive natural Cerrado areas into grain monoculture (Strassburg et al. 2017). More recently, new threats have also been added to the environment disturbance scenario, such as climate changes, infectious diseases, emerging contaminants, e-commerce and invasions, light and noise (Reid et al. 2019).

In Brazil, the concept of Water bodies Framework by Class of Use (WFCU) based on a series of water quality parameters has been used since 2005. According to the Framework, water bodies – or stretches of them – are designated under five different classes (Table 1). The WFCU's objective is to ensure that water bodies present water quality compatible with the designated best uses defined for each class. Thus, management actions can be directed towards the restoration and maintenance of places where better conditions are expected. Although the better water quality classes foresee the preservation of aquatic assemblages and ecosystems, in practice few biological parameters are referenced in the legislation, such as the thermotolerant coliforms, chlorophyll *a*, and cyanobacteria density (Brasil 2005). A similar approach is used in India, where water bodies are also classified into five classes according to their intended uses, but in contrast of Brazil the biological indicators used in India, such as fish and insects, are considered part of the assessment criteria (Singh & Saxena 2018). Thus, there is an intention in India to comprehensively describe the quality of a freshwater ecosystem, while in Brazil only few States, such as São Paulo (CETESB 2012), or isolated monitoring programs carry out some type of biomonitoring.

From an ecological point of view, a healthy river has "ecological integrity" (Boulton 1999), which means the capability of supporting and maintaining a balanced, integrated, adaptive community of organisms having a species composition, diversity, and functional organization comparable to that of natural habitats of the region (Karr 1999). Biological assemblages and ecosystem processes may be good tools to measure the effects of the complex mechanisms of environmental degradation and their interactions (Karr 1999; Ruaro & Gubiani 2013). Thus, changing from a water quality approach to an ecosystem integrity approach is a necessary key-change face to the pressures that aquatic ecosystems have been suffering.

Advancing in the concept of ecological integrity assessment, Fairweather (1999) pointed that the political, social and economic aspects cannot be dissociated from the idea of river health. Considering the values defined by society in relation to water, the uses and the historical relationship of the community with the rivers in its region are essential not only for defining management strategies but also but also to involve society in the whole process (Anderson et al. 2019). The OECD (Organization for Economic Co-Operation and Development) have proposed a model based on three types of indicators: pressure, condition, and response (P-C-R; OECD 2013). “Pressure” indicators (P) address human threats to the environment, such as removing vegetation, launching pollutants, and physically altering water courses. “Condition” (C) or “State” indicators address the performance of the environment. “Response” indicators (R) are based on society's reactions to trends in any of the other indicators, to improve or correct problems, and may include the elaboration of public policies, social engagement and management actions.

Ecological integrity approaches taking into account aspects of human pressures on ecosystems and the “society x water bodies” interaction are becoming a trend. More recently, Luo et al. (2018) proposed a new framework to access the river health status, based on the harmony theory, which considers the balance between river ecosystem integrity and human service demand by the harmony degree (HD) (Zuo et al. 2016). In Australia, the well-known Australian River Assessment System (AUSRIVAS; Simpson & Norris 2000) has been questioned, precisely because it does not consider the anthropic pressures on ecosystems. A recent study suggested the necessity of diagnostic methods to identify the stressors causing ecological impact rather than merely to infer impact intensity and assign quality ratings to assessment sites (Chessman 2021).

Faced with such a variety of realities in terms of human stressors, natural characteristics, management capacity and data availability, applying a monitoring program becomes complex and challenging if there is no guiding framework (Pinto & Maheshwari 2014). In countries with continental dimensions, such as Brazil, the framework is even a necessity to facilitate the understanding of the results at the managerial level (Sadat et al. 2020). Also, a well-defined framework allows for the standardization of a process that can be applied in larger scales, as long as the necessary adaptations are made. Despite all the difficulties that may arise from the implementation of biomonitoring, after an initial research phase these programs considerably reduce analysis costs and provide relevant information for watershed management (Buss et al. 2003).

Considering the absence of a river health framework and the current model of Waterbodies Framework by Class of Use (WFCU), the three main objectives of this study were: 1) to outline a comprehensive methodology for assessing the river health status and the suitability of the river conditions to their respective demanded best uses (class of use); 2) to access the ecological integrity of the streams in the study area; 3) to propose a river health framework to support management decisions on watersheds and freshwater issues.

Table 1. Current Water bodies Framework by Class of Use which presents five classes (0 to 4) and the respective uses foreseen for each one of them.

Classes	Designated Best Uses
0 (special)	Human supply after disinfection Preservation of the natural balance of aquatic assemblages Preservation of aquatic environments in full-protected areas
1	Human supply after simplified treatment Protection of the aquatic biological assemblages Primary contact recreation Irrigation of raw-consumed vegetables and fruits developed close to the soil and/or no-cooked and peeled eaten Protection of biological aquatic assemblages in Indigenous lands
2	Human supply after conventional treatment Protection of aquatic assemblages Primary contact recreation Irrigation of vegetables, fruit plants and parks, gardens, sports and leisure fields, with which the public may come into direct contact Aquaculture and fishing
3	Human supply after conventional or advanced treatment Irrigation of tree, cereal and forage crops Amateur fishing Secondary contact recreation Animal water supply
4	Navigation Landscape harmony

2. Material and Methods

2.1. Study area

The Brazilian central plateau over the federal capital (Distrito Federal) was studied. The region hosts headwaters of three major Brazilian river basins: Tocantins-Araguaia, Paranaíba and São Francisco (Figure 1). The predominant land uses are natural vegetation, agriculture and urban occupation. Its average altitude is 1000 m and the predominant vegetation is the Brazilian savannah (Cerrado). The climate is characterized by two well defined seasons, dry (April to

September) and wet (October to March), average temperature and annual precipitation around 20°C and 1700 mm, respectively, but the spatial distribution of precipitation is uneven across the area.

We selected 50 study sites for which we had abiotic and biotic data available for the dry season (August/September) of 2018.

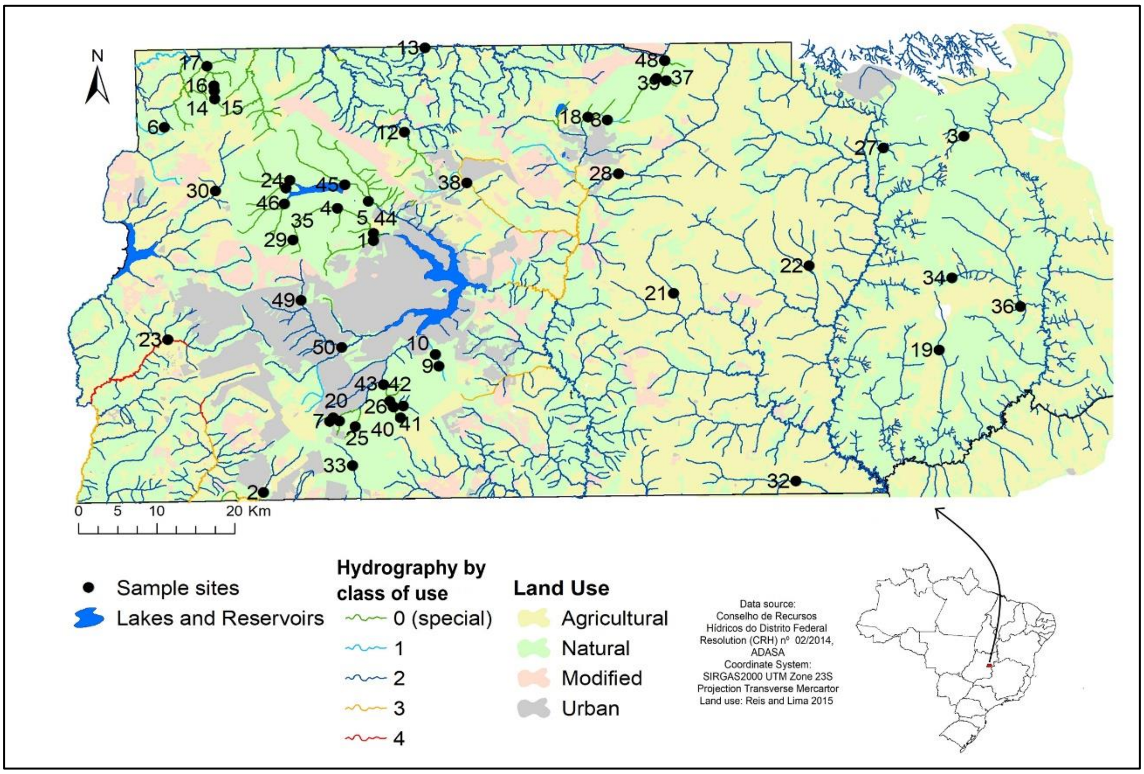

Figure 1. Study area, sample sites and the hydrography coloured according to the class of use determined by the Waterbodies Framework by Class of Use.

