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Programa de Pós-graduação em Ecologia, Conservação e Manejo da Vida Silvestre

Distúrbios antrópicos em escala local causam danos a assembleias de bioindicadores bentônicos na bacia hidrográfica do rio Pandeiros, MG

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Belo Horizonte, 31 de março de 2021

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Distúrbios antrópicos locais causam danos a assembleias de bioindicadores bentônicos em uma bacia hidrográfica protegida

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“O real não está na saída nem na chegada: ele se dispõe para a gente é no meio da travessia”

“...e que verte no Rio Pandeiros – esse tem cachoeiras que cantam, e é d’água tão tinto, que papagaio voa por cima e gritam, sem acordo: - É verde! É azul! É verde! É verde!...”

(João Guimarães Rosa – Grande Sertão: Veredas)

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Resumo

As crescentes alterações nos ecossistemas aquáticos causadas pelas intervenções humanas são o motivo da transformação rápida e irreversível nos ecossistemas globais. Essas não apenas causam diminuição na disponibilidade água para os usos humanos, como também provocam modificações hidrológicas em ecossistemas aquáticos, perda de habitat para espécies aquáticas e, consequentemente declínio da biodiversidade. Por isso se faz necessário o estudo, desenvolvimento e consolidação de metodologias eficazes para avaliação ambiental e subsidiar ações de conservação de ecossistemas aquáticos em bacias hidrográficas. Assembleias de invertebrados aquáticos têm sido frequentemente utilizados como bioindicadores de qualidade de águas, em múltiplas perspectivas: (i) aplicação de índices multimétricos que integram múltiplos atributos biológicos e ecológicos de assembleias; (ii) aplicação de modelos que avaliem a probabilidade de estressores antropogênicos impactarem a biota aquática; (iii) como indicadores ecológicos através da análise de grupos específicos. O objetivo dessa tese foi avaliar a condição ecológica na bacia do rio Pandeiros (MG/Brasil), respondendo às seguintes perguntas: a) Índices multimétricos bentônicos podem ser utilizados em avaliações de integridade biótica independentemente dos locais onde foram desenvolvidos?; b) A condição biológica é afetada por distúrbios humanos em áreas protegidas e prioritárias para a conservação da biodiversidade?; c) Os gêneros da família Chironomidae e suas características funcionais são eficazes para avaliar o estado ecológico em riachos Neotropicais? Verificamos que dez índices multimétricos foram eficientes para avaliar a condição ecológica em uma bacia com condições ambientais moderadamente alteradas devido a estressores locais. Os distúrbios avaliados (% pastagem, índice de Distúrbio Integrado (IDI), índice de impacto na zona ripária (W1_hall), Baixa Estabilidade Relativa do Leito (LRBS), % substrato fino) foram identificados em 20-40% da extensão da bacia, com a presença de substratos finos e distúrbios antropogênicos integrados (IDI e W1_hall) sendo as ameaças mais importantes relacionadas às baixas pontuações do índice multimétrico (MMI) de condição biológica. Foram observadas relações entre variáveis ambientais (% areia, imersão, % pasto, % agricultura, % finos, W1_hall e cobertura do dossel) e características da assembleia de Chironomidae (predadores, herbívoros, minadores, engolfadores e tamanho do corpo). Esse estudo possibilitou a padronização de metodologias e ferramentas eficazes para avaliações ecológicas, mesmo em uma bacia hidrográfica com baixo distúrbio. Além disso, identificamos os principais distúrbios estressores e pressões associados às más condições biológicas na bacia hidrográfica do rio Pandeiros e avaliamos a magnitude desses distúrbios estressores e pressões. Esse estudo forneceu evidências de que mesmo em áreas protegidas, os distúrbios locais degradam a condição biológica, indicando a importância das ações locais para a conservação e reabilitação de uma condição ecológica equilibrada em bacias hidrográficas tropicais.

Palavras-chave: Macroinvertebrados; Índice de integridade biótica; Cerrado; biomonitoramento; unidade de conservação; características.

Abstract

The increasing changes in aquatic ecosystems caused by human interventions are the reason for the rapid and irreversible transformation of global ecosystems. These not only cause a decrease in water availability for human uses, but also cause hydrological changes in aquatic ecosystems, loss of habitat for aquatic species and, consequently, a decline in biodiversity. That is why it is necessary to study, develop and consolidate effective methodologies for environmental assessment and to subsidize the actions of conservation of aquatic ecosystems in watersheds. Aquatic invertebrate assemblages have often been used as water quality bioindicators, in multiple perspectives: (i) application of multimetric indices in biotic integrity assessments that integrate multiple biological and ecological attributes of assemblies; (ii) application of models that assess the probability of anthropogenic stressors to impact aquatic biota; (iii) as ecological indicators through the analysis of especific groups. The objective of this thesis was to assess the ecological condition in the Pandeiros River basin (MG / Brazil), answering the following questions:
a) Can benthic multimetric indices be used regardless of where they were developed? b) Is the biological condition affected by human disturbances in protected and priority areas for the conservation of biodiversity? c) Are the genera of the organisms of the Chironomidae and its functional characteristics effective to assess the ecological status in Neotropical streams? We found that ten multimetric indices were effective in assessing the ecological condition in a basin with moderately altered environmental conditions and only local stressors. The evaluated disorders (% pasture, IDI (Integrated Disturbance Index), W1_hall (impact index in the riparian zone), LRBS (Low Relative Bed Stability), % fines) occurred in 20-40% of the basin extension, with the presence of thin substrates and integrated anthropogenic disturbances (IDI and W1_hall) being the most important threats to low MMI scores of biological condition. We found strong relationships between environmental variables (% sand, immersion, %pastures, % agriculture, % fines, W1_hall, and canopy cover) and characteristics of the Chironomidae assembly (predators, herbivores, miners, engulfers and body size). This study enabled the standardization of effective methodologies and tools for ecological assessments, even in places with low disturbance, in addition to identifying the main stressors and pressures associated with the poor biological conditions present in the watershed of the Pandeiros River Basin and assessing the magnitude of these stressors and pressures. This study provided evidence that even in protected areas, local disturbances degrade the biological condition, indicating the importance of local actions for the conservation and rehabilitation of a balanced ecological condition in tropical watersheds.

Keywords: Macroinvertebrates; Index of biotic integrity; Cerrado; bioassessment; conservation unit; traits.

Sumário

Introdução

Embora sua importância seja indiscutível, os ecossistemas aquáticos estão sendo ameaçados por atividades antrópicas como urbanização, industrialização, alterações nos tipos de usos e cobertura do solo e construção de barragens (Von Sperling, 2012). Consequentemente há perda de água de boa qualidade para o uso humano, modificações hidrológicas em riachos, perda de habitat de espécies aquáticas, declínio da biodiversidade e facilitação da introdução de espécies não nativas (Dudgeon, 2006; Linares et al., 2018; Reid et al., 2018). Por essas razões é necessário o desenvolvimento e aperfeiçoamento de ferramentas que possibilitem o diagnóstico de condições ambientais de ecossistemas aquáticos, que possam subsidiar a proposição de soluções para o manejo e conservação de bacias hidrográficas (Balderas et al., 2016; Revenga et al., 2005; Sánchez-Bayo & Wyckhuys, 2019).

A água é recurso essencial para a existência humana, sendo os rios e riachos como suas principais fontes (Benda et al., 2005). Além disso, ecossistemas aquáticos continentais são responsáveis por abrigar elevada diversidade de espécies, muitas dessas endêmicas e sensíveis (Strayer & Dudgeon, 2010). Os ecossistemas aquáticos ainda fornecem uma série de bens e serviços, como por exemplo, suprimento de água para usos doméstico, industrial e agropecuário, geração de energia, navegação e lazer (Callisto et al., 2019a; Malmqvist & Rundle, 2002).

Os distúrbios em ecossistemas gerados por atividades antrópicas são o motivo da transformação rápida e irreversível de ecossistemas em diferentes partes do planeta (Barnosky et al., 2012). Distúrbios são definidos como "algum evento que altera a estrutura dos ecossistemas, comunidades ou populações, resultando em alterações na disponibilidade de recursos, nos substratos e no ambiente físico" (White & Pickett, 1985). As mudanças nos ecossistemas causadas por distúrbios antrópicos são graduais e declinam lentamente. Muitas vezes, dependendo de sua intensidade (Davies & Jackson, 2006) e resiliência do ecossistema. O modelo conceitual proposto por Davies & Jackson (2006) mostra de que forma os aspectos biológicos respondem à transição de um ambiente natural até a condição degradada. O entendimento desse gradiente de condições ambientais é útil na identificação dos danos, além de possibilitar a previsão de condições futuras, direcionando ações para a recuperação e diminuição das causas desse distúrbio.

Os ecossistemas fluviais são organizados de forma hierárquica dividida em diversas escalas de observação (Frissell et al., 1986). Em menor escala estão os micros hábitats, representados pelos substratos que formam o leito dos rios; os hábitats são caracterizados por fluxos de água lentos (remansos) e rápidos (corredeiras); trecho e segmento dos riachos representam as extensões longitudinais definidas por características das zonas ripárias e dos vales dos riachos. Todas essas escalas em conjunto, formam a bacia hidrográfica, a maior escala (Allan et al., 1997). Além dos diferentes níveis de distúrbios que podem gerar diferentes tipos de impactos em assembleias biológicas, a escala em que o distúrbio afeta também pode variar, e as consequências serão diferentes em escalas distintas. A possibilidade de predizer como variáveis ecológicas mudam ao longo de múltiplas escalas é fundamental para a interpretação de informações em escalas maiores, e para comparar dados mensurados em diferentes regiões (de Morais et al., 2017; Martins et al., 2018; Turner et al., 1989).

Para cada escala de observação, há métodos para avaliar de que forma o distúrbio está afetando a integridade do ecossistema. Em maior escala, a bacia hidrográfica pode ser caracterizada em relação ao tipo de uso que se faz do solo no seu entorno. Algumas das mudanças ambientais mais importantes ocorrem na escala espacial da paisagem (O'Neill et al., 1997). Em média escala, no caso de trecho e segmento e interações com a zona ripária, as características físicas desses ambientes são caracterizadas em métricas de habitat físico (Kaufmann et al., 1999), que incluem todos os atributos estruturais que influenciam ou possibilitam a manutenção de organismos em um ecossistema aquático (Peck et al., 2006). A diminuição da diversidade de habitas físicos pode levar à simplificação de assembleias de organismos aquáticos (Busch & Lary, 1996) por isso a caracterização desses habitats é de fundamental importância para a avaliação de condições ambientais (Gerritsen et al., 2011). Modificações nos hábitats físicos são consideradas como fatores estressores de ecossistemas aquáticos (Karr et al., 1986), pois os hábitats incorporam aspectos físicos e químicos às interações bióticas (Barbour et al., 1999). Em menor escala, pode-se avaliar os organismos associados ao substrato, como a assembleia de macroinvertebrados bentônicos (Bonada et al., 2006; Callisto et al., 2019b). A abordagem de comunidade, definida como um grupo de várias populações de espécies interagindo entre si e ocorrendo juntas no espaço (Ricklefs, et al., 2016), busca entender padrões na estrutura e comportamento de grupos de indivíduos e de suas interações (Begon et al., 2006). No entanto, as amostras biológicas podem não representar toda a comunidade, sendo comumente empregado o uso do termo assembleia, que sugere um corte metodológico, cujo foco está em determinados grupos dentro da comunidade. Geralmente é definida como "grupos taxonômica e filogeneticamente relacionados dentro de uma comunidade biológica" (Stroud et al., 2015).

Comunidades ou assembleias biológicas que respondem a impactos antrópicos e alterações no meio são consideradas indicadoras biológicas ou bioindicadoras (Heink & Kowarik, 2010). Para que uma comunidade possa ser considerada bioindicadora, ela deve atender a alguns critérios, como: fácil mensuração, sensibilidade a estressores, resposta previsível ao distúrbio, antecipação de mudanças iminentes no ambiente, previsibilidade de mudanças que possam ser evitadas por ações de manejo, integração com as mudanças ao longo dos gradientes ambientais, resposta conhecida a distúrbios naturais de origem antrópica, baixa variabilidade de resposta a estressores (Dale & Beyeler, 2001; Evans & Guariguata, 2008). Essas características possibilitam que a abordagem de bioindicadores possua vantagens em relação as abordagens que utilizam apenas parâmetros abióticos para a avaliação de condições ecológicas (Karr, 1999), pois indicadores biológicos refletem o componente temporal na avaliação ecológica (Holt, 2010).

As avaliações ambientais utilizando bioindicadores se tornaram cada vez mais completas, no sentido de representar como um todo a comunidade presente no ambiente. Essa abordagem deu origem a índices que, integram medidas variadas da comunidade bioindicadora, e que possuem capacidade de refletir os distúrbios antrópicos, os chamados índices de integridade biótica (IBI – *Integrity Biotic Index*) (Buss et al., 2015; Niemi & McDonald, 2004; Ruaro et al., 2020). A integridade biótica pode ser definida como sendo a capacidade de manutenção e suporte de comunidades biológicas preservando sua composição, diversidade e estrutura funcional de forma comparável às características naturais da região (Karr, 1981). Com o objetivo de desenvolver metodologias capazes de quantificar e avaliar a integridade biótica em ecossistemas aquáticos, além de entender como os distúrbios afetam as comunidades biológicas, Karr (1981) propôs o Índice de Integridade Biótica. Esse índice é composto por uma combinação de vários atributos ou métricas biológicas que refletem os distúrbios antrópicos em um gradiente de condições ambientais (Karr et al., 1986).

A partir do trabalho pioneiro de Karr surgiram novos índices e adaptações com o intuito de aumentar o potencial de avaliação ecológica do índice (Ruaro et al., 2020). Foram incorporados critérios estatísticos na seleção de métricas (Hering et al., 2006; Stoddard et al., 2008), site de referência ou locais menos impactos ("*least disturbed*") (Fierro et al., 2018; Ligeiro et al., 2013; Martins et al., 2018; Silva et al., 2017; Stoddard et al., 2008), correção de métricas para variação ambiental (Chen et al., 2014; Fierro et al., 2018; Macedo et al., 2016). Apesar da proposta de índices ter sido proposta incialmente para peixes, gradativamente novas propostas utilizando outros grupos, como por exemplo diatomáceas (Delgado et al., 2010), aves (Bryce, 2006), anfíbios (Stapanian et al., 2015) e macroinvertebrados (Chen et al., 2017; Couceiro et al., 2012; Fierro et al., 2018; Macedo et al., 2016; Silva et al., 2017; Stoddard et al., 2008).

Uma vantagem da utilização da abordagem de índices de integridade é sua capacidade de integrar atributos biológicos variados de comunidades em uma pontuação que indica a condição biológica em um sítio amostral (Fierro et al., 2018; Hughes et al., 1998). As abordagens multimétricas utilizando os macroinvertebrados têm sido a mais utilizada para avaliação da qualidade de água (Bonada et al., 2006; Ruaro et al., 2020). Os índices multimétricos em grande escala foram desenvolvidos em muitos países e continentes (Baptista et al., 2007; Chen et al., 2014; Fierro et al., 2018; Hering et al., 2006; Jun et al., 2012; Klemm et al., 2003; Moya et al., 2011; Silva et al., 2017; Stoddard et al., 2008), no entanto, essa abordagem tem sido pouco utilizada para avaliação de ecossistemas aquáticos tropicais, apesar desses locais abrigarem elevada diversidade biológica (Chen et al., 2017). No Brasil, a maioria dos estudos utilizam peixes como indicador biológico (de Carvalho et al., 2017; Terra et al., 2013) e macroinvertebrados bentônicos (Baptista et al., 2013, 2007; Macedo et al., 2016; Oliveira et al., 2011; Pereira et al., 2016; Silva et al., 2017). Há ainda tentativas recentes de integrar diferentes comunidades biológicas em um único índice (Chen et al., 2017).

No entanto, ainda há lacunas de conhecimento quanto à padronização de métodos e limitações quanto à aplicabilidade de índices em larga escala (Silva et al., 2017), grande quantidade de diferentes índices foram desenvolvidos e não foram testadas suas eficiências e aplicabilidade em outros locais.

Além das abordagens de índices multimétricos, outros métodos buscam identificar riscos para a condição biológica avaliando variáveis estressoras. As abordagens de risco relativo (RR), extensão relativa (ER) e Risco Atribuível (RA) são utilizadas pela Agência de Proteção Ambiental dos Estados Unidos (US-EPA) para relatar as condições regionais e nacional de riachos em seu programa de monitoramento nos EUA (Angradi et al., 2011; USEPA, 2016). Esta abordagem baseia-se na capacidade de fornecer associações quantificáveis entre os principais estressores (métricas de distúrbio) e respostas biológicas (Paulsen et al., 2008). O RR descreve a probabilidade de condição biológica boa X ruim, dada a presença / ausência de condição estressora baixa X alta. A ER fornece a magnitude em que a condição de alto estressor é encontrada em uma região. Essa abordagem é obtida através de metodologia de amostragem aleatória, utilizando delineamento probabilístico para seleção de locais (Van Sickle & Paulsen, 2008). O Risco Atribuível (RA) é expresso como uma combinação de um ER e seu RR. Se RA ≠ 0, qualquer aumento em um estressor ER ou em seu RR também aumentará seu RA. Por outro lado, diminuições em ER ou RR irão diminuir RA (Van Sickle & Paulsen, 2008).

O uso de metodologia probabilística é importante pois garante representatividade na região estudada, permitindo que as características físicas, químicas e biológicas reflitam as condições ecológicas da região ou bacia como um todo (Herlihy et al., 2000, 2008). Além disso, essa abordagem tem outras vantagens incluindo (i) ser uma ferramenta econômica, pois permite inferir de forma precisa e confiável a condição ecológica de grandes áreas com base em um número mínimo de locais (Paulsen et al., 2008; Stevens & Olsen, 2004); (ii) permitir estimativa estatística sobre o comprimento do fluxo de toda a bacia hidrográfica (Herlihy et al., 2000); (iii) por ser uma abordagem aleatória, evita seleção de locais de amostras por conveniência, evitando conclusões tendenciosas em estudos de avaliação ecológica (Dobbie & Negus, 2013; Jiménez-Valencia et al., 2014).

Grupos de invertebrados aquáticos têm sido frequentemente utilizados como bioindicadores de qualidade de água em múltiplas perspectivas, incluindo a análise de grupos relevantes. Organismos pertencentes à família Chironomidae (Diptera, Insecta) possuem grande potencial de bioindicação (Rosenberg, 1992), não somente por possuírem distribuição em larga escala e elevada diversidade de gêneros em sua composição taxonômica, mas também pelas suas respostas morfológicas potencias a mudanças ambientais e globais (Nicacio & Juen, 2015; Roque et al., 2010). Apesar de sua importância ecológica relevante, em muitos estudos em riachos e ecossistemas lacustres, as larvas de Chironomidae ainda são ignoradas ou negligenciadas com a sua identificação mantida ao nível de família e subfamílias (Serra et al., 2016). Chironomidae é um grupo amplamente diverso com exigências e características ecológicas diferentes, e se identificado em nível de gêneros ou espécies, possui potencial para melhorar os sinais fornecidos por ferramentas de avaliação ecológica, quer em avaliações estruturais taxonômicas ou em avaliações funcionais indiretas (Serra et al., 2016). Informações taxonômicas e características de larvas de Chironomidae ao nível de gêneros fornecem uma clara segregação de tipos de rios, devido à alta variedade de preferências ecológicas de diferentes gêneros (Serra et al., 2017). Os fatores ambientais atuam como filtros que selecionam as características que apresentam melhor desempenho dentro das condições bióticas e abióticas locais (Firmiano et al., 2021; Poff, 1997; Statzner & Moss, 2004). A identificação dessas características auxilia a compreensão das relações entre estressores locais e a biodiversidade, indicando os estressores que mais influenciam as alterações nas assembleias biológicas (Castro et al., 2018; Dolédec & Statzner, 2010).

Estudos de avaliação de qualidade ambiental em ecossistemas aquáticos no Brasil têm sido realizados em sua maioria nos biomas Cerrado, Mata Atlântica e Amazônia, mas ainda são insuficientes. O Cerrado é o segundo maior bioma brasileiro, sendo atualmente um dos mais ameaçados por atividades antrópicas (Strassburg et al., 2017; Wantzen, 2003). Taxas de desflorestamento no cerrado foram 2,5 vezes superiores àquelas observadas no bioma amazônico (de 2002 a 2011) (Strassburg et al., 2017), e a relação área protegida / Cerrado dentro das bacias hidrográficas é insignificante (0,8–4% em 10 das 11 bacias), bem abaixo da taxa nacional de 28,44% das áreas de conservação no Brasil (Latrubesse et al., 2019). A constante ameaça a esse bioma associada ao elevado endemismo de espécies faz do cerrado um “hotspot” de biodiversidade (Myers et al., 2000). Do ponto de vista hidrológico, o cerrado desempenha um papel fundamental na dinâmica de recursos hídricos, uma vez que abriga nascentes de importantes bacias hidrográficas brasileiras (por exemplo, a bacia do rio São Francisco), contribuindo com 43% das águas continentais fora da bacia Amazônica Strassburg et al., 2017). O cerrado também abriga um grande número de nascentes e riachos de pequeno porte, o que faz desse bioma o berço das águas, exercendo, portanto, importante papel na manutenção e conservação da biota aquática(Latrubesse et al., 2019). No entanto, a constante pressão sobre esses ecossistemas tem resultado em mudanças na composição da biota e na estrutura e funcionamento dos habitats aquáticos (Rausch et al., 2019) . O grande potencial hídrico do cerrado propiciou a construção de empreendimentos hidrelétricos que trouxeram impactos diretos de sua implementação (p.ex. realocação de pessoas, deterioração da qualidade da água, perda de patrimônio genético, desestruturação de comunidades aquáticas, alterações climáticas, etc.) e efeitos indiretos como, por exemplo, adensamentos populacionais e expansão de atividades agropecuárias no entorno (Von Sperling, 2012).

O bioma Cerrado compreende uma grande diversidade de regiões e fisionomias, uma delas compreende a região alagada e as veredas do rio Pandeiros, localizadas no norte do estado de Minas Gerais. Estão entre as áreas prioritárias para conservação do bioma Cerrado, sendo também considerada de Importância Biológica Especial, por constituírem-se em ambientes únicos no estado e possuir alta riqueza de espécies (Drummond et al., 2005). A maior parte de seu território compõe a Área de Proteção Ambiental Estadual do rio Pandeiros (APAERP) (431.401,14 hectares), criada por meio da Lei Estadual nº 11.901/1995, cuja região pantanosa foi definida pelo Decreto Estadual nº 43.910/2004 Refúgio Estadual da Vida Silvestre do Rio Pandeiros. Está localizada nos municípios de Januária, Bonito de Minas e Cônego Marinho, sendo a maior Unidade de Conservação do Estado de Minas Gerais (Instituto Estadual de Florestas, 2019). A bacia do rio Pandeiros possui uma pequena barragem hidrelétrica, desativada desde 2007 (Linares et al., 2018). A legislação brasileira classifica as unidades de conservação em vários tipos, segundo o Sistema Nacional de Unidades de Conservação da Natureza

– SNUC. Segundo a Lei n.º 9.985, de 18 de julho de 2000, Área de Proteção Ambiental (APA) é uma extensa área natural destinada à proteção e conservação dos atributos bióticos (fauna e flora), estéticos ou culturais ali existentes. Como unidade de conservação da categoria uso sustentável, a APA permite a ocupação humana. O Refúgio de Vida Silvestre, pertence a categoria de Unidades de Proteção integral, é admitido apenas o uso indireto dos recursos naturais.

Apesar de sua grande importância, a proteção atual dos Cerrado é fraca: áreas públicas protegidas cobrem apenas 7,5% do bioma e, de acordo com as leis brasileiras em vigor, apenas 20% de terras privadas devem ser destinados à conservação (Strassburg et al., 2017). Em áreas protegidas, ainda influenciadas por atividades antrópicas, os impactos locais podem afetar a qualidade ambiental geral da bacia e, consequentemente, sua condição biológica. Além disso, mesmo baixos níveis de distúrbios podem ter efeitos marcantes em várias espécies com restrições de habitat (Barlow et al., 2018), como as aquáticas por exemplo. Ainda nesse contexto, o elemento fundamental para conservação em regiões tropicais é a presença de áreas protegidas, pois elas limitam as pressões demográficas e estressores locais. No entanto, a rede de áreas protegidas atual nos ambientes tropicais é insuficiente, não abrange os ecossistemas de água doce, e possuem gestão e fiscalização pouco eficientes (Azevedo-Santos et al., 2018; Dala-Corte et al., 2020; Leal et al., 2020). Um recente estudo mostrou que o processo de antropização da APA Pandeiros alterou as condições naturais na área do Refúgio da Vida Silvestre (dos Santos et al., 2020). Nesse sentido, expandir e fortalecer as unidades de conservação existentes deve ser uma prioridade para qualquer estratégia de conservação dos ecossistemas aquáticos tropicais (Sundar et al., 2020), caso contrário, será impossível limitar o efeito de estressores locais e evitar novas perdas de biodiversidade (Pouzols et al., 2014).

