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Avaliação de Condições Ecológicas em Riachos no Cerrado: Bases para sua Conservação

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Tese apresentada ao Programa de Pós-Graduação em Ecologia, Conservação e Manejo de Vida Silvestre do Instituto de Ciências Biológicas da Universidade Federal de Minas Gerais como parte dos requisitos para a obtenção do título de Doutor em Ecologia.

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Sumário

Resumo

Ecossistemas aquáticos continentais estão entre os mais ameaçados por atividades antrópicas em escala global. A elevada demanda por água tem resultado em um processo contínuo de degradação, levando à perda de biodiversidade nestes ecossistemas. No cerrado brasileiro, a intensa expansão do agronegócio resulta em desflorestamento, perda de habitat, introdução de espécies, erosão do solo, poluição de ecossistemas aquáticos, entre outros. Neste contexto, o objetivo geral desta tese foi avaliar as condições ecológicas em riachos no cerrado através do desenvolvimento de ferramentas efetivas, visando a conservação e o manejo eficiente destes recursos. Através de um desenho amostral probabilístico, foram selecionados riachos pertencentes às unidades hidrológicas de quatro empreendimentos hidrelétricos (Nova Ponte, Três Marias, Volta Grande e São Simão). As amostragens de assembleias de macroinvertebrados e a caracterização de habitat físicos seguiram o protocolo padronizado da Agência de Proteção Ambiental norte americana (US-EPA) adaptado para riachos no cerrado. O primeiro capítulo teve como objetivo avaliar a eficiência do esforço amostral para estimar a riqueza taxonômica de macroinvertebrados obtida através de amostragens em escalas local e de bacia. Além disso, foram determinados quais fatores ambientais estão associados a um maior esforço amostral necessário para caracterização da riqueza taxonômica em escala local. Verificou-se que o esforço amostral varia com a escala de estudo e com a resolução taxonômica avaliada.

Métricas que descrevem a heterogeneidade e estrutura de habitat, distúrbios antrópicos na zona ripária e qualidade de água explicaram um percentual significativo do esforço necessário para caracterização da riqueza de macroinvertebrados. No segundo capítulo, o objetivo foi desenvolver um índice multimétrico (MMI) capaz de refletir impactos antropogênicos utilizando métricas de assembleias de macroinvertebrados bentônicos. Etapas do desenvolvimento do MMI incluíram: 1) seleção de sítios de referência e impactados; 2) classificação e seleção de métricas biológicas; 3) seleção do melhor modelo MMI; 4) atribuição de classes de condições ecológicas; 5) avaliação das relações entre o MMI e estressores antropogênicos; 6) avaliação de condições ecológicas em escalas local e regional. O MMI final foi capaz de distinguir entre sítios impactados e sítios de referência, respondendo a impactos antrópicos relacionados a usos do solo, qualidade de água e estrutura física de habitat. No terceiro capítulo, foi avaliada a associação entre estressores antropogênicos e a condição biológica representada pelo MMI, em escalas local (em cada unidade hidrológica) e regional. Para tanto foi utilizada a abordagem de risco relativo (RR) e extensão dos estressores (RE), que avaliam respectivamente a severidade e magnitude de estressores para a condição biológica. Verificou-se que em escala regional turbidez, % de sedimentos finos e % de agricultura são os principais estressores associados a piores condições biológicas. Em escala local, % de pastagem e nitrogênio total estão associados a piores condições biológicas mas ocorrem em extensões reduzidas. De maneira geral, avaliando juntos o RR e o RE, viu-se que mitigar o impacto do excesso de sedimentos no leito de riachos pode melhorar consideravelmente a condição biológica. Os produtos desta tese visam fornecer subsídios para gestores e tomadores de decisão para implementação de programas de monitoramento e políticas ambientais cujo foco seja a conservação de ecossistemas aquáticos.

Palavras-chave: Habitat físico, uso do solo, esforço amostral, índices multimétricos, macroinvertebrados, risco relativo, EPT, bioindicadores, cerrado.

Abstract

Freshwater ecosystems are among the most threatened by anthropogenic activities on a global scale. The high demand for water has resulted in an ongoing process of degradation leading to the loss of biodiversity in these ecosystems. In the Brazilian savanna the intense expansion of agribusiness results in deforestation, habitat loss, introduction of exotic species, soil erosion, water pollution, among others. The general objective of this thesis was to evaluate the ecological conditions of savanna streams through the development of effective tools to efficiently conserve and manage these resources. Through a probabilistic sampling design, streams belonging to the hydrological units of four hydroelectric projects were selected (Nova Ponte, Três Marias, Volta Grande and São Simão). Macroinvertebrate sampling and physical habitat characterization followed the standardized protocol of the US Environmental Protection Agency (US EPA) adapted for savanna streams. The objective of the first chapter was to evaluate the efficiency of macroinvertebrate taxonomic richness sampling effort obtained through samplings at local and basin scales. In addition, it was determined which environmental factors are associated with a greater sampling effort required to characterize the taxonomic richness on a local scale. It was verified that the sample effort varies with the scale of study and with the evaluated taxonomic resolution. Metrics describing habitat heterogeneity and structure, anthropogenic disturbances in the riparian zone and water quality explained a significant amount of the effort required to characterize the macroinvertebrate richness. In the second chapter the objective was to develop a multimetric index (MMI) capable of reflecting anthropogenic impacts using benthic macroinvertebrate assemblage metrics. Steps of MMI development included: 1) selection of least- and most-disturbed sites; 2) classification and selection of biological metrics; 3) selection of the best MMI model; 4) assignment of condition classes; 5) evaluation of the relationships between MMI and anthropogenic stressors; 6) assessment of ecological conditions at local and regional scales. The final MMI was able to distinguish between least- and most-disturbed sites, responding to anthropogenic impacts related to land use, water quality, and physical habitat structure. In the third chapter, the association between anthropogenic stressors and the biological condition represented by MMI was evaluated at local (in each hydrological unit) and regional scales. For that, the relative risk (RR) and relative stressor extent (RE) approach was used, which evaluates the importance and magnitude of a given stressor for the biological condition, respectively. It was verified that in regional scale turbidity, % of fine sediment and % of agriculture are the main stressors associated with poor biological conditions. In local scale % of pasture and total nitrogen are also associated with poor biological conditions but occurring in small extensions. In general, evaluating RR and RE together, it was found that reducing the impact of excess sediment on the streambed can greatly improve the biological condition. The products of this thesis aim to provide subsidies for managers and decision makers to implement monitoring programs and environmental policies focused on the conservation of aquatic ecosystems.

Keywords: Physical habitat, land uses, sample effort, multimetric indices, macroinvertebrates, relative risk, EPT, bioindicators, savanna.

Introdução

Embora ocupem um pequeno percentual da superfície total da Terra (aproximadamente 1%), os ecossistemas aquáticos continentais são responsáveis por abrigar uma elevada diversidade de espécies, muitas destas endêmicas e sensíveis a alterações no meio (Revenga et al., 2005; Strayer e Dudgeon, 2010). Além disso, ecossistemas aquáticos são essenciais para o estabelecimento de populações humanas, uma vez que fornecem água para uso doméstico, industrial e agropecuário, geração de energia, navegação e lazer (Malmqvist e Rundle, 2002; Revenga et al., 2005).

O acelerado crescimento da população humana e a intensa demanda por água tem resultado em séria, e muitas vezes irreversível, degradação de ecossistemas aquáticos e sua biota (Abell et al., 2008; Naiman e Turner, 2000). Atividades antrópicas tem levado a perda de habitat, modificações nos fluxos naturais de rios, poluição das águas, exploração da fauna e introdução de espécies (Dudgeon et al., 2006). Como forma de mitigar esses impactos antrópicos, faz-se necessário o desenvolvimento de ferramentas de avaliação de condições ecológicas em ecossistemas aquáticos (Balderas et al., 2016). Dessa forma, forncendo subsídios para implementação de políticas de manejo cujo foco seja a conservação da integridade e dos serviços promovidos por esses ecossistemas (Revenga et al., 2005).

Nesta introdução geral são apresentadas as bases conceituais ecológicas que nortearam esta tese. O conceito de **distúrbio** (e termos correlatos como **estresse** e **perturbação**) e como distúrbios de origem antrópica influenciam as condições biológicas são fundamentais para o entendimento das transformações que ocorrem nos ecossistemas. O conceito de **organização hierárquica de sistemas fluviais** estabelece como as diversas escalas de observação influenciam na estruturação de comunidades aquáticas. **Níveis hierárquicos em ecologia** descrevem a organização estrutural e dinâmica de elementos da natureza. Um enfoque maior é dado nos conceitos de **comunidades** (e **assembleias**), e no papel de comunidades de macroinvertebrados bentônicos como **indicadores biológicos**. Por fim, é abordado o conceito de **integridade biótica** e o papel de índices de integridade biótica como ferramenta de avaliação de condições ecológicas em riachos no contexto do bioma cerrado.

Distúrbio

Os ecossistemas estão em constante mudança em resposta às alterações no meio circundante, sejam elas de origem natural (p. ex. inundações, secas, fogo) ou antrópicas (Odum, 1969). Na literatura, essas alterações têm sido associadas aos termos *distúrbio*, *perturbação* e *estresse* como forma de entender os efeitos e mecanismos de respostas dos ecossistemas frente às alterações causadas no ambiente (Borics et al., 2013).