2.2. Indexes development steps

The methodology adopted to generate the river health index (hereafter Tropical Waters Health Index, TWHI) and the Suitability to the Class of Use (SCU) index was based on the P-C-R approach (OECD 2013) and the Harmony Degree method (Luo et al. 2018), following steps described below and summarized in Figure 2.

2.3. Index system

The Tropical Waters Health Index (TWHI) was divided into three layers: pressure, condition, and response. For each layer, attributes and indicators were chosen in order to represent relevant aspects of the study area (Table 2).

Changes in land use were considered the main pressures on water courses, since they aggregate multiple stressors on aquatic ecosystems (Allan 2004). The Land Use Index (LUI, Eq. 1, adapted from Rawer-Jost et al. 2004) applied for the upstream catchment; and the percentage of riparian vegetation clearing (30m each-bank along upstream) were selected as indicators.

$$LUI = 4x\ \%\ CAT\_urb + 2x\ \%\ CAT\_agr + \%\ CAT\_mod \quad \text{(Eq. 1)}$$

Where LUI is the Land Use Index; CAT_urb, CAT_agr and CAT_mod are, respectively, the percentage of urban, agricultural and pasture, and other uses (allotment, exposed soil, eucalyptus plantations) in the upstream catchment.

The "condition" indicators encompass hydrological, water quality and ecological attributes (Table 2). Hydrological aspects are fundamental, especially when dealing with headwater streams, where the streamflows are naturally low, such as in the study area. Hydrological indicator is related to the deviance (to up or down) of the reference streamflow. Minimum, maximum and average historical streamflow values for the studied period (August/September) were considered as references (GDF 2012). Conductivity and phosphorous were selected as water quality indicators since they are important water quality parameters usually related to human impacts, especially urbanization, in the study area (Silva et al. 2011; Fonseca et al. 2014). Biological indicators related to periphytic diatoms - Percentage of *Eunotia* and Trophic Diatom Index (TDI, Kelly 1998); macroinvertebrate assemblages - Percentage of Oligochaeta and Hirudinea, Percentage of Ephemeroptera, Plecoptera, Trichoptera, and Macroinvertebrates abundance; and algal biomass production (based on chlorophyll *a* production) were selected as stream ecological indicators because they responded to a set of human disturbances in the study area (Chapter 3).

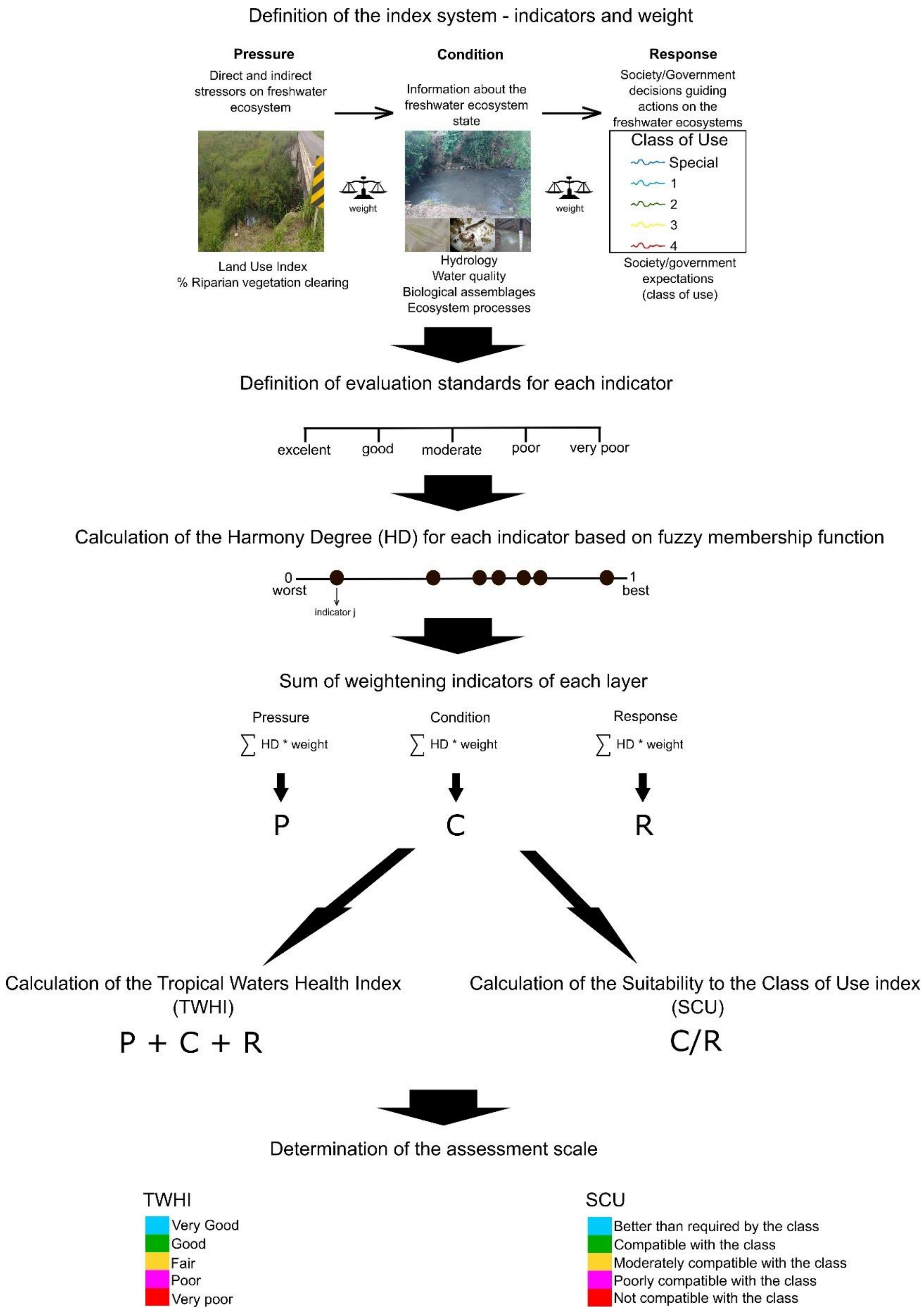

Figure 2. Scheme of the calculation of the Tropical Waters Health Index (TWHI) and the Suitability to the Class of Use Index (SCU) based on the P-C-R approach and the Harmony Degree.

The class of use determined for each watercourse by the official Water bodies Framework by Class of Use (WFCU) was considered as the response indicator. The class of use is related to the designated best uses intended by the stakeholders, not necessarily constituting the current quality of the river, but the expected quality. The class of use reflects the expectations of society and government in relation to the water bodies, and for their determination, technical, economic, social and political aspects are considered.

2.4. Calculation weight of each indicator

We argue that pressure, condition and response together may represent the healthiness of an aquatic ecosystem, so we distributed equal weight among the three layers (~0.33) and into each layer the weight was divided equally among the indicators (Table 2).

2.5. River health assessment criteria

2.5.1. Determination of the Evaluation Standards

To determine the classification criteria of river health it is usually necessary to carry out threshold research and analyse the values of the indicators among different rivers (Sadat et al, 2020). We used different sources of references considering official guidelines and previous studies in the study area. For land use and water ecological attributes, we considered as thresholds values identified in specific models developed in previous studies carried out at the same sites (Chapters 1 and 2). Water quality references were based on national guidelines (Brasil 2005) and in previous studies (Chapters 1 and 2). The reference streamflows were extracted from the Integrated Water Resources Management Plan for Distrito Federal (GDF 2012), considering as reference the historical monthly average, maximum and minimum streamflows of August and September (Table 2). For the “Response”, the classes of use were considered the standard values. The evaluation standard nodes were classified as Excellent, Good, Moderate, Poor, and Very poor (Table 2).