As abordagens e ferramentas de avaliação da condição biológica descritas são frequentemente utilizadas por pesquisadores e também gestores ambientais (USEPA, por exemplo), e ressaltam a importância do pluralismo de metodologias e a necessidade uma variedade de abordagens que se integrem e se complementem: (1) a partir da perspectiva de índices multimétricos, que integram múltiplos atributos biológicos e ecológicos destas assembleias; (2) através de modelos que usam a probabilidade da presença de estressores antropogênicos impactarem a biota; (3) como indicadores ecológicos através da análise de grupos relevantes.

Objetivo geral e Hipóteses

Considerando a importância da água e bacias hidrográficas como sua fonte principal, o objetivo desse trabalho foi avaliar a condição ecológica de uma bacia hidrográfica no Cerrado (rio Pandeiros), respondendo às seguintes perguntas:

Pergunta 1 – Índices multimétricos bentônicos podem ser utilizados em avaliações de integridade biótica independentemente do local para onde foram desenvolvidos?

Hipótese 1 - Índices multimétricos bentônicos desenvolvidos em vários locais distintos são eficientes para a avaliação da qualidade ambiental em outras bacias hidrográficas semelhantes.

Predições - A estrutura e composição dos macroinvertebrados bentônicos refletem os distúrbios na bacia hidrográfica, respondendo a um gradiente de condições ambientais.

Pergunta 2 – A condição biológica é afetada por distúrbios humanos em áreas protegidas e prioritárias para a conservação da biodiversidade?

Hipótese 2 – A presença de distúrbios humanos (variáveis estressoras) em escala local representa risco negativo para a condição biológica.

Predições – Locais que possuem condição ruim em relação às variáveis estressoras do ambiente, tem maior risco probabilístico de apresentar uma condição biológica ruim.

Pergunta 3 - Os gêneros dos organismos da família Chironomidae (Diptera, Insecta) e suas características funcionais são eficazes para avaliar o estado ecológico em riachos Neotropicais?

Hipótese 3 – A composição e características funcionais dos gêneros de Chironomidae são alterados negativamente pelos distúrbios antrópicos.

Predições - Gêneros de Chironomidae mais sensíveis, tem sua abundância diminuída na presença de distúrbios antropogênicos; os distúrbios antrópicos atuam como filtros, alterando as características funcionais das assembleias.

Metodologia geral

Área de estudo

A bacia do rio Pandeiros está localizada na região norte do estado de Minas Gerais, Brasil, no bioma Cerrado e possui uma área de 431.401,14 ha (Figura 1), faz parte de importante bacia hidrográfica brasileira: a bacia do rio São Francisco. O clima na região da bacia do rio Pandeiros é tropical quente e úmido, com estação seca bem acentuada e verão chuvoso (Alvares et al., 2013). A região alagada e as veredas do rio Pandeiros estão entre as áreas prioritárias para conservação do bioma do Cerrado, sendo também considerada de Importância Biológica Especial, por constituir-se em ambiente único no estado e possuir alta riqueza de espécies (Drummond et al., 2005). A maior parte de seu território compõe a Área de Proteção Ambiental Estadual do Rio Pandeiros (APAERP), criada por meio da Lei Estadual nº 11.901/1995, cuja região pantanosa foi estabelecida pelo Decreto Estadual nº 43.910/2004 como Refúgio Estadual da Vida Silvestre do Rio Pandeiros (Instituto Estadual de Florestas, 2019). É a maior Unidade de Conservação do Estado de Minas Gerais, abrangendo integralmente a bacia do rio Pandeiros, a qual se insere nos municípios de Januária, Bonito de Minas e Cônego Marinho.

Definição dos sítios amostrais

Os locais de amostragem foram selecionados através do uso de procedimentos espacialmente balanceados seguindo um desenho de levantamento aleatório e sistemático, de acordo com o método usado pela US-EPA em seu *National Rivers and Streams Survey* (Olsen & Peck, 2008; USEPA, 2013) e amplamente utilizado para avaliar outros riachos do Cerrado brasileiro (Callisto, et al., 2014; 2019; Macedo et al., 2018, 2016; Silva et al., 2017). Após ordenação da malha hidrográfica segundo classificação de Strahler (1957), foi realizado um sorteio balanceado de pontos em riachos de 3ª a 5ª ordens a uma distância mínima de 1 km entre eles. A amostragem foi realizada em riachos considerados "*wadeable*", ou seja, capazes de serem atravessados por um adulto mediano com a lâmina d'água até a altura do peito (Kaufmann et al., 1999), e em riachos "*non wadeable*", onde a metodologia para rios não vagueáveis foi aplicada

(USEPA, 2013). Foram sorteados pontos amostrais distantes a até 35 Km dos limites do reservatório de Pandeiros, totalizando assim 40 sítios de amostragem.

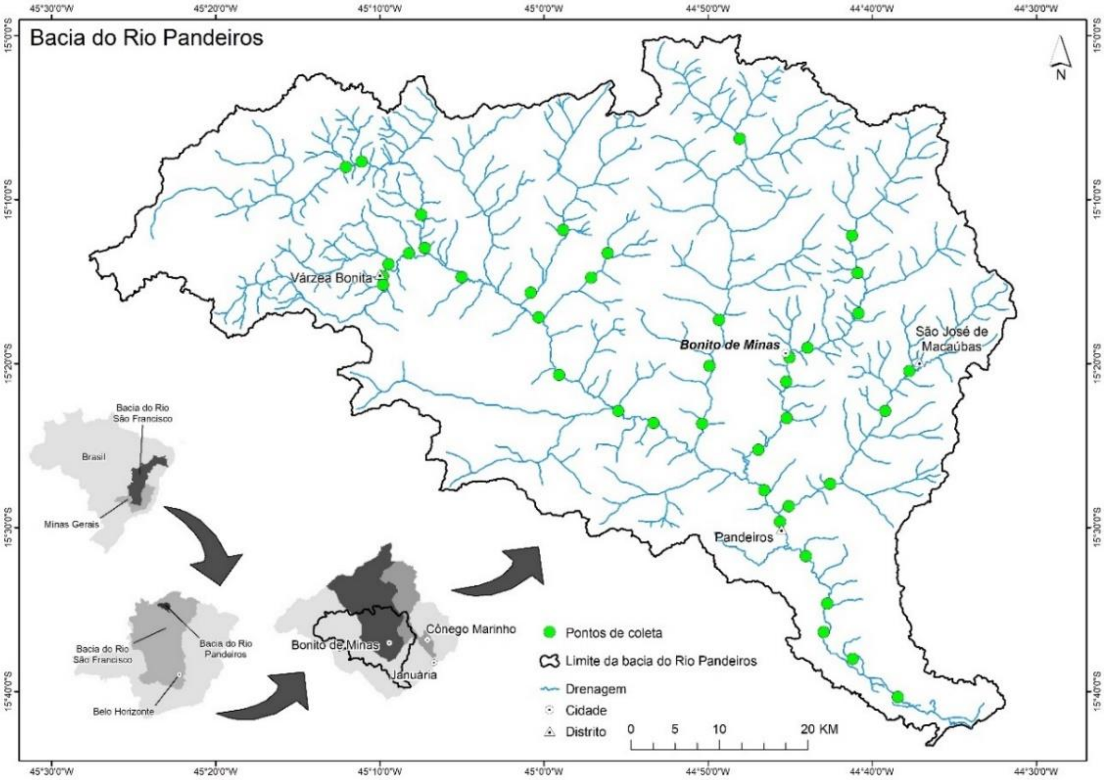

Figura 1 - Localização da bacia do rio Pandeiros e pontos amostrais selecionados

Reconhecimento e validação dos sítios amostrais

Para confirmar se o conjunto de locais de amostragem selecionados previamente são acessíveis, um reconhecimento de campo foi necessário (Figura 2). Nesta fase, os sites foram verificados quanto a acessos e fluxos hidrológico; os sítios amostrais deveriam ter ordens e dimensões de largura e profundidade semelhantes (Kaufmann et al., 1999); e deveriam ser acessíveis e de amostragem possíveis de serem realizados. Também nessa visita foi realizada a comunicação com as comunidades ribeirinhas locais para autorização de entrada nas propriedades (quando houvesse) e esclarecendo os objetivos da pesquisa para os moradores da região. Além disso foram mapeados os trajetos que seriam utilizados posteriormente na coleta. Aqueles que não foram selecionados por algum motivo (riachos secos, não *wadeable*, acesso negado etc.) foram substituídos por sites reservas, também selecionados previamente.

Figura 2 – Reconhecimento dos sítios amostrais: a) comunicação com a população local, b) avaliação de acesso, c) riacho seco, d) acesso negado, e) riacho sem fluxo contínuo, f) riacho selecionado para a amostragem.

Avaliação do uso e ocupação do solo

A avaliação do uso e cobertura do solo foi baseada na classificação supervisionada e avaliação post-hoc de imagens digitais, onde as classes foram atribuídas a pixels de imagens de satélite, criando padrões homogêneos aos quais diferentes classes de uso e cobertura do solo estão associadas (Hughes et al., 2019; Macedo et al., 2014; Santos et al., 2017). As imagens utilizadas neste trabalho foram do satélite Landsat-8, sensor OLI, cena orbital 219/71 e 219/70, para o ano de 2016, disponibilizadas pelo INPE (Instituto Nacional de Pesquisas Espaciais) (http: // www. dgi.inpe.br).

Amostragens em campo

As amostragens nos trechos de rios da bacia hidrográfica do Rio Pandeiros (MG) foram realizadas no final do período seco (abril e junho/2016), em que variações no fluxo das águas são relativamente menores e as abundâncias de macroinvertebrados são maiores devido à exposição de habitats e microhabitats (Melo & Froehlich, 2001) e há uma maior estabilidade do leito. Em cada sítio a amostragem foi realizada em um trecho proporcional à largura do riacho. A extensão deste trecho foi estabelecida multiplicando a média da largura do riacho por 40, de tal forma que o comprimento do trecho do riacho a ser amostrado foi de, no mínimo, 150 metros. Em cada trecho foram estabelecidos 11 transectos transversais (perpendiculares ao trecho do riacho) demarcados de "A" a "K", definindo 10 seções, onde foram realizadas medidas de habitats físicos e coleta de macroinvertebrados bentônicos (Peck et al., 2006) (Figura 3).

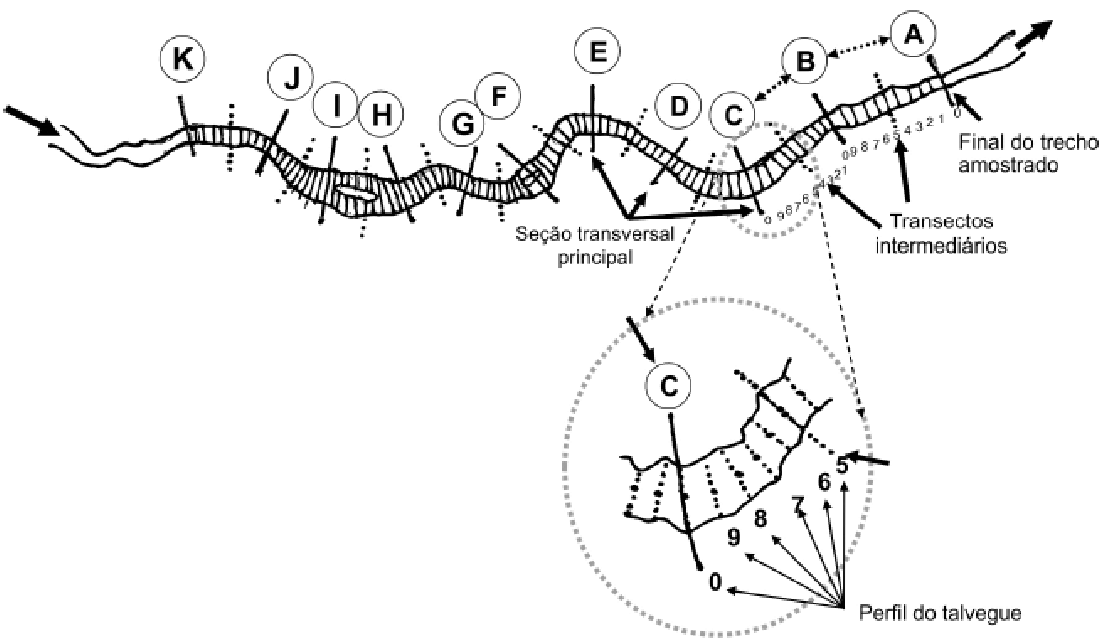

Figura 3 – Esquema representativo de 11 transectos transversais 10 seções definidas dentro do trecho mínimo amostrado em um riacho (Peck et al., 2006)

A caracterização de habitats físicos foi realizada conforme descrito no manual "*Surface Waters: Western Pilot Study Field Operations Manual for Wadeable Streams*", proposto por Peck et al. (2006) e adaptado para os riachos do Cerrado (Callisto, et al., 2014) e *"Field Operations Manual Non-wadeable*" (USEPA, 2013). Considerando o trecho amostrado uma série de medições foram realizadas em cada transecto (A, B, C etc.) e em cada seção (AB, BC, CD etc.) (Figura 3 acima), avaliando as características do canal (profundidade, largura molhada, altura do leito sazonal, altura da incisão, inclinação de margens, sinuosidade, declividade etc.), características do habitat (tamanho e imersão do substrato, tipos de habitat, complexidade), características da vegetação ripária (sombreamento do leito e margens, densidade de estratos vegetais etc.) e influência humana (presença de estradas, lixo, plantações, pastagens etc.) (Figura 4). Posteriormente foram calculadas métricas de habitats físicos conforme Kaufmann et al. (1999).

Figura 4 – Aplicação do protocolo de avaliação de características do hábitat físico: a) aferição da profundidade, b) altura do leito sazonal, c) distâncias e sinuosidade, d) declividade, e) imersão do substrato, f) cobertura do dossel.

Seguindo Peck et al. (2006), em cada local de amostragem, a qualidade da água (temperatura (° C), pH e condutividade (µS / cm) foram determinadas (Figura 5). As amostras de água foram levadas para o laboratório e sólidos totais (ppm), turbidez (UNT), oxigênio dissolvido (mg / L), alcalinidade total (mEq / L CO_2), nitrogênio total (mg

/ L), fósforo total (ug / L) e clorofila (ug / L) foram determinados por meio de métodos padrão (APHA, 1998).

Figura 5 – Mensuração de parâmetros físicos e coleta de água para avaliação dos parâmetros químicos de qualidade da água.

Coleta e identificação de macroinvertebrados bentônicos

Em cada um dos 11 transectos, demarcados de “A” a “K”, foi realizada uma amostragem das comunidades de macroinvertebrados bentônicos, totalizando 11 subamostras por riachos e 440 subamostras no total por bacia. Um coletor do tipo “*kick-net*” (30 cm de abertura, 500 µm de malha, área de 0,09 m^2) foi utilizado na coleta dos organismos bentônicos (Figura 6). Cada amostra foi colocada em um saco plástico e fixada com 50 ml de formol tamponado. As amostras foram levadas ao laboratório de Ecologia de Bentos/UFMG, onde foram lavadas sobre peneira de 500 µm de malha. A triagem foi realizada em bandejas sobre caixa de luz e os invertebrados identificados em microscópio estereoscópico (32x) com o auxílio de chaves de identificação (Hamada et al., 2019; Merritt & Cummins, 1996; Mugnai et al., 2010; Pérez, 1988). A identificação foi realizada até o nível de família, exceto de Bivalvia, Hydracarina, Hirudinea, Nematoda, Collembola e Oligochaeta. Os exemplares foram depositados na Coleção de Referência de Macroinvertebrados Bentônicos do ICB/UFMG.

Figura 6 – Coleta de macroinvertebrados utilizando um coletor do tipo "*kick-net*", ou Rede D, em 11 transectos do trecho amostral, seguindo o padrão tipo zigue zague: margem esquerda – centro - margem direita.

Capítulo 1

Are multiple multimetric indices effective for assessing ecological condition in tropical basins?

Isabela Martins, Diego Rodrigues Macedo, Robert M. Hughes, Marcos Callisto

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Are multiple multimetric indices effective for assessing ecological condition in tropical basins?

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Are multiple multimetric indices effective for assessing ecological condition in tropical basins?

Abstract

The quality and availability of water resources in tropical watersheds are threatened by increased multiple use demands by human populations. Therefore, there is a need for cost-effective ecological indicators of water body status and trends. Multimetric indices (MMIs), based on responses of biological assemblages to anthropogenic disturbances, are excellent examples of such indicators and they have been applied globally. However, creating new MMIs for each water body or study area requires considerable analytical effort and hinders our ability to make regional or global comparisons. Therefore, we tested the effectiveness of 17 published benthic macroinvertebrate MMIs for assessing the environmental quality of a tropical anthropogenically least-disturbed river basin in the Neotropical Savanna (Brazilian Cerrado) biome. We tested those MMIs through use of macroinvertebrate data sampled at 40 stream sites in the Pandeiros River basin, Brazil. Disturbances in the basin were related to local factors such as pasture, garbage, and cropland in stream riparian areas. Index performance was tested by comparing precision, bias, responsiveness and sensitivity to anthropogenic pressures and stressors. Ten indices performed satisfactorily in evaluating the environmental condition of the basin. Therefore, we do not recommend developing new benthic MMIs for rapid environmental quality assessments. On the other hand, we do recommend using standard data collection methods for evaluating conditions throughout the biome.

Keywords: Macroinvertebrates; wadeable streams; Index of biotic integrity; Cerrado biome; bioassessment; anthropogenic disturbance.

Introduction

The increasing demand for water uses by humans affects the quality and availability of water resources (Gangloff et al., 2016) and threatens global aquatic biodiversity (Reid et al., 2018). In some regions this becomes particularly important, as in the Brazilian Cerrado (Neotropical Savanna) biome. Although this biome contains important hydrographic basins, has high biodiversity, high endemism, and covers 2 million km2, it is one of the most threatened biomes in South America (Strassburg et al., 2017). Despite the great importance of the Cerrado as a biodiversity hotspot (Myers et al., 2000), its current protection is insufficient: public protected areas cover only 7.5% of the biome (Strassburg et al., 2017). The Cerrado biome has historically been neglected by the Brazilian government, resulting in its devastation through intense land use change, with only 20% remaining of its original natural area (Strassburg et al., 2017). Aquatic ecosystems in this biome are largely threatened by habitat fragmentation, sedimentation, flow regulation (dam construction), water pollution and biological invasions (Callisto et al., 2019; Linares et al., 2017; Macedo et al., 2018; Reid et al., 2018; Sánchez-Bayo & Wyckhuys, 2019). Because of its great biological importance and current threats, ecological studies in the Cerrado are justified to ensure that management actions are effective and based on widely used and validated scientific studies and methodologies. Therefore, identifying the major anthropogenic changes in aquatic ecosystems and understanding how they affect biological conditions are important steps in the assessment of Cerrado environmental quality (Revenga et al., 2005).

Environmental quality assessment of tropical aquatic ecosystems is critically important for the management and conservation of water resources and for the protection of aquatic biodiversity (Sánchez-Bayo & Wyckhuys, 2019) for several reasons. The Tropics support over three quarters of global biodiversity (Barlow et al., 2018). In addition, the risk of biodiversity losses because of anthropogenic disturbances (e.g., urbanization, agriculture, pasture, mining, dams) is increasing in tropical regions (Dirzo et al., 2014). Studies in neotropical regions have become more frequent but remain insufficient for understanding their biodiversity (Barlow et al., 2018; Hortal et al., 2015). This is especially important in freshwater ecosystems where there are higher rates of degradation and loss of species than in terrestrial or marine ecosystems (Reid et al., 2018).

Unlike assessments of water chemistry or physical habitat structure, biological assessments of water bodies are direct measures of biological condition that integrate both long- and short-term and small- and large-extent anthropogenic disturbances (Davies & Jackson, 2006; Hughes, 2019; Karr & Dudley, 1981). Assessments of water chemistry, physical habitat structure, and landscape or riverscape condition typically explain less than half the variability in biological condition (Hughes, 2019; USEPA, 2016) and are extremely sensitive to sampling effort and natural variability (Hughes, 2019). Unlike species richness or tolerance or diversity indices, multimeric indices (MMIs) integrate multiple biological attributes of aquatic macroinvertebrate assemblages (Hughes et al., 1998) and have been used to evaluate water body quality globally (Buss et al., 2015; Ruaro & Gubiani, 2013). MMIs are robust tools for assessing aquatic ecosystem status and trends (Buss et al., 2015; Ruaro & Gubiani, 2013; USEPA, 2016) because they can discriminate the effects of different types of anthropogenic pressures and stressors (Hering et al., 2006; Lunde & Resh, 2012; USEPA, 2016). Therefore, they are considered one of the best approaches for aquatic ecosystem biomonitoring and bioassessment (Bonada et al., 2006; Ruaro & Gubiani, 2013).

In Brazil, indices were developed in different biomes, including Amazonia (Couceiro et al., 2012), Cerrado (Ferreira et al., 2011; Macedo et al., 2016; Saito et al., 2015; Silva et al., 2017), Atlantic Forest (Baptista et al., 2007; Oliveira et al., 2011) and Pampas (Melo et al., 2015). However, this approach has not yet been standardized for evaluating tropical aquatic ecosystems nationally (Buss et al., 2015) despite their high biological diversity (Barlow et al., 2018). There are difficulties in extending this approach because there is no legal provision for its use at the national level, which would require defining and standardizing the tools used for this purpose (Macedo et al., 2016; Ruaro and Gubiani, 2013; Silva et al., 2017).

Despite the large number of MMIs available in the literature, they have not been evaluated for their efficacy and applicability in places other than those where they were developed (Ruaro & Gubiani, 2013; Silva et al., 2017). Although several researchers have tested alternative MMIs or metrics to arrive at the best final index based on their data sets, they did not test other MMIs developed in different places. Therefore, we carefully selected existing MMIs, calculated their metrics, and performed statistical tests to validate their reliability as measured by their precision, bias, responsiveness and sensitivity to anthropogenic pressures and stressors (Chen et al., 2019).

The maintenance or improvement of water quality, ecological health, and biodiversity are major societal goals. Therefore, the objective of this work was to test the applicability of seventeen existing MMIs to our study area, evaluate the metrics used, and conduct an environmental quality assessment in an environmentally protected area. We tested the hypothesis that those benthic MMIs are efficient in assessing environmental quality, regardless of where they were developed. We assumed that the indices were accurate, lacked a natural variability bias, and responded to anthropogenic disturbances, making them useful for evaluating environmental quality. To meet our objectives, we evaluated how anthropogenic disturbances affected water quality, physical habitat structure, and benthic macroinvertebrate assemblage MMIs.

Material and Methods

Study Area

The Pandeiros River basin is in northern Minas Gerais state, Brazil (Figure 1) located in the Brazilian Cerrado biome. The basin area is 3.960 km2 and the flooded areas (palm swamp, wetlands, and marginal lagoon complexes) of the Pandeiros River are priority areas for conservation in the biome (Drummond et al., 2005). They are designated of Special Biological Importance, being unique environments in an otherwise semi-arid region (Azevedo et al., 2009). The entire Pandeiros River Basin is an Environmental Protection Area, the largest Conservation Unit in the state of Minas Gerais (IEF - Instituto Estadual de Florestas, 2019).

The Pandeiros River is a strategic tributary on the left bank of the São Francisco River and of fundamental importance for the protection of that basin (Azevedo et al., 2009). The climate is semi-arid, and the basin drains mostly sandy soils, but many Pandeiros tributaries are perennial, which makes the river network critically important annually and during long-term droughts (IEF, 2019). The basin also has a small hydropower dam, decommissioned since 2007 and slated for future removal, which is unprecedented so far in South America (Linares et al., 2018, 2020).