Embora não haja um consenso na literatura, Pickett & White (1985) propuseram a definição de distúrbio como sendo *qualquer evento que rompe a estrutura dos ecossistemas, comunidades ou populações, resultando em alterações na disponibilidade de recursos, nos substratos e no ambiente físico.* O termo estresse, por sua vez, apresenta um número ainda maior de ambiguidades e inconsistências em estudos ecológicos e, muitas vezes, é erroneamente usado como sinônimo de distúrbio (Borics et al., 2013). Embora ambos os termos estejam associados à degradação de ecossistemas, a distinção entre distúrbio e estresse é bem entendida através dos seus efeitos sobre os sistemas ecológicos (Cain et al., 2013). Enquanto distúrbio é geralmente causador de danos severos em ecossistemas, estresse está associado a

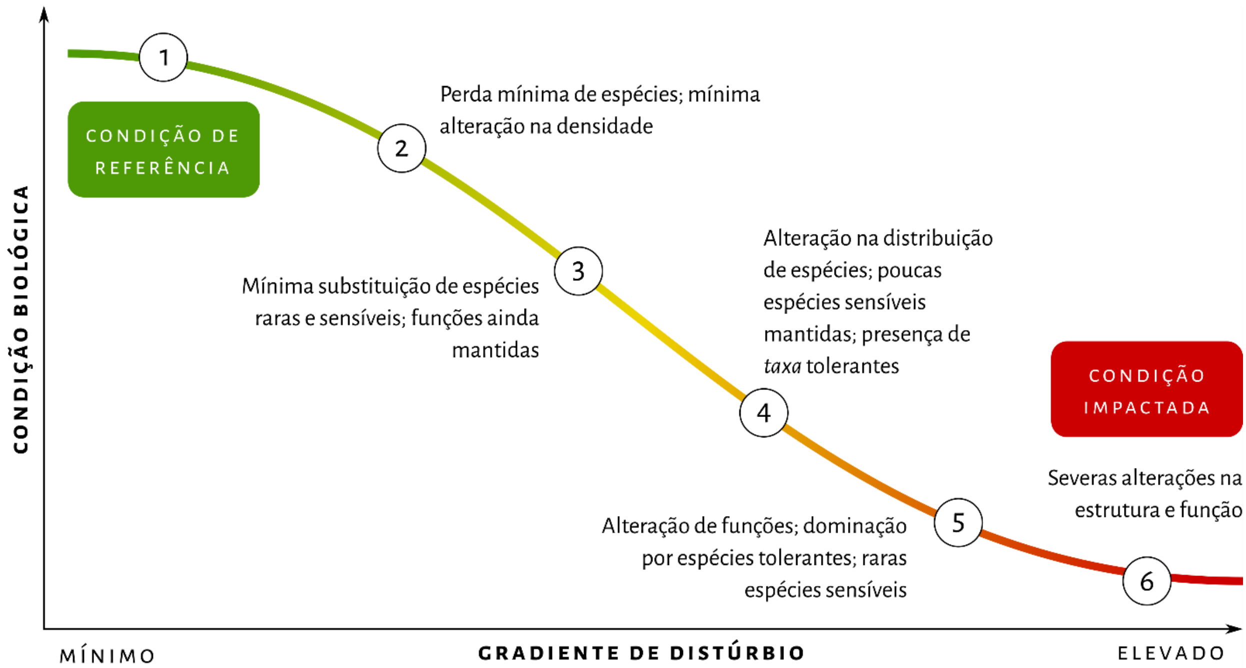


Figura 1. Representação dos estágios de mudanças na condição biológica de um ecossistema frente a um gradiente de distúrbio ambiental devido a atividades antrópicas (adaptada de Davies & Jackson 2006).

fatores limitantes de crescimento e reprodução de indivíduos não envolvendo mortalidade (Cain et al., 2013). O termo perturbação, por fim, trata da resposta de um componente ou sistema ecológico mediante distúrbios ou outro processo ecológico (Rykiel, 1985). Dessa forma, enquanto distúrbio e estresse são agentes causadores de danos aos sistemas ecológicos, perturbação trata de seus efeitos.

Distúrbios de origem antrópica têm sido responsáveis por intensa e contínua transformação nos ecossistemas de maneira rápida e muitas vezes irreversível (Barnosky et al., 2012; Hong e Lee, 2006). As principais causas de perda de biodiversidade estão associadas a atividades antrópicas como a degradação do solo, fragmentação de habitat, poluição, introdução de espécies exóticas e mudanças climáticas (Hong e Lee, 2006; Sala et al., 2000).

Como forma de interpretar mudanças nos ecossistemas frente a distúrbios antrópicos, Davies & Jackson (2006) propuseram um modelo conceitual que descreve claramente como a condição biológica de um ecossistema declina mediante um gradiente de distúrbio (Figura 1). Aspectos biológicos acompanham a transição de um ambiente em condições de referência (1) até a condição impactada, incluindo perda de espécies (2), mudanças na densidade de organismos (3), substituição de espécies sensíveis (4), dominância por espécies tolerantes (5), até por fim, um cenário com severas alterações na estrutura e função de comunidades (6). O entendimento dessa relação de distúrbio *versus* condição biológica permite identificar e proteger áreas em boas condições ecológicas e, da mesma forma, diagnosticar e recuperar áreas em estágios avançados de degradação.

Organização Hierárquica de Ecossistemas Fluviais

As principais ameaças aos ecossistemas aquáticos são decorrentes de atividades humanas em múltiplas escalas (Allan, 2004; Allan et al., 1997; Roth et al., 1996). Conforme proposto por Frissell et al. (1986), os sistemas fluviais podem ser compreendidos através de uma organização hierárquica dividida em diversas escalas espaciais de observação: bacia hidrográfica (maior escala), segmento, trecho, habitat (corredeiras e remansos), e micro-habitat (menor escala) (Figura 2). As bacias hidrográficas, maior unidade de estudo, são formadas por segmentos e trechos de riachos. Estes, por sua vez, referem-se às extensões longitudinais definidas por características das zonas ripárias e dos vales dos riachos. Os habitats são unidades hidrogeomórficas caracterizadas por fluxos de água lentos (remansos) e rápidos (corredeiras). Os micro-habitat são caracterizados pelos substratos presentes no leito de rios (p. ex. detritos foliares, siltes, areias, seixos, cascalhos e musgos) (Allan et al., 1997). Nesta tese o termo unidade hidrológica foi utilizado no intuito de caracterizar uma área de drenagem pré-estabelecida entre os níveis de bacia hidrográfica e segmentos de rio (Ferreira et al., 2017; Seaber et al., 1987)

O sistema hierárquico fluvial está arranjado de maneira aninhada, ou seja, níveis superiores controlam as características expressas nos níveis inferiores, o inverso não sendo verdadeiro (Frissell et al., 1986). Assim, fatores geofísicos, diferentes usos dos solos e impactos de ocupação humana em escalas de bacia hidrográfica afetam a estrutura da vegetação ripária, o regime de vazão, a carga de nutrientes e sedimentos nos leitos

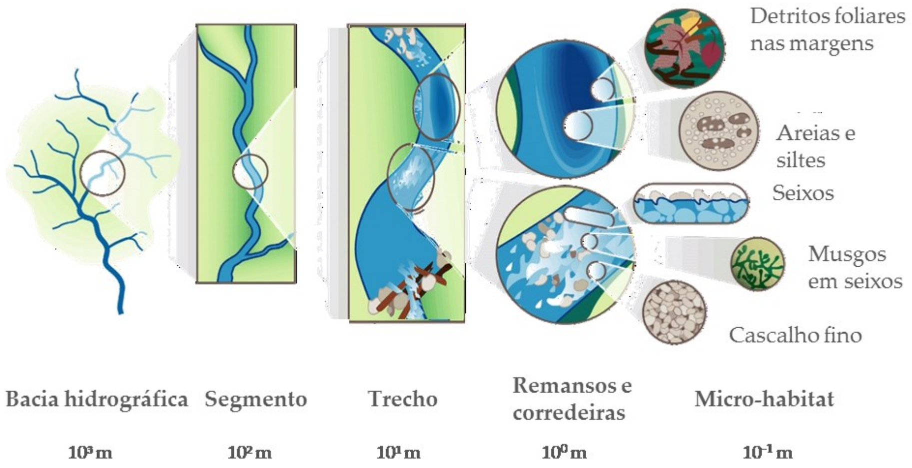


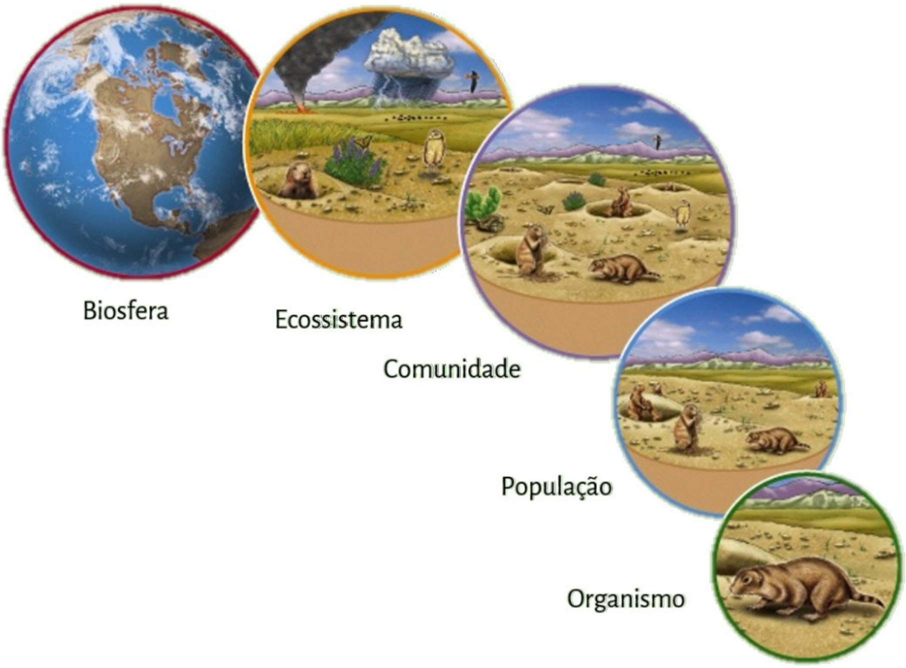
Figura 2. Esquema dos níveis hierárquicos de ecossistemas fluviais (Frissell et al. 1986)

dos rios e a qualidade e disponibilidade de habitat (Allan, 2004; Macedo et al., 2014a). Por sua vez, alterações na qualidade e disponibilidade de habitat físicos são fatores que influenciam diretamente a estrutura e composição de comunidades aquáticas (Maddock, 1999). A organização hierárquica permite avaliar e determinar a importância dos vários fatores ambientais e antrópicos agindo nas diversas escalas espaciais influenciando as comunidades aquáticas.

Níveis Hierárquicos em Ecologia

Em ecologia, a organização estrutural e dinâmica dos elementos bióticos e suas interações com o ambiente podem ser entendidas através de níveis hierárquicos (Odum e Barrett, 2005). Em cada nível hierárquico são descritos sistemas ecológicos que vão de organismos — menor escala e unidade fundamental em ecologia — até biosfera, a maior escala e que compreende todos os organismos vivos interagindo com o ambiente físico (Ricklefs, 2008). Organismos da mesma espécie vivendo em conjunto define o nível hierárquico de população. Subsequentemente, comunidade refere-se às várias populações interagindo entre si, ecossistema representa a conexão de diversas comunidades com o ambiente e a biosfera compreende todos os ecossistemas e processos globais (Ricklefs, 2008)

Figura 3. Hierarquia dos níveis de organização ecológica (Figura adaptada de Ricklefs, 2008).