Table 2. Tropical Waters Health Index (TWHI) system and evaluation standards. (*) cost indicator – the higher the value the worst the condition, (**) efficiency indicator – the higher the value the better the condition.
mean = historical mean, min = historical minimum, max = historical maximum

Index System					Evaluation Standards				
Layer	**Attribute**	**Indicator**	**Weight**	**CODE**	**Excellent (a)**	**Good (b)**	**Moderate (c)**	**Poor (d)**	**Very poor (e)**
Pressure	Land Use	Land Use Index *	0.167	LUI	50	100	150	200	250
		Riparian vegetation clearing (%) *	0.167	RIP_clear	5	10	15	20	25
Condition	Hydrology	Natural streamflow deviance (%)	0.037	S_dev	mean	min or max	-10% min or +10% max	-20%min or +20% max	-30% min or +30% max
	Water Quality	Electrical conductivity (µS/cm) *	0.037	Cond	50	80	110	140	170
		Phosphorous (mg/L) *	0.037	PO4	0.025	0.05	0.075	0.1	0.125
	Water Ecology	Trophic Diatom Index *	0.037	TDI	30	40	50	60	70
		% abundance of genus Eunotia **	0.037	Eunotia	80	60	40	20	10
		Invertebrates abundance (ind/0.45m^2) *	0.037	Inv_Abund	200	300	500	1000	1500
		% abundance of EPT (Ephemeroptera, Plecoptera and Trichoptera **	0.037	EPT	40	30	20	10	5
		% abundance of Oligochaeta and Hidrudinea *	0.037	Oli_Hir	5	10	20	30	40
		Algal biomass (Chlorophyll *a* production ug/m^2) *	0.037	Chl	1	3	5	7	9
Response	Designated best uses	Class of use *	0.334	Uses	0	1	2	3	4

2.5.2. Harmony degree (HD)

Following the steps suggested by Luo et al. (2018), the harmony degree was calculated taking into account the five nodes of the evaluation criteria (Table 2) and the fuzzy membership function. The harmony degree for the value (*x*) of a single indicator (*j*) was defined by a fuzzy membership function ($\mu_k(x)$) with $\mu_k \in [0, 1]$ and the harmony degree was enumerated by a linear interpolation (Eq. 2; Luo et al. 2018), where HD_j is the harmony degree of the j^{th} indicators at time t and $HD_j \in [0, 1]$; a_j, b_j,c_j,d_j, and e_j are the evaluation standards values of j^{th} indicator (Table 2). Efficiency (or positive) indicators are those for which higher values are better for the environment (e.g., % of EPT), while cost (or negative) indicators are those for which higher values are worse for the environment (e.g., Land Use Index).

We used opposite formulas (Eq. 2) to calculate the harmony degree of the cost and efficiency indicators, so that the harmony degree is standardized with values ranging from 0 (indicative of the worst situation) to 1 (indicative of the best situation). To the natural streamflow deviance (S_dev) was used the "cost indicator" formulae to values above the best condition and "efficiency indicator" formulae to values under the best condition, because in this case more is worse and less is worse as well.

To the response layer, as the classes of use are discrete and non-continuous values, it was not necessary to apply the interpolation, and the harmony degree was determined just using the intervals from 0.2 to 1. Class 0 (special), 1, 2, 3 and 4 have harmony degree equal to 1, 0.8, 0.6, 0.4 and 0.2, respectively. Class 4 was not assigned a zero-value due to the calculations of the suitability to the class of use index, which function's denominator is the response layer and this cannot be zero.

Eq. 2 (Sadat et al. 2020)

For efficiency indicator

$$HD_j = \begin{cases} 1 & x_j \geq a_j \\ 0.8 + 0.2 \times \left[\dfrac{x_j - b_j}{a_j - b_j}\right] & b_j \leq x_j < a_j \\ 0.6 + 0.2 \times \left[\dfrac{x_j - c_j}{b_j - c_j}\right] & c_j \leq x_j < b_j \\ 0.3 + 0.3 \times \left[\dfrac{x_j - d_j}{c_j - d_j}\right] & d_j \leq x_j < c_j \\ 0.3 \times \left[\dfrac{x_j - e_j}{d_j - e_j}\right] & e_j \leq x_j < d_j \\ 0 & x_j < e_j \end{cases}$$

For cost indicator

$$HD_j = \begin{cases} 1 & x_j \leq a_j \\ 0.8 + 0.2 \times \left[\dfrac{x_j - a_j}{b_j - a_j}\right] & b_j \geq x_j > a_j \\ 0.6 + 0.2 \times \left[\dfrac{x_j - b_j}{c_j - b_j}\right] & c_j \geq x_j > b_j \\ 0.3 + 0.3 \times \left[\dfrac{x_j - c_j}{d_j - c_j}\right] & d_j \geq x_j > c_j \\ 0.3 \times \left[\dfrac{x_j - d_j}{e_j - d_j}\right] & e_j \geq x_j > d_j \\ 0 & x_j > e_j \end{cases}$$

2.5.3. Determination of the river health index

The Tropical Waters Health Index (TWHI) is the weighted average of the harmony degree of collected single indexes calculated above. TWHI is now obtained by the following equation:

Eq. 3.

$$TWHI(t) = \sum_{j=1}^{m} w_j . HD_j(t)$$

Where TWHI(t) is the tropical waters health index at time t, TWHI(t) ∈ [0,1]. The closer to 1 the value is, the better the integrity/health of the aquatic ecosystem; w_j is the weight of the j^{th} indicator, m is the river health indicators.

A five-grade assessment scale from 0 to 1 with 0.2 increments was established to describe the river health status as Critical, Poor, Fair, Good, and Very Good (Table 3).

Table 3. Five grades of the Tropical Waters Health Index (TWHI) assessment scale.

TWHI	Health Status	Remarks
> 0.8 – 1.0	Very good	Close to reference river conditions
> 0.6 – 0.8	Good	A small difference from reference river condition
> 0.4 – 0.6	Fair	Moderately different from reference river
> 0.2 – 0.4	Poor	The large difference from reference river condition
0.0 – 0.2	Critical	Significantly different from reference river condition

2.6. Determination of the suitability to the class of use index (SCU)

To check if the water ecosystem conditions are coherent with the expected designated best uses (class of use) we applied the ratio between the weightening harmony degree of the "condition" layer by the "response" layer (Eq. 4). If the “condition” value is higher than the “response” value, the classification is considering a worse condition than the freshwater ecosystem really has. Otherway, if the “condition” value is lower than “response” so the stream/river classification is better than the freshwater ecosystem presents. This is a simplified way to verify the suitability of the ecosystem condition to the current class of use. A five-grade assessment scale from 0 to 1 was established to describe the SCU (Table 4).

Eq. 4.

$$SCU = HD_{cond} / HD_{resp}$$

Where,

$$HD_{cond} = \sum_{j=1}^{c} w_j.HD_j(t)$$ c = condition indicators, w_j is the weight of the j^{th} indicator

and

$$HD_{resp} = w_j.HD(t)$$ w_j is the weight of the response indicator

Table 4. Suitability of the ecosystem health conditions to the class of use (SCU) based on the ratio Condition/Response.

SCU	SCU status	Suitability to the class of use
> 1.0	BTR	Better condition than required by the class
> 0.8 – 1.0	C	Compatible with the class of use
> 0.6 – 0.8	MC	Moderatly compatible with the class of use
> 0.4 – 0.6	PC	Poorly compatible with the class of use
0.0 – 0.4	NC	Not compatible with the class of use

2.7. Framework for stream ecosystem health assessment

This study proposes the evaluation of water bodies under two aspects, the first in which the demands of society are considered part of the health index assessment, understanding that the classes of use guide management actions and contribute so much for the current condition of water bodies; the second in which the condition of the river is confronted with the respective class of use.

The core of the method remains on the elaboration of the index system, determination of the evaluation standards, the harmony degree calculation, the weighting of the indicators, the calculation onf the health index (TWHI) and the suitability to the class of use index (SCU). But after the indexes calculations it is necessary to determine the acceptable limits for each index. In this study, following Sadat et al. (2020), the limit of 0.4 (Table 3) for TWHI was considered the minimum acceptable condition. Bellow this value it is necessary to define steps to improve the condition of the aquatic ecosystem. Between 0.6 and 0.4 the situation is acceptable but requires attention, and above 0.6 the stream is in good condition of ecological integrity.

To the suitability to the class of use (SCU), we determined three ranges of values. Values above 1 indicate that the condition of the river is better than the proposed class, between 1 and 0.6 there is some degree of compatibility between condition and class of use, and less than 0.6 indicates that actions are necessary to reach the proposed uses.

The framework indicates for each index (TWHI and SCU) general actions required. However, for each combination of results between the two indices we proposed more specific management actions.

3. Results

3.1. Harmony degree of indicators

Considering all the study sites, the lower harmony degree average values were observed for the following indicators: natural streamflow deviance (S_dev; 0.502), the percentage of *Eunotia* (Eunotia; 0.657), and the percentage of the EPT group (EPT; 0.418). On the other hand, those that presented higher values were the Trophic Diatom Index (TDI; 0.899), percentage of Oligochaeta and Hirudinea (Oli_Hir; 0.922) and Algal biomass (Chl; 0.922) (Figure 3, Supplementary material).