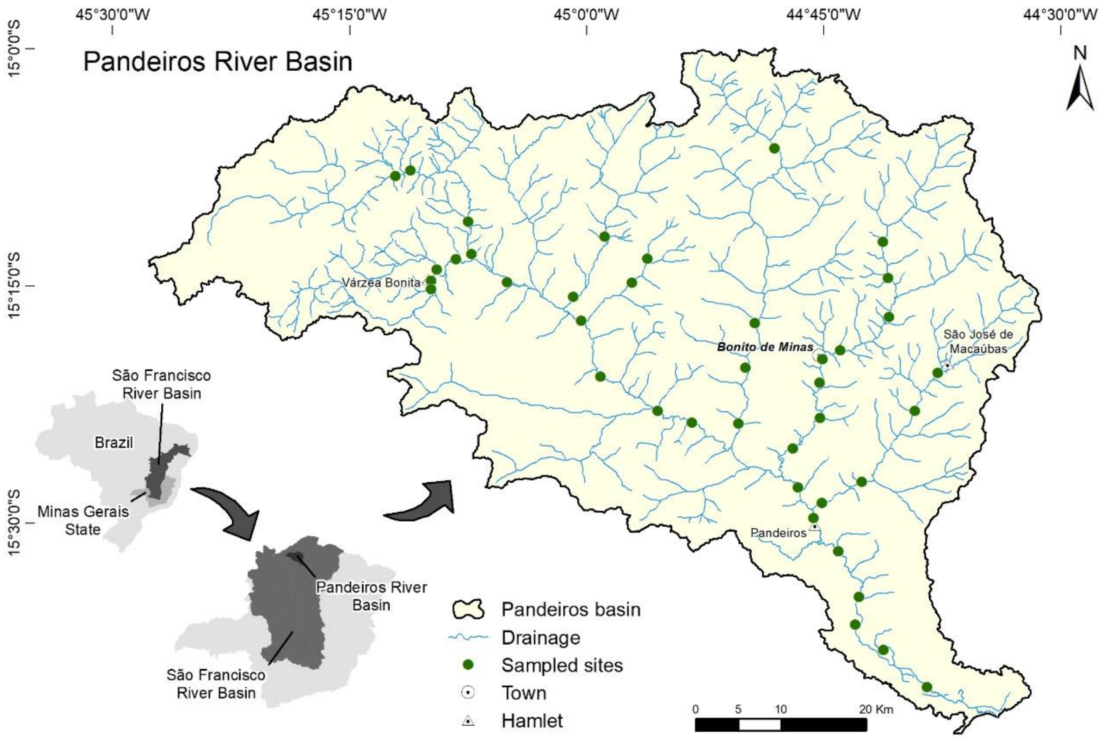

Figure 1 – Location of the sampling sites in the Pandeiros River basin.

Selection of stream sites and sample sections

Forty stream sites were selected through use of a spatially balanced randomized survey design developed by the United States Environmental Protection Agency (Olsen & Peck, 2008) and also widely used for assessing other Brazilian Cerrado streams (Callisto et al., 2014; Callisto et al., 2019; Macedo et al., 2016, 2018; Silva et al., 2017) and Atlantic Forest streams (Jiménez-Valencia et al., 2014; Terra et al., 2016). Such a design allows one to obtain an unbiased sample and to infer results from a relatively small number of sites to the entire stream population from which the sample was drawn, with known confidence intervals (Jiménez-Valencia et al., 2014; Silva et al., 2017; USEPA, 2016). This is not possible with ad hoc or systematic survey designs. After sorting the basin by Strahler (1957) stream orders, a balanced selection of stream sites was carried out for 3rd to 5th order streams on a 1: 100,000 map with a minimum distance of 1 km between them. We sampled sites deemed wadeable, i.e., capable of being safely crossed by an adult with the water depth up to breast height (Kaufmann et al., 1999).

We sampled sites during the dry season (April/June 2016) when water flow variations are relatively small, there is greater bed stability, habitats and microhabitats are more accessible, and macroinvertebrate abundances are high (Hughes & Peck, 2008; Melo & Froehlich, 2001).

Benthic macroinvertebrate sampling

Each stream site was 40 x its mean width, with a minimum length of 150 meters. In each site, 11 transects (perpendicular to the stream) were marked, defining 10 sections where physical habitat was measured and benthic macroinvertebrates collected (Peck et al., 2006; USEPA, 2016). In each transect, marked "A" to "K", benthic macroinvertebrates were sampled, totaling 11 sub-samples per site and 440 sub-samples in total. A D-frame kick-net (500 µm mesh, 0.9 m2 area) was used to sample benthic organisms. Each sample was placed in a plastic bag and fixed with 50 ml of formaldehyde. The samples were taken to the UFMG's (Universidade Federal de Minas Gerais) Benthos Ecology laboratory, where they were washed on a 500 µm mesh screen. The washed material was placed in clear trays on a light box and the invertebrates were identified through use of a stereoscopic microscope (32x) with identification keys (Fernández and Domínguez, 2001; Merritt and Cummins, 1996; Mugnai et al., 2010; Pérez, 1988). Identification was performed to family, except for Bivalvia, Hydracarina, Hirudinea, Nematoda, Collembola and Oligochaeta. All specimens were identified and deposited in the Reference Collection of Benthic Macroinvertebrates of ICB / UFMG (Instituto de Ciências Biológicas / Universidade Federal de Minas Gerais).

Anthropogenic stressor and pressure metrics to test the MMIs

Water quality and physical habitat stressors

Following Peck et al. (2006), at each sampling site, water quality (temperature (ºC), pH, and conductivity (µS/cm) were determined through use of a multimeter (YSI Model 650). Water samples were taken to the laboratory and total solids (ppm), turbidity (UNT), dissolved oxygen (mg/L), total alkalinity (mEq/L CO2), total nitrogen (mg/L), total phosphorus (ug/L), and chlorophyll (ug/L) were determined via standard methods (APHA, 1998).

Physical habitat structure, such as hydrological, geomorphological, riparian vegetation, and anthropogenic impacts were evaluated through use of the US-EPA protocol (Peck et al., 2006; USEPA, 2016), adapted for use in the Cerrado biome and widely used in Cerrado stream assessments (Callisto et al., 2014; Callisto et al., 2019; de Carvalho et al., 2017; de Castro et al., 2017; Macedo et al., 2016, 2018; Martins et al., 2018; Silva et al., 2017; Silveira et al., 2018). A series of measurements were performed on each transect and in each section between transects. Channel characteristics (e.g., wetted depth, height and width, bank-full height and width, incision height, margin slope, sinuosity, channel slope, etc.), habitat characteristics (e.g., substrate size and embeddedness, habitat types and complexity), riparian vegetation characteristics (e.g., shading of the bed and margins, density of plant strata, etc.) and human influences (e.g., presence of roads, trash, plantations, pastures, etc.) were measured. The physical habitat data were converted to metrics according to Kaufmann et al. (1999; 2009).

Estimates of anthropogenic impacts included the complexity of riparian vegetation and the degree of substrate sedimentation by sand and fines (clay and silt; Xembed), the presence and proximity of anthropogenic impacts in the riparian zone (W1_Hall), and multilayer woody vegetation cover (Xcmgw) as described in Kaufmann et al. (1999). W1_Hall summarizes the amount of evidence from eleven types of disturbances (walls/ dikes/ revetments; buildings; pavement; roads/railroads; pipes; landfills/trash; parks/lawns; row crops; pasture/range/hay fields; logging operations; mining activities) at each bank along the 11 transects at each site. The values were weighted according to their proximity to the stream (Kaufmann et al., 1999). In addition, we calculated the relative bed stability (LRBS), according to Kaufmann et al (2009). Those physical habitat metrics were used in previous studies and proved effective for evaluating environmental quality in Cerrado streams (Macedo et al., 2016; Silva et al., 2017).

Land use pressures

Evaluation of land use and cover was based on supervised classification and post-hoc evaluation of digital images, where classes were assigned to pixels of satellite images, creating homogeneous patterns to which different classes of land use and cover are associated (Hughes et al., 2019; Santos et al., 2017). The images used in this work were from the Landsat-8 satellite, sensor OLI, orbit scene 219/71 e 219/70, for the year 2016, made available by INPE (Instituto Nacional de Pesquisas Espaciais) (http://www.dgi.inpe.br).

Selection of multimetric indices and biological metrics calculation

We performed a bibliographic search in the Web of Science, Scopus, and SciElo, on the terms IBI (index of biotic integrity), multimetric index, and macroinvertebrates. We found 32 papers that included indices developed in different parts of the world, from the year 2002 to 2018. We selected 17 papers that described indices with potential to be applied in the Cerrado biome, considering the reproducibility of the index and whether the metrics used to elaborate each index could be calculated from our data. We discarded studies that included metrics not obtained in our study, such as insect genera and environmental quality indices specific to each region (e.g. Lunde and Resh, 2012; Melo et al., 2015; Mondy et al., 2012; Pond et al., 2013; Saito et al., 2015; Shi et al., 2017; Weigel and Dimick, 2011). Biological metrics based on the final metrics chosen for each index were calculated from Pandeiros data (Table 1, Supplementary Material - Table S1). Each index was calculated and applied as described by its authors, including their processes for defining floor and ceiling values, standardization and scoring, and assessment thresholds. We had only one exception to the authors' index development. If the original index metrics did not consider correction for natural variability (e.g., catchment area, channel slope, or climate data), we determined if that correction was needed for our data.

Table 1 – Final indices evaluated.

Region	Biome	Authors
South America		
Brazil	Amazonian Forest	Couceiro et al. (2012)
	Atlantic Forest	Baptista et al. (2007)
		Oliveira et al. (2011)
	Cerrado	Ferreira et al. (2011)
		Macedo et al. (2016)
		Silva et al. (2017)
Chile	Mediterranean Shrub	Fierro et al. (2018)
Central America		
Panamá	Rain Forest	Helson and Willians (2013)
North América		
USA	Temperate Broadleaf Forest	Klemm et al. (2003)
	Mediterranean Shrub	Ode et al. (2005)
Europe		
Belgium	Temperate Broadleaf Forest	Gabriels et al. (2010)
Africa		
Ethiopia	Savanna	Lakew and Moog (2015)
		Mereta et al. (2013)
Asia		
China	Rainforest	Chen et al. (2014)
		Li et al. (2010)
Vietnam	Rainforest	Nguyen et al. (2014)
South Korea	Temperate Broadleaf Forest	Jun et al. (2012)

Data analyses

Classification and validation of least disturbed sites

Selection of reference sites is a first step in any MMI development (Hughes et al., 1986). In the Pandeiros River basin, we used the concept of least-disturbed sites (Martins et al., 2018, Stoddard et al., 2008), because no place on the planet can be considered totally pristine as a result of atmospheric contaminants and anthropogenic climate change (Hughes, 1995; Hughes, 2019). Those sites are where the best biological, water quality and physical habitat conditions are found, considering the current state of the landscape (Stoddard et al., 2006). To calculate locations with least anthropogenic disturbance, we used the Integrated Disturbance Index (IDI; Ligeiro et al., 2013), which is calculated from local (LDI – Local Disturbance Index) and catchment (CDI – Catchment Disturbance Index) anthropogenic impacts. For evaluating the LDI, we used the metric

W1_Hall, which summarizes the amount of evidence observed in the channel and in the riparian zone. Those values are weighted according to the proximity of the observation from the stream channel, calculated as described in Kaufmann et al. (1999). The CDI was based on the % of human land uses in the catchment of each site, weighted by the potential of degradation that each has on the aquatic ecosystem (CDI=4 x % urban + 2x % agriculture + % pasture) (Ligeiro et al., 2013).

Through examination of an ordered IDI plot, stream sites with the lowest IDI values were considered least disturbed, and those with the highest IDI values were considered most-disturbed. This procedure has been used in a series of multimetric indices in South America (e.g. Chen et al., 2017; de Carvalho et al., 2017; Fierro et al., 2018; Macedo et al., 2016; Terra et al., 2013).

To test whether the macroinvertebrate assemblages responded to impacts and to validate sites as least-disturbed, a Mann-Whitney test (U-Test) was performed. To do so we used the biological metrics of total richness and richness and percentage of sensitive organisms (Ephemeroptera, Plecoptera and Trichoptera - EPT) versus the IDI, W1_Hall, and the metrics comprising W1_Hall in one-to-one analyses. To assess whether the disturbances affected water quality, we performed a Pearson correlation analysis between each of the nine water quality variables and the IDI, one by one. In addition, we used thresholds defined in the Brazilian national environmental law (CONAMA – Conselho Nacional do Meio Ambiente 357/2005) to evaluate water quality (Resolução No 357, de 17 de Março de 2005, 2005).

Natural variability

We used multiple linear regression to assess the influence of natural variability (catchment area, elevation, slope, temperature and rainfall) on the biological metrics comprising each MMI. The analysis was performed only for the sites classified as least disturbed. Significant results ($r2>0.70$; $p<0.05$) were corrected by subtracting the predicted metric values obtained by regression from each raw value (residual value = observed - expected) (Cao et al., 2007; Chen et al., 2014; Klemm et al., 2003; Stoddard et al., 2008).

MMI performance tests

To verify if the indices calculated from our data were effective in evaluating Pandeiros sites, we conducted five statistical analyses (Figure 2), as suggested in many MMI development procedures (e.g., Chen et al., 2014; Hering et al., 2006; Klemm et al., 2003; Macedo et al., 2016; McCormick et al., 2001; Ruaro and Gubiani, 2013; Silva et al., 2017; Stoddard et al., 2008). 1) Precision was assessed through use of the Coefficient of Variation (CV) based on the MMI scores calculated from the least-disturbed sites. The lower the CV, the more precise the MMI (Chen et al., 2014). 2) Bias was determined by evaluating the degree to which MMIs were influenced by natural variation. To do so we performed Pearson correlations between the MMI and the natural variables, one by one (Cao et al., 2007; Hawkins et al., 2010). 3) Spatial independence was evaluated by measuring the degree of spatial autocorrelation or the degree to which the MMI score at one site was influenced by that of a neighboring site (Anselin and Bera, 1998). In other words, this test assesses the degree to which an MMI could distinguish nearby sites. 4) MMI responsiveness assessed the degree to which the disturbance classes (good, fair, poor) were significantly different from each other. To do so, we performed analysis of variance (ANOVA) with Bonferroni correction to test for differences between the disturbance classes given for each index and their respective boxplots (Vander Laan & Hawkins, 2014). 5) Index sensitivity relative to the disturbance metrics was tested via multiple linear regressions between each index and the anthropogenic disturbance metrics (land use and cover, water quality and physical habitat metrics; Macedo et al., 2016). We considered models significant that were Bonferroni corrected.

The normal distribution of each metric (land use and cover, physical habitats and water quality variables) was first determined using the Kolmogorov-Smirnov test; those that were not normally distributed were treated with square root arcsine (percentage data) or log (x + 1) (other types of data) (Gotelli & Ellinson, 2013). Redundant metrics were eliminated if correlated >0.70; we retained the metric with the highest ecological relevance for the macroinvertebrate assemblages and those that are more intuitively understood (Little et al., 1999).

The models were validated through use of analyses of normality, homoscedasticity (Gotelli & Ellinson, 2013) and spatial autocorrelation of residuals

(Anselin & Bera, 1998; Diniz-Filho et al., 2008). All MMIs that passed the five tests were considered valid for our data. To assess similarity of response between the MMIs, a Pearson correlation test was performed between the results of each of the validated indices, one by one.

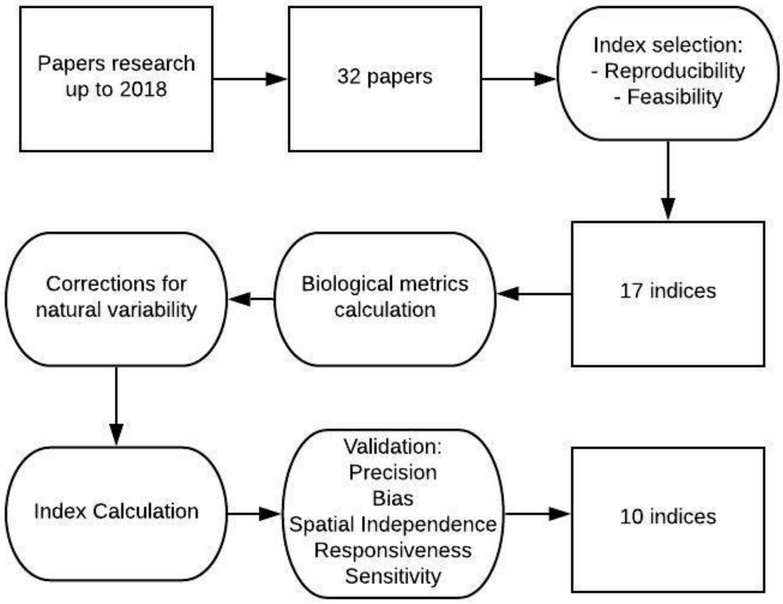

Figure 2 – Steps for index selection, metric calculation and validation.

Results

Benthic macroinvertebrates

In total, 32,271 organisms and 82 taxa were identified. The most abundant families were Chironomidae (41%), Hydrobiidade (11%), Elmidae (7%) and Leptohyphidae (4.3%). Three non-native mollusk species were found: *Corbicula fluminea* (Corbiculidae), *Melanoides tuberculata* (Thiaridae) and *Limnoperna fortunei* (Mytilidae).

Anthropogenic pressure measures

The water quality parameters analyzed (Table S2) were within the limits established for Class 1 waters in Brazilian national legislation. Waters in this class can be used for human consumption after simple treatment, protection of aquatic communities, primary contact recreation, and irrigation of vegetables and fruits (Brasil, 2005). No correlation was observed between water quality parameters and the IDI as demonstrated by Pearson correlation analysis (Table S3). Only total alkalinity was negatively correlated with the IDI ($r2 = -0.32$, $p < 0.05$).

Riparian woody vegetation cover (Xcmgw) and substrate embeddedness (Xembed) were not significantly affected by anthropogenic impacts ($p > 0.05$) and were not correlated with the IDI (Table S4). However, the presence and proximity of anthropogenic impacts in the riparian zone (W1_Hall), which was a component of the IDI, was highly correlated with the IDI, indicating that IDI scores were driven by LDI (W1_Hall) scores.

The evaluation of land use and cover (catchment) types showed a high proportion (65.08%) of natural savanna vegetation in the basin, followed by pasture (33.44%), agriculture (1.43%), and urban (0.02%).

Least-disturbed sites selection

Based on the IDI, 7 stream sites were deemed highly altered, 26 sites were in intermediate condition, and 7 sites were considered least-disturbed (Figure 3). The classification of least-disturbed sites (IDI scores) was validated through the U-test (least-disturbed sites versus most-disturbed sites: $U = 0.00$; z adjusted = -3.09839; $p = 0.002$). EPT richness was significantly different between least- and most-disturbed sites ($U = 10.00$; z adjusted = 2.547529; $p = 0.012355$) and the organisms were significantly affected by catchment disturbance (IDI: $U = 0,00$; z adjusted = -3.46410; $p = 0.001$), and local disturbance (w1_Hall: $u = 0,00$; z adjusted = -3.46410; $p = 0.000532$). The key local disturbance metrics were pasture (45%), litter/garbage in the channel or channel margins (20%), and riparian agriculture (10%).

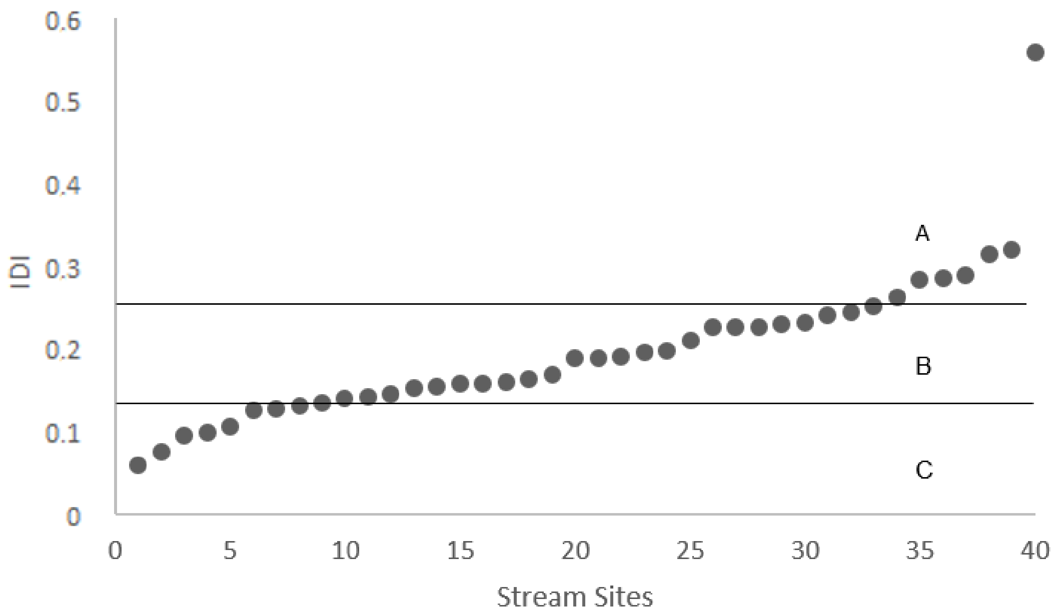

Figure 3 – Integrated Disturbance Index (IDI) values in stream sites in the Pandeiros River basin. IDI values range from 0 to 1, with 0 sites having the best environmental quality. Cut-off values for site classification: A) most-disturbed: IDI ≥ 0.26; B) intermediate: 0.13 ≤ IDI ≤ 0.25; C) least-disturbed: IDI ≤ 0.12.

Multimetric indices

Of the 17 MMIs evaluated, seven failed one or more of our validation steps (Table 2). In the evaluation of precision, five of the 17 indices showed a Coefficient of Variation above 15% among reference sites. None of the indices indicated natural variability bias because there was no correlation between the MMIs and the natural variation metrics. Only one index (Chen et al., 2014) indicated spatial autocorrelation in the index scores. We believe this resulted from the absence of a metric that is sensitive to differences among least disturbed sites. One index (Gabriels et al., 2010) responded weakly to disturbances and was eliminated. The remaining indices showed significant differences between the different disturbance classes defined for each index. The indices of Chen et al. (2014) and Lakew and Moog (2015) did not classify stream sites into quality classes (good, intermediate, poor) according to the scales defined by the authors, so it was not possible to perform the variance tests (Supplementary Material - Figure S1). Four indices (Couceiro et al., 2012; Gabriels et al., 2010; Lakew & Moog, 2015; Mereta et al., 2013) were insensitive to stressor or pressure variables because they violated normal and homoscedasticity assumptions and lacked residual normality. At the end of the five validation steps, ten of the 17 MMIs passed all tests and were correlated with various stressor and pressure variables (Table 3). Except for the Fierro et al (2018) MMI, all the index results were strongly or moderately correlated with each other when calculated through use of the Pandeiros data (Table 4).

Table 2 – Comparative values of index tests. (*significant result, F = Failed, P = Passed).

	Precision		Bias	Space Independence			Responsiveness		Sensitivity		
MMIs	CV			Moran-I	p		Anova (p)		R^2	p	Residual validation
Klemm et al. (2003)	19.01	F	No	0.02	0.83	P	<0.001*	P	0.28	0.004*	P
Ode et al. (2005)	7.25	P	No	-0.05	0.62	P	<0.001*	P	0.27	0.016*	P
Baptista et al. (2007)	4.36	P	No	-0.09	0.38	P	<0.001*	P	0.29	0.003*	P
Li et al. (2010)	0.93	P	No	-0.09	0.36	P	<0.001*	P	0.32	0.007*	P
Gabriels et al. (2010)	53.08	F	No	-0.11	0.3	P	0.71	F	0.08	0.752	F
Oliveira et al. (2011)	24.53	F	No	-0.05	0.72	P	<0.001*	P	0.38	0.001*	P
Ferreira et al. (2011)	6.32	P	No	-0.02	0.82	P	<0.001*	P	0.20	0.030*	P
Couceiro et al. (2012)	11.14	P	No	-0.15	0.12	P	<0.001*	P	0.17	0.006*	F
Jun et al. (2012)	10.39	P	No	-0.11	0.28	P	<0.001*	P	0.30	0.012*	P
Mereta et al. (2013)	7.55	P	No	-0.02	0.88	P	<0.001*	P	0.50	0.000*	F
Chen et al. (2014)	95.82	F	No	0.22	0.01*	F	-	F	0.31	0.002*	P
Nguyen et al. (2014)	7.00	P	No	-0.11	0.22	P	<0.001*	P	0.24	0.010*	P
Helson and Willians (2013)	13.39	P	No	-0.09	0.37	P	<0.001*	P	0.36	0.003*	P
Lakew and Moog (2015)	29.76	F	No	0.09	0.38	P	-	F	0.22	0.002*	F
Macedo et al. (2016)	8.10	P	No	-0.08	0.48	P	<0.001*	P	0.38	0.001*	P
Silva et al. (2017)	8.96	P	No	-0.08	0.41	P	<0.001*	P	0.25	0.004*	P
Fierro et al. (2018)	13.19	P	No	-0.1	0.35	P	<0.001*	P	0.37	0.003*	P

Table 3 – The MMIs selected, including their regressions with various stressors and pressures.