(Figura 3). Diferentes autores consideram, entre os níveis de ecossistema e biosfera, os níveis de paisagem e bioma. Para Odum & Barret (2005), paisagem refere-se à área heterogênea composta de um agregado de ecossistemas em interação e que se repetem de maneira similar em toda a sua extensão; e bioma, no nível acima, como um grande sistema regional caracterizado por clima, solo e/ou vegetações específicas.

Embora possam existir divergências nas formas de classificação, o arranjo hierárquico é uma forma conveniente e prática de subdividir e estudar, de maneira holística, interações complexas na natureza (Ahl e Allen, 1996).

Comunidades e Indicadores Ecológicos

Cada nível hierárquico apresenta características de processos e estruturas únicas, permitindo abordagens sob a perspectiva de sistemas ecológicos específicos no intuito de responder a questões ecológicas (Ricklefs, 2008). A abordagem de comunidades, por exemplo, busca entender padrões na estrutura e comportamento de grupos de indivíduos e suas interações (Begon et al., 2006). Embora o conceito de comunidade não seja um consenso na literatura (Tabela 1; Fauth et al., 1996; Stroud et al., 2015), de maneira geral, uma comunidade pode ser definida como *grupo de populações de espécies que interagem e ocorrem juntas no espaço* (Stroud et al., 2015), onde são considerados apenas os componentes bióticos e as relações entre eles (Eichhorn, 2016).

Tabela 1. Definições de *comunidade* retiradas de glossários de livro texto em ecologia (adaptado de Stroud et al. 2015).

Definição	Fonte
Grupo de populações de plantas e animais num determinado local; unidade ecológica utilizada em sentido amplo para incluir grupos de vários tamanhos e graus de integração.	Krebs (1985)
Um conjunto de plantas e animais que interagem em um local compartilhado.	Freedman (1989)
Uma associação de populações interagindo, geralmente definida pela natureza de sua interação ou local onde vivem.	Ricklefs (1990)
Um grupo de organismos que vivem juntos e em que as diferentes espécies e indivíduos interagem uns com os outros.	Tudge (1991)
Um grupo de plantas e animais que interagem habitando uma determinada área.	Smith (1992)
Grupo de populações de plantas e animais em um determinado local; usado em sentido amplo para se referir a unidades ecológicas de vários tamanhos e graus de integração.	Stiling (1996)
Uma associação de populações interagindo, geralmente definida pela natureza de sua interação ou pelo local onde vive.	Ricklefs & Miller (1999)
A componente biótica viva total de um ecossistema, incluindo plantas, animais e microorganismos.	Calow (2009)
Um conjunto de populações que interagem, formando um grupo identificável dentro de um bioma.	Arora and Kanta (2009)
Um grupo de espécies vivendo juntas e interagindo através de processos ecológicos como competição e predação.	Levinton (2009)
Todas as espécies de organismos encontradas em uma área definida, ao longo do tempo ecológico.	Dodds (2009)
Uma associação de espécies ou todos os organismos que interagem vivendo em uma determinada área.	Molles (2010)
Coleção de espécies encontradas em um determinado lugar.	Morin (2011)

Outro termo comum em estudos ecológicos refere-se às assembleias biológicas. Devido ao fato de nem toda amostra biológica representar, necessariamente, toda a comunidade, o termo assembleia sugere um recorte metodológico para estudos, cujo foco esteja em determinados grupos (Eichhorn, 2016). Assim como ocorre com o conceito de comunidades, não há um consenso para a definição de assembleias (Fauth et al., 1996), embora seja largamente aceita como g*rupos taxonômica e filogeneticamente relacionados dentro de uma comunidade biológica* (Stroud et al., 2015).

Assim, estudos ecológicos muitas vezes têm seu foco voltado para determinados grupos taxonomicamente relacionados (p. ex. assembleias de peixes em riachos). Macroinvertebrados bentônicos, embora nem sempre compartilhem da mesma filogenia ou unidade taxonômica (p. ex. insetos, moluscos, anelídeos), são frequentemente referidos como *assembleias*. Esta é uma forma eficiente de distinguir este grupo de organismos através de um recorte funcional em termos do uso de um compartimento específico em ecossistemas aquáticos - o compartimento bentônico.

Comunidades ou assembleias biológicas que respondem a impactos antrópicos e alterações no meio são consideradas indicadoras biológicas (ou bioindicadoras) (Rosenberg e Resh, 1993). Suas características biológicas e ecológicas, mensuradas qualitativa ou quantitativamente, refletem o estado de um sistema ecológico, possibilitando estabelecer relações de causalidade, antever mudanças futuras no ambiente e obter um diagnóstico de condições ecológicas (Dale e Beyeler, 2001).

O uso de bioindicadores como ferramenta potencial de avaliação de integridade ecológica deve atender aos seguintes critérios (Dale e Beyeler, 2001): (1) fácil mensuração; (2) sensibilidade a estressores; (3) resposta previsível a stress; (4) antecipação de mudanças iminentes no ambiente; (5) previsibilidade de mudanças que possam ser evitadas por ações de manejo; (6) integrado com as mudanças ao longo de gradientes ambientais; (7) resposta conhecida a distúrbios naturais e de origem antrópica; (8) baixa variabilidade de respostas a estressores.

As características acima colocam a abordagem com indicadores biológicos em vantagem às abordagens tradicionais que utilizam apenas parâmetros abióticos para avaliação de condições ecológicas (Karr, 1999). Indicadores biológicos refletem, através do seu tempo de vida ou tempo de residência, o componente temporal na avaliação ecológica de um local. Isso permite integrar condições ambientais passadas, presentes e futuras, diferente de medidas físicas e químicas que apenas caracterizam condições ecológicas no momento da amostragem (Holt, 2010).

Historicamente, o uso de bioindicadores para avaliação de condições ecológicas deu-se através de parâmetros baseados em espécies isoladamente (p. ex. presença de determinada espécie indicadora) ou através de métricas simplificadas de comunidades (p. ex. riqueza taxonômica, índices de diversidade) (Niemi e McDonald, 2004). No entanto, tais medidas não representam como um todo a comunidade biológica presente no ambiente avaliado (Niemi e McDonald, 2004). Assim, Karr (1981) desenvolveu uma ferramenta de avaliação de condições ecológicas baseada em um índice que integra medidas múltiplas de assembleias bioindicadoras, os chamados Índices de Integridade Biótica.

Índice de Integridade Biótica como Ferramenta de Avaliação de Condições Ecológicas: Histórico, Aplicações e Abordagens

O termo *integridade biótica* surgiu em 1972 com a lei federal norte americana Clean Water Act, que estabelecia a restauração e manutenção da integridade química, física e biológica de recursos hídricos nos EUA. O termo foi então definido por Frey (1977) e adaptado por Karr (1981), como sendo a c*apacidade de manutenção e suporte de comunidades biológicas preservando sua composição, diversidade e estrutura funcional de forma comparável às características naturais da região.*

Visando o desenvolvimento de metodologias eficazes para quantificar e avaliar a integridade biótica de ecossistemas aquáticos Karr (1981) propôs o Índice de Integridade Biótica (do inglês *Index of Biotic Integrity*, IBI). Um IBI, ou índice multimétrico (MMI), propõe em um único índice a síntese de atributos ou métricas biológicas que reflita distúrbios antrópicos em ecossistemas aquáticos ao longo de um gradiente de condições ambientais (Karr et al., 1986).

Em sua versão inicial, um IBI foi desenvolvido utilizando 12 métricas que descrevem características de composição e riqueza de espécies, composição trófica, comportamento reprodutivo e condições específicas de assembleias de peixes (Karr, 1981). Nessa abordagem, as métricas recebem um escore de acordo com o que seria esperado em situações onde há ausência de distúrbio antrópico e a condição ecológica é descrita por meio de classes qualitativas que variam de excelente a muito pobre (Karr, 1981).

O estabelecimento de condições de referência é um componente fundamental na avaliação da integridade biótica e desenvolvimento de MMIs (Elias et al., 2016; Feio et al., 2014; Whittier et al., 2007). Os sítios de referência são descritos como locais minimamente alterados por atividades antrópicas e com características próximas à condição natural. No entanto, nem sempre é possível identificar os sítios minimamente alterados devido à intensificação da pressão antrópica sobre os ecossistemas (Stoddard et al., 2006; Whittier et al., 2007). Para contornar esse problema são selecionados dentro de um gradiente de distúrbio sítios que se encontram em condições menos alteradas (*least disturbed*), utilizados como sítios de referência (p. ex. Ligeiro et al., 2013; Martins et al., 2017).

O trabalho pioneiro de Karr (1981) tornou-se referência como ferramenta de avaliação de integridade biótica (Ruaro e Gubiani, 2013), servindo como base para adaptações e desenvolvimento de novos índices com o intuito de aumentar seu potencial de avaliação ecológica, conservação e manejo de ecossistemas aquáticos (Klemm et al., 2003). Ao longo dos anos foram incorporados critérios estatísticos na seleção de métricas (Hering et al., 2006; Stoddard et al., 2008), definição de sítios de referência (Bailey et al., 2004; Herlihy et al., 2008; Ligeiro et al., 2013; Whittier et al., 2007), avaliação de métodos de escores (Blocksom, 2003), uso de desenho amostral probabilístico (Herlihy et al., 2000) e correção de métricas para variação ambiental (Carvalho et al., 2017; Chen et al., 2014; Macedo et al., 2016; Pereira et al., 2016).

Além de ser desenvolvido e aplicado para assembleias de peixes (p. ex. Carvalho et al. 2017, Casatti et al. 2009, Hughes et al. 1998, Pont et al. 2009, Terra et al. 2013) o IBI foi também adaptado para diatomáceas (p. ex. Delgado et al. 2010, Fore 2003), aves (p. ex. Bryce 2006, Bryce et al. 2002), anfíbios (p. ex. Stapanian et al. 2015) e macroinvertebrados (p. ex. Kerans & Karr 1994, Lunde & Resh 2012, Macedo et al. 2016, Moya et al. 2011, Pereira et al. 2016). Nesse último caso, a utilização de assembleias de macroinvertebrados bentônicos como ferramenta para o desenvolvimento de índices multimétricos justifica-se principalmente devido à sua capacidade de resposta a mudanças ambientais (Karr e Chu, 1998). Esses organismos exibem preferências quanto à utilização de recursos alimentares (Ferreira et al., 2017; Graça, 2001; Tomanova et al., 2006), habitat físico (Kaufmann et al., 2014; Maddock, 1999; Nerbonne e Vondracek, 2001; Silva et al., 2016) e qualidade de água (Cao et al., 1996; Lenat, 2011) respondendo a distúrbios em ecossistemas aquáticos, através de alterações em sua estrutura e composição (McCabe e Gotelli, 2000).