In general, the highest TWHI values were accompanied by an equivalent distribution among the three layers of indicators (pressure, condition, and response) and by the contribution of several condition indicators (Figure 3). The absence of any indicator means its contribution was zero, i.e., it contributed to reducing the value of TWHI. Sites that presented the lowest TWHI values (bellow 0.4) were 2, 23, 38 and 50. Among them, sites 2, 23 and 50 are characterized by low contributions of the pressure layer indicators, i.e., they are more subject to the action of land uses in the catchment and/or riparian corridor. Also, they presented low contribution for some of the condition indicators. Site 38, despite showing relatively good contribution from pressure indicators, it fails to present harmony degree zero or very close to zero for almost all condition indicators. In sites 12 and 13, despite presenting a balance between the three layers, all have moderated values, dropping the value of the index. Sites 21, 22 and 32 are negatively influenced by pressure, but still have good condition and moderated response. In site 28, despite the low contributions of the pressure and response layers, the condition layer had a good contribution of all indicators, which ensured a still reasonable TWHI value (0.6).

3.2. Tropical Waters Health Index (TWHI) and Suitability to the Class of Use Index (SCU)

Among the study sites, most of them (31) presented TWHI status “Very Good”, 10 were classified as “Good”, 5 were “Fair”, 3 were “Poor” and 1 site was “Critical” (Figure 4A). Sites classified as “Good” and “Very Good” were located in areas with a predominance of natural vegetation, whereas sites with “Fair” status were predominant in agricultural areas. “Poor” and “Critical” status sites were related to urban areas (Figure 4A).

SCU-wise, 12 sites presented better conditions than expected for their class of use, 19 sites presented condition compatible with the class of use, 17 were moderately compatible, 1 poorly compatible and 1 not compatible (Figure 4B).

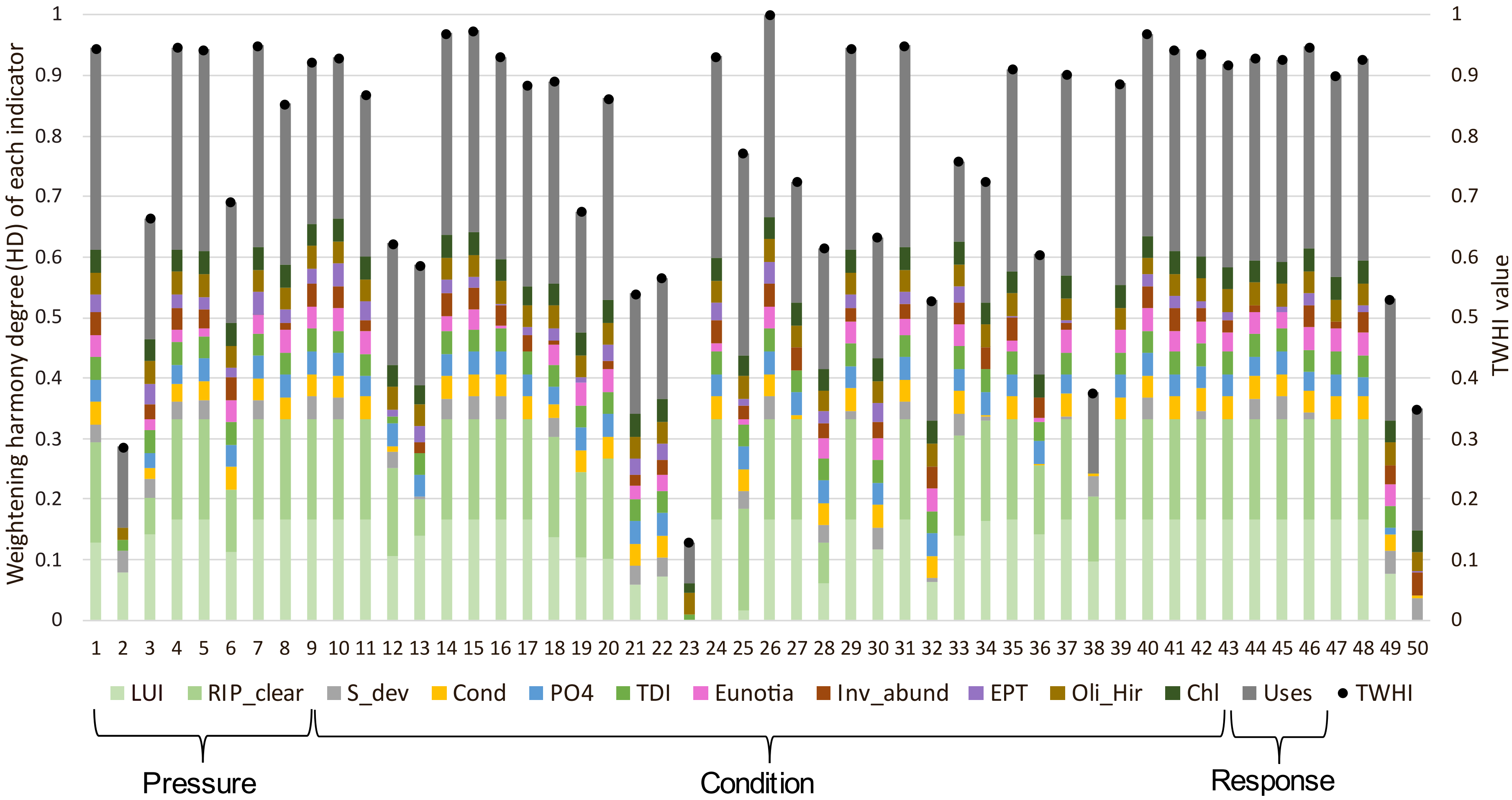

Figure 3. Weightening harmony degree (HD) of each indicator and the Tropical Waters Health Index (TWHI, black point). Indicators are agrouped by layer (pressure, condition, response) on the bottom of the figure.

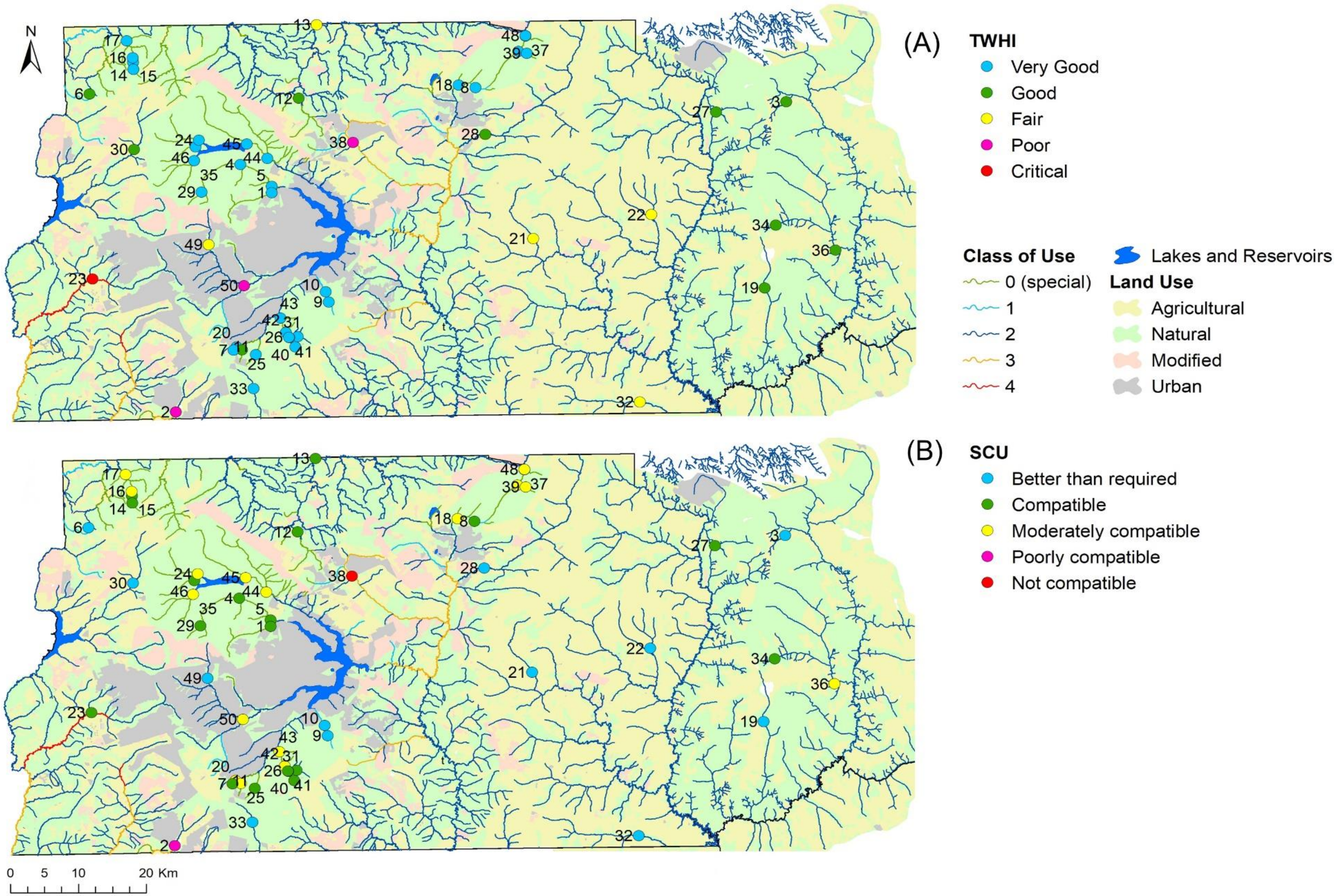

Figure 4. Spatial distribution of Tropical Waters Health Index (TWHI) and Suitability to the Class of Use (SCU). Number of sites 1 -50 (see TWHI and SCU values for each sample site in Supplementary Material I).