Index	r^2	Variables and β (std) values		
Macedo et al. (2016)	0.38	Nitrogen: -0.32	Natural (%): 0.57	Alkalinity: 0.47
Fierro et al. (2018)	0.37	Fines (%): 0.44	Pheophytin: 0.34	Urban (%): -0.32
Helson & Willians (2013)	0.36	Turbidity: -0.38	Natural (%): 0.44	pH: 0.31
Li et al. (2010)	0.32	Turbidity: -0.33	Natural (%): 0.34	Nitrogen: -0.34
Jun et al. (2012)	0.30	Nitrogen: -0.40	Natural (%): 0.41	pH: 0.34
Baptista et al. (2007)	0.29	Turbidity: -0.41	Natural (%): 0.33	
Ode et al. (2005)	0.27	Turbidity: -0.40	Natural (%): 0.36	
Silva et al. (2017)	0.25	Turbidity: -0.44		
Nguyen et al. (2014)	0.24	Turbidity: -0.29	Natural (%): 0.38	
Ferreira et al. (2011)	0.20	Natural (%): 0.42	pH: 0.38	

Table 4 – Pearson's correlation between the final indices that were validated by the tests. Significant correlations are marked with *.

	Fierro et al 2018	Macedo et al 2016	Jun et al 2012	Mereta et al 2013	Baptista et al 2007	Nguyen et al 2014	Li et al 2010	Ode et al 2005	Helson & Willians 2013	Ferreira et al 2011
Fierro et al 2018	1.00									
Macedo et al 2016	-0.07									
Jun et al 2012	0.03	0.79*								
Mereta et al 2013	0.20	0.56*	0.62*							
Baptista et al 2007	-0.05	0.61*	0.83*	0.42*						
Nguyen et al 2014	-0.07	0.77*	0.75*	0.59*	0.75*					
Li et al 2010	-0.05	0.78*	0.90*	0.53*	0.87*	0.85*				
Ode et al 2005	-0.03	0.63*	0.60*	0.41*	0.69*	0.83*	0.72*			
Helson & Willians 2013	-0.06	0.54*	0.78*	0.29	0.92*	0.70*	0.85*	0.62*		
Ferreira et al 2011	0.01	0.63*	0.86*	0.42*	0.87*	0.73*	0.85*	0.59*	0.90*	
Silva et al 2017	-0.14	0.67*	0.84*	0.44*	0.86*	0.78*	0.95*	0.68*	0.90*	0.86*

Discussion

From the total of seventeen indices tested, ten were considered effective for assessing the environmental condition of Pandeiros basin stream and river sites. That is, they were not influenced by natural variability, were spatiality independent, and had good responsiveness and sensitivity to identify anthropogenic disturbances. Those ten indices were effective in a basin with moderately altered environmental conditions (Callisto et al., 2019) and only local stressors, indicating that the indices were sensitive and responsive even to low disturbance gradients. The biological indicators (MMIs) and the disturbance indicators (IDI) indicated the mostly local (LDI, W1_Hall) effects of riparian pasture, trash, and agriculture.

Natural landcover predominates in the Pandeiros River basin, but locally significant anthropogenic impacts influenced environmental and biological quality at some sites (Figure S2). We found that the basin has higher quality environmental conditions when compared to other studies using the IDI (e.g. Fierro et al., 2018; Ligeiro et al., 2013; Macedo et al., 2016; Silva et al., 2017; Terra et al., 2013), reflecting the relatively good ecological condition and low environmental fragility in this basin (Callisto et al., 2019). Such protected areas are critical to limit anthropogenic pressures and the effects of local stressors (Barlow et al., 2018). As observed, human impacts in the basin are evidenced at local scales, that is, the protection status of the area is limiting large-extent anthropogenic pressures; however, it is not limiting local stressors. Other studies also have shown a decrease in biodiversity, even in protected areas (Hallmann et al., 2017; Sánchez-Bayo & Wyckhuys, 2019).

Benthic MMIs reflect anthropogenic disturbances, with decreasing scores as disturbance increases (Ruaro and Gubiani, 2013; Silva et al., 2017; Stoddard et al., 2008). We observed that natural variability among the evaluated sites was only relevant at the metric level, and after their correction, it was not relevant in the MMI scores that we calculated for the Pandeiros River basin, as reported in other studies (Fierro et al., 2018). Such corrections for natural variability increase the accuracy, responsiveness and sensitivity of MMIs, thereby improving their performance across large regions in China (Chen et al., 2014, 2019), Bolivia (Moya et al., 2011), the United States (Stoddard et al., 2008), and Brazil (de Carvalho et al., 2017; Macedo et al., 2016; Pereira et al., 2016; Silva et al., 2017).

Our validation steps offer useful insights for MMI development and testing. The indices were tested in an area covered by natural vegetation that has low to moderate human impact (Callisto et al., 2019), and this impact was evidenced only on a local scale. The indices tested under these conditions have some limitations as to their reproducibility; however, the ten indices that passed all validation stages proved to be extremely efficient for detecting even moderate and local impacts. In general, most indices were eliminated by lack of precision as indicated by high coefficients of variation in their reference areas, even when their metrics were corrected for natural variability. Therefore, we recommend considering reference area heterogeneity in an initial screening step (Martins et al., 2018) and correcting for it when appropriate (Fierro et al., 2018; Ruaro et al., in press). Some indices were eliminated because of the lack of normality of their residuals, meaning that they had a tendency for greater predictive error at low or high MMI scores. Because scores at those extremes are often deemed most important in local and regional risk assessments (Paulsen et al., 2008; Silva et al., 2017), residual evaluation can be a critical validation step. Except for the Lakew and Moog (2015) MMI, all indices that were eliminated were developed for tropical or temperate forests. However, other indices that were also developed in these biomes performed well in our analyses, suggesting that index construction or metric selection were more important factors than biome. Spatial autocorrelation was not a preponderant factor for index elimination, only one index was eliminated in this phase (Chen et al., 2014). The index elaborated by Chen et al. (2014) consists of 4 metrics that describe only richness and composition (Trichoptera_taxa richness, Ephemeroptera and Plecoptera taxa richness, Total_insect taxa richness, % Ephemeroptera, Plecoptera and Trichoptera individuals). Many authors recommend that indices be composed of metrics that represent the multiple biological aspects of an assemblage (Hering et al., 2006; Huang et al., 2015; Karr & Chu, 1999; Stoddard et al., 2008), such as richness, composition, diversity, dominance, tolerance, feeding groups, mobility and breathing types. An index composed of only four metrics focused on taxa richness is likely to be highly sensitive to natural taxa distributions, and therefore be spatially autocorrelated for purely natural reasons. The final MMI correlation analysis showed that the selected indices are moderately or highly correlated with each other, except for Fierro et al. (2018), presumably because that index is the only one that included a total macroinvertebrate density metric. Density and abundance metrics are not commonly used in MMIs because density and abundance vary greatly with location, season, collection method, and species counted (Aguiar et al., 2015; Hughes et al., 1998).

The tolerance of benthic organisms does not vary significantly between different regions and different climates (Jacobsen et al., 2008), which in part justifies the applicability of several indices to our study area. Therefore, any of the ten MMIs validated in this study are likely to be effective in discriminating most-disturbed stream sites from least-disturbed sites or reference conditions because of the integrated response of biological assemblage metrics (Hughes et al., 1998; Stoddard et al., 2008). The MMIs developed from several macroinvertebrate metrics that represent different assemblage structural and functional characteristics have better performance than MMIs that fail to do so (Hering et al., 2006; Silva et al., 2017; Stoddard et al., 2008). Thus, several dimensions of biological systems are incorporated into a single index, which increases their ability to reflect anthropogenic disturbances in aquatic ecosystems (Karr & Chu, 1999).

Among the metrics selected to calculate MMIs used in this study, taxonomic richness and sensitivity/tolerance metrics stand out. Taxonomic richness reflects a key component of taxonomic diversity (Baptista et al., 2007) and MMI construction (Ruaro et al., in press). The percentage and richness of Ephemeroptera, Plecoptera and Trichoptera (EPT) are widely used because these organisms are sensitive to various types of anthropogenic impacts (Ferreira et al., 2011; Firmiano et al., 2017; Klemm et al., 2003; Li et al., 2014; Macedo et al., 2016; Mereta et al., 2013; Pescador et al., 1995; Ruaro et al., in press; Silva et al., 2017; Stoddard et al., 2008).

Diversity indices, such as the Shannon-Wiener diversity index (Gabriels et al., 2010; Helson and Williams, 2013; Jun et al., 2012; Li et al., 2010; Oliveira et al., 2011; Silva et al., 2017) and the Margalef diversity index (Helson & Williams, 2013; Mereta et al., 2013; Nguyen et al., 2014) are also frequently used in MMIs. These indices integrate assemblage taxonomic richness and dominance or evenness.

Functional attributes or traits are also commonly used in MMIs (Chen et al., 2019; Moya et al., 2011; Saito et al., 2015; Silva et al., 2017; Stoddard et al., 2008) because of their ability to detect anthropogenic disturbances independently of taxonomic composition (Tomanova & Usseglio-Polatera, 2007).

In the United States, Europe and Australia, there is widespread use of biological indicators to assess continental-scale aquatic conditions (Barbour et al., 1999; Davies et al., 2010; Hering et al., 2006; USEPA, 2016) because of legal statutes. However, in most South American countries, there are no such statutes, which is reflected in the small number of studies on the development and application of MMIs for evaluation in national or continental programs (Buss et al., 2015; Ruaro and Gubiani, 2013). In Latin America, interest in developing and testing rapid assessment tools has increased over the past decade, but few studies have tested and standardized methods and indices that are central to the development of a systematic and effective biomonitoring program (Buss et al., 2015). However, multimetric indices can be used effectively to support environmental managers in national and continental water body monitoring programs (Hering et al., 2006; Moya et al., 2011; Pont et al., 2006; Ruaro and Gubiani, 2013; USEPA, 2016). Despite those international examples, Brazil still lacks a standardized national approach to evaluate and maintain the quality of its watersheds (Buss et al., 2015). Anthropogenic disturbances have become increasingly frequent in the Cerrado biome (Strassburg et al., 2017) and environmental catastrophes, such as mine tailings dam failures, have recently occurred (Silveira et al., 2019). Such chronic and acute pollution has considerably degraded the water quality of Cerrado river basins. Thus, it is necessary to apply fast and efficient tools, such as those developed in this study, for the monitoring and diagnosis of environmental quality in the Cerrado biome.

To be effective and used at national and continental levels, MMIs must use comparable metrics that represent key ecological parameters of aquatic assemblages (Stoddard et al., 2008; Ruaro and Gubiani, 2013; Ruaro et al., in press). However, in many aquatic ecosystem studies, metrics were adapted for specific regional conditions and are difficult to compare globally (e.g., Pond et al., 2013). This lack of standardization hinders using MMIs in water resource management (Ruaro & Gubiani, 2013). Other challenges to wide use of MMIs include the lack of standardized sampling, ignorance of all factors that may influence aquatic assemblages, and determination of sensitive metrics that are applicable at regional scales (Stoddard et al., 2008). Contrary to the implicit assumptions indicated by the many published MMIs (e.g., Ruaro and Gubiani, 2013), we found that several relatively simple existing indices, composed of a few metrics replicable in any region and easily calculated with a sufficiently robust data set, were effective for assessing stream sites at basin extents. However, this does not mean that any single MMI will suffice for all sites globally because we found several inappropriate MMIs for our study area. Nonetheless, we did find a set of metrics (taxa richness, diversity, sensitivity/tolerance, function) that should be considered for application and perhaps for moving towards a more standardized MMI that would facilitate national, continental, or global comparisons of site status and trends (Buss et al., 2015; Moya et al., 2011; Ruaro et al., in press; Stoddard et al., 2008). Furthermore, the correlations among MMIs (Table 4) indicate that those MMIs were consistent for assessing water body condition at the sampling sites, meaning that any one of those indices might suffice for a rapid assessment of water body condition. The selection and development of such biological indicators is very important for decision making (Nõges et al., 2009). If used in conjunction with other water body assessment and forecasting tools (Alizadeh et al., 2018; Chen and Chau, 2016; Hughes, 2019; Olyaie et al., 2015; Shamshirband et al., 2019), they can be used effectively as a basis for protecting and rehabilitating degraded environments (Statzner & Bêche, 2010).

Conclusions

This study indicated that ten indices, originally developed in multiple continents, were effective in evaluating ecological conditions in the Pandeiros River basin. Therefore, it is not necessary to elaborate new benthic MMIs for environmental quality assessments in each neotropical river basin. Instead, we recommend developing standard sampling and processing methods so that published indices can be used in national scale evaluations. In addition, this study offers an approach for standardizing and using MMIs in future evaluations of environmental quality in other neotropical basins. Lastly, even in protected areas, we observed that local disturbances degraded biological condition, indicating the importance of local actions for conserving and rehabilitating water resources in this and similar basins. As long as the particularities of our study area are observed, such as the presence of large areas of natural vegetation and mostly local anthropogenic impacts, our conclusions can be extended to other regions. In our case, there was a disturbance gradient, but on a local scale, because the study was conducted in a well-preserved area protected by national laws. For future applications of this metric testing approach, it would be useful to include more sampling sites, consider a wider diversity of river basins, and assess a stronger disturbance gradient in the Cerrado and multiple neotropical biomes by collaborating with other aquatic ecologists nationally and globally.

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Capítulo 2

Major risks to aquatic biotic condition in a Neotropical Savanna river basin

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Keywords:	conservation unit, macroinvertebrates, Cerrado, bioassessment, biotic indices, streams

Major risks to aquatic biotic condition in a Neotropical Savanna river basin

Isabela Martins, Diego Rodrigues Macedo, Robert M. Hughes, Marcos Callisto

Abstract

Conditions in freshwater ecosystems are responsible for maintaining biodiversity and other ecosystem services. Identifying and understanding how anthropogenic disturbances affect biotic condition are important steps in rehabilitating and protecting environmental quality. The Relative Risk (RR), Relative Extent (RE) and Attributable Risk (AR) approaches are used to determine ecosystem conditions in ecological monitoring programs conducted across large spatial extents. Our study was conducted in the Pandeiros River basin, which is a protected area in Minas Gerais, Brazil, that contains 233 km of mapped streams that were perennial and accessible. Field sampling was conducted in the dry period (April and June 2016) at 40 randomly selected sites. 10 Multimetric Indices (MMIs), previously determined to be sensitive in this river basin, were calculated. All the physical habitat disturbance metrics were significantly correlated with the multimetric indices. The risk of finding poor MMI scores was 1.6 to 1.7 times higher at sites with a high Integrated Disturbance Index (IDI) or Local Disturbance Index (LDI) score. Pasture was the most extensive disturbance, affecting 40.8% of the stream length, followed by 40.1% for low bed stability, 29% for fine substrates (< 16 mm), 24.4% for high IDI scores, and 21.7% for high LDI scores. This is useful to know for 5 reasons: 1) Standardized MMIs can assess environmental quality. 2) MMIs clarify that both catchment and local disturbances may represent serious risks to aquatic assemblages. 3) MMIs indicate which disturbances represent the most risk by comparing MMI scores against disturbance scores. 4) MMI risk assessments facilitate choosing the most appropriate mitigation actions. 5) Our results suggest environmental conservation actions for similar river basins.

Keywords: conservation unit, macroinvertebrates, Cerrado, bioassessment, biotic indices, streams

Introduction

The increased human demands for water affect the quality and availability of this resource and threaten aquatic biodiversity (Reid et al., 2018). Identifying the most threatening anthropogenic disturbances of these ecosystems and understanding how they affect biotic condition are important steps in improving environmental quality and proposing recovery options (Sánchez-Bayo & Wyckhuys, 2019; Feio et al., 2021).

The relative risk (RR), relative extent (RE) and attributable risk (AR) approaches are used by the United States Environmental Protection Agency (USEPA) to report regional and national aquatic ecosystem conditions in its national-extent biomonitoring and bioassessment program (USEPA, 2016a, b, c). They have also been implemented by some USA state biomonitoring programs (e.g., Merrick, 2015; Mulvey et al., 2009; Rowe et al., 2009) and in Brazilian research (Jiménez-Valencia et al., 2014; Silva et al., 2018). This approach is based on its ability to provide quantifiable associations between major anthropogenic disturbance metrics and biological responses (Paulsen et al., 2008). The RE provides the magnitude in which high disturbance levels occur in a region or basin. The RR describes the probability of good vs. poor biological condition, given the presence/absence of low vs. high disturbance levels. The AR is the percentage reduction in the regional extent (RE) of a poor biological condition, if the stressor is eliminated (Van Sickle & Paulsen, 2008). By implementing a probabilistic survey design for site selection, results can be statistically inferred from a relatively small set of sampled sites to an entire channel network across a basin, region or nation (Van Sickle & Paulsen, 2008). This is important because it ensures representation across the entire studied area, allowing the physical, chemical and biological characteristics of the sampled sites to reflect the ecological conditions of the region or basin as a whole (Herlihy et al., 2008, 2020; Mulvey et al., 2009; Silva et al., 2018). Also, probability survey designs have three other advantages: 1) They are economical because they allow accurate and reliable inferences to the ecological condition of large areas based on a minimum number of probabilistic sampled sites (Paulsen et al., 2008). 2) They allow rigorous statistical estimation of the channel length of the entire river basin with known confidence limits (Herlihy et al., 2000). 3) Because they are random approaches, sites are not selected for convenience, thereby avoiding biased conclusions in ecological assessment studies, including in studies across difficult and unroaded subtropical and subarctic terrains (Hughes; et al., 2020; Jiménez-Valencia et al., 2014).

Relative risk and extent approaches have included metrics that quantify instream and riparian physical characteristics (Kaufmann et al., 1999). In general, physical habitat includes all structural attributes that influence or enable the maintenance of organisms in an aquatic ecosystem (Peck et al., 2006). The decrease in physical habitat diversity can lead to the simplification of biological communities, therefore, its assessment is of fundamental importance for assessing ecological conditions (Barbour et al., 1999).

In addition to physical habitat assessment, it is essential to evaluate the biological condition of entire aquatic ecosystems. The Index of Biotic Integrity approach proposed by Karr (1981), has been widely used for water quality assessment (Ruaro et al., 2020). Multimetric Indices (MMIs), which are variants of Karr's index, are composed of a combination of various biological attributes or metrics that reflect anthropogenic disturbances along a gradient of environmental disturbance (Karr et al., 1986). Macroinvertebrate assemblages are commonly and effectively used in environmental monitoring programs globally (Buss et al., 2015; Feio et al., 2021) because they respond to environmental conditions and integrate physical, chemical, and biological aspects of ecosystems (Bonada et al., 2006).

Because of their usefulness in assessing environmental quality, over 400 MMIs have been developed globally (Ruaro et al., 2020). However, such a diversity of indices hinders making regional, let alone global, comparisons and condition assessments across the various studies (Buss et al., 2015). Therefore, Martins et al. (2020) assessed the efficacy of MMIs developed in different regions and continents in the Pandeiros River basin and showed that 10 MMIs passed all validation stages and were extremely effective for assessing anthropogenic impacts. However, a knowledge gap remained regarding their applicability, combined with a probabilistic survey, in environmental diagnostic studies. Therefore, we sought to assess the relative risk of various types of anthropogenic disturbances on those 10 MMI scores in a river and its tributaries in a priority area for biodiversity conservation. The results of such studies can be used to inform managers of conservation units and agencies responsible for conserving aquatic ecosystems.

Materials and Methods

Study Area

The study area included the entire 3,960 km^2 Pandeiros River basin. The basin is located in Minas Gerais state, Brazil, in the Cerrado (Neotropical Savanna) biome (Figure 1). The basin is considered an area of Special Biological Importance because it is a unique environment (Azevedo et al., 2009), having flooded regions (wetland and marginal lagoon complexes) and palm swamps. Both are among the international priority areas for biome conservation. Most of the basin area (85.7%) is part of the Rio Pandeiros State Environmental Protection Area (APAERP) (IEF, 2019). The region's climate is tropical, with an April-September dry season (Aw climate; Alvares et al., 2013), so the perennial flow regime of most Pandeiros tributaries is of great importance to guarantee water supply for local human populations. However, most 1st and 2nd order streams mapped at 1:100,000 scale in the basin were dry or inaccessible (Figure 1).

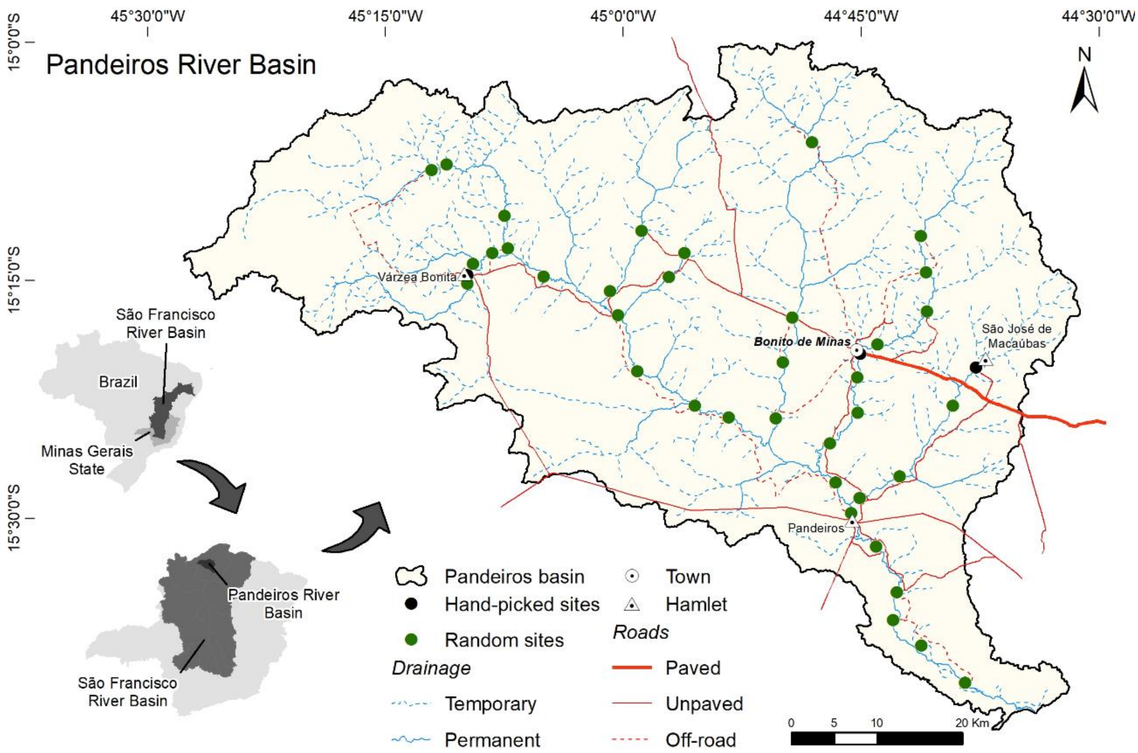

FIGURE 1. Location of sample sites in the Pandeiros River basin, Minas Gerais

Survey design and sampling

Stream and river sites were selected through use of spatially balanced procedures employing a random and systematic survey design, following the method used by the USEPA in its National Rivers and Streams Survey (Olsen & Peck, 2008) adapted for Cerrado aquatic ecosystems (Callisto et al., 2014). To ensure a gradient of ecological conditions, some presumably degraded sites were handpicked (Whittier et al., 2007). A random set of 40 potential sampling sites and an additional set of substitute sites were selected to ensure that we had a final set of 40 because we assumed that some sites initially selected would be dry, inaccessible, or have access denied (Macedo et al., 2014). Each site was at least 1 km from any other to minimize spatial autocorrelation and it received a weight, proportional to the inverse of its selection probability. That probability is the length of the entire channel network that represents the entire target population i.e., the entire stream length in that stream order. We used those weights to balance the number of sites across third, fourth and fifth order streams and rivers to ensure that most sites were not in the more abundant lower-order streams. The weights were also used to estimate the extent of the stream environmental and biological conditions and their relative risks to the biota (Van Sickle et al., 2006). The sites chosen manually were not used to make extent estimates because they had zero weights. But both probabilistic and handpicked sites were considered for establishing thresholds for metrics and MMIs (Van Sickle et al., 2006). To confirm the set of sampling sites (target length), field reconnaissance was required. In this phase, sites were checked for access and flows; those that were not sampled for any reason (dry, non-wadeable, access denied, etc.) were substituted for by sites having the same weights (Silva et al., 2018).