Apesar de países tropicais abrigarem elevada biodiversidade aquática, estes estão entre os que apresentam menor número de estudos para avaliar a condição ecológica de seus ecossistemas aquáticos (Ruaro e Gubiani, 2013). Parte disso justifica-se pela falta de apoio financeiro (Bozzetti e Schulz, 2004), baixo conhecimento ecológico e taxonômico das espécies indicadoras, dificuldade em encontrar áreas de referência (Casatti et al., 2009), além da falta de legislação específica para avaliações biológicas de qualidade de água (Buss et al., 2016; Ruaro e Gubiani, 2013). No Brasil, a maioria dos estudos a respeito do desenvolvimento e aplicação de IBI utiliza como indicador biológico assembleias de peixes (Araujo et al., 2003; Bozzetti e Schulz, 2004; Carvalho et al., 2017; Casatti et al., 2009; Terra et al., 2013) e macroinvertebrados bentônicos (Baptista et al., 2013, 2007; Couceiro et al., 2012; Macedo et al., 2016; Oliveira et al., 2011; Pereira et al., 2016), em sua maioria, nos biomas mata atlântica, cerrado e amazônia. No entanto, ainda observam-se importantes lacunas quanto à padronização de métodos, limitações quanto à aplicabilidade em larga escala e na definição de critérios para estabelecimento de áreas de referência.

Estimativas de Riqueza

Estudos que descrevem a riqueza taxonômica de macroinvertebrados bentônicos em riachos de cabeceira são ainda escassos, o que torna difícil tirar conclusões a respeito dos padrões de distribuição da biodiversidade em riachos (Clarke et al., 2008). Comparações entre estudos nem sempre são possíveis devido às diferentes técnicas de amostragem (Gotelli e Colwell, 2001) e desenho experimental altamente variável. Além disso, outro problema que envolve comparação de riqueza taxonômica entre diferentes locais surge quando a curva de acumulação de espécies não atinge uma assíntota, significando que o número de amostras coletadas ainda não foi suficiente para representar o conjunto de unidades taxonômicas presentes em uma dada área ou ecossistema (Gotelli e Colwell, 2001). Clarke et al. (2008) também sugerem o uso de estimadores de riqueza para avaliar o quanto uma dada comunidade terá sido adequadamente amostrada.

A contagem do número de diferentes indivíduos em uma determinada área, através da riqueza taxonômica, é uma maneira simples e intuitiva de caracterização da diversidade de comunidades biológicas (Gotelli e Colwell, 2001). A riqueza de espécies é influenciada por variações naturais no ambiente e por distúrbios de origem antrópica (Rosenberg e Resh, 1993). É uma métrica amplamente utilizada em estudos de biodiversidade (Wilsey et al., 2005), uma importante ferramenta para gestão de áreas protegidas (Melo, 2008), e um conceito fundamental em ecologia de comunidades.

Considerando que o número de espécies em uma dada comunidade é limitado, a riqueza total de espécies poderia teoricamente ser determinada para qualquer comunidade (Walther e Morand, 1998). No entanto, a realização de um censo ou um inventário completo requer esforços extraordinários, muitas vezes inviáveis por limitações taxonômicas incluindo espécies ainda não descritas (Chao, 2006). Além disso, o aumento no número de amostras necessário para obter uma estimativa real (ou próxima disso) de uma comunidade pode também resultar em aumento dos custos necessários para obtenção dos dados em campo (Bartsch et al., 1998).

Curvas de acumulação de espécies (ou curvas do coletor) são formas simples de avaliar como a riqueza de espécies varia de acordo com o esforço amostral, onde o número de *taxa* geralmente cresce assintoticamente com o aumento no número de amostras (Cao et al., 2001). Quando a curva atinge a estabilização e não é observado incremento na riqueza de com o aumento do esforço amostral, todas as espécies terão sido amostradas. Assim, essas curvas permitem estimar o número esperado de espécies em um conjunto de amostras e estimar o mínimo necessário de amostras para caracterização de uma comunidade (Bady et al., 2005; Bartsch et al., 1998).

Stout & Vandermeer (1975), comparando riachos em diferentes latitudes, sugeriram que riachos tropicais abrigam maior riqueza de espécies de insetos aquáticos em relação a riachos temperados. No entanto devido ao pequeno tamanho das amostras coletadas, a riqueza observada nos trópicos era sub-estimada. Além do mais, devido à constante captura de *taxa* raros, nem sempre é possível observar uma estabilização na curva de acumulação de espécies de macroinvertebrados bentônicos em riachos tropicais (Schneck e Melo, 2010).

Sendo assim, a impossibilidade de mensurar toda a riqueza local torna necessário o uso de estimadores de riqueza para preencher as lacunas deixadas por inventários incompletos (Walther e Morand, 1998). Dentre as formas de se obter estimativas da riqueza, os estimadores não paramétricos são amplamente utilizados (Gotelli e Colwell, 2010). Estes estimadores utilizam informações do número de espécies raras, mas que não foram detectadas nas amostras coletadas para aproximar ao número de espécies presentes (Gotelli e Colwell, 2010). Diversos modelos não paramétricos disponíveis na literatura foram testados e comparados para diversas comunidades biológicas e seu uso indicado em algumas situações (Hellmann and Fowler, 1999, com comunidades de plantas lenhosas; Hughes et al., 2002, com peixes; Melo and Froehlich, 2001, com invertebrados aquáticos; Ricetti and Bonaldo, 2008, com aranhas; Walther and Morand, 1998, com parasitas).

Avaliação de risco relativo e extensão relativa de estressores

A intensificação de pressões antrópicas sobre ecossistemas aquáticos faz com que seja necessário o desenvolvimento de métodos de avaliação ambiental no intuito de melhorar a gestão, conservação e reabilitação de recursos aquáticos (Buss et al., 2015; Helson e Williams, 2013; Ruaro e Gubiani, 2013). Em particular, é essencial identificar os principais estressores que influenciam, de forma direta ou indireta, os ecossistemas de água doce.

Nos Estados Unidos (EUA), a Agência de Proteção Ambiental (EPA) utiliza a abordagem de risco relativo (RR) e extensão relativa (RE) para relatar condições regionais e nacionais de riachos, rios, lagos e zonas húmidas (Angradi et al., 2011; Paulsen et al., 2008; USEPA, 2016a, 2016b, 2016c). O fundamento desta abordagem está na sua capacidade de fornecer associações quantificáveis entre os principais estressores e as respostas biológicas (Van Sickle et al., 2006; Van Sickle e Paulsen, 2008). O RR descreve a probabilidade de se observar uma condição biológica boa versus ruim dada à presença versus ausência de estressores em níveis elevados. O RE fornece a magnitude na qual estressores em elevados níveis foram encontrados em determinada região. As abordagens RR e RE são descritas em termos de medidas discretas (condições "boas" e "ruins") ao invés de medidas contínuas. Elas fornecem estimativas de risco que são facilmente interpretadas e familiares para o público em geral (Van Sickle et al., 2006, Van Sickle e Paulsen, 2008). Além disso, as abordagens RR e RE visam dar suporte aos tomadores de decisão através do direcionamento de esforços visando a reabilitação dos ecossistemas aquáticos e mitigação dos estressores mais fortemente associados a condições biológicas ruins (Hughes et al., 2000).

Estimativas precisas de RR e RE podem ser obtidas por meio de amostragens aleatorizadas (Van Sickle e Paulsen, 2008). O uso de desenho amostral probabilístico é fortemente recomendado para seleção de sítiosamostrais em estudos de avaliações de condições ecológicas em riachos por várias razões (Dobbie e Negus, 2013; Herlihy et al., 2000; Olsen e Peck, 2008; Stevens e Olsen, 2004). 1) Este desenho assegura a representatividade sobre a região estudada (Herlihy et al., 2008, 2000), onde as características físicas, químicas e biológicas refletem a condição ecológica (Macedo et al., 2014a). 2) É uma ferramenta econômica que permite inferências confiáveis e precisas para áreas geográficas maiores através de um número menor de sites (Paulsen et al., 2008) 3) Este desenho permite estimativas estatísticas sobre a extensão de riachos de toda a população alvo de interesse (Herlihy et al., 2000). 4) Finalmente, essa abordagem aleatória evita conclusões tendenciosas quando a seleção do sítios de amostragem é baseada na conveniência, fato muito comum em estudos de avaliação ecológica (Dobbie, et al., 2008; Dobbie e Negus, 2013; Jiménez-Valencia et al., 2014).

Um programa de monitoramento bem projetado fornece resultados confiáveis e válidos em relação a questões ambientais de interesse (Dobbie e Negus, 2013; Paulsen et al., 2008). No entanto, a prática ainda é negligenciada no Brasil e na maioria dos outros países da América do Sul (Jiménez-Valencia et al., 2014; Macedo et al., 2014b), onde a biodiversidade é alta (Barlow et al., 2016; Brook et al., 2006; Myers et al., 2000) e mudanças ambientais generalizadas estão ocorrendo rapidamente (Barlow et al., 2016; Brook et al., 2006; Hernández et al., 2016; Vörösmarty et al., 2010). Existe uma necessidade urgente de melhorar os métodos de seleção de sítes para obter dados de qualidade que ofereçam subsídios para o emprego de melhores práticas de gerenciamento no intuito de proteger e reabilitar condições ecológicas em riachos. Este é especialmente o caso do cerrado, um bioma altamente ameaçado, que sofre com a substituição de sua cobertura vegetal natural e expansão de atividades de pastagem e agricultura (Hunke et al., 2015; Ratter et al., 1997; Strassburg et al., 2017).

Bioma cerrado: contexto de estudos de avaliação de condições ecológicas

Apesar de sua importância global como um *hotspot* de biodiversidade (Myers et al., 2000), o cerrado apresenta um número reduzido de estudos de avaliação de condições ecológicas. Esse fato é especialmente preocupante tendo em vista o acelerado processo de degradação que este bioma sofre (Hunke et al., 2015).