3.3. River health and suitability to the class of use assessment framework

Our framework proposal was built up on the basic concepts of P-C-R and Harmony Degree approaches. The activities flow involved steps from data collection/selection to management actions for the different range values of TWHI and SCU (Figure 5).

The framework predicted the interaction between the two main indices and feedback vectors since management decisions should impact new pressure/condition / response profiles.

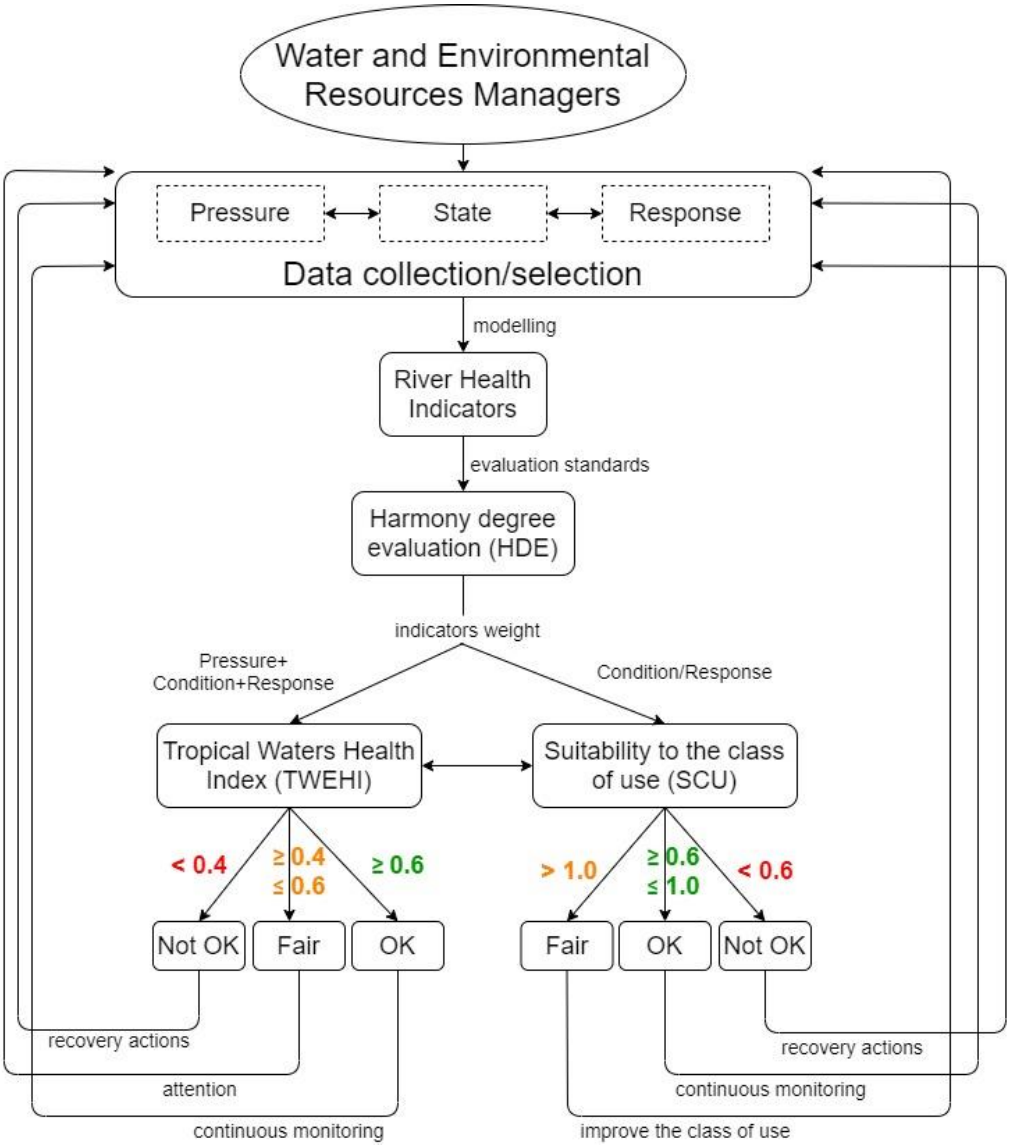

Figure 5. Framework for river health assessment and suitability to the class of use to support water and watershed management.

We proposed specific management actions for each combination TWHI x SCU (Table 4). The most common result (31 sample sites) suggested continuing monitoring since the health status was equal or higher than “Good” (TWHI > 0.6) and the conditions were at least moderately compatible with the class of use but not better than expected ($0.6 < SCU \leq 1$).

In 12 sites the SCU presented BTR (“Better condition than required by the class) status. In those cases, different strategies have been suggested for each corresponding TWHI. If the TWHI was "Very Good" or "Good" the recommendation was to improve the class of use that have been adopted, unless the current class is already 0 (special). In case of TWHI equal to "Fair", actions are necessary to improve the river health conditions, although it is suitable to the uses for which it was intended according to the current classification.

Two sites presented “Poor” TWHI status and SCU poor compatible or not compatible. One site presented “Poor” TWHI status and SCU moderately compatible. Although our framework suggested attention for SCU moderately compatible, the poor health condition induces the recommendation of strong recovery actions in the three sites.

In two sites the health condition was “Good” but the SCU was moderately or poor compatible. In these cases, we suggested some attention and minimal or moderated recovery measures, respectively.

Finally, one site presented critical health status but compatible conditions to its class of use. From the point of view of ecosystem health, it needs strong recovery actions, but in terms of suitability to the class of use it is suitable since it is not intended for uses that demand good water quality (class 4).

Table 4. Detected combinations of TWHI and SCU in the study sites, number of sites in each situation, site codes and management actions proposed. BTR = Better condition than required by the class, C = Compatible with the class of use, MC = Moderately compatible with the class of use, PC = Poorly compatible with the class of use, NC = not compatible with the class of use.

TWHI	**SCU**	**Number of sites**	**Site codes**	**Management actions**
Very good	BTR	3	9, 10, 33	Improve class of use if it is not special (0)
Very good	C	14	1, 4, 5, 7, 8, 11, 14, 15, 26, 29, 31, 40, 41, 46	Continuous monitoring
Very good	MC	14	16, 17, 18, 20, 24, 35, 37, 39, 42, 43, 44, 45, 47, 48	Continuous monitoring
Good	BTR	5	3, 6, 19, 28, 30	Improve class of use if it is not special (0)
Good	C	3	12, 27, 34	Continuous monitoring
Good	MC	2	25, 36	Need some attention and minimal recovery actions
Fair	BTR	4	21, 22, 32, 49	Need some actions to recovery aquatic ecosystem health, but from the point of view of "class of use" it is better than required
Fair	C	1	13	Need some actions to recovery aquatic ecosystem health, but from the point of view of "class of use" it is compatible
Poor	MC	1	50	Need some recovery actions
Poor	PC	1	2	Need strong recovery actions
Poor	NC	1	38	Need very strong recovery actions
Critical	C	1	23	Managers should decide if improve the class of use and invest in strong recovery actions or keep it in a critical situation but suitable to the worst class of use.

4. Discussion

The results showed that the water bodies in the study region were, in general, in good ecosystem health status. Sites in poor or critical situations were located in urbanized regions, while those with status equal to “Fair” or equal/higher than “Good” were, predominantly, in agricultural and preserved areas, respectively. It was expected and not surprising since the pressures from urbanization are proven to be harmful to aquatic and terrestrial environments across the planet (Murray et al. 2019). Moreover, how to coordinate the relationships between urbanization and eco-environment is a complex problem to be solved (Fang et al. 2017). Agricultural occupation, although to a lesser extent, also causes damage to aquatic ecosystems, especially in small streams (Szöcs et al. 2017). This occur due to the removal of natural vegetation from the upstream drainage area and riparian forest or due to the chemical pollutant’s diffuse inputs (e.g., fertilizers and pesticides; Clapcott et al. 2012; Szöcs et al. 2017).