During April and June 2016, we sampled 15 3rd order sites, 13 4th order sites and 12 5th order sites for a distance equal to 40 times their mean wetted width, with a minimum length of 150 meters (Hughes & Peck, 2008). In each site, 11 transverse transects (perpendicular to the stream flow) were established defining 10 sections, where physical habitat structure and biota were sampled (Peck et al., 2006; USEPA, 2016b). For details on sampling, identification, calculation of indices and results see Martins et al., 2020.

Anthropogenic disturbances

The quantification of types of land use and cover was carried out using supervised classification of digital images, whereby classes were assigned to pixels of satellite images, creating homogeneous patterns to which different classes of land use and cover are associated (Santos et al., 2017). We used 2016 imagery from the Landsat-8 satellite, sensor OLI (30-m spatial resolution), orbit scene 219/71 and 219/70 made available by INPE (Instituto Nacional de Pesquisas Espaciais, 2016). The anthropogenic land use classes included human settlements, row crop agriculture, and pasture that were calculated as the percent of each class in the total catchment, as described in Callisto et al. (2014).

We selected disturbance variables based on the results from other studies in Brazil (Jiménez-Valencia et al., 2014; Macedo et al., 2016; Martins et al., 2020; Silva et al., 2018). We used the concept of least-disturbed or minimally disturbed (Martins et al., 2018; Stoddard et al., 2006) because there were no pristine sites in the basin. To identify these sites, we used IDI (Integrated Disturbance Index) scores, which were calculated from local anthropogenic disturbances (LDI - Local Disturbance Index) and total catchment disturbances (CDI - Catchment Disturbance Index) for each site (Ligeiro et al., 2013).

We measured 3 additional disturbance indicators: 1) Percent fine substrates (< 16 mm) included fine gravel, sand, silt, and clay. 2) Bed stability estimated the relationship between the average geometric diameter of the bed substrate and the critical theoretical diameter that the flow and channel might support, indicating % excess fine sediment. 3) Percent pasture in the total catchment, the dominant land use in the Pandeiros basin, was determined from satellite images (Macedo et al., 2014).

Selection of multimetric indices

Ten different MMIs (Table 1) had been tested and validated to be effective in assessing biological quality in the Pandeiros River basin (Martins et al., 2020). Each MMI was built following the original procedures described in its publication; their metrics are described in Appendix 1. To standardize and classify the indices used in this study, we defined thresholds by anthropogenic disturbances based on the distribution of each of the 10 MMI scores in the least-disturbed sites. Seven least-disturbed sites were classified according to their IDI values (see below), as described in Martins et al. (2020) and were the same sites for all MMIs. Each MMI had a different range of values, so we scored each one as: MMI scores < 5th percentile of the IDI distribution of reference sites equaled poor and MMI scores > 25th percentile equaled good (Table 1). Sites classified as fair were combined with poor sites to create a not-good class for subsequent risk analyses (Silva et al., 2017).

TABLE 1. Multimeric Indices (MMIs) used, references, development locations, and threshold values for biological condition classification in the Pandeiros River basin.

MMI	Reference	Location	Poor	Good
MMI_Baptista	Baptista et al. (2007)	Brazil – Atlantic Forest	< 20.1	> 27
MMI_Ferreira	Ferreira et al. (2011)	Brazil - Cerrado	< 22.7	> 25.5
MMI_Macedo	Macedo et al. (2016)	Brazil - Cerrado	< 50	> 59
MMI_Silva	Silva et al. (2017)	Brazil - Cerrado	< 56	> 70
MMI_Helson	Helson and Williams (2013)	Panamá – Rainforest	< 5.04	> 7.08
MMI_Fierro	Fierro et al. (2018)	Chile - Mediterranean Shrub	< 3.33	> 3.98
MMI_Ode	Ode et al. (2005)	USA – Mediterranean Shrub	< 66.78	> 68.64
MMI_Li	Li et al. (2010)	China – Rainforest	< 5.25	> 6.31
MMI_Nguyen	Nguyen et al. (2014)	Vietnam – Rainforest	< 0.55	> 0.58
MMI_Jun	Jun et al. (2012)	South Korea – Temperate Broadleaf Forest	< 29.5	> 36

Anthropogenic disturbance thresholds

Disturbance thresholds are generally based on regional distributions of values observed in least-disturbed sites (Herlihy et al., 2020; Kaufmann et al., In Review). Using an approach similar to that used for biological condition, we defined sites with fine substrates, % total catchment pasture, and IDI having > 75th percentile of the distribution in least-disturbed sites as being in not-good condition. Sites with percentages < 75th percentile were considered as being in good condition (Van Sickle and Paulsen, 2008). The LDI sites with values < 1 were classified as good and those > 1 were classified as not-good (Silva et al., 2018). The substrate stability thresholds were based on their score distributions, with values less than -1.5 being classified as not-good, and higher values considered as good (Table 2) (Kaufmann et al., 2009).

TABLE 2. Disturbance metric thresholds.

	good	not good
% Fines (substrates < 16 mm)	< 93	> 93
Local Disturbance Index (LDI)	< 1	> 1
Bed Stability	> -1.5	< -1.5
% Pasture (in the total watershed)	< 40%	> 40%
Integrated Disturbance Index (IDI)	< 0.23	> 0.23

Relative Extent (RE), Relative Risk (RR) and Attributable Risk (AR) Analyses

Relative extent (RE) measures extents across a study area, as represented by the stream length and proportion with high disturbance scores of each predictor variable used. Proportions are obtained as a sum of the sample weights of the sites found with high disturbance scores divided by the sum of all the weights of the sites (expressed in % channel length) (Van Sickle & Paulsen, 2008).

Relative risk (RR) was used to assess the severity of the disturbances previously selected to affect MMI scores (for each of the 10 MMIs used) and the relative extent (RE) of those disturbances (Van Sickle & Paulsen, 2008). Relative risk was based on conditional probability obtained from a 2 x 2 contingency table, in which all possible situations of having a good or not-good MMI condition were obtained, given a site's high or low disturbance value. The analysis uses the concept of conditional probability to measure relative risk and is calculated as:

$$RR = \frac{\Pr\,(MMIp|ng)}{\Pr\,(MMIp|g)} \tag{1}$$

the numerator is the probability of finding poor biological condition (MMIp) at a site where the disturbance indicates not-good environmental condition (ng). The denominator is the probability of finding poor biological condition at a site, where the disturbance indicates good environmental condition (g). A RR equal to or < 1 indicates the absence of an association between the biological indicator and the disturbance. For an RR > 1, we interpreted the value as how many times more likely a not-good MMI condition would occur, given the high disturbance compared to the low disturbance level. We calculated 95% confidence intervals for RR estimates; for the RR to be significant, the lower bound of the 95% confidence interval of RR must also have been > 1.

The attributable risk (AR) is expressed as a combination of a RE and its RR. If AR ≠ 0, any increase in a stressor RE or in its RR will also increase its AR. Conversely, decreases in RE or RR will decrease AR. We report 100 x AR as the % reduction that could be achieved by eliminating a stressor (Van Sickle & Paulsen, 2008). The RR and RE confidence intervals were obtained using R statistical software version 2.2.1 (R Development Core Team, 2005) and the R spsurvey package (version 2.9).

Results

The total mapped perennial stream length in the Pandeiros River basin is 431 km, from this, 233 km (C.I.= 3.04km) (or 54% of the total length) constituted the target length, defined as perennial, accessible, 3rd to 5rd order and with flowing water. A total of 84 sites were visited, 40 were sampled and 44 were not. The main reasons sites were not sampled were lack of access (34%), including absence of owner, locked gates, GPS error, or inaccessible roads for our vehicles or by walking. Dry 3rd order sites mapped as permanent represented 12% of the total. The number of sites classified by IDI as good, fair and poor was 7, 26 and 7, respectively.

Regarding relative extent (RE), percent pasture is the most widespread disturbance in the basin, present in 40.8% of the target stream length, followed by low streambed stability, present in 40.1% of the target stream length. Percent fine substrates (< 16 mm) were found in 29%, high IDI scores in 24.4%, and high LDI in 21.7% of the target stream length in the Pandeiros River basin (Figure 2).

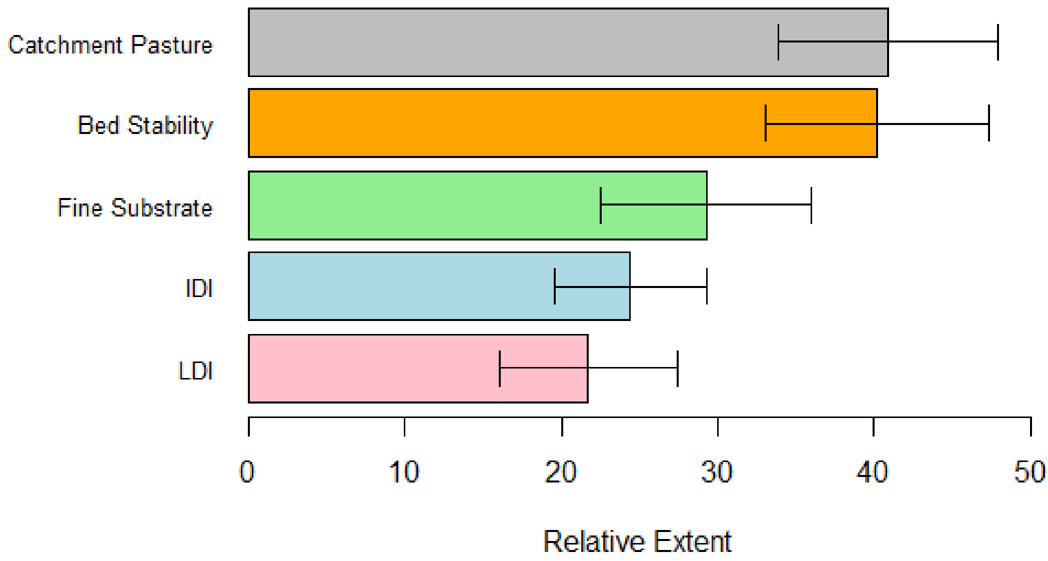

FIGURE 2. Relative extent of disturbances (with 95% confidence intervals) in the Pandeiros River basin.

The disturbance metrics evaluated (LDI, IDI, % fine substrate and % pasture) were associated (RR > 1 and lower bound of CI >1) with two or more of the MMIs (Table 3). For example, high LDI and IDI scores were the greatest risks for low MMI_Macedo and MMI_Silva scores (Table 3). In other words, the risk of finding a poor MMI score with a high IDI or LDI score was 1.6-1.7 times higher than for sites where the IDI or LDI did not exceed thresholds. The risk of finding low MMI_Silva and MMI_Ode scores in the presence of high % fines was 1.2 - 1.6 times higher. In the presence of pasture, the risk was 1.2 - 1.6 higher for finding low scores from the indexes of MMI_Jun, MMI_Baptista and MMI_Silva (Figure 3).

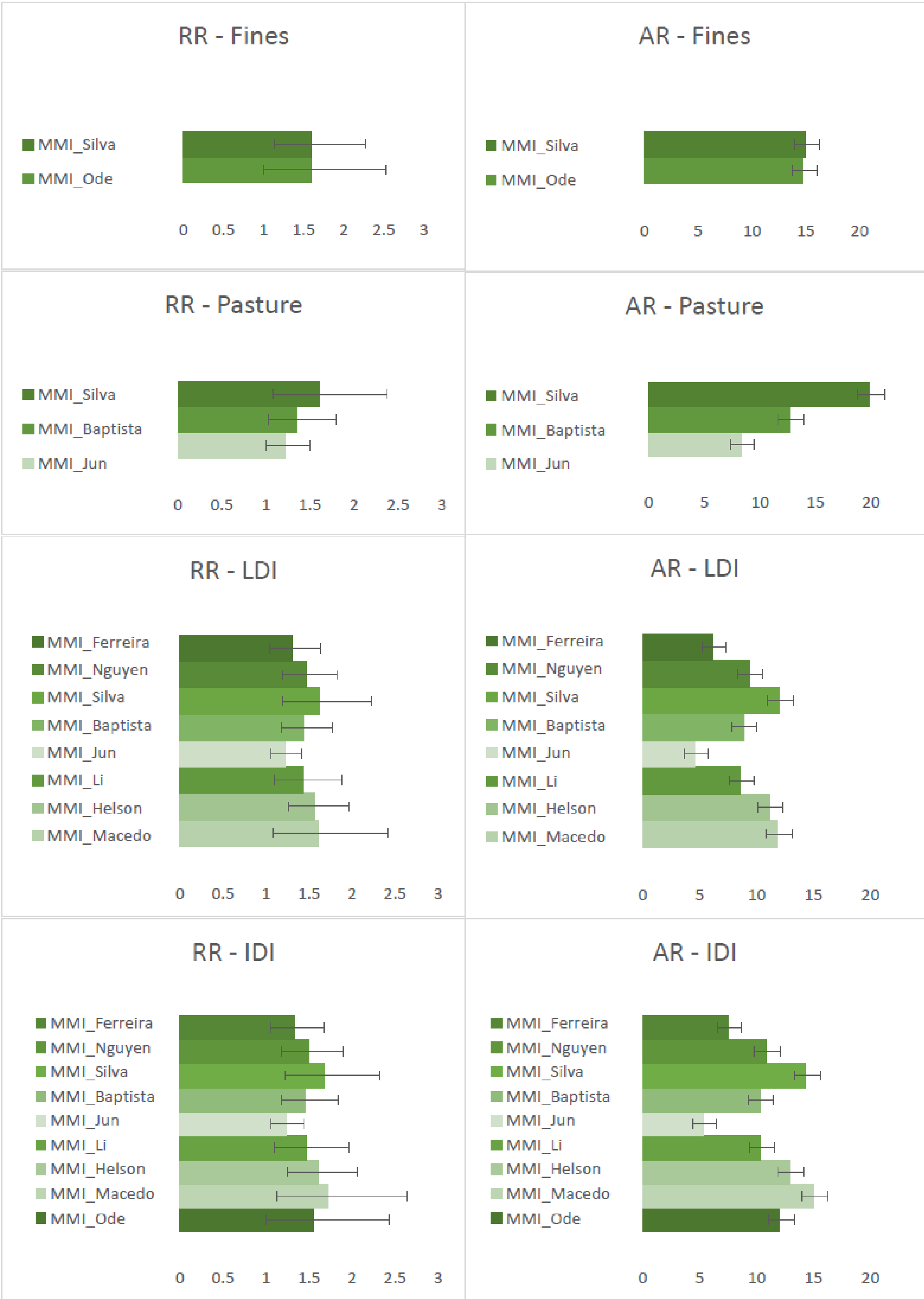

FIGURE 3. Relative Risk (RR), 95% Lower Confidence Intervals (LCI) of the indices (MMI) that are significantly related to the disturbances assessed in the Pandeiros River basin and their respective Attributable Risks and Confidence Intervals.

Attributable Risk assessments offer insights into possible cost-effective management options (Table 3, Figure 3). Elimination of excessive levels of fine substrate could decrease the risk of low MMI scores by 14% (MMI_Ode, MMI_Silva). Elimination of the pasture pressure would result in 19%, 12% or 8% decrease in the risk of finding low MMI_Silva, MMI_Baptista and MMI_Jun scores, respectively. Eliminating local riparian disturbances (LDI) could allow decreases of 4.5-12% in the risk of finding low scores for the significant indices. Similarly, eliminating catchment plus local disturbances (IDI) could allow decreases of 5 to 15% in the risk of finding low scores. Only IDI and LDI (0.91) and % fines and bed stability (-0.52) were highly or moderately correlated (Appendix 2).

TABLE 3. Values of Relative Risk (RR), 95% Lower Confidence Intervals (LCI) for each calculated multimetric index (MMI). RR values > 1 and 95% LCIs > 1 represent a negative influence of the disturbance on an MMI score (bold).

	% Fine Substrate		LDI		Bed Stability		% Pasture		IDI	
	RR	LCI	RR	LCI	RR	LCI	RR	LCI	RR	LCI
MMI_Macedo	0.88	0.5	**1.61**	**1.08**	0.72	0.19	0.99	0.57	**1.72**	**1.12**
MMI_Fierro	0	n.a.	0	n.a.	1.12	0.19	1.91	0.34	0	n.a.
MMI_Helson	1.10	0.75	**1.57**	**1.26**	0.79	0.55	1.08	0.79	**1.60**	**1.25**
MMI_Li	1.11	0.76	**1.43**	**1.09**	0.73	0.48	1.27	0.90	**1.47**	**1.10**
MMI_Jun	1.04	0.81	**1.22**	**1.05**	0.91	0.71	**1.22**	**1.00**	**1.23**	**1.05**
MMI_Baptista	1.24	0.93	**1.44**	**1.17**	0.81	0.57	**1.35**	**1.02**	**1.46**	**1.17**
MMI_Ode	**1.59**	**1.00**	1.44	0.92	1.09	0.68	0.84	0.52	**1.55**	**1.00**
MMI_Silva	**1.60**	**1.13**	**1.62**	**1.18**	1.04	0.70	**1.60**	**1.08**	**1.68**	**1.21**
MMI_Nguyen	1.27	0.94	**1.47**	**1.19**	0.83	0.59	1.21	0.88	**1.49**	**1.18**
MMI_Ferreira	1.24	0.93	**1.30**	**1.03**	1.02	0.75	1.08	0.82	**1.33**	**1.05**

Discussion

The 10 MMIs, combined with a probabilistic survey allowed us to assess ecological condition in an environmental protection area and to estimate the risks of each disturbance contributing to poor MMI scores. The disturbances (% catchment pasture, IDI, LDI, streambed stability, % fine substrate) that we evaluated occurred in 20-40% of the target stream length, with the presence of anthropogenic disturbances

(IDI, LDI and pasture) being the most important threats to poor biological condition. Nonetheless, our results were limited by the 40 sample sites, which can create statistically unstable RR estimates (Van Sickle & Paulsen, 2008).

In the presence of large amounts of fine substrate, the risk for poor MMI scores was 1.59 (MMI_Ode) to 1.60 (MMI_Silva) times greater. And this disturbance was present in 29% of the target stream length. In aquatic ecosystems, the presence of fine substrates in stream beds is one of the most important threats to their ecological condition (Burdon et al., 2013). This is because fine sediments reduce habitat availability for macroinvertebrate assemblages, directly compromising their structure, composition, and function (Beermann et al., 2018).

Anthropogenic disturbances in the riparian zone (LDI) represented a risk for poor MMI scores that varied from 1.22 (MMI_Jun), to 1.62 (MMI_Silva) and represented 21.7% of the target stream length. This type of disturbance, although local, can alter habitats and biota (Kaufmann & Hughes, 2006; Kaufmann et al., In Review). Changes in soil conditions, vegetation, and other factors directly reflect the aquatic-terrestrial interactions (Naiman et al., 2000) and meta-ecosystem services (Callisto et al., 2019). In other studies, macroinvertebrate abundance was predominantly affected by local land use (Allan, 2004) and only 1.4-6.5% reduction in riparian vegetation coverage was associated with the loss of sensitive macroinvertebrate species (Brito et al., 2020; Dala-Corte et al., 2020).

Low bed stability represented a risk of producing poor MMI scores that varied from 0.72 (MMI_Macedo), to 1.12 (MMI_Fierro) and represented 40.1% of the target stream length. However, the lower confidence intervals indicated a statistically insignificant effect on MMI scores. Lower streambed stability values suggest that there are ongoing landscape or channel erosion processes (Kaufmann et al., 2009). Other studies have found that this process is intensified by reduced vegetation cover resulting from agricultural activities (de Castro et al., 2017; Leal et al., 2018; Leitão et al., 2018).

The most extensive land use impact was % total catchment pasture, representing 40.8% of the target stream length and a risk for poor MMI scores varying from 1.60 (MMI_Silva), 1.35 (MMI_Baptista) and 1.22 (MMI_Jun). Diffuse disturbances, such as pasture, contribute to excess fine sediments, nutrients, and pollutants in freshwater ecosystems (Allan, 2004; Hughes et al., 2019). As the extent of cattle grazing increases in river basins, there is an increase in pollutants and sediments, as well as channel degradation, which affects the habitat available to organisms (Beschta et al., 2013), particularly when that grazing occurs in riparian zones (Kauffman et al., 1997).

In the presence of high levels of anthropogenic disturbances measured by the IDI, the risk for poor MMI scores varied between 1.23 (MMI_Jun), to 1.72 (MMI_Macedo). Considering that poor IDI condition is present in 24.4% of the target stream length, it is a considerable concern for the management and conservation of biological condition. High IDI values were also associated with increased risk of biological changes related to human activities in other Cerrado streams (Ligeiro et al., 2013). Bed instability and excess fine sediments are associated with disturbances in the catchment and riparian zone, thereby reducing MMI scores and sensitive taxa (Brito et al., 2020; Dala-Corte et al., 2020).

In all field studies based on correlative relationships, one can ask whether the observed relationships are truly causal, only correlated by chance, or driven by some unmeasured variable. This is particularly a concern when the relative risk values are only slightly greater than one, when relatively small proportions of observed biological variability are explained by the study variables, and if the sample size is relatively small (40 sites in our case). In these cases, it is useful to use a weight-of-evidence approach based on six factors for supporting conclusions (Kaufmann et al., 1992; Kaufmann & Hughes, 2006).

1) Is there a clear scientific mechanism for the relationship? We found that landscape pressures (as measured by increased IDI and LDI scores) were associated with increased % fine substrate at the sites as well as the condition of the macroinvertebrate assemblages (measured by several MMIs) at those sites. The four most sensitive MMIs to our disturbance measures all include a taxa richness or diversity metric as well as an EPT (Ephemeroptera, Trichoptera, Plecoptera) metric (Martins et al., 2020). Those metrics are also the most commonly used macroinvertebrate MMI metrics globally (Ruaro et al., 2020). In other words, what humans do to the land produces stressors (fine

sediments) that negatively affect the benthic macroinvertebrates living on stream bottoms.

2) What is the statistical rigor of the study design? We used a probability survey to ensure our sites were statistically representative of an entire river basin. This is a much more rigorous study design than ad hoc site selection, which tends to be biased by convenience or ease of sampling, or a disturbance gradient design, which is biased along a single presumed disturbance (Stevens & Olsen, 2004).

3) What is the statistical strength of the observed relative risk associations? Those associations and their confidence intervals were only slightly above one in our study (Table 3). However, this is not unusual for similar field studies conducted in the USA and Brazil. USEPA (2020) reported relative risk scores of 1.4-1.8 for four measures of physical habitat structure, yet that study was based on data from 1,853 sites. Studies employing probability designs with far fewer sites (20-190) in Brazil reported relative risk scores of 1.9-2.5 for watershed and riparian disturbance (Jiménez-Valencia et al., 2014; Silva et al., 2018). Those Brazilian scores reflected much stronger landscape disturbance gradients than were observed in our study and therefore had much stronger relative risk scores.

4) What alternative or unmeasured explanations might exist for explaining the study relationships? Frequently, unmeasured disturbances or substantial and unmeasured natural gradients, such as channel slope, lithology, or climate confound observed disturbance-biology patterns (Macedo et al., 2014; Silva et al., 2017; Stoddard et al., 2008). We are aware of no other anthropogenic disturbances in the minimally disturbed Pandeiros basin nor are there any large natural background gradients (Azevedo et al., 2009).