Mais da metade de seus originais 2 milhões de km² de área foram transformados para usos antrópicos nas últimas décadas (Klink e Machado, 2005). Estudos recentes mostram que as taxas de desflorestamento no cerrado foram 2,5 vezes superiores àquelas observadas no bioma amazônico de 2002 a 2011 (Strassburg et al. 2017), enquanto esforços para sua conservação são ainda limitados, pois apenas 2,2% de sua área encontram-se sob proteção legal (Klink e Machado, 2005). Projeções recentes demonstram que o cerrado pode vir a ser extinto até 2050 (Strassburg et al., 2017).

Das principais atividades, o agronegócio é o que mais movimenta o setor econômico no cerrado através de pastagens e plantações de cana de açúcar, soja, milho, café, feijão, entre outros (Hunke et al., 2015). Como consequência dessa super exploração do cerrado, observam-se a perda de cobertura vegetal nativa (savanas, florestas, campos), fragmentação de habitat, invasão de espécies exóticas, perda da biodiversidade, erosão do solo, poluição de ecossistemas aquáticos, alterações no regime natural de fogo e mudanças climáticas regionais (Klink e Machado, 2005).

Do ponto de vista hidrológico, o cerrado desempenha um papel fundamental na dinâmica de recursos hídricos. Ele é responsável por abrigar porções de 10 regiões hidrográficas brasileiras: Tocantins (65% da área desta região hidrográfica está no Cerrado), São Francisco (57%), Paraguai (50%), Paraná (49%), Parnaíba (46%), Atlântico Nordeste (46%), Atlântico Leste (8%), Amazônia (4%), Atlântico Sudeste (1%) e Nordeste Oriental (<1%). Além disso, abriga aproximadamente 90% das nascentes da bacia do Rio São Francisco (Oliveira et al., 2014). Essas bacias fornecem água para suprir demandas da indústria, agricultura, navegação, turismo e geração de energia hidrelétrica (Oliveira et al., 2014). Além disso, o cerrado também possui 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 (Oliveira et al., 2014).

No entanto, a constante pressão sobre esses ecossistemas tem resultado em mudanças na composição da biota e na estrutura de habitat aquáticos (Ferreira et al., 2017; Macedo et al., 2014a). O grande potencial hídrico do cerrado propiciou a construção de empreendimentos hidrelétricos que trouxeram impactos diretos a partir de sua implementação (p. ex. realocação de pessoas, deterioração da qualidade da água, perda de patrimônio genético, desestruturação de comunidades aquáticas, alterações climáticas) e efeitos indiretos como, por exemplo, adensamento populacional, expansão de atividades agropecuárias no entorno, entre outros (Von Sperling, 2012).

Esses fatores fazem com que seja necessário o desenvolvimento de ferramentas efetivas visando a gestão de bacias hidrográficas. Nesta tese, a avaliação das relações do habitat físico e de esforço amostral (Cap. 1), o desenvolvimento do índice de integridade biótica (Cap. 2) e a avaliação de condições ecológicas em riachos no cerrado (Cap. 3) fornecerão subsídios para implementação de programas de biomonitoramento, cujo enfoque seja a conservação da biodiversidade e manejo de ecossistemas aquáticos.

Objetivo Geral

Avaliar condições ecológicas em riachos no cerrado a fim de fornecer bases para sua conservação.

Objetivos Específicos

Esta tese foi dividida em 3 capítulos que abordam aspectos complementares visando alcançar o objetivo geral.

No capítulo 1, *The role of physical habitat and sampling effort on estimates of benthic macroinvertebrate taxonomic richness at basin and site scales*:

a. avaliar a eficiência do esforço amostral da riqueza taxonômica obtida através de amostragens em escalas local e de bacia;
b. determinar quais características ambientais estão associadas a um maior esforço amostral necessário para caracterização da riqueza taxonômica de macroinvertebrados bentônicos em escala local (riachos).

No capítulo 2, *An improved macroinvertebrate multimetric index for the assessment of wadeable streams in the neotropical savanna*:

a. desenvolver um índice multimétrico robusto utilizando macroinvertebrados bentônicos para avaliar a integridade ecológica de riachos no cerrado;
b. avaliar a condição ecológica em unidades hidrológicas de empreendimentos hidrelétricos em região neotropical;

c. avaliar a relação do índice multimétrico proposto com distúrbios antrópicos;
d. extrapolar a condição ecológica de riachos para o comprimento total de riachos.

Finalmente, no capítulo 3, *Assessing the extent and relative risk of aquatic stressors on stream macroinvertebrate assemblages in the neotropical savanna*:

a. estimar a extensão de riachos perenes e acessíveis.
b. avaliar, individualmente, a importância – ou risco relativo (RR), e a magnitude – ou extensão (RE) de estressores para a condição biológica (representada pelo MMI) em escalas local (em cada unidade hidrológica) e regional.

Metodologia Geral

Estrutura da Tese

Esta tese de doutorado foi desenvolvida no âmbito do projeto CEMIG – Companhia Energética de Minas Gerais/Peixe-Vivo, intitulado: Desenvolvimento de índices de integridade biótica para avaliação de qualidade ambiental e subsídio para restauração de habitat em áreas de soltura de alevinos. Realizado entre os anos de 2009 e 2013, este projeto contou com a colaboração de instituições nacionais (Universidade Federal de Minas Gerais, Universidade Federal de Lavras, Pontifícia Universidade Católica de Minas Gerais, Centro Federal de Educação Tecnológica de Minas Gerais) e internacionais (Oregon State University, United States Environmental Protection Agency). O objetivo principal foi traduzir, aplicar e validar metodologias de avaliação de qualidade ambiental desenvolvidas e utilizadas pela Agência de Proteção Ambiental Norte-Americana, adaptando-as ao contexto de riachos no cerrado brasileiro (Callisto et al., 2014). As amostragens foram realizadas em unidades hidrológicas de quatro empreendimentos hidrelétricos da CEMIG: Nova Ponte (MG), Três Marias (MG), Volta Grande (MG–SP) e São Simão (MG–GO).

Dando continuidade a esse projeto, foi desenvolvido o projeto de Pesquisa e Desenvolvimento (P&D ANEEL/CEMIG, GT–487), intitulado

Desenvolvimento de Índices de Integridade Biótica: macroinvertebrados bentônicos como indicadores de qualidade de água em bacias hidrográficas de empreendimentos hidrelétricos da CEMIG em Minas Gerais (2013-2017). Nessa etapa do projeto foi realizada a re-amostragem nos tributários e no reservatório de Nova Ponte com o intuito de validar as metodologias, avaliar a estabilidade de métricas e verificar possíveis alterações na diversidade de habitat e integridade biótica naquela bacia amostrada pela primeira vez em 2009. Através deste projeto também foi possível definir e amostrar novas áreas de referência na unidade hidrológica da UHE Nova Ponte (bacia do Rio Araguari, regiões do Parque Nacional da Serra da Canastra e Serra do Salitre). Ao final, novas redes de colaboração foram firmadas com instituições nacionais (Universidade Estadual da Paraíba, Universidade Federal do Pará, Universidade de Brasília) e internacionais (Universidade de Lyon, Universidade de Barcelona, Universidade de País Basco, Universidade de Coimbra) além dos antigos parceiros.

Esta tese está dividida em três capítulos, dos quais dois foram publicados e o terceiro encontra-se em formato de manuscrito a ser submetido a uma revista científica internacional. O primeiro capítulo intitulado *The role of physical habitat and sampling effort on estimates of benthic macroinvertebrate taxonomic richness at basin and site scales* tratou das relações entre os hábitats físicos e as estimativas de riqueza de macroinvertebrados bentônicos em escalas local e de bacia, publicado na revista *Environmental Monitoring and Assessment* em 2016. No segundo capítulo, *An improved macroinvertebrate multimetric index for the assessment of wadeable streams in the neotropical savana*, publicado na revista *Ecological Indicators* em 2017, foi proposto um índice multimétrico baseado em macroinvertebrados bentônicos para riachos no cerrado. Esse índice foi utilizado no terceiro capítulo, *Assessing the extent and relative risk of aquatic stressors on stream macroinvertebrate assemblages in the Brazilian Savanna*, para avaliação das associações entre estressores antropogênicos e a condição biológica em riachos no cerrado, através de desenho amostral probabilístico.

Parte desta tese foi desenvolvida na Oregon State University (OSU) e Agência de Proteção Ambiental Norte Americana (US-EPA) com apoio do Programa de Doutorado-Sanduíche no Exterior (PDSE-CAPES, processo

99999.006833/2015-02), no período de setembro de 2015 a agosto de 2016, sob co-orientação do professor Alan T. Herlihy e colaboração do professor Robert M. Hughes.

Em cada capítulo serão apresentadas as metodologias específicas (número de amostras, detalhamento taxonômico, área amostrada, variáveis ambientais, etc.) conforme a abordagem utilizada. Abaixo é apresentada a metodologia geral desta tese.

Área de Estudo

O estudo foi realizado em riachos a montante de quatro empreendimentos hidrelétricos da CEMIG: Nova Ponte, Volta Grande e São Simão, localizados na bacia do Rio Paraná; e Três Marias, na bacia do Rio São Francisco. A área de estudos compreende um total de 45.180 km²

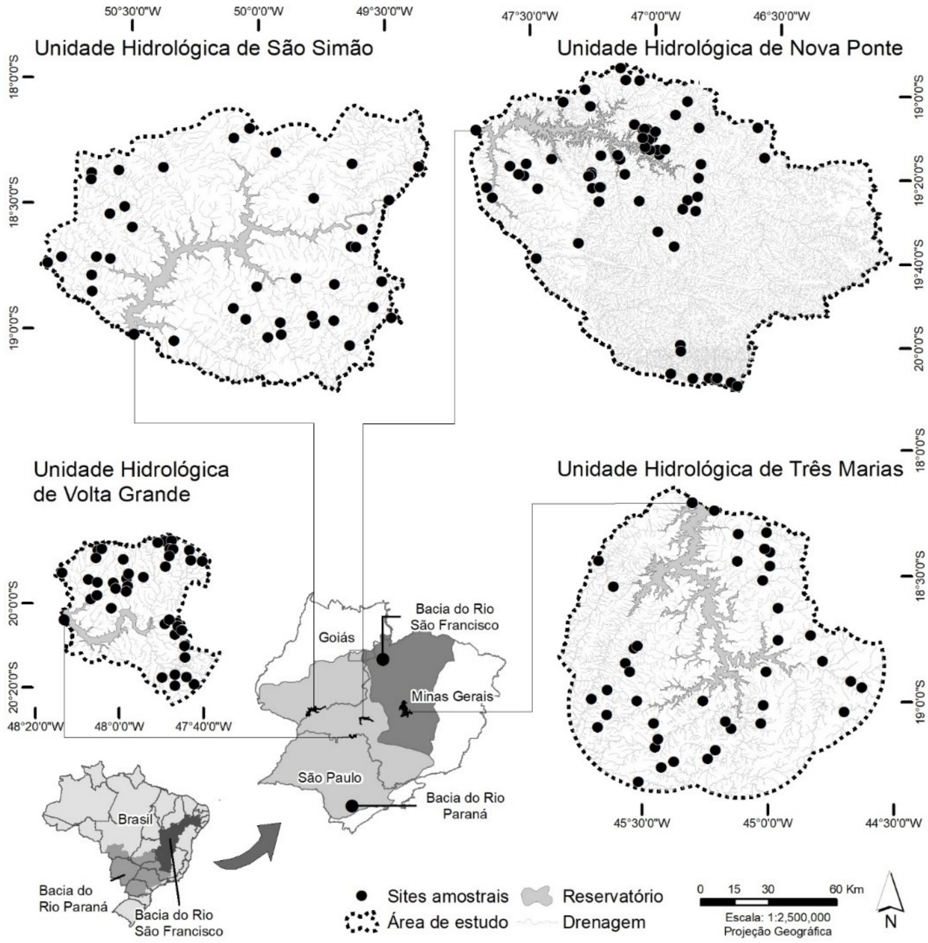


Figura 4. Pontos amostrais nas unidades hidrológicas de Nova Ponte, Três Marias, Volta Grande e São Simão.