The fact that highest TWHI values have been associated with a balance in the relative contribution of the three layers considered - pressure, condition, response - demonstrates the coherence of this approach. The conditions of an ecosystem are the result of the natural characteristics and pressures that it suffers. This, in turn, leads to human management actions and decisions that also end up corroborating the current condition. The imbalance of the three layers with lower TWHI values show us potential problems and enables directing solutions. The removal of riparian vegetation and changes in land use in the upstream catchment can lead to changes in the aquatic ecosystem (Allan 2004). Even though in terms of water quality, biology, and hydrology there is still a good condition, it is expected that at some point they would respond to pressures. Perhaps such changes have not yet been perceived due to the resistance and resilience of ecosystems (Sarremejane et al. 2020). However, it is also clear in some places that the high pressure has already caused changes in several aspects of the condition. Therefore, the level of intervention must be even higher to restore an environmental balance. A response layer with a low contribution to the index can also be interpreted as an alert because if we have expected little of a given water body in terms of quality, then little will be done for its preservation. This can lead, in the long run, to increased pressures and worsening condition indicators.

Among the condition indicators, the deviation from natural streamflows was the indicator which presented lower harmony degree values. This indicates a widespread water pressure in the study area. It may be related to the greatest water crisis (drought) recorded in the region between 2016 and 2017, with the river flows and accumulated precipitation still recovering along 2018 (Lima et al. 2018). Even preserved sites have suffered this impact, clearly demonstrating a response to a regional scale phenomenon. In biological terms, specific indicators of diatoms, macroinvertebrates and algal biomass showed high or low contribution depending on each location. The diversity of responses for these indicators was important from an ecological point of view, since this may indicate the influence of different stressors, opening possibilities for targeted recovery actions (Castro-Català et al. 2020; Waite et al. 2019).

Our proposal incorporated indicators related to different attributes of a river ecosystem (hydrology, water quality, biology) and was able to indicate whether the conditions were compatible with the class, not in terms of designated best uses, but in terms of environmental quality status. Most of the study sites presented conditions at least moderately compatible with the class of use and, in some cases, even better than required by the class, which indicates a tendency to classify the rivers according to their current uses and water quality and with no prospect of improving or creating more daring goals. Most countries and regions have adopted the practice of having river health status' target as similar as possible to reference sites (e.g., European Water Framework Directive, US Clean Water Act, Freshwater Environmental Health Monitoring Programme – Queensland, Australia) that is, pristine sites (or as close as possible to their natural conditions; Marshall & Negus 2018). From an environmental point of view, this practice makes more sense, since, even if the current conditions are not the best, the goal is always getting closer to the ecological integrity.

Results indicating "Better conditions than required by the class" can be understood as a "permission" to the impact since even if its quality deteriorates, the water body will still be suitable for its class and no preservation measure would be applied. Conditions that are compatible or moderately compatible with the class may indicate that rivers that should have good conditions do have them, but also that rivers that may have bad conditions also have them. In the latter case, low condition requirements can also be understood as the "acceptance" of the impact. This kind of government strategy moves current management away from sustainable development goals (SDGs), such as maintaining healthy and productive ecosystems, and the sustainability of biodiversity and ecosystem services through better management, valuation, measurement, conservation, and restoration (Griggs et al. 2013).

Only two sites presented inferior conditions ("poor" or "not compatible") to their respective classes. Both are located downstream of point-source of treated sewage releases (Sobradinho and Alagado; Caesb 2021) and are inserted in urban areas. Although low demand water quality class (class 3) was defined for these rivers (GDF 2014), the environmental conditions are even worse. Currently, the assessment of suitability to the class of use in the study region is based on only a few physical, chemical, and microbiological parameters (GDF

2014). According to this current approach, the two rivers showed results “adequate” and “in accordance with” to the class of use in 2018 (Adasa 2021), same year we carried out the fieldwork of this study. The water quality variables’ instability and the restricted response to only a few stressors can lead to misinterpretations from an environmental point of view (Gatti 2016). The inclusion of biological and hydrological indicators in the assessment of the suitability to the class of use allowed better visualization of the river conditions (Sadat et al. 2020) concerning their classes, even though the classification by classes of use is, in itself, an outdated tool.

The combination of indexes (TWHI and SCU) made it possible to visualize the rivers under two different aspects: health and suitability to the class of use. The first considers a comprehensive approach and is concerned with current and future environmental issues, the second is restricted to the designated best uses and has an anthropocentric bias. The thresholds and actions proposed in the framework tend to broaden the vision and help managers in decision making. It makes possible to check that although the conditions of a river are adequate from the point of view of the current legislation, the real situation in environmental terms is not good.

The results of this study allow for a reflection on the real intention of classifying rivers according to the designated best uses. Would the conditions adopted for each class be sufficient to guarantee the uses attributed to them? Would rivers with conditions compatible with class 4 be useful for navigation and landscape composition, for example? One of the great effects of deforestation and urbanization is the silting up (Franz et al. 2014) which leads to a reduction in the water column and may cause problems for navigation. In addition, the rivers in the study region are not even navigable, which would not even make sense to include such use. For landscape composition, is a river expected to smell bad, with turbid and lifeless waters? To what extent are the uses defined for each class not just masking the real purpose of the framework that would be to accept rivers with low water quality and that, therefore, do not need to be protected/recovered? The current approach is distorted since it is known that a healthy river provides benefits far beyond those described in the table of priority uses, and an unhealthy river has far more restrictions on use than those considered. Ecosystems provide four types of service: provisioning (e.g., food), regulating (e.g., water quality regulation and pollination), cultural (e.g., recreation) and supporting (e.g., nutrient cycling) (Millennium Ecosystem Assessment, MA, 2005). These ecosystem services are generated from numerous interactions (Harrison et al. 2014) and biodiversity is expected to have direct and/or indirect effects on them (Teixeira et al. 2019).

In addition to the ecological gap present in the Waterbodies Framework by Class of Use (WFCU), it also brings great implementation difficulties. Some of these issues are related to the lack of data, reduced number of monitoring points, diversity of legislation between states, differences in the detection of polluting sources, and lack of institutional articulation from local to national levels (Souza & Pizella, 2020). Thus, the current framing of water bodies in Brazil, beyond do not contribute to a healthy aquatic ecosystem, is still hardly applicable, measurable, and comparable.

In terms of functionality, the advantage of the proposed framework is its rigid math, but its flexibility in relation to indicators and thresholds. This admits the methodology to be adapted to different realities, allowing a wide diffusion of its use and at the same time comparability between the results. In a country with environmental, social and political characteristics that are so different in its territory (Veiga & Magrini 2013), this flexibility and ease of communication of results (guaranteed by colour scales and value ranges) are essential for the model to be applied in large scale (Flint et al. 2017). At the same time, the composition of the indicators allows a thorough assessment of the managers in relation to which problems must be tackled. Meeting the expectations of the various stakeholders, in a cooperative approach, based on scientific evidence and effective communication is a fundamental part of the success of a water resources management framework (Bunn et al. 2010).

Brazil has an internationally prominent role in terms of biodiversity and water resources (Agostinho et al. 2005) and, in addition, has many studies on freshwater bioindicators and environmental pressure thresholds in its various biomes (e.g., Brito et al. 2020; Dala-Corte et al. 2020; Firmiano et al. 2017; Ligeiro et al. 2013). But the restoration of freshwater ecosystems has barely been discussed in the country and is still a topic largely restricted to the academic community. Brazilian authorities need to rethink opportunities for depollution, following trends in several other countries (Azevedo-Santos et al. 2021). The proposed framework is a valuable tool for conducting a self-evaluation on the current model adopted, identifying its discrepancies in relation to an approach committed to environmental sustainability. Brazil has all the potential for leadership in sustainable management models for aquatic environments and this work presented one more tool to facilitate this endeavour.

5. Conclusions

River health assessment is a management tool useful to restore the health status of rivers, protect their ecological and social service functions, and create an environment in which people are in harmony with nature (Zhao et al. 2019). The Tropical Waters Health Index (TWHI) developed with the principles of the pression-condition-response and the harmony degree could fully reflect the ecological status of streams and more accurately identified their problems. This comprehensive but at the same time segmented index (TWHI), allows actions aimed at maintaining biodiversity and balancing ecological functions. Although we have simplified the indicators considerably when compared to other studies (Singh & Saxena 2018), they were sufficient to draw an overview of the region's water bodies health status. The simplicity of determining and communicating the results of a river health index makes its implementation effective and accessible by the managers and other stakeholders involved (Flint et al. 2017). The proposal to evaluate the suitability to the classes of use, considering the ecological conditions of the water body, brought an improvement in relation to the current model (based only on some physical, chemical, and microbiological parameters). The suitability of most study sites demonstrates the tendency to classify the streams into classes according to their current conditions, although in two cases poor health status and distance from the class of use were evident. The acceptability of poor conditions in the freshwater ecosystem is not compatible with sustainable development, therefore, it is a management tool that should be revised. The proposed framework allowed to put both approaches side by side, allowing the manager to be aware that a river suited to its demanded best uses (class of use) does not always represent a good ecological condition. In the long term, it is suggested that the current approach focused on classes by demanded best uses be shifted to a holistic approach of river health, with the main goal of reach environments as close as possible to their natural conditions – or with good ecological status (Maes et al. 2020). It is also recommended that any methodologies, including this one, be continually revised and improved, as has been done even in countries where there is already a consolidated assessment of the ecological integrity of streams, as in the case of Australia (Chessman 2021).