5) To what degree does one study agree with or contradict similar studies? Our results conform with a large body of evidence indicating that land uses that remove the natural vegetation of catchments and riparian zones lead to increased stream sedimentation and degradation of benthic macroinvertebrate assemblages (Allan, 2004; Beschta et al., 2013; Callisto et al., 2019; Herlihy et al., 2020; Hughes, 2019; Kaufmann et al., 2009; Wood & Armitage, 1997).

6) Lastly, is there any evidence that mitigating a major disturbance can reduce its impact on ecosystems? In this case, improved management of livestock grazing or pasturing does reduce stream sedimentation and improve the condition of benthic macroinvertebrates (Agouridis et al., 2005; Quinn et al., 2009; Weigel et al., 2000). The attributable risk (AR) results in our study indicate that decreasing the disturbances that we measured could reduce the risks of finding low MMI scores by up to 19%.

The identification of disturbances that represent the greatest risk to biological condition is essential for assessment and management purposes, especially in protected areas worldwide. However, our study basin has low levels of anthropogenic disturbances (Callisto et al., 2019) compared with those in other Cerrado hydrologic units that have been studied (e.g., Ligeiro et al., 2013; Macedo et al., 2016; Silva et al., 2017). Protected areas, such as the Rio Pandeiros State Environmental Protection Area (APAERP), are of fundamental importance to limit anthropogenic disturbances (Barlow et al., 2018; Leal et al., 2020), thereby maintaining basin environmental quality.

Composite measures, such as MMIs, are useful for detecting the overall degradation of aquatic ecosystems. MMIs combined with probabilistic analyses of relative risk and relative extent are important tools for decision making (Nõges et al., 2009) and for implementing more cost-effective measures for protecting high-quality systems and rehabilitating degraded ecosystems (Statzner & Bêche, 2010). Probabilistic studies like ours can help policymakers and managers identify important local and regional disturbances and estimate the possible benefits of their remediation (Van Sickle & Paulsen, 2008). In protected areas, still influenced by human activities, local impacts can still affect overall basin environmental quality and consequently its biological condition (Barlow et al., 2018). Creating new protected areas and improving existing ones should be a priority for any strategy for conserving tropical aquatic ecosystems (Sundar et al., 2020).

The results of our study also support the use of standard sampling methods and MMIs in neotropical environmental quality assessments because all 10 MMIs had similar responses to a set of five common disturbance metrics. They also indicated which disturbances were associated with the most risk to poor MMI scores, which disturbances when eliminated would most decrease risk, and thereby those that should be primarily monitored and mitigated. Our results indicate that managers in the Pandeiros basin (and other Cerrado basins) should focus on reducing erosion and sedimentation through better livestock management across the river basin, but particularly in riparian zones.

Conclusions

We found that it was possible to identify the main disturbances associated with the poor biological conditions present and to assess the extent, relative risks and attributable risks of those disturbances. The Pandeiros River basin is an important tributary in the São Francisco River basin. Therefore, it is necessary to focus river rehabilitation efforts on reducing key landscape disturbances that generate risks to losing good biological condition. Our scientific information has been presented to the pertinent state and national environmental agencies, electrical company, riverine citizens, and members of the river basin committee to support them in rehabilitation efforts. Improved pasture management, avoiding erosion, and reduced siltation of river courses are key priorities for better freshwater ecological condition in the entire river basin. This joint management effort offers an example for other tropical river basins globally.

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Capítulo 3

Anthropogenic impacts influence the functional traits of Chironomidae assemblages in a neotropical savanna river basin

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Abstract

Increased demands for water affect its quality and availability and threaten biodiversity. In freshwaters, the Chironomidae represent ~50% of macroinvertebrate individuals and have great potential to improve ecological assessment tools. Incorporating trait-based approaches in those tools can further improve how we assess the effects of human disturbances on aquatic macroinvertebrate assemblages. Given that chironomid genera have different degrees of sensitivity to anthropogenic disturbances, we expected that composition, structure, and functional characteristics of chironomid genera would be affected by anthropogenic disturbances in a neotropical savanna river basin. We used nine traits in 32 categories related to Chironomidae functional roles. Out of 6147 individuals distributed in three subfamilies, we identified 52 chironomid genera collected from 30 randomly selected stream sites. The index of functional divergence was lower in places with greater anthropogenic disturbance of riparian vegetation. A RLQ matrix analysis revealed a significant relationship between genera abundance and environmental variables as well as with biological traits. We observed a positive relationship between Tanypodinae, which are mainly engulfer predators, with average embeddedness, % sand, and catchment pasture. Three Chironomidae genera (*Stenochironomus, Endotribelos, Beardius*) were positively related to miner habit, herbivore feeding strategy and larger body size. We found that physical habitat structure and food resources were the most important factors structuring Chironomidae assemblages in the study sites and that chironomid genera were effective for assessing basin ecological status.

Key Words: traits, macroinvertebrates, monitoring, ecological assessment, Cerrado, bioindicators

Introduction

Rivers are important because they provide ecosystem services, such as water supply for domestic, industrial, and agricultural use, power generation, navigation and recreation (Callisto et al. 2019b). In addition, they are home to a great diversity of species (Strayer and Dudgeon 2010). Increasing demands for water affects its quality and availability and threatens aquatic biodiversity (Gangloff et al. 2016; Reid et al. 2018). Important steps for preserving water quality and maintaining biodiversity are identifying human pressures and stressors and understanding how they affect biological condition (Sánchez-Bayo and Wyckhuys 2019). In some regions, this becomes particularly important, such as in the Neotropical Savanna (Cerrado). Although this biome is home to important springs and hydrographic basins, housing high biodiversity and endemism and covering 2 million km2, it is one of the most threatened biomes in South America (Strassburg et al. 2017; Latrubesse et al. 2019).

Simplification of aquatic habitats resulting from degradation processes alters the structure of aquatic communities (Collen et al. 2014; Agra et al. 2021). Benthic macroinvertebrate assemblages respond to environmental changes resulting from anthropogenic activities, which is why they are commonly used in aquatic environmental assessment studies (Karr and Chu 1999; Ruaro et al. 2020). These organisms exhibit preferences regarding food acquisition and type, physical habitat preferences and water quality (Ferreira et al. 2015) and they respond to aquatic ecosystem disturbances through changes in their structure, composition, and function. Those characteristics give biological indicators an advantage over traditional water quality assessments, which do not detect the effects of altered flow regimes and physical habitats (Karr 1981). Studies that use biological indicators and assessments of site and landscape variables have been more robust and have better responses (Roque et al. 2010; Herlihy et al. 2020).

In freshwaters, the Chironomidae represent ~50% of macroinvertebrate assemblage individuals (Serra et al. 2016) and have great value as bioindicators because they are widely distributed, taxonomically and functionally diverse, and responsive to environmental changes (Rosenberg 1992; Puntí et al. 2009; Nicacio and Juen 2015). Chironomids play fundamental roles in processing organic matter, scraping leaf detritus

(Callisto et al. 2007), consuming fine particles of organic matter (Callisto and Graça 2013), and transferring energy and nutrients to the invertebrates, fish and birds that prey upon them (Serra et al. 2016). Despite their ecological importance and diversity in most freshwater ecosystems (Nicacio and Juen 2015), chironomid larvae are usually only identified to family or subfamily in ecological studies (Poff et al. 2006). However, if identified to genus, they have the potential to improve the signals provided in ecological assessments (Morais et al. 2010; Serra et al. 2016). Although chironomids have great potential as bioindicators, their functional responses remain little explored in the Neotropics (Gomes et al. 2018; Saulino and Trivinho-Strixino 2018a.,b; Jovem-Azevêdo et al. 2019; Pereira et al. 2020). This is because their identification to genus is difficult and time-consuming, especially in tropical aquatic ecosystems (Rosenberg 1992; Roque et al. 2010).

Trait-based approaches have been successfully used to assess the effects of anthropogenic disturbances on aquatic macroinvertebrate assemblages (Dolédec and Statzner 2010; Kuzmanovic et al. 2017; Castro et al. 2018; Firmiano et al. 2021), including some focused on Chironomidae assemblages (Serra et al. 2016, 2017; Jovem-Azevêdo et al. 2019). Traits are generally defined as any measurable characteristics at the individual level that directly or indirectly affect general fitness or performance (Violle et al. 2007). Change in performance can affect population demographics, which in turn can affect the structure and dynamics of the community and the functioning of the ecosystem (Villéger et al. 2008). Different environmental factors act as filters by selecting species with a set of traits that determine the ability of individuals to coexist in a local community and allow them to persist under specific environmental conditions (Poff et al. 2006; Castro et al. 2018). Anthropogenic stressors are additional environmental filters that can alter the expected functional structure of assemblages observed under natural conditions (Floury et al. 2017). Anthropogenic disturbances can cause instability in habitat structure and select organisms that have specific functional characteristics and high abundance, such as resistant and generalist taxa (Poff 1997; Statzner and Bêche 2010; Li et al. 2019). Identifying assemblage traits filtered by specific environmental conditions enable mechanistic understanding of cause-effect relationships, indicating the stressors most likely responsible for biological impairment

(Berger et al. 2018; Firmiano et al. 2021). Furthermore, considering that traits are stable across large spatial extents and natural environmental gradients, they offer a more reliable assessment of ecological condition than taxonomic composition, which varies naturally even within small spatial extents (Dolédec et al. 1996; Mouillot et al. 2013; Chen et al. 2019).

Despite these promising perspectives, knowledge gaps remain regarding how Chironomidae functional traits relate to specific environmental characteristics arising from anthropogenic stressors. Thus, we wanted to know which chironomid genera are effective for assessing ecological status in Cerrado streams and to understand how anthropogenic stressors affect traits and shape the functional structure of Chironomidae assemblages in neotropical streams. Given that chironomid genera have different degrees of sensitivity to anthropogenic disturbances, we hypothesized that the taxonomic and functional structure of Chironomidae genera would be negatively affected by the anthropogenic disturbances identified in the watershed. We expected to find trait combinations that are selected by specific stressors acting as environmental filters and to identify cause-effect relationships.

Methods

Study Area

The Pandeiros River basin is in the northern region of the state of Minas Gerais, Brazil, in the Cerrado biome and has an area of 3,960 km2. (Figure 1). Occurring in an area of “Special Biological Importance”, it is a unique environment, having shrubby wetland, marginal lagoon and palm swamp complexes (Azevedo et al. 2009). The basin has a tropical savanna climate, with mean annual temperatures of 22 ºC, annual precipitation close to 1000 mm, and a water deficit between April and September (Alvares et al. 2013). Therefore, it is an international priority area for biome conservation (Drummond et al. 2005). Most of the basin area (85.7%) is part of the Rio Pandeiros State Environmental Protection Area (Instituto Estadual de Florestas 2019). It has less anthropogenic disturbance than other basins in the biome (Macedo et al. 2018; Callisto et al. 2019a,), making it fundamentally important for revitalizing the São Francisco River basin (Azevedo et al. 2009). Because of the region's semi-arid climate (Instituto Estadual de Florestas 2019) the basin is also important for the perennial flow regime of most of its tributaries.

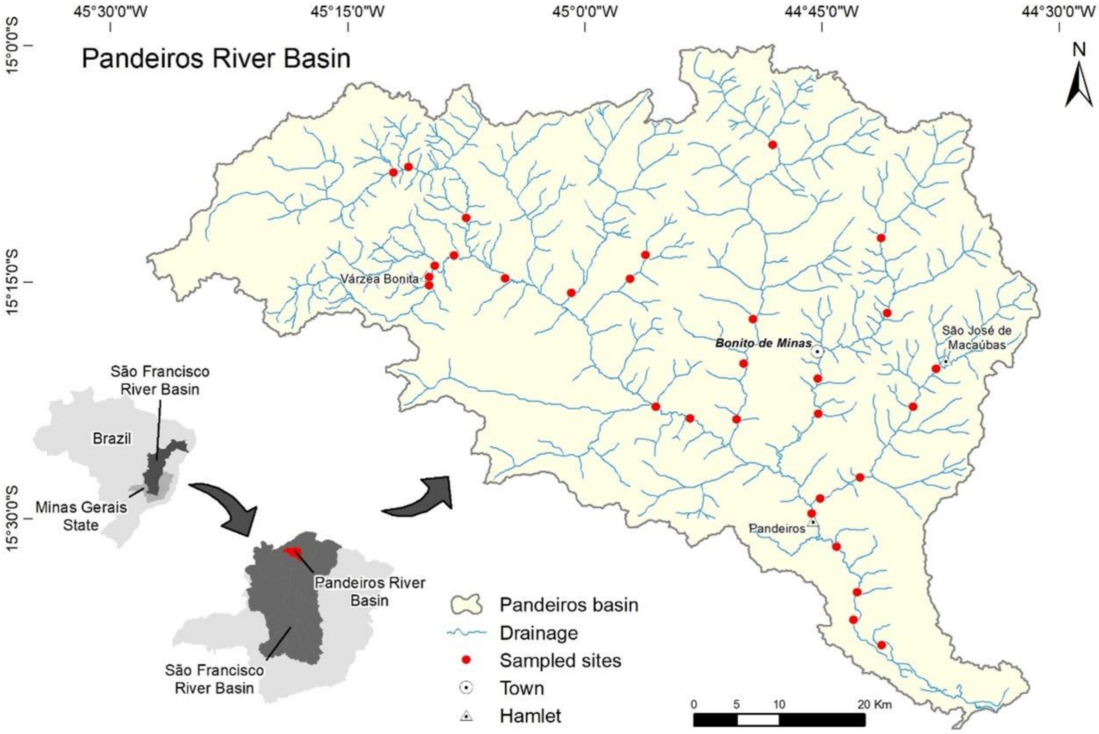

Figure 1 - Location of the Pandeiros River basin and the sample sites.

Survey design and physical habitat

The sample sites were selected through use of spatially balanced procedures following a random and systematic survey design, according to the method used by the USEPA in its National Rivers and Streams Survey (Olsen and Peck 2008). We sampled 30 sites at the beginning of the dry period (April to June 2016) in 3rd to 5th order wadeable streams (Strahler 1957). Site lengths were proportional to 40 x the mean width of each site, with a minimum length of 150 meters. In each site, 11 transverse transects (perpendicular to the channel flow) were established defining 10 sections, where physical habitat measurements were performed (Peck et al. 2006; USEPA 2020).

The physical habitat metrics were calculated based on Kaufmann et al. (1999). From a list of potential anthropogenic stressors and pressures assessed by the physical habitat protocol and satellite images, plus results from other Cerrado studies, we selected 12 environmental indicators. The 10 local site variables were natural cover, leaf bank cover, canopy cover over the channel, riparian canopy cover, channel slope, % sand substrate, % substrate embeddedness, % fine substrates (< 16mm), average site depth, and W1_hall. W1_hall (Riparian Human Disturbance Index) is the proximity weighted total of anthropogenic pressures observed in the channel and riparian zone. We also determined two catchment disturbance indicators: % catchment agriculture and % catchment pasture.

Catchment anthropogenic pressures

We used digital land use and cover maps for measuring catchment pressures. The quantification of types of land use and cover was carried out using supervised classification of digital images, whereby classes are assigned to the pixels of the satellite images, creating homogeneous patterns to which different classes of land use and cover are associated (Santos et al. 2017). We used 2016 imagery from the Landsat-8 satellite, sensor OLI, orbit scene 219/71 and 219/70 made available by INPE (Instituto Nacional de Pesquisas Espaciais) (http://www.dgi.inpe.br). The anthropogenic land use classes included urban areas, row crop agriculture, and pasture and were calculated as the percent of each class in the total site catchment, as described in Macedo et al. (2014).

Integrated anthropogenic pressures

For quality assessments across a gradient of environmental conditions, it is necessary to establish reference conditions for comparison and standardization (Stoddard et al. 2008). We used the concept of "least-disturbed" or minimally disturbed (Stoddard et al. 2006; Martins et al. 2018), because there were no pristine sites in the basin (Hughes et al. 1986). To identify these sites, we used IDI (Integrated Disturbance Index) scores that were calculated from local anthropogenic pressures (LDI - Local Disturbance Index) and catchment pressures (CDI - Catchment Disturbance Index). The

CDI was based on the % of human land uses in the site's total catchment, weighted by the potential degradation that each land use class has on aquatic ecosystems (CDI = 4x % urban + 2x % agricultural + % pasture) (Ligeiro et al. 2013). The LDI (W1_hall) summarizes the amount of anthropogenic disturbances observed in the channel and the riparian zone for 11 types of disturbances. The disturbances were walls/dikes/revetments, buildings, pavement, roads/railroads, pipes, landfills/trash, parks/lawns, row crops, pasture/range/hay fields, logging operations, and mining activities. Each disturbance was assessed on both sides of the channel and at each of the 11 transects (Ligeiro et al. 2013).

Chironomidae sampling

We collected chironomids at each of the 11 transects (Peck et al. 2006) per site by use of a D-frame kick-net (500 µm mesh, 0.9 m2 area). Each sample was placed in a plastic bag and fixed with 50 ml of formaldehyde. The samples were taken to the UFMG (Universidade Federal de Minas Gerais) Benthos Ecology laboratory, where they were washed on a 500 µm mesh screen. The washed material was placed in transparent trays on a light box and each chironomid individual was separated and later identified to genus (Trivinho-Strixino 2011, Hamada et al. 2019). Each individual was photographed in a stereomicroscope (Leica M80) equipped with a digital camera (Leica IC 80 HD). The length of each photographed specimen was measured using Motic Image Plus 2.0 software. All specimens were deposited in the Reference Collection of Benthic Macroinvertebrates at the UFMG Institute of Biological Sciences.

Chironomidae traits

Traits that are associated with species morphology, behavior, and life history strategies were used for analyzing the functional structure of the Chironomidae assemblages (Armitage et al. 1995, Trivinho-Strixino 2011). The trait categories were based on studies carried out in the neotropics (Butakka et al. 2016; Saulino et al. 2017; Jovem-Azevêdo et al. 2019; Pereira et al. 2020) and when not available, the search was expanded to studies carried out in other locations (USEPA 2012, Serra et al. 2017) (Table 1).

Nine traits in 32 categories related to the functional role of genera were used. Body size was obtained by direct measurement of individuals, from the cephalic capsule to the last segment of the body, excluding the cephalic and terminal appendices. Then, individuals were grouped into body size classes (Table 1). Regarding feeding or trophic habits, the larvae were divided into five classes according to their food preferences and eating habits. The trophic food groups are associated with the organic matter available to the species, allowing us to infer the trophic dynamics in ecosystems. The feeding strategy categories were analyzed based on the size and type of organic particles ingested and reflect the adaptation of genera to capture available food, which varies with the taxonomic composition of the assemblages (Tomanova et al. 2006). The trait table is in Supplementary Material 1.

Table 1 – Description of traits used, codes and references.

Trait	Category	Codes	References
Tube construction	Tube absent	TUBNON	(Serra et al. 2016)
	Tube without shape, unorganized	TUBUNO	
	Tube rigid/ Case like	TUBRIG	
Hemoglobin	Hemoglobin Present	HBPRES	(Serra et al. 2016; Saulino et al. 2017; Jovem-Azevêdo et al. 2019)
	Hemoglobin Absent	HBNONE	
Substrate relation	Free living	FREELV	(USEPA, 2012; Serra et al., 2016)
	Burrower	BURROW	
	Miner	MINER	
	Fixed	FIXED	
Body size	<2.5mm	SIZE1	(Serra et al. 2016)
	>2.5-5mm	SIZE2	
	>5-10mm	SIZE3	
	>10-20mm	SIZE4	
	>20-40mm	SIZE5	
Feeding/trophic habits	Fine sediment eater	DEFEE	(USEPA, 2012; Jovem-Azevêdo et al., 2019; Saulino et al., 2017; Serra et al., 2016)
	Shredder	SHR	
	Scraper grazer	SCR	
	Filterer	FFEEDT	
	Predator	PRED	
Feeding strategy	Filters	FI	

	Gatherer Herbivore Engulfer	GA HE EN	(Butakka et al. 2016; Saulino et al. 2017; Jovem-Azevêdo et al. 2019)
Habit	Sprawler Silk tube Climber Miner	SP ST CL MI	(USEPA, 2012; Jovem-Azevêdo et al., 2019; Saulino et al., 2017)
Pseudopods	Elongated Short Absent	EL SH AB	(Trivinho-Strixino 2011; Jovem-Azevêdo et al. 2019)
Lauterborn organs	Present Absent	PR AB	(Trivinho-Strixino 2011; Jovem-Azevêdo et al. 2019)

Data analyses

We calculated four functional diversity indices using the relative abundance of taxa in each trait category. 1) Functional richness (FRic) represents the amount of functional space filled by the assemblage (Villéger et al. 2008). 2) Functional divergence (FDiv) represents how abundance is spread along a functional characteristic axis within the range occupied by the assemblage. Many groups having greater than average abundances indicate greater functional divergence (Villéger et al. 2008). 3) Functional evenness (FEve) describes the evenness of abundance distribution in a functional trait space (Villéger et al. 2008). 4) Functional dispersion (FDis) represents the dispersion of species in the space of characteristics from the centroid of all species weighted by their relative abundances (Laliberte and Legendre 2010). After testing the normality of residuals and homoscedasticity, we used linear regression to assess the degree to which the functional indices were affected by the IDI values.

To assess associations between trait categories and local environmental variables, we applied RLQ and fourth-corner methods. RLQ produces three tables: environmental (R), taxa abundance (L), and trait (Q) tables. RLQ aims to identify the main co-structures between traits and environmental characteristics weighted by taxa abundances (Dolédec et al. 1996) and provides classification scores to summarize the joint structure between the three tables. The fourth-corner method primarily tests relationships between individual characteristics and the environment (that is, one characteristic and one environmental variable at a time) (Dray et al. 2014). We standardized local environmental metrics (mean = 0 and standard deviation = 1) before running all analyses. Overall significance was assessed via a global Monte-Carlo test using 9999 random permutations of the table rows of R (sites, model 2) and of the rows of Q (species, model 4). A combination of RLQ and fourth-corner analyses was used to evaluate the significance of associations between traits and combinations of environmental variables identified by RLQ. Significance was tested using a permutation procedure with model 6, which is a combination of models 2 (permutation of sites) and 4 (permutation of species). We used 9999 permutations and the false discovery rate adjustment (FDR) method to correct P-values for multiple-test comparisons (Dray et al. 2014). All analyzes were performed in R (R Core Development Team 2016) with vegan (Oksanen et al. 2017), FD (Laliberté et al. 2014) and ade4 (Chessel et al. 2004) packages.

Results

We identified 6147 individuals in 52 chironomid genera distributed in 3 subfamilies. The body size of the organisms ranged from 1.5 to 7.2 mm (($\bar{x}$ = 3.3, SD = 1.02). Functional richness (FRic: $F_{1,28} = 0.13$, $P = 0.72$), evenness (FEve: $F_{1,28} = 0.21$, $P = 0.64$), and dispersion (FDis: $F_{1,28} = 0.41$, $P = 0.52$) did not significantly differ among the anthropogenic disturbance categories. Functional divergence index scores were significantly lower in sites with greater anthropogenic disturbances (FDiv: $F_{1,28} = 5.54$, $P = 0.02$) (Figure 2).

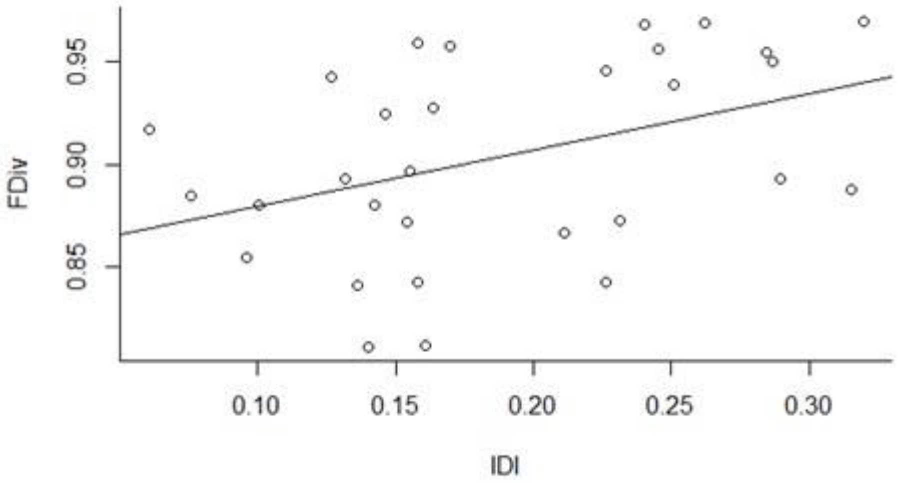

Figure 2 – Linear regression between the Functional Divergence Index (FDiv) and the Integrated Disturbance Index (IDI).