(a) (b) (c)

Figura 5. Exemplos de sítios amostrados nas unidades de Nova Ponte, Três Marias, Volta Grande e São Simão em condições de referência (a), intermediárias (b) e impactadas (c).

(Figura 4). Os riachos foram amostrados uma vez em cada área (referida adiante como *unidade hidrológica*; ver Ferreira et al. 2017, Seaber et al. 1987), durante o período seco entre 2009 e 2012. Riachos da unidade hidrológica de Nova Ponte foram re-amostrados em 2013, para avaliar a variabilidade interanual de métricas biológicas através de uma análise *signal-to-noise* (ver Capítulo 2). Além disso, em 2014 foi realizada uma amostragem adicional na unidade hidrológica de Nova Ponte a fim de identificar riachos em condições de referência (Martins et al., 2017) (ver Cap. 2).

A área de estudo é localizada no bioma cerrado apresentando clima regional úmido tropical, marcado pelas estações chuvosa (outubro a março) e seca (abril a setembro). A precipitação média anual varia entre 1200 e 1800 mm e a temperatura entre 22°C e 27°C (Hunke et al., 2015; Ratter et al., 1997). A vegetação é composta por árvores de pequeno porte, vegetação rasteira e matas ciliares ao longo de cursos d'água (Klink e Machado, 2005; Quesada et al., 2008; Urbanetz et al., 2013).

Figura 6. Exemplos de sítios em condições de referência amostrados em áreas protegidas na unidade hidrológica de Nova Ponte.

Desenho Amostral

Em cada unidade hidrológica, a área potencial de amostragem foi definida dentro de um *buffer* de 35 km a partir dos limites de cada reservatório (Macedo et al., 2014b). Nessa área, a rede de drenagem foi mapeada em uma escala de 1:100.000 e os cursos d'água classificados conforme hierarquia proposta por Strahler (1957).

Os pontos de amostragem foram selecionados seguindo um desenho probabilístico, sistemático e espacialmente balanceado (Herlihy et al., 2000; Olsen e Peck, 2008; Stevens e Olsen, 2004), o que garantiu uma distribuição uniforme e representativa da região (Herlihy et al., 2000). Foram considerados para sorteio apenas riachos de 1ª a 3ª ordens e 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). Além dos pontos sorteados em cada unidade hidrológica foram escolhidos pontos em riachos nos extremos de condições ecológicas (referência e impactados, ver Cap. 2), totalizando 40 pontos de amostragem em cada unidade hidrológica (Figura 5). Somados a esses, 31 pontos adicionais foram selecionados em áreas protegidas de Nova Ponte (Figura 6) (ver Martins et al. 2017; Cap. 2) totalizando 191 pontos amostrais.

Definição de Trechos Amostrais

Em cada ponto de amostragem, a definição do comprimento do trecho amostrado foi obtida através da média da largura molhada multiplicada por 40 (Peck et al., 2006), respeitando um mínimo de 150 metros de rio a serem amostrados (Figura 7). Esse trecho foi então dividido igualmente em 11 transectos (marcados de a a k), onde foi aplicado o protocolo de caracterização de hábitat físico (Peck et al., 2006). Através desse protocolo foram obtidas medidas do canal (profundidade, largura molhada, altura do leito sazonal, altura da incisão, inclinação de margens, sinuosidade, declividade, etc), características dos substratos e fluxos de água (tamanho e imersão do substrato, frequeência e tipos de fluxo), características da vegetação ripária (sombreamento do leito e margens, densidade de estratos vegetais, etc) e influência humana (presença de estradas, lixo, plantações, pastagens, etc). Posteriormente foram calculadas métricas de habitat físicos conforme Kaufmann et al. (1999). Adicionalmente foi realizada mensuração de parâmetros físicos e químicos na coluna d'água: oxigênio dissolvido, turbidez, condutividade, sólidos totais dissolvidos, alcalinidade total e concentrações de nitrogênio e fósforo totais (APHA, 1998).

A caracterização dos diferentes tipos de usos do solo foi realizada na sub-bacia de drenagem a montante do ponto de amostragem, por meio de

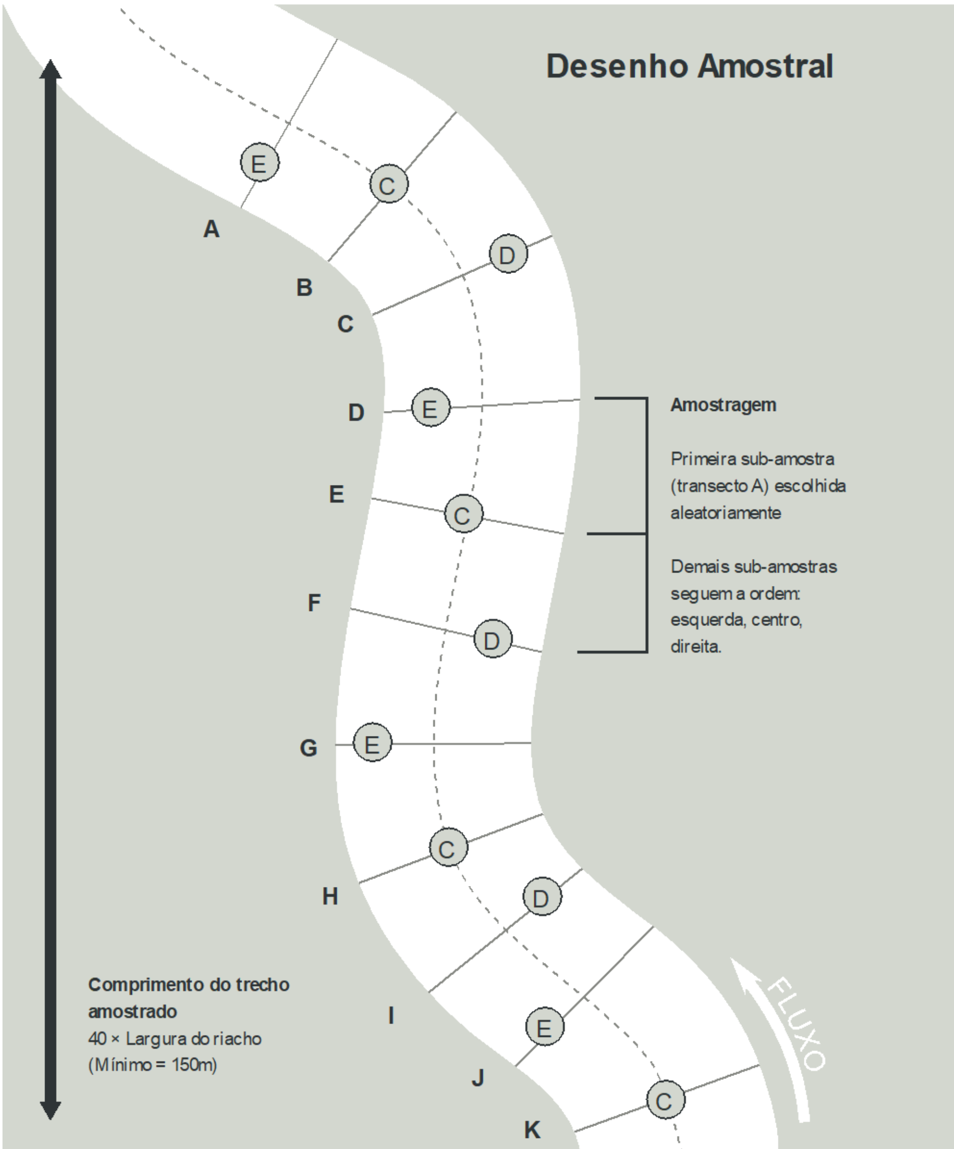


Figura 7. Representação esquemática de definição do trecho amostral e da amostragem das assembleias de macroinvertebrados bentônicos. Figura adaptada do manual *Surface Waters: Western Pilot Study Field Operations Manual for Wadeable Streams*, Peck et al. (2006).

interpretação manual de imagens de alta resolução (Google, 2016) juntamente com imagens multiespectrais do satélite TM Landsat (ver Macedo et al. 2014a, 2014b). Ao final, foram obtidos percentuais de cobertura natural do solo (% de vegetação natural) e usos antrópicos (% de área urbana, % pastagem, % agricultura e % de plantações de eucalipto).

Caracterização das Assembleias de Macroinvertebrados Bentônicos

Em cada um dos 11 transectos, demarcados de A a K, foi realizada a amostragem dos macroinvertebrados bentônicos. A primeira sub-amostra foi selecionada de maneira aleatória no transecto A e as amostras subsequentes seguiram o padrão esquerda, centro e direita, totalizando 11 sub-amostras (Figura 7). 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. 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 (Costa et al., 2006; Fernández e Domínguez, 2001; Merritt et al., 2008; Mugnai et al., 2010; Roldán, 1999). A identificação foi realizada até o nível de gênero para os macroinvertebrados das ordens Ephemeroptera, Plecoptera e Trichoptera (EPT), sendo os demais organismos identificados até o nível de família, exceto Bivalvia, Hydracarina, Hirudinea, Nematoda, Collembola, e Oligochaeta (Figura 8).