Acknowledgments

This work was supported by the Fundação de Amparo à Pesquisa do Distrito Federal (FAP-DF), Aquariparia Project (edital 05/2016-Pró-Águas; Proc. no.: 193.000716/2016), that allowed the execution of fieldwork and laboratory analyses; the Conselho Nacional de Desenvolvimento Cientifico e Tecnológico (CNPq) through research fellowship to José Francisco Gonçalves Jr (Proc. no.: 310641/2017-9); and the Agência Reguladora de Águas, Energia e Saneamento Básico do Distrito Federal (ADASA) that in addition to the financial support to Campos C.A. also offered logistical support of vehicles for the fieldwork.

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SUPPLEMENTARY MATERIAL

Table S1. Harmony Degree (HD), TWHI and SCU of each indicator/site.

Sample site	Code	LUI	RIP_clear	S_dev	Cond	PO4	TDI	Eunotia	Inv_abund	EPT	Oli_Hir	Chl	Uses	TWHI	SCU
Acampamento	1	0,77	1,00	0,80	1,00	1,00	1,00	1,00	1,00	0,79	1,00	1,00	1	0,95	0,95
Alagado	2	0,47	0,00	1,00	0,00	0,00	0,47	0,00	0,00	0,00	0,53	0,00	0,4	0,29	0,55
Areia	3	0,84	0,37	0,82	0,48	0,68	1,00	0,54	0,65	0,89	1,00	1,00	0,6	0,66	1,31
Bananal_I	4	1,00	1,00	0,74	0,84	0,83	1,00	0,56	1,00	0,60	1,00	1,00	1	0,95	0,84
Bananal_II	5	1,00	1,00	0,80	0,87	1,00	1,00	0,35	0,86	0,57	1,00	1,00	1	0,94	0,83
Barreiro	6	0,68	0,62	0,00	1,00	1,00	1,00	1,00	1,00	0,43	1,00	1,00	0,6	0,69	1,37
Bonito	7	1,00	1,00	0,81	1,00	1,00	1,00	0,83	0,00	1,00	1,00	1,00	1	0,95	0,85
Brejinho	8	0,99	1,00	0,00	1,00	1,00	1,00	1,00	0,30	0,59	1,00	1,00	0,8	0,85	0,95
Cab_Veado_I	9	1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00	0,72	1,00	1,00	0,8	0,92	1,21
Cab_Veado_II	10	1,00	1,00	0,92	1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00	0,8	0,93	1,24
Capetinga	11	1,00	1,00	0,00	1,00	0,89	1,00	1,00	0,48	0,86	1,00	1,00	0,8	0,87	1,00
Contagem_I	12	0,63	0,88	0,72	0,29	1,00	0,32	0,00	0,00	0,31	1,00	1,00	0,6	0,62	0,85
Contagem_II	13	0,84	0,36	0,09	0,00	1,00	0,94	0,00	0,48	0,75	1,00	0,80	0,6	0,59	0,93
D_Irmaos_I	14	1,00	1,00	0,89	1,00	1,00	1,00	0,68	1,00	0,62	1,00	1,00	1	0,97	0,91
D_Irmaos_III	15	1,00	1,00	0,98	1,00	1,00	1,00	0,87	1,00	0,45	1,00	1,00	1	0,97	0,92
D_Irmaos_IV	16	1,00	1,00	1,00	1,00	1,00	1,00	0,15	0,93	0,04	1,00	1,00	1	0,93	0,79
D_Irmaos_V	17	1,00	1,00	0,00	1,00	1,00	1,00	0,00	0,73	0,33	1,00	0,82	1	0,88	0,65
Fumal	18	0,82	1,00	0,83	0,63	0,76	1,00	0,89	0,21	0,54	1,00	1,00	1	0,89	0,76
Fundo	19	0,62	0,85	0,00	1,00	1,00	1,00	1,00	0,00	0,24	1,00	1,00	0,6	0,68	1,15
Gama	20	0,60	1,00	0,00	1,00	1,00	1,00	1,00	0,38	0,70	1,00	1,00	1	0,86	0,78
Jardim	21	0,36	0,00	0,81	1,00	1,00	1,00	0,58	0,48	0,73	1,00	1,00	0,6	0,54	1,40
Lagoinha	23	0,44	0,00	0,82	1,00	1,00	1,00	0,71	0,66	0,70	1,00	1,00	0,6	0,57	1,46
Melquior	24	0,00	0,00	0,00	0,00	0,00	0,25	0,00	0,00	0,00	0,99	0,43	0,2	0,13	0,92
Milho_Cozido	25	1,00	1,00	0,00	1,00	1,00	1,00	0,38	1,00	0,78	1,00	1,00	1	0,93	0,79
Onca_FAL	26	0,10	1,00	0,80	1,00	1,00	1,00	0,23	0,60	0,31	1,00	0,94	1	0,77	0,76
Onca_IBGE	27	1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00	1	1,00	1,00

Pindaiba	28	1,00	1,00	0,00	0,18	1,00	1,00	0,00	1,00	0,01	1,00	1,00	0,6	0,73	0,96
Pipiripau	29	0,36	0,41	0,78	1,00	1,00	1,00	0,90	0,69	0,52	0,88	1,00	0,6	0,62	1,43
Poco_Dagua	30	1,00	1,00	0,36	1,00	1,00	1,00	0,99	0,58	0,60	1,00	1,00	1	0,95	0,83
Rodeador	32	0,70	0,00	1,00	1,00	1,00	1,00	1,00	0,71	0,85	1,00	1,00	0,6	0,63	1,58
Roncador	33	1,00	1,00	0,75	1,00	1,00	1,00	0,67	0,70	0,54	1,00	1,00	1	0,95	0,85
S_Bernardo	34	0,37	0,00	0,19	1,00	1,00	1,00	0,99	1,00	0,01	1,00	1,00	0,6	0,53	1,33
Saia_Velha	35	0,83	1,00	1,00	1,00	1,00	1,00	1,00	0,97	0,68	1,00	1,00	0,6	0,83	1,60
Salobra	36	0,98	1,00	0,19	0,09	1,00	1,00	0,00	1,00	0,01	1,00	1,00	0,6	0,73	0,98
Santa_Maria	37	1,00	1,00	0,00	1,00	1,00	1,00	0,50	1,00	0,09	1,00	1,00	1	0,91	0,73
Santo_Inacio	38	0,85	0,69	0,00	0,08	1,00	0,84	0,22	0,89	0,00	0,00	1,00	0,6	0,61	0,74
Serrinha	39	1,00	1,00	0,12	1,00	0,83	1,00	1,00	0,34	0,09	1,00	1,00	1	0,90	0,71
Sobradinho	40	0,58	0,64	0,92	0,08	0,00	0,02	0,00	0,00	0,00	0,00	0,00	0,4	0,38	0,28
Tabatinga	41	0,99	1,00	0,00	1,00	1,00	1,00	1,00	0,00	0,00	1,00	1,00	1	0,89	0,66
Taquara_I	42	1,00	1,00	0,93	1,00	1,00	1,00	1,00	1,00	0,50	0,73	1,00	1	0,97	0,90
Taquara_II	43	1,00	1,00	0,00	1,00	1,00	1,00	0,91	1,00	0,55	1,00	1,00	1	0,94	0,83
Taquara_III	44	1,00	1,00	0,35	1,00	1,00	1,00	0,99	0,61	0,32	1,00	1,00	1	0,94	0,80
Taquara_IV	45	1,00	1,00	0,00	1,00	1,00	1,00	0,84	0,54	0,41	1,00	1,00	1	0,92	0,75
Torto	46	1,00	1,00	0,90	1,00	0,86	1,00	1,00	0,32	0,00	1,00	1,00	1	0,93	0,78
Tres_Barras	47	1,00	1,00	1,00	1,00	1,00	1,00	0,77	0,00	0,25	1,00	1,00	1	0,93	0,78
Vargem_Grande	48	1,00	1,00	0,25	1,00	0,83	1,00	1,00	1,00	0,52	1,00	1,00	1	0,95	0,84
Ver_Grande_I	49	1,00	1,00	0,00	1,00	1,00	1,00	1,00	0,32	0,00	1,00	1,00	1	0,90	0,70
Ver_Grande_II	50	1,00	1,00	0,00	1,00	0,84	1,00	1,00	0,90	0,31	1,00	1,00	1	0,93	0,78
Vic_Pires_I	51	0,46	0,00	1,00	0,74	0,28	1,00	0,93	0,89	0,00	1,00	1,00	0,6	0,53	1,26
Vic_Pires_II	52	0,00	0,00	1,00	0,10	0,00	0,00	0,00	1,00	0,08	0,85	1,00	0,6	0,35	0,74