The global RLQ test revealed a significant relationship between abundance of genera and environmental variables (model 2, P = 0.01), as well as abundance of genera and biological traits (model 4, P = 0.01). The cross-variance between traits and environmental variables can be summarized by the first two RLQ axes (60.6% and 23.7% for axis 1 and 2, respectively). These axes were responsible for 86% of the variability of the environmental variables and 83% of the variance of the traits table (Figure 3).

Figure 3– Axis 1 and axis 2 of the RLQ analysis of 30 stream sites in the Pandeiros basin. (a) Chironomidae scores; (b) environmental variable scores; c) trait scores. The values for 'd' indicate grid sizes for scale comparison between the four figures. Each type of circle represents a significant relationship between the variables analyzed.

Regarding the first RLQ axis, we observed a positive relationship between *Tanypus, Nilotanypus, Larsia, Labrundinia, Djalmabatista, Denopelopia, Ablabesmyia, Monopelopia, Procladius and Fittkauimyia* with average substrate embeddedness, % sand substrate, and % catchment pasture. Most of these taxa are predators (PRED) with an engulfer (EN) feeding strategy, and the greater the amount of stream sand and catchment pasture, the greater the abundance of those chironomid genera.

The positive relationship of RLQ axis 1 with *Stenochironomus, Endotribelos and Beardius*, which have a miner habit (MI), herbivorous feeding strategy (HE) and larger body size, were related to riparian and channel vegetation cover (Figure 3). The presence of *Polypedilum* was related to % catchment agriculture, % site fine sediments, and W1_hall.

We also assessed the relationships between individual traits and the two RLQ environmental axes and individual environmental variables and the two RLQ trait axes by combining both RLQ and fourth-corner analysis. The first environmental axis (AxcR1, combination of environmental variables) was positively correlated with a herbivorous feeding strategy (Figure 4a). The first RLQ trait axis (AxcQ1, combination of traits) was positively related to riparian and channel canopy cover (Figure 4b).

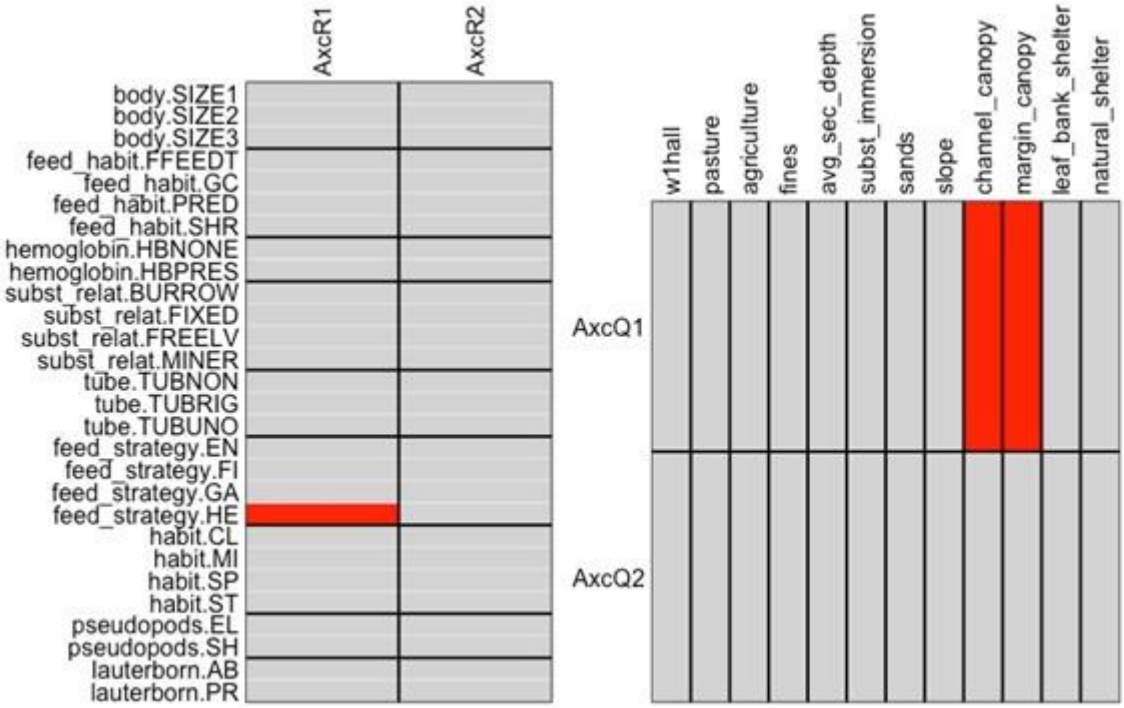

Figure 4 - Significant relationships (P-adjusted <0.05) between (a) the RLQ environmental axes and individual traits and (b) between the RLQ trait axes and environmental variables. Red indicates positive correlations between factors. Non-significant relationships are labeled in grey.

Discussion

We found strong relationships between environmental variables (local and catchment) and Chironomidae assemblage traits (predators, herbivores, miners, engulfers and body size). The most important trait for structuring Chironomidae assemblages was herbivorous feeding strategy, positively related with riparian and channel canopy cover. Functional divergence (FDiv) increased with increased disturbance (IDI), but functional richness (FRic), uniformity (FEve) and dispersion (FDis) showed no relationship with IDI scores. These results, taken together, suggest a high divergence of chironomid functional traits between sites with low versus high anthropogenic disturbance.

We also found that the structure of habitat and food resources were important factors structuring Chironomidae assemblages, as did Specziár et al. (2018). The abundance of Tanypodinae was positively associated with higher percentages of sand, embeddedness by fine sediments, and amount of catchment pasture. Tanypodinae species are widely distributed, occupying a wide variety of habitats (Cortelezzi et al. 2020) and they are known to be more tolerant of intermediate flow conditions (Puntí et al. 2009). These organisms are predators, one of the most important components of assemblages, because they strongly influence food chain structure (Saulino and Trivinho-Strixino 2018a) and are often related to human disturbances (Feio et al. 2015). Consequently, they lead to changes in ecological processes, because they are directly linked to energy transfer processes within ecosystems (Sih et al. 2010). Genera of this subfamily have been associated with high nitrogen concentrations, low dissolved oxygen concentrations (Cortelezzi et al. 2020), drought (Jovem-Azevêdo et al. 2019) and pesticides (Kuzmanovic et al. 2017). This indicates that they are tolerant to ecological changes as observed in this study. Some characteristics of these organisms may explain this tolerance. They can change their eating habits depending on the availability of food items (Butakka et al. 2016; Jovem-Azevêdo et al. 2019). Their body shape, longer pseudopods, retractable antennae and fused eyes also make them more mobile and hydrodynamically efficient (Trivinho-Strixino 2014; Saulino and Trivinho-Strixino 2018a).

Elevated percentages of sand and substrate embeddedness often indicate anthropogenic changes in aquatic ecosystems. In another study conducted in the same river basin (Martins et al. 2020, 2021), the authors observed that increased levels of fine substrate were associated with greater relative risk for poor biological condition. In aquatic ecosystems, the presence of fine substrates in stream beds is one of the most important threats to their ecological condition (Bryce et al. 2010; Burdon et al. 2013). This is because fine sediments reduce the availability of habitat for macroinvertebrate assemblages, directly compromising their structure, composition, and function (Wood and Armitage 1997; Angradi 1999; Matthaei et al. 2010; Buendia et al. 2013; Beermann et al. 2018). Fine sediments also have been found to be important stressors of macroinvertebrate condition in regional and national assessments in the USA (Van Sickle et al. 2006; Paulsen et al. 2008; Herlihy et al. 2020, USEPA 2020), Cerrado (Silva et al., 2018a), and Amazonia (Leitão et al. 2018). Excess riverbed fine substrates are associated with human activities that increase erosion, such as agriculture, pasture, roads, and deforestation (Kaufmann et al. 2009; Burdon et al. 2013; Strassburg et al. 2017; Brito et al. 2020; Dala-Corte et al. 2020).

The RLQ and fourth corner analyses showed positive relationships between riparian canopy cover and herbivorous organisms such as *Endotribelos, Beardius* (shredders) and *Stenochironomus* (miner). In addition, these characteristics were related to larger sized chironomids. Riparian vegetation is essential for important ecological processes in aquatic ecosystems, such as providing allocthonous nutrients, temperature balance and habitat heterogeneity. The absence or minimization of riparian vegetation reduces and homogenizes biological diversity (Castro et al. 2018; Firmiano et al. 2021) and eliminates sensitive species (Martins et al (In Review).; Oliveira-Junior et al. 2015; Brito et al. 2020; Dala-Corte et al. 2020). In addition, riparian vegetation is an important component in herbivory processes in aquatic ecosystems. Herbivores play an important ecological role in determining the energy flow from primary producers to higher consumers (Wood et al. 2017). Freshwater herbivorous macroinvertebrates are composed mostly of scrapers (algae grazers) and shredders (leaf, wood and debris fragmenters) (Saulino et al. 2020). Shredders have fundamental importance in fragmenting coarse particulate organic matter (CPOM) present on the streambed into fine particulate organic matter (FPOM) (Graça 2001, Boyero et al. 2015). Low canopy coverage can cause changes in the functional composition of chironomid assemblages (Cañedo-Argüelles et al. 2016). Riparian canopy cover was the main factor responsible for the functional structure of least-disturbed sites (Castro et al. 2018) and was positively correlated with more sensitive chironomid genera (Sensolo et al. 2012). On the other hand, the absence of riparian vegetation had a negative effect on abundance and richness of specialist organisms in wood processing, such as *Endotribelos, Beardius and Stenochironomus* (Valente-Neto et al. 2015). These results reinforce the importance of riparian vegetation for the functional structure of Chironomidae assemblages and other aquatic assemblages (Dala-Corte et al. 2020).

The largest Chironomidae larvae were significantly enhanced by the presence of riparian vegetation. Relative to the sizes of chironomids reported in the literature (Serra et al. 2016), we found intermediate sizes. In general, smaller larvae are benefited by high temperatures, low rainfall, and anthropogenic disturbances (Feio et al. 2015; Jovem-Azevêdo et al. 2019). However, some traits, such as body size, are still not well described for Chironomidae, even though this trait is of great importance for other macroinvertebrates in discriminating various types of anthropogenic impact (Dolédec and Statzner 2008). Body size is linked with key ecological functions of macroinvertebrates (production ⁄ biomass, production ⁄ respiration) (Robson et al. 2005). Greater effort is needed to describe some traits for Chironomidae (body size, voltinism and forms of resistance) (Serra et al. 2017) in the Neotropics.

Percent catchment agriculture, % fines, and W1_hall were related to the presence of *Polypedilum*. This cosmopolitan genus is generally associated with sandy substrate, silt and aquatic macrophytes (Cenzano and Würdig 2006) and has a general detritivore food habit (Higuti and Takeda 2002; Amorim et al. 2004). The genus includes species tolerant to a wide range of environmental conditions (Silva et al. 2018b), such as eutrophication (Saito and Fonseca-Gessner 2014), moderate concentrations of dissolved inorganic nitrogen, and low levels of dissolved oxygen (Cranston et al. 1997; Roque et al. 2010; Cortelezzi et al. 2020). This is because *Polypedilum* contain large amounts of hemoglobin and can store oxygen (Trivinho-Strixino 2011). These characteristics help explain its relationship with anthropogenic disturbance metrics.

Riparian zone disturbance, as indicated by W1_hall, can alter habitats and biota (Death and Joy 2004; Kaufmann and Hughes 2006; Bryce et al. 2010). The riparian zone strongly influences the organization, diversity, and dynamics of aquatic communities (Gregory et al. 1991; Allan 2004). Changes in soil conditions, vegetation and other factors directly reflect terrestrial aquatic interactions (Naiman et al. 2000) and services provided by the riparian meta-ecosystem (Callisto et al. 2019b). Thus, it is essential to consider the important role of the riparian zone in the organization, diversity, and dynamics of aquatic communities.

Chironomid genera proved to be effective for assessing ecological status in the Pandeiros River basin and some environmental characteristics were fundamental for structuring chironomid assemblages. As predicted, chironomid traits and functional indices were affected by the anthropogenic pressures identified in the catchment and the resultant stressors measured at the sites. This approach allowed us to identify cause-effect relationships, such as the reduced herbivorous feeding strategy associated with reduced riparian and channel canopy cover. Despite the relatively low levels of basin disturbance, we observed that riparian vegetation and substrate size were particularly important for structuring chironomid assemblages. The Chironomidae is a very diverse family, and its functional relationships are still little explored in the Neotropics (Saulino and Trivinho-Strixino 2018a). However, its predominance in sandy environments where EPT (Ephemeroptera, Plecoptera, Trichoptera) are uncommon, such as the Pandeiros River basin, make it an important tool for ecological assessment of places with these characteristics (Li et al. 2014). Thus, this study adds an important contribution to that knowledge and consolidated those aspects that are most important for maintaining aquatic ecosystem condition in the Pandeiros River basin.

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Conclusões

Vários índices multimétricos (MMI) bentônicos originalmente desenvolvidos em diferentes continentes foram testados e validados. Desses, 10 foram eficazes na avaliação das condições ecológicas da bacia do rio Pandeiros. Portanto, não é necessário elaborar novos MMIs bentônicos para avaliações da qualidade ambiental em cada bacia hidrográfica neotropical. Em vez disso, recomendamos o desenvolvimento de métodos de amostragem e processamento padrão para que os índices publicados possam ser usados em avaliações em escala nacional. Além disso, esse estudo oferece uma abordagem para padronizar e usar MMIs em futuras avaliações da qualidade ambiental em outras bacias neotropicais. Mesmo em uma área protegida, foi observado que os distúrbios locais degradam a condição biológica, indicando a importância das ações locais para a conservação e reabilitação dos recursos hídricos desta e de bacias semelhantes.

Foi possível identificar os principais distúrbios associados às más condições biológicas presentes e avaliada a extensão desses distúrbios (% substratos finos, pastagem, IDI e LDI). Dessa forma, é necessário concentrar os esforços na reabilitação da bacia do rio Pandeiros e seus afluentes e redução destes principais distúrbios locais que geram riscos à perda de boas condições biológicas. Os principais riscos para a comunidade de macroinvertebrados foram a presença de pastagem e a presença de substratos finos, dessa forma, o manejo aprimorado das pastagens, evitando a erosão e reduzindo o assoreamento dos cursos dos rios, são as principais prioridades para melhorar a condição ecológica na bacia do rio Pandeiros.

Os gêneros de Chironomidae e suas características funcionais mostraram-se eficazes para avaliar a condição ecológica da bacia do rio Pandeiros e algumas características ambientais foram fundamentais para a manutenção dessas assembleias. Como previsto, as características e índices funcionais dos Chironomidae foram afetados negativamente pelas pressões antrópicas identificadas na bacia hidrográfica e os estressores resultantes locais. Apesar dos níveis relativamente baixos de perturbação da bacia, observamos que a vegetação ripária e o tamanho do substrato foram particularmente importantes para estruturar as assembleias, sendo a presença de gêneros resistentes associados a impactos como pastagem e % substratos finos. A vegetação riparia esteve relacionada com organismos herbívoros e de maior tamanho. Os Chironomidae são uma família muito diversa, e suas relações funcionais ainda são pouco exploradas na região Neotropical. Seu predomínio em ambientes arenosos, como a bacia do rio Pandeiros, os torna uma importante ferramenta para avaliação ecológica de locais com essas características. Assim, esse estudo agrega importante contribuição a esse conhecimento e consolida os aspectos mais importantes para a manutenção das condições dos ecossistemas aquáticos na bacia hidrográfica do rio Pandeiros.

Esse estudo possibilitou uma avaliação ampla e integrada de vários aspectos ecológicos das comunidades de macroinvertebrados indicadores de qualidade ambiental na bacia do rio Pandeiros. (i) Na avaliação de índices multimétricos, que integram múltiplos atributos biológicos e ecológicos dessas assembleias; (ii) modelos que utilizam a probabilidade da presença de estressores antropogênicos impactarem a biota; (iii) e o uso de indicadores ecológicos através da análise de grupos específicos, foram identificadas pressões locais, e características importantes para manutenção da biodiversidade aquática na bacia. Além disso, (iv) foram identificados os principais distúrbios estressores e pressões associados às más condições biológicas presentes na bacia hidrográfica do rio Pandeiros. Esse estudo forneceu evidências de que mesmo em áreas protegidas, os distúrbios locais degradam a condição biológica, indicando a importância das ações locais para a conservação e reabilitação de uma condição ecológica equilibrada, em escala maior, como de bacia hidrográfica.

Perspectivas futuras

- Desenvolvimento de métodos de amostragem e processamento padrão para que os índices avaliados possam ser utilizados em avaliações de extensão nacional.
- Avaliar índices multimétricos previamente existentes para outros grupos taxonômicos e em outras biomas.
- Aplicação dessa abordagem em um maior número de locais, considerando uma diversidade maior de bacias hidrográficas e avaliar um gradiente de perturbação no Cerrado e em vários biomas neotropicais.

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Anexos

Anexo 1 - Material Suplementar do Capítulo 1

Tables

Table S1 – MMI metrics and the authors and regions where the indices were developed.

Metrics	MMIs	Regions
Taxonomic Composition		
% EPT	Ferreira et al. (2011)	Brazil – Neotropical Savanna
	Oliveira et al. (2011)	Brazil – Atlantic Forest
	Couceiro et al., 2012;	Brazil – Amazon Forest
	Jun et al., 2012	South Korea
	Chen et al., 2014	China
% EPT (Minus Baetidae+Caenidae+Hydropsychidae	Lakew and Moog, 2015	Ethiopia – semi-arid
% Trichoptera	Helson and Willians, 2014	Panama
EPT Abundance	Nguyen et al., 2014	Vietnam
	Helson and Willians, 2014	Panama
	Fierro et al., 2018	Chile - mediterranean
% Plecoptera	Oliveira et al., 2011	Brazil – Atlantic Forest
% Coleoptera+Odonata+EPT	Lakew and Moog, 2015	Ethiopia – semi-arid
% Coleoptera	Baptista et al., 2007	Brazil – Atlantic Forest
% Chironomidae	Jun et al, 2012	South Korea
% Oligochaeta+Chironomidae	Ferreira et al., 2011	Brazil – Neotropical Savanna
	Lakew and Moog, 2015	Ethiopia – semi-arid
% Diptera	Baptista et al., 2007	Brazil – Atlantic Forest
% Non-Insects	Klemm et al., 2003	USA
	Ode et al., 2005	USA – Semi-arid
% Insects	Nguyen et al., 2014	Vietnam
% Gastropoda	Silva et al., 2017	Brazil – Neotropical Savanna
% Oligochaeta	Ferreira et al., 2011	Brazil – Neotropical Savanna
% Odonata	Macedo et al., 2016	Brazil – Neotropical Savanna
% Mollusca+Diptera	Oliveira et al., 2011	Brazil – Atlantic Forest
Chironomidae/Diptera	Helson and Willians, 2014	Panama
	Oliveira et al., 2011	Brazil – Atlantic Forest
EPT/Chironomjdae	Couceiro et al., 2012	Brazil – Amazon Forest
Hydropsychidae/Trichoptera	Oliveira et al., 2011	Brazil – Atlantic Forest
Total Density	Fierro et al., 2018	Chile - mediterranean
Taxonomic richness		
Total Richness	Chen et al., 2014	China
	Mereta et al., 2013	Ethiopia - Wetlands
	Lakew and Moog, 2015	Ethiopia – semi-arid
	Nguyen et al., 2014	Vietnam
	Li et al., 2010	China
	Ferreira et al., 2011	Brazil – Neotropical Savanna
	Gabriels et al., 2010	Belgium
	Jun et al., 2012	South Korea
	Baptista et al., 2007	Brazil – Atlantic Forest

	Oliveira et al., 2011	Brazil – Atlantic Forest
EPT Richness	Lakew and Moog, 2015	Ethiopia – semi-arid
	Chen et al., 2014	China
	Macedo et al., 2016	Brazil – Neotropical Savanna
	Baptista et al., 2007	Brazil – Atlantic Forest
	Gabriels et al., 2010	Belgium
	Couceiro et al., 2012	Brazil – Amazon Forest
	Ode et al., 2005	USA – Semi-arid
Trichoptera Richness	Couceiro et al., 2012	Brazil – Amazon Forest
	Oliveira et al., 2011	Brazil – Atlantic Forest
	Kelmm et al., 2003	USA
	Li et al., 2010	China
	Chen et al., 2014	China
EP Richness	Chen et al., 2014	China
Ephemeroptera Richness	Macedo et al., 2016	Brazil – Neotropical Savanna
	Silva et al., 2017	Brazil – Neotropical Savanna
	Klemm et al., 2003	USA
	Li et al., 2010	China
Plecoptera Richness	Klemm et al., 2003	USA
Ephemeroptera+Trichoptera Richness	Mereta et al., 2013	Ethiopia - Wetlands
Sensitive Taxa Richness	Gabriels et al., 2010	Belgium
	Couceiro et al., 2012	Brazil – Amazon Forest
Ephemeroptera+Odonata+Trichopera Richness	Mereta et al., 2013	Ethiopia - Wetlands
Coleoptera Richness	Ode et al., 2005	USA – Semi-arid
Diptera Richness	Fierro et al., 2018	Chile - mediterranean
Feeding groups		
Predator Richness	Ode et al., 2005	USA – Semi-arid
	Fierro et al., 2018	Chile - mediterranean
% Predators	Macedo et al., 2016	Brazil – Neotropical Savanna
% Collector-Gatherers	Jun et al., 2012	South Korea
	Lakew and Moog, 2015	Ethiopia – semi-arid
	Ferreira et al., 2011	Brazil – Neotropical Savanna
	Couceiro et al., 2012	Brazil – Amazon Forest
Ratio Filterers/Scrapers	Jun et al., 2012	South Korea
% Shredders	Baptista et al., 2007	Brazil – Atlantic Forest
	Couceiro et al., 2012	Brazil – Amazon Forest
	Oliveira et al., 2011	Brazil – Atlantic Forest
	Helson and Willians, 2014	Panama
	Lakew and Moog, 2015	Ethiopia – semi-arid
% Collector-Filterers	Mereta et al., 2013	Ethiopia - Wetlands
Collector-Filterer Richness	Klemm et al., 2003	USA
% Collector-Gatherers+Collector-Filterers	Ode et al., 2005	USA – Semi-arid
% Scrapers	Helson and Willians, 2014	Panama
	Silva et al., 2017	Brazil – Neotropical Savanna
	Li et al., 2010	China
Tolerance		
% Intolerant or Sensitive	Ode et al., 2005	USA – Semi-arid
	Silva et al., 2017	Brazil – Neotropical Savanna
% Tolerant	Ode et al., 2005	USA – Semi-arid
Mean Tolerance	Gabriels et al., 2010	Belgium
Hilsenhoff 88	Lakew and Moog, 2015	Ethiopia – semi-arid
Saprobiotic Index	Jun et al., 2012	South Korea

Macroinvertebrate Tolerance Index	Klemm et al., 2003	USA
BMWP	Baptista et al., 2007	Brazil – Atlantic Forest
	Ferreira et al., 2011	Brazil – Neotropical Savanna
	Nguyen et al., 2014	Vietnam
	Mereta et al., 2013	Ethiopia - Wetlands
ASPT	Lakew and Moog, 2015	Ethiopia – semi-arid
	Macedo et al., 2016	Brazil – Neotropical Savanna
Dominance		
% 5 taxa dominant	Klemm et al., 2003	USA
	Jun et al., 2012	South Korea
Mobility and Breathing		
Number of Burrowers	Li et al., 2010	China
Temporary Attached Richness	Silva et al., 2017	Brazil – Neotropical Savanna
Gill Respiration Taxa Richness	Silva et al., 2017	Brazil – Neotropical Savanna
Diversity		
Shannon's Index	Gabriels et al., 2010	Belgium
	Jun et al., 2012	South Korea
	Li et al., 2010	China
	Oliveira et al., 2011	Brazil – Atlantic Forest
	Helson and Willians, 2014	Panama
	Silva et al., 2017	Brazil – Neotropical Savanna
Margalef's Index	Mereta et al., 2013	Ethiopia - Wetlands
	Helson and Willians, 2014	Panama
	Nguyen et al., 2014	Vietnam

Table S2 – Water quality parameters in the Pandeiros River sites.