(a)

(b)

(c)

(d)

(e)

(f)

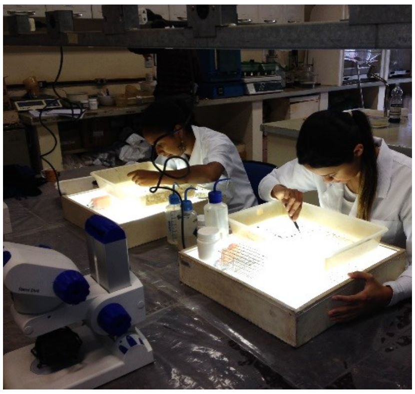
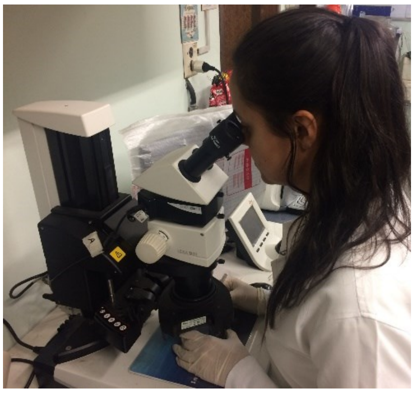
(g)

(h)

(i)

Figura 8. Etapas da amostragem em campo: aplicação do protocolo de caracterização de habitat físico em (a) e (b); mensuração de parâmetros de qualidade de água em (c) e (d); amostragem das assembleias de macroinvertebrados bentônicos em (e) e (f). Etapas em laboratório: lavagem de amostras em (g); triagem dos organismos em (h); identificação em (i).

Capítulo 1

The Role of Physical Habitat and Sampling Effort on Estimates of Benthic Macroinvertebrate Taxonomic Richness at Basin and Site Scales

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The role of physical habitat and sampling effort on estimates of benthic macroinvertebrate taxonomic richness at basin and site scales

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Abstract Taxonomic richness is one of the most important measures of biological diversity in ecological studies, including those with stream macroinvertebrates. However, it is impractical to measure the true richness of any site directly by sampling. Our objective was to evaluate the effect of sampling effort on estimates of macroinvertebrate family and Ephemeroptera, Plecoptera, and Trichoptera (EPT) genera richness at two scales: basin and stream site. In addition, we tried to determine which environmental factors at the site scale most influenced the amount of sampling effort needed. We sampled 39 sites in the Cerrado biome (neotropical savanna). In each site, we obtained 11 equidistant samples of the benthic assemblage and multiple physical habitat measurements. The observed basin-scale richness achieved a consistent estimation from Chao 1, Jack 1, and Jack 2 richness estimators. However, at the site scale, there was a constant increase in the observed number of taxa with increased number of samples. Models that best explained the slope of site-scale sampling curves (representing the necessity of greater sampling effort) included metrics that describe habitat heterogeneity, habitat structure, anthropogenic disturbance, and water quality, for both macroinvertebrate family and EPT genera richness. Our results demonstrate the importance of considering basin- and site-scale sampling effort in ecological surveys and that taxa accumulation curves and richness estimators are good tools for assessing sampling efficiency. The physical habitat explained a significant amount of the sampling effort needed. Therefore, future studies should explore the possible implications of physical habitat characteristics when developing sampling objectives, study designs, and calculating the needed sampling effort.

Keywords Sampling efficiency · Biodiversity · Richness estimators · Physical habitat

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Introduction

At the assemblage level, the most straightforward and frequently used measure of biodiversity is taxonomic richness, i.e., the number of taxa found in a given locality (Gotelli and Colwell 2001; Brown et al. 2007). Being an intuitive measure of assemblage diversity, taxonomic richness is well understood by researchers, managers, and the general public (Basualdo 2011). It is an important metric for composing biological indices (Klemm et al. 2003; Macedo et al. 2016), ecological

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models (Arita and Vázquez-Domínguez 2008), macroecological and biogeographic studies (Oberdorff et al. 1995; Gaston 2000; Heino 2011), and for developing conservation strategies (Meir et al. 2004; Freemark et al. 2006). Patterns that determine biodiversity aspects are better studied in terrestrial than in aquatic ecosystems (Heino 2002). Rivers and streams exhibit a particular distribution pattern of biological diversity among taxonomic groups and among regions (Ligeiro et al. 2010; Hughes et al. 2012; McGarvey and Terra 2015). Those variations are associated with differences in physical habitat characteristics, water quality, colonization history, and frequency and magnitude of disturbances (Clarke et al. 2008; Ligeiro et al. 2010; McGarvey and Terra 2015).

Because the number of species in a given assemblage is limited, total species richness theoretically could be determined for any assemblage (Walther and Morand 1998). However, conducting a complete species inventory often is limited by overwhelming costs to obtain the data (Bartsch et al. 1998; Melo and Froehlich 2001) and there is also a lack of adequate taxonomic keys for most assemblages in many regions (Chao 2005; Hughes and Peck 2008). Especially for aquatic invertebrate assemblages, the measurement of taxonomic richness is still an obstacle in ecological studies (Allan and Flecker 1993). This situation is worse in tropical ecosystems than in temperate waters (Heino 2002).

The number of taxa generally increases asymptotically with increased number of samples or individuals counted, and species accumulation curves (or collection curves) are simple ways to assess how species richness varies with sampling effort (Cao et al. 2001; Gotelli and Colwell 2001; Hughes et al. 2012). When the curve stabilizes, i.e., when no significant increase in richness is observed with an increase in the sampling effort, almost all species will have been sampled. Thus, such curves allow estimates of the minimum number of samples or individuals required for adequate characterization of an assemblage (Bartsch et al. 1998; Bady et al. 2005). This dependency of species richness in relation to sampling effort has hindered proper comparisons among studies and identification of broad global patterns of species distributions. Stout and Vandermeer (1975), comparing streams in different latitudes, suggested that tropical streams harbor greater species richness of aquatic insects relative to temperate streams; however, because of small sample sizes, observed richness in the tropics is usually underestimated.

Species accumulation curves are directly influenced by the capture of rare species, because the greater the number of rare species, the steeper the observed accumulation curve (Melo and Froehlich 2001). The definition of rare species will depend mostly on the spatial and temporal scale of the study (Mao and Colwell 2005). Species that occur in low numbers or in low frequencies in sample units play an important role for detecting ecological changes (Leitão et al. 2016) and are critical for bioassesment studies (Cao et al. 1998). Because of the constant capture of rare taxa, it is rarely possible to observe stabilization in accumulation curves of benthic macroinvertebrates in tropical streams (Melo and Froehlich 2001; Schneck and Melo 2010). Given the impossibility of collecting all species from a site, richness estimators are used to fill the gaps left by incomplete inventories (Walther and Morand 1998). Among the ways to estimate richness, nonparametric estimators are widely employed (Gotelli and Colwell 2010). These estimators use the number of rare species that could not be detected consistently in multiple samples to try to predict the total number of species (Gotelli and Colwell 2010).

Understanding macroinvertebrate diversity is especially important for Cerrado (neotropical savanna) streams. Macroinvertebrates are considered a good ecological indicator, being sensitive to environmental changes in the physical, chemical, and biological components of aquatic ecosystems (Bonada et al. 2006). The Cerrado is the second largest neotropical biome (Wantzen 2003), originally covering about 20 % of Brazilian territory. It is a global terrestrial biodiversity hotspot (Myers et al. 2000) and is highly threatened by rapidly expanding anthropogenic activities (Wantzen et al. 2006). Different watershed land uses and cover can alter riparian zone structure and flow regimes, increase loadings of sediments, nutrients, and other pollutants, and reduce the availability of habitats for aquatic biota (Wang et al. 1997; Allan 2004). Because of its close proximity to aquatic organisms, the variety and variability of habitats have a strong influence on aquatic biodiversity (Allan 2004). Others have demonstrated how physical habitat complexity can explain stream macroinvertebrate assemblage composition and structure (Allan 2004; Ferreira et al. 2014; Macedo et al. 2014). However, less attention has been given to learning how those environmental characteristics can influence the amount of sampling effort necessary to characterize stream sites in an adequate manner.

Considering the importance of both within- and among-site heterogeneity for assessing macroinvertebrates in Cerrado streams (Ligeiro et al. 2010; Silva et al. 2014), the first objective of our study was to evaluate the sampling efficiency of taxonomic richness obtained in benthic macroinvertebrate samples at site and basin spatial scales. We were particularly interested in analyzing sampling effort in terms of the number of samples obtained (subsamples at the local scale and stream sites at the basin scale), in this way evaluating the patterns of areal richness, also known as richness density (sensu Hulbert 1971; Ligeiro et al. 2013a). This operational aspect of taxonomic richness is very often employed in ecological studies and corresponds simply to the number of species found in a certain area of habitat sampled, independently of the number of individuals counted. Richness density is related to numerous ecological phenomena including habitat heterogeneity, ecosystem productivity, isolation, climatic stability, and disturbance effects (Oberdorff et al. 1995; Gotelli and Colwell 2001; Ligeiro et al. 2013b). Our second objective was to determine which habitat characteristics are associated with increased site-scale sampling effort. Because species-level taxonomic keys are poorly developed for neotropical benthic macroinvertebrates, to answer both questions we assessed the effects at two taxonomic levels: macroinvertebrate family richness and Ephemeroptera, Plecoptera, and Trichoptera (EPT) genera richness.