CONSIDERAÇÕES FINAIS

Nesta tese apresentamos uma avaliação abrangente de fatores abióticos e bióticos, estruturais e funcionais, de ecossistemas de riacho, com a finalidade de traçar um *framework* para avaliação da integridade ecológica. Uma das grandes motivações desta tese foi a constatação da distância entre o monitoramento de qualidade das águas que é feito hoje no Brasil em comparação com diversas regiões e países do mundo, onde a visão holística sobre o ecossistema aquático já vem sendo consolidada (Southerland & Stribling 1995 - EUA; Tamai 2000 – Japão; Parsons 2015 - Austrália; Clapcott et al. 2018 - Nova Zelândia; Santos et al. 2021 - Europa). Considerando que o conhecimento básico sobre as características dos ecossistemas deve ser a primeira etapa de uma abordagem integrativa, realizamos um extenso trabalho de campo, englobando regiões de cabeceira de três bacias hidrográficas de relevância nacional (Paranaíba, Tocantins-Araguaia e São Francisco), 52 locais de amostragem, duas estações climáticas distintas (chuva e estiagem), características abióticas dos riachos e de suas áreas de drenagem, duas comunidades biológicas (diatomáceas perifíticas e macroinvertebrados) e diversos processos ecossistêmicos. Nas primeiras análises exploratórias (Capítulo I) identificamos que as diferenças entre locais protegidos ou não por lei se sobressaiam às diferenças entre bacias e entre estações climáticas, mas que essas diferenças foram mais nítidas na bacia com maior percentual de urbanização (Bacia do Paranaíba). Separando os locais em categorias "protegido" e "não protegido" conseguimos detectar valores medianos para diversas variáveis físicas e químicas. A necessidade de áreas de proteção ambiental, especialmente em regiões mais urbanizadas, ficou clara, embora também se tenha percebido que nem sempre áreas protegidas são livres de impactos. Assim, a categorização dos locais tende a mascarar a visualização de um gradiente de condições que está mais próximo da realidade (Ligeiro et al. 2013; Rezende et al. 2014).

Para avaliar os efeitos dos gradientes naturais e de distúrbios antrópicos sobre as duas comunidades biológicas e processos ecológicos, foram elaborados dois capítulos. No primeiro (Capítulo II) avaliamos as alterações da composição das comunidades biológicas, por meio de análises multivariadas e Análise de Táxons Indicadores de Limiares (TITAN; Baker & King 2013) em gradientes ambientais. No segundo (Capítulo III), por meio de modelos do tipo *Boosted Regression Trees* (BRT; Elith et al. 2008), avaliamos como métricas biológicas estruturais e funcionais respondem aos gradientes ambientais. Ambas as abordagens permitiram a identificação de limiares ecológicos para os diversos gradientes de impactos, mas o primeiro possibilitou também a identificação de táxons sensíveis e tolerantes para cada gradiente, enquanto o segundo permitiu a visualização da forma não linear das respostas de cada métrica biológica bem como a contribuição de cada preditor na variância observada. Dentre as métricas biológicas consideradas no Capítulo III, cinco delas, relacionadas ao percentual de táxons sensíveis e tolerantes a impactos antrópicos, foram identificadas no capítulo anterior. Verificamos que variáveis de qualidade da água (nitrato, fosfato e condutividade) e o uso do solo no corredor ripário e na área de drenagem foram os principais preditores das alterações nas comunidades biológicas. Em ambos os capítulos, identificamos limiares ecológicos mais restritivos do que os valores de referência hoje aplicados em legislação, além da condutividade, que foi um preditor de grande relevância e que sequer é considerada na legislação federal Brasileira. Macroinvertebrados, diatomáceas e processos ecossistêmicos apresentaram respostas complementares aos distúrbios antrópicos, reforçando a importância de se considerar diferentes componentes dos ecossistemas em programas de monitoramento (Schiller et al. 2017; Wagenhoff et al. 2017).

Com as informações sobre as melhores métricas resposta e preditores de condições ambientais, passamos para o desenvolvimento de um índice multimétrico que facilitasse e padronizasse a comunicação sobre a saúde dos riachos (Ruaro et al. 2020). Também demonstramos um caminho para avaliação da adequação ao Enquadramento, considerando condições ecológicas hoje negligenciadas. Além disso, delineamos um *framework* que resume todas as etapas até aqui, facilitando a aplicação na gestão das águas e bacias hidrográficas. O Índice de Saúde de Águas Tropicais (*Tropical Waters Health Index* – TWHI), como foi chamado, foi composto por camadas que representam as pressões antrópicas (P; uso do solo), as condições dos ecossistemas (C; hidrologia, qualidade da água, biologia) e as respostas da sociedade/governo (classes de uso), em uma abordagem que integra os corpos hídricos ao contexto social. O índice se mostrou muito coerente com a realidade, apresentando os menores valores em riachos inseridos em áreas urbanas (bacia do Paranaíba), seguido por aqueles que se encontram em áreas agrícolas (bacia do Preto) ou com alguma modificação no uso do solo (locais isolados nas bacias do Paranaíba e Maranhão). Os locais com maiores valores de TWHI foram identificados em áreas protegidas ou com pouco impacto. Por outro lado, o Índice de Adequação ao Enquadramento (*Suitability to the Class of Use* – SCU) mostrou a grande maioria dos locais com valores igual ou superior ao limiar de "moderadamente adequados" às classes de Enquadramento, ou até melhores do que o previsto. Isto indicou uma tendência à classificação dos corpos hídricos de acordo com as condições atuais e não pensando em uma melhoria da qualidade das águas e do ecossistema. Algumas sugestões de ações de gestão foram propostas para cada combinação de resultados TWHI x SCU, como por exemplo: continuidade do monitoramento, necessárias ações de recuperação, elevação da classe de uso.

Como perspectivas futuras, esperamos que o monitoramento da qualidade das águas avance em direção à abordagem integrativa em diferentes escalas, incluindo a nacional, e considere o objetivo de melhorar a qualidade dos ecossistemas aquáticos até o mais próximo possível das condições naturais. O Brasil é considerado um país continental, apresentando regiões com as mais diversas características até mesmo dentro de um mesmo bioma (Sano et al. 2019) e uma ampla diversidade de impactos antrópicos. Ainda assim, é possível a detecção de padrões e de bons bioindicadores, construindo protocolos de alcance nacional, ou até multinacional, como a Diretiva Quadro Europeia (European Directive 2000). Em países em desenvolvimento, como é o caso do Brasil, outro grande desafio além das proporções do território, são as limitações financeiras (Fernandes et al. 2017). Portanto, mais uma das grandes vantagens do biomonitoramento é a possibilidade de redução de custos por meio de um intervalo maior entre as coletas. Isto pois os bioindicadores, em geral, apresentam respostas mais estáveis, uma vez que não se modificam rapidamente diante de eventos que alteram momentaneamente a qualidade da água. Além disso, sofrem efeito cumulativo dos impactos que atingem não apenas o corpo hídrico diretamente, mas também indiretamente - como as alterações no uso do solo (Allan 2004). Assim, ainda que levemente mais trabalhoso nos aspectos de coleta e análise, o biomonitoramento acaba incorrendo em menores custos a longo prazo, além de ser mais abrangente em termos de respostas a diferentes impactos. Mais uma vantagem desta abordagem é a maior integração entre meio ambiente e recursos hídricos, objetos geralmente tratados separadamente, inclusive por órgãos diferentes, mas que estão intimamente relacionados. Um dos fundamentos da Política Nacional de Recursos Hídricos (Lei Federal Nº 9.433, Brasil 1997) diz que "*a gestão dos recursos hídricos deve sempre proporcionar o uso múltiplo das águas*". Assim, não há razões para que o uso primordial de manter o equilíbrio da estrutura e funções ecossistêmicas, que beneficiam a todos, inclusive o ser humano, seja esquecido ou deixado em segundo plano. É olhando para a qualidade do ecossistema que se tem a garantia dos recursos em qualidade e quantidade para as gerações atual e futura.

Este trabalho apresentou uma direção e um caminho para a avaliação da integridade ecológica de riachos. Mas sabemos que, apesar do grande esforço amostral e análises realizadas, o universo do monitoramento é ainda maior. Assim, não encerramos o assunto, mas somamos a todo conhecimento existente, e abrimos inúmeras possibilidades para o futuro.

In every respect, the valley rules the stream

H.B.N. Hynes (1975)

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