Stream sites	Water temp (°C)	pH	Conductivity (µS/cm)	Total solids (ppm)	Turbidity (NTU)	DO (mg/L)	Alkalinity (mEq/L CO2)	Total Nitorgen (mg/L)	Total Phosphorus (ug/L)	Chlorophyll (ug/L)
1	23.10	6.79	173.90	67.80	2.94	6.77	157.70	0.05	3.64	0.00
2	22.00	8.21	85.50	36.60	6.31	8.63	793.40	0.04	7.65	0.36
3	22.00	8.10	148.60	61.90	23.70	8.29	1399.00	0.03	6.04	0.00
4	22.50	4.03	18.92	6.87	8.16	6.85	30.17	0.04	10.85	1.42
5	23.00	7.48	115.70	47.40	10.07	8.46	891.70	0.03	9.25	0.36
6	25.10	7.43	58.70	22.90	24.30	4.40	73.65	0.06	3.64	7.10
7	22.40	6.76	175.10	67.40	1.73	7.11	152.30	0.04	3.64	0.71
8	25.00	7.58	96.70	34.90	33.00	7.78	71.86	0.06	6.04	0.00
9	22.80	6.42	12.98	0.30	5.09	6.94	-0.50	0.06	4.44	0.71
10	22.60	6.60	23.90	8.70	11.54	8.63	14.44	0.03	11.66	0.00
11	19.60	8.15	82.30	36.10	3.49	8.60	761.80	0.03	12.46	0.00
12	23.30	7.61	32.30	0.00	2.18	6.94	36.40	0.07	12.46	0.71
13	18.10	8.27	80.20	30.40	2.86	8.29	779.60	0.03	10.85	0.71
14	25.00	7.60	218.00	46.00	8.84	8.12	847.40	0.05	7.65	2.13
15	20.20	9.06	182.10	75.20	8.09	10.32	434.30	0.03	6.84	1.07
16	23.60	8.40	62.00	0.00	1.66	9.14	50.46	0.06	4.44	0.36
17	25.00	7.75	207.00	95.20	0.11	6.51	222.70	0.04	9.25	1.07
18	23.30	8.10	43.50	0.00	11.54	7.28	29.02	0.05	12.46	0.00

19	25.00	7.87	275.00	106.90	22.50	6.43	257.40	0.06	9.25	0.00
20	23.50	7.36	226.00	0.98	6.46	7.61	224.30	0.06	2.84	0.00
21	22.90	6.80	87.30	32.90	9.29	7.44	89.06	0.06	6.04	1.42
22	30.00	6.40	29.80	11.16	18.23	7.61	36.67	0.06	2.03	0.36
23	24.80	8.10	319.00	114.30	0.10	7.11	325.60	0.04	4.44	0.71
24	22.70	8.30	44.80	0.00	17.98	7.44	21.29	0.05	7.65	0.71
25	24.50	7.35	25.30	33.10	4.18	7.61	233.50	0.05	2.84	0.00
26	23.00	7.67	533.00	231.00	8.91	3.38	312.40	0.08	18.87	2.84
27	19.80	8.87	81.20	37.50	4.04	7.78	792.70	0.04	8.45	3.22
28	24.30	5.94	7.28	0.00	3.90	7.61	32.07	0.04	4.44	9.00
29	19.20	7.68	87.30	0.00	8.47	8.63	837.30	0.04	3.64	0.36
30	21.90	7.45	47.30	0.00	1.95	7.78	28.10	0.05	14.06	0.88
31	25.00	8.75	13.95	5.99	1.59	7.44	63.50	0.04	9.25	2.13
32	25.00	6.39	24.90	9.27	4.21	7.78	38.69	0.04	2.03	1.42
33	21.90	7.60	92.70	0.00	14.68	7.95	686.10	0.03	4.44	0.37
34	25.00	7.73	215.00	99.20	0.11	7.28	187.10	0.04	6.84	0.71
35	25.00	8.05	96.00	41.20	9.94	7.78	958.30	0.03	10.05	0.36
36	21.30	7.29	3.91	184.60	7.96	3.72	331.80	0.03	4.44	0.36
37	19.90	7.28	95.40	0.00	5.30	9.14	812.60	0.03	4.44	0.00
38	16.00	7.60	136.30	8.70	5.63	8.63	1123.00	0.04	9.25	1.07
39	21.60	7.39	40.60	15.52	16.85	7.61	131.60	0.06	11.66	0.00
40	20.40	7.76	115.70	0.00	7.05	6.60	764.40	0.04	18.07	0.36

Table S3 – Results of Pearson correlations between water quality variables and the IDI.

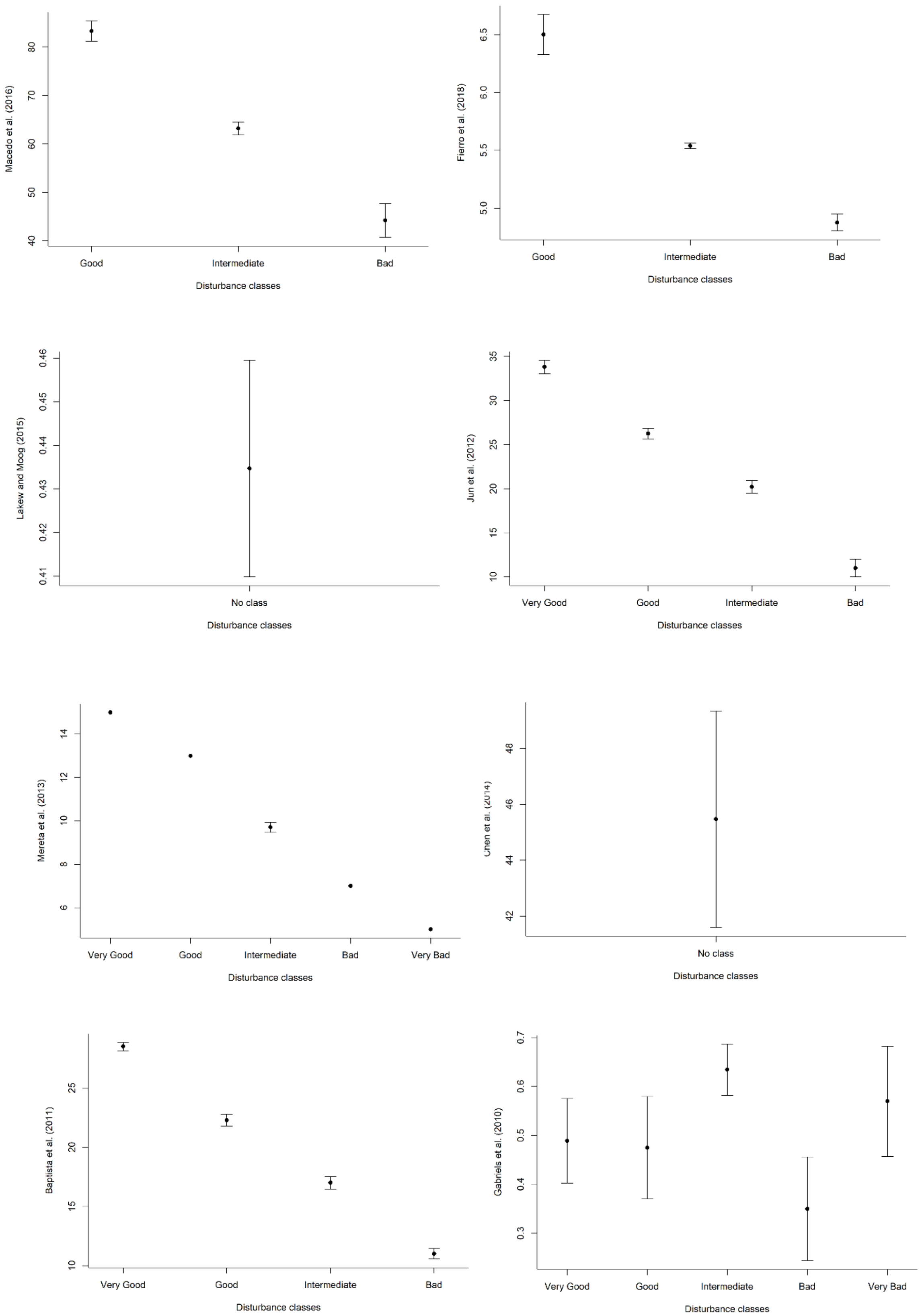
	Temp (°C)	pH	Cond (µS/cm)	Total solids (ppm)	Turbidity (NTU)	OD (mg/L)	Alkalinity (mEq/L CO2)	Total nitrogen (mg/L)	Total phosphorus (ug/L)	Chlorophyll (ug/L)
IDI	0.05	-0.13	-0.25	0.05	0.27	-0.20	-0.32*	0.13	-0.14	-0.03

Table S4 – Pearson correlations between physical habitat metrics and the IDI.

	XCMGW	XEMBED	LRBS	IDI
XCMGW	1.00	-0.44*	0.00	-0.24
XEMBED	-0.44*	1.00	-0.19	0.14
LRBS	0.00	-0.19	1.00	-0.04
IDI	-0.24	0.14	-0.04	1.00

Figures

Figure S1 – MMI boxplots as calculated and classified for each index according to their authors.



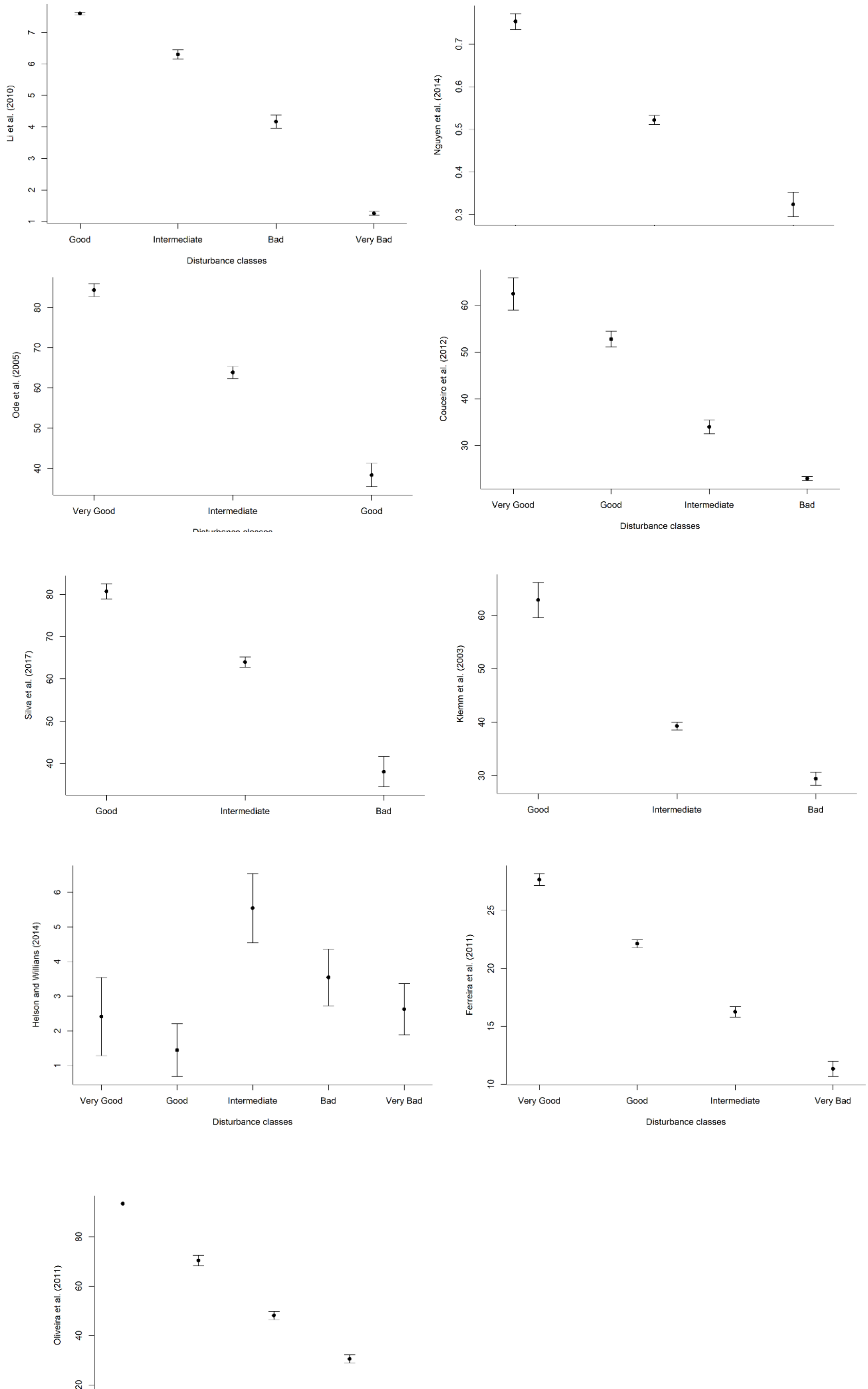
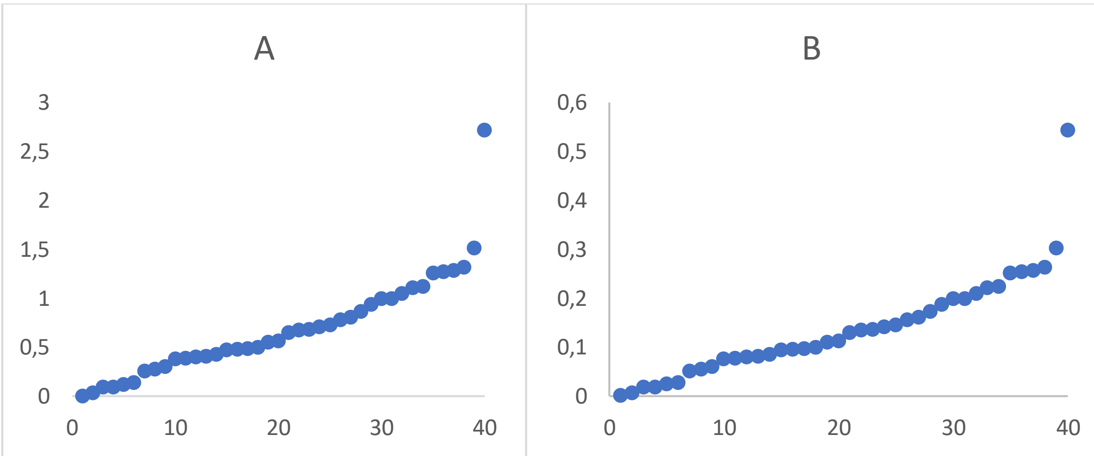

Li et al. (2010)
Good
Intermediate
Bad
Very Bad
Disturbance classes
Nguyen et al. (2014)
Ode et al. (2005)
Very Good
Intermediate
Good
Couceiro et al. (2012)
Very Good
Good
Intermediate
Bad
Disturbance classes
Silva et al. (2017)
Good
Intermediate
Bad
Klemm et al. (2003)
Good
Intermediate
Bad
Helson and Willians (2014)
Very Good
Good
Intermediate
Bad
Very Bad
Disturbance classes
Ferreira et al. (2011)
Very Good
Good
Intermediate
Very Bad
Disturbance classes
Oliveira et al. (2011)

Figure S2 – A) Graph in ascending order of the W1_HALL metric scores. which is calculated from site-scale anthropogenic disturbance. Values near the source indicate low anthropogenic disturbance. B) Graph in ascending order of the IDI (Integrated Disturbance Index) scores. which is calculated from the integration of local and catchment index values. Values close to source indicate low anthropogenic disturbance.



Anexo 2 - Material Suplementar do Capítulo 2

Appendix 1

MMI	MMI_Jun	MMI_Baptista	MMI_Nguyen	MMI_Li	MMI_Ode
Metrics	Richness	Richness	Richness	Richness	%Collectors
	%EPT	EPT richness	EPT abundance	Ephemeroptera richness	%Non-insect
	% 5 dominant taxa	%Diptera	%Insects	Trichoptera richness	% tolerant taxa
	% Chironomidae	%Coleoptera	BMWP	% Scrapers	Coleoptera richness
	Shannon diversity	BMWP	Margalef diversity	Number of clinger taxa	Predator richness
	% Collector-gatherers	%Shredders		Shannon diversity	%Intolerant
	Ratio Filtr/Scrapers				EPT richness
	Saprobiotic index				
MMI	MMI_Helson	MMI_Ferreira	MMI_Macedo	MMI_Silva	MMI_Fierro
Metrics	EPT abundance	Richness	Ephemeroptera Richness	Ephemeroptera Richness	Diptera richness
	%Trichoptera	%Oligochaeta	EPT Richness	%Gastropoda	Predator richness
	Margalef diversity	%Chironomidae + Oligochaeta	%Odonata	Shannon diversity	Total density
	Shannon diversity	%EPT	Average tolerance per taxon	%Sensitive Taxa	EPT abundance
	%Scrapers	%Collector-gatherers	%Predators	%Scraper individuals	
	%Shredders	BMWP		Temporarily attached taxa richness	
	Chiro/Diptera			Respiration taxa richness	

Appendix 2

	Disturbance correlations.				
	LRBS	LD1	IDI	Fines	Pasture
LRBS	1.00	0.01	-0.04	-0.52	0.12
LDI	0.01	1.00	0.91	-0.03	0.30
IDI	-0.04	0.91	1.00	0.14	0.19
Fines	-0.52	-0.03	0.14	1.00	-0.05

Pasture	0.12	0.30	0.19	-0.05	1.00

Anexo 3 - Material Suplementar do Capítulo 3

Chironomidae	Body size (mm)	Body size (Serra)	Feeding _trophic	hemoglobin	Substrate relation	Tube construction	Fedding_ strategy	habit	pseudopods	Lauterborn organs	tubules
Ablabesmyia	3,555	SIZE2	PRED	HBNONE	FREELV	TUBNON	EN	SP	EL	AB	AN
Aedokritus	2,910	SIZE2	GC	HBPRES	BURROW	TUBNON	EN	SP	SH	PR	AN
Apedilum	1,980	SIZE1	GC	HBPRES	BURROW	TUBNON	GA	ST	SH	PR	AB
Asheum	2,455	SIZE1	GC	HBPRES	BURROW	TUBNON	GA	ST	SH	AB	AN
Beardius	3,174	SIZE2	SHR	HBPRES	BURROW	TUBNON	HE	ST	SH	PR	AN
Brundiniella	7,260	SIZE3	PRED	HBNONE	BURROW	TUBNON	EN	SP	EL	AB	AN
Chironomus	4,517	SIZE2	FFEEDT	HBPRES	BURROW	TUBRIG	GA	ST	SH	PR	ABD/AN
Cladopelma	3,644	SIZE2	PRED	HBPRES	BURROW	TUBNON	EN	SP	SH	AB	AN
Cladotanytarsus	4,402	SIZE2	GC	HBPRES	BURROW	TUBRIG	FI	CL	SH	PR	AN
Clinotanypus	3,856	SIZE2	PRED	HBPRES	BURROW	TUBNON	EN	ST	EL	AB	AN
Coelotanypus	5,159	SIZE3	PRED	HBNONE	BURROW	TUBNON	EN	ST	EL	AB	AN
Constempellina	2,293	SIZE1	GC	HBPRES	BURROW	TUBRIG	FI	CL	SH	PR	AN
Corynoneura	2,040	SIZE1	PRED	HBNONE	BURROW	TUBNON	GA	SP	EL	AB	AN
Cricotopus	2,536	SIZE2	SHR	HBNONE	MINER	TUBUNO	GA	ST	EL	PR	AN
Cryptochironomus	4,045	SIZE2	PRED	HBNONE	BURROW	TUBNON	GA	SP	SH	PR	AN
Cyphomella	4,057	SIZE2	GC	HBPRES	BURROW	TUBNON	GA	ST	SH	AB	AN
Denopelopia	2,878	SIZE2	PRED	HBNONE	FREELV	TUBNON	EN	SP	EL	AB	AN
Dicrotendipes	3,486	SIZE2	GC	HBPRES	BURROW	TUBUNO	GA	SP	SH	PR	ABD/AN
Djalmabatista	3,832	SIZE2	PRED	HBNONE	FREELV	TUBNON	EN	SP	EL	AB	AN
Endotribelos	3,307	SIZE2	SHR	HBPRES	BURROW	TUBUNO	HE	ST	SH	PR	AN
Fissimentum	4,768	SIZE2	GC	HBPRES	BURROW	TUBNON	GA	SP	SH	PR	AN
Fittkauimyia	3,863	SIZE2	PRED	HBNONE	BURROW	TUBNON	EN	SP	EL	AB	AN
Goeldichironomus	2,613	SIZE2	GC	HBPRES	BURROW	TUBNON	GA	ST	SH	PR	ABD/AN
Labrundinia	2,970	SIZE2	PRED	HBNONE	FREELV	TUBNON	EN	SP	EL	AB	AN

Larsia	3,265	SIZE2	PRED	HBNONE	FREELV	TUBNON	EN	SP	EL	AB	AN
Lauterborniella	2,257	SIZE1	GC	HBPRES	FREELV	TUBRIG	GA	CL	SH	PR	AN
Lopescladius	2,568	SIZE2	GC	HBNONE	BURROW	TUBNON	GA	SP	EL	PR	AN
Monopelopia	3,650	SIZE2	PRED	HBNONE	BURROW	TUBNON	EN	SP	EL	AB	AN
Nilotanypus	3,498	SIZE2	PRED	HBNONE	FREELV	TUBNON	EN	SP	EL	AB	AN
Nilothauma	3,438	SIZE2	GC	HBPRES	BURROW	TUBNON	GA	ST	SH	PR	AB
Onconeura	2,120	SIZE1	GC	HBNONE	BURROW	TUBNON	GA	ST	EL	PR	AN
Oukuriella	3,095	SIZE2	GC	HBPRES	BURROW	TUBNON	GA	ST	SH	PR	AN
Parachironomus	3,093	SIZE2	GC	HBPRES	BURROW	TUBUNO	GA	SP	SH	PR	AN
Paralauterborniella	3,915	SIZE2	FFEEDT	HBPRES	BURROW	TUBUNO	GA	ST	SH	PR	AN
Parametriocnemus	3,556	SIZE2	GC	HBNONE	BURROW	TUBNON	GA	SP	EL	PR	AN
Paratendipes	3,517	SIZE2	GC	HBPRES	BURROW	TUBUNO	GA	ST	SH	PR	AN
Pelomus	3,421	SIZE2	GC	HBPRES	BURROW	TUBNON	GA	ST	SH	AB	AN
Phaenopsectra	3,352	SIZE2	GC	HBPRES	FIXED	TUBUNO	GA	CL	SH	PR	AN
Polypedilum	3,073	SIZE2	GC	HBPRES	MINER	TUBUNO	GA	CL	SH	PR	AN
Procladius	5,321	SIZE3	PRED	HBPRES	FREELV	TUBNON	GA	SP	EL	AB	AN
Pseudochironomus	2,190	SIZE1	GC	HBPRES	BURROW	TUBNON	EN	SP	SH	PR	AN
Rheocricotopus	1,500	SIZE1	GC	HBNONE	FIXED	TUBRIG	GA	SP	EL	PR	AN
Riethia	3,937	SIZE2	GC	HBPRES	BURROW	TUBNON	EN	SP	EL	PR	AN
Saetheria	3,296	SIZE2	GC	HBPRES	BURROW	TUBNON	GA	ST	SH	PR	AB
Stempellina	2,119	SIZE1	GC	HBPRES	BURROW	TUBRIG	FI	CL	SH	PR	AN
Stenochironomus	5,381	SIZE3	SHR	HBPRES	MINER	TUBNON	HE	MI	SH	PR	AN
Stictochironomus	1,900	SIZE1	SHR	HBPRES	BURROW	TUBRIG	GA	ST	SH	PR	AN
Tanypus	3,427	SIZE2	PRED	HBPRES	FREELV	TUBNON	EN	SP	EL	AB	AN
Tanytarsus	2,488	SIZE1	GC	HBPRES	BURROW	TUBRIG	FI	CL	SH	PR	AN
Xestochironomus	3,440	SIZE2	GC	HBPRES	BURROW	TUBNON	GA	ST	SH	AB	AN
Zavreliella	3,668	SIZE2	GC	HBPRES	BURROW	TUBRIG	EN	ST	SH	PR	AN
Zavrelimyia	3,150	SIZE2	PRED	HBPRES	FREELV	TUBNON	EN	SP	EL	AB	AN