Materials and methods

We conducted our study in wadeable Cerrado biome streams in the Upper São Francisco Basin (Minas Gerais State, southeastern Brazil) above Três Marias reservoir (Fig. 1). The basin climate is defined by a rainy season from October to March and a dry season from April to September, with mean annual precipitation

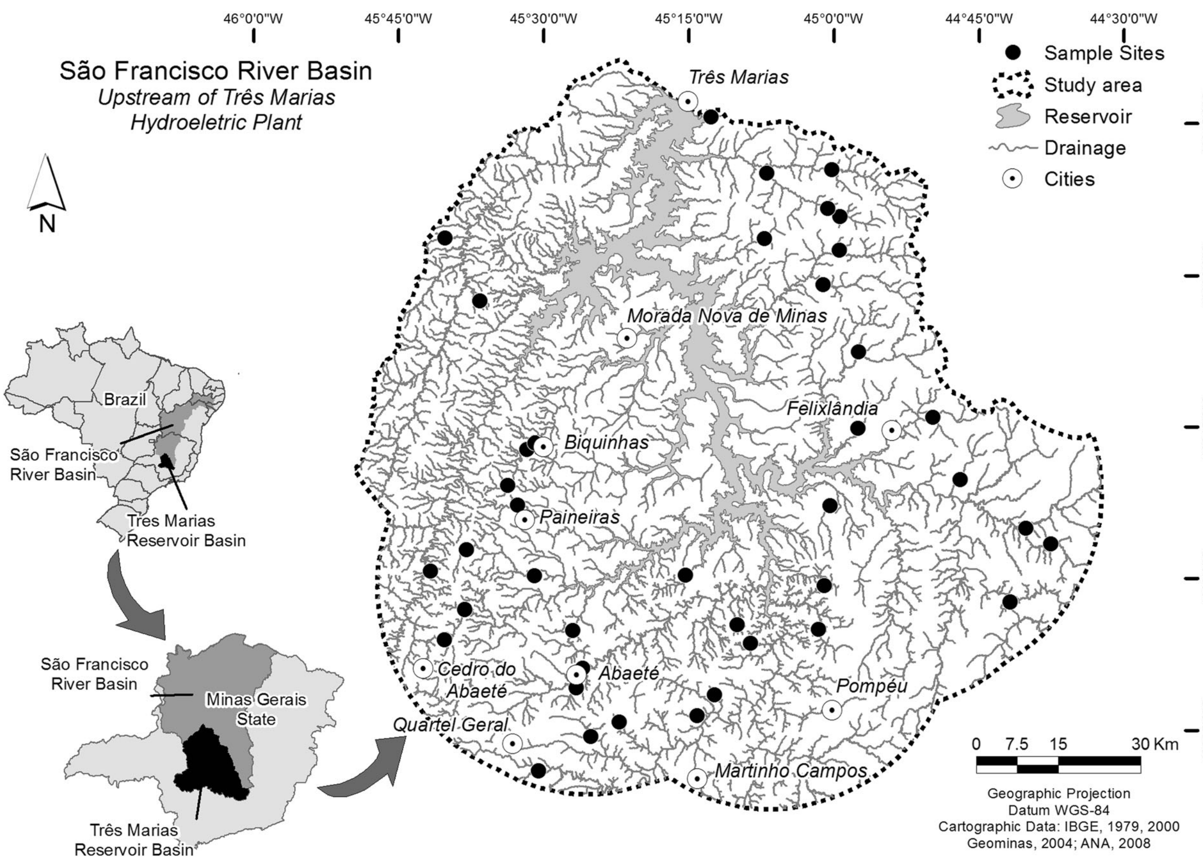

Fig. 1 Location of Três Marias Reservoir and stream sites sampled

ranging from 1200 to 1800 mm and mean annual temperature of 23 °C (Brasil 1992).

Field sampling was conducted in the end of the dry season (September 2010) once in each of 39 stream sites ranging from first to third order (1:100,000 scale maps) randomly selected by using the spatially balanced unequal probability survey design described by Olsen and Peck (2008). The length of each site was equal to 40 times the mean wetted width, with a minimum length of 150 m, to encompass the habitat complexity of the site, and 11 equidistant subsamples of benthic macroinvertebrates were collected following Peck et al. (2006). The subsamples were obtained systematically through a zigzag sampling pattern along the site, ensuring the sampling of multiple types of habitats and microhabitats. We used a D-net (30-cm mouth width, 0.09-m^2 area, 500-μm mesh) to collect macroinvertebrates. The samples were fixed with 10 % formalin and taken to the laboratory, where all macroinvertebrates were sorted and identified through use of a stereomicroscope (×100 magnification). Most invertebrates were identified to family level through use of identification keys (Pérez 1988; Merritt and Cummins 1996; Fernández and Domínguez 2001; Costa et al. 2006; Mugnai et al. 2010). Some noninsect invertebrates, like Hydracarina and Oligochaeta, were not identified to family. These groups accounted for very few of the total number of individuals collected (fewer than 5 %) and therefore were included in the family-level analyses. We identified EPT individuals to genus, allowing us to interpret results at two taxonomic levels: total macroinvertebrate families and EPT genera.

Also following Peck et al. (2006), we recorded many measurements of physical habitat characteristics in the sites, including stream channel morphology (e.g., slope, sinuosity, depth, wetted and bankfull width, incision, bank angle), habitat features (substrate size, flow types, presence of wood in the channel), riparian structure (canopy cover, vegetation type), and human alterations in the channel and riparian zones (e.g., presence of buildings, pasture, crops, roads, trash). Physical habitat metrics were then calculated according to Kaufmann et al. (1999). Some water quality characteristics were also measured once in each site: pH, electrical conductivity, total dissolved solids, dissolved oxygen, turbidity, total alkalinity, total nitrogen, and total phosphorus.

To estimate basin-scale sampling effort for the 39 sites, we evaluated the performance of three nonparametric richness estimators: the incidence-based first-order Jackknife and second-order Jackknife (hereafter Jack 1 and Jack 2, Burnham and Overton 1978) and the abundance-based Chao 1 (Chao 1984). The Jack 1 and Jack 2 richness estimators use counts of taxa that are present only in one (uniques) or two (duplicates) samples. The Chao 1 estimator bases its calculation on the number of taxa represented by just one or two individuals (singletons and doubletons). Those estimators have fundamentally different conceptual bases, but all three account for the presence of rare species in the samples. They were previously reported as producing good and reliable estimation of true taxa richness (Melo and Froehlich 2001; Martínez-Sanz et al. 2010; Basualdo 2011). We constructed curves of observed richness and estimated richness (Jack 1, Jack 2, and Chao 1) considering both total families and EPT genera richness using EstimateS 8.0 (Colwell 2006). One thousand randomizations were employed in the analyses.

To evaluate site-scale sampling effort, we constructed taxa accumulation curves for each of the 39 sites sampled, given the observed richness found in 1 to 11 subsamples. We represented the variation of the observed richness among the sites for each sampling effort by using boxplots. To assess the influence of physical habitat metrics on site-scale sampling effort, we first computed the observed richness at each subsample as a proportion of the total richness observed in each site, in this way avoiding biased slope calculations resulting from small or high richness values. Then, we obtained the logarithmic equation of the accumulation curve of each site:

$$y = a^*\ln(x) + b$$

where y is the expected richness, x is the sampling effort (number of subsamples collected), and b is a fitting constant. We used the slope of the curves (a value) to describe the sampling efficiency of the site. The greater the slope, the greater was the amount of sampling effort necessary to represent local richness. The sampling curves of all sites fitted well to the logarithmic equation (R^2 values > 0.95). We replicated this procedure for both total families and EPT genera datasets, also using EstimateS 8.0 (Colwell 2006), with 1000 randomizations in all cases.

We then performed multiple regression analysis (best-subset procedure) to determine the degree to which our predictor variables (metrics of disturbance, physical habitat structure, and water chemistry; Table 1)

Table 1 Candidate predictor metrics used in the best-subset multiple linear regression analysis

Metric categories and names	Code
Land use	
Percentage of agriculture	%agr
Percentage of pasture	%pas
Riparian human disturbance (proximity weighted index)	
Buildings	w1h_bldg
Pavement	w1h_pvmt
Trash and landfill	w1h_ldfl
Pasture	w1h_pstr
Logging	w1h_log
Channel morphology	
Mean (width/depth)	xwd_rat
Mean slope	xslope
Mean depth	xdepth
Mean bankfull width	xbkf_w
Mean width x mean depth	xwxd
Channel sinuosity (m/m)	sinu
Bankfull (width/depth)	bkf_wdrat
Mean water volume/m^2	v1w_msq
Bed substrate	
Substrate % cobbles (diameter 64–250 mm)	pct_cb
Percentage of coarse substrate (>16 mm)	pct_bigr
Percentage of fines (silt and clay)	pct_fn
Mean substrate embeddedness	xembed
Coarse litter (%)	pct_bf
Flow type	
Percentage of pools	pct_pool
Percentage of fast water	pct_fast
Shelter	
Natural fish cover in the stream (all)	xfc_nat
Brush and small debris fish cover (areal proportion)	xfc_brs
Undercut banks (areal proportion)	xfc_ucb
Anthropogenic fish cover (%)	pfc_ant
Riparian	
Riparian canopy (>5 m high) cover	xc
Riparian canopy (>5 m high) presence	xpcan
Total riparian cover (all vegetation layers)	xcmg
Mean mid-channel canopy density	xcdenmid
Water chemistry	
Negative log hydrogen ion concentration	pH
Material in suspension (NTU)	turb
Dissolved oxygen (mg/L)	do
Total nitrogen (mg/L)	ntotal

explained the variation in the slope values. In other words, we also evaluated the degree that habitat metrics influenced the amount of sampling effort required. We considered models generated with up to four explanatory variables, equivalent to 10 % of the number of sites analyzed, in this way avoiding model overfitting. Assumptions of homocedasticity and normality of errors were respected. We excluded correlated metrics that indicated high redundancy ($p \geq |0.8|$). Corrected Akaike information criteria (AICc) were used to select the best models. The analyses were performed in Systat 13 (Systat Software Inc 2009).

Results

We collected a total of 69,726 individuals, belonging to 80 taxa. Of those, 15,137 organisms were EPT, distributed in 65 genera. Family and EPT richness varied from 14 to 42 taxa and 1 to 36 genera per site, respectively.

At the basin scale, family and EPT patterns differed slightly. We observed that family-richness sampling effort achieved a consistent representation of the total richness because the curves of observed richness and all three estimators approached asymptotes (Fig. 2a). Regarding EPT genera richness, we observed a continuous increase in richness with increased number of sampled sites and proportionately less consistency of the estimators (Fig. 2b).

At the site scale, the average number of taxa observed increased continuously with increasing number of subsamples collected per site, both for total families and EPT genera (Fig. 3a, b). The wide range of richness values in each sampling effort reveals different richness patterns among sites. The variation in the slopes of the accumulation curves were moderately but significantly correlated with site-scale physical and water chemistry metrics ($R^2 = 0.369$ and 0.317, for macroinvertebrate families and EPT genera, respectively, Table 2). For the sampling effort of families, the significant metrics were dissolved oxygen, bankfull width/depth, mean natural fish cover (all natural instream habitat cover index), and mean bankfull width. Similar results were observed for EPT genera: dissolved oxygen, bankfull width/depth, mean brush and small debris fish cover (fraction of reach area covered by brush and small debris), and proximity of logging (Table 2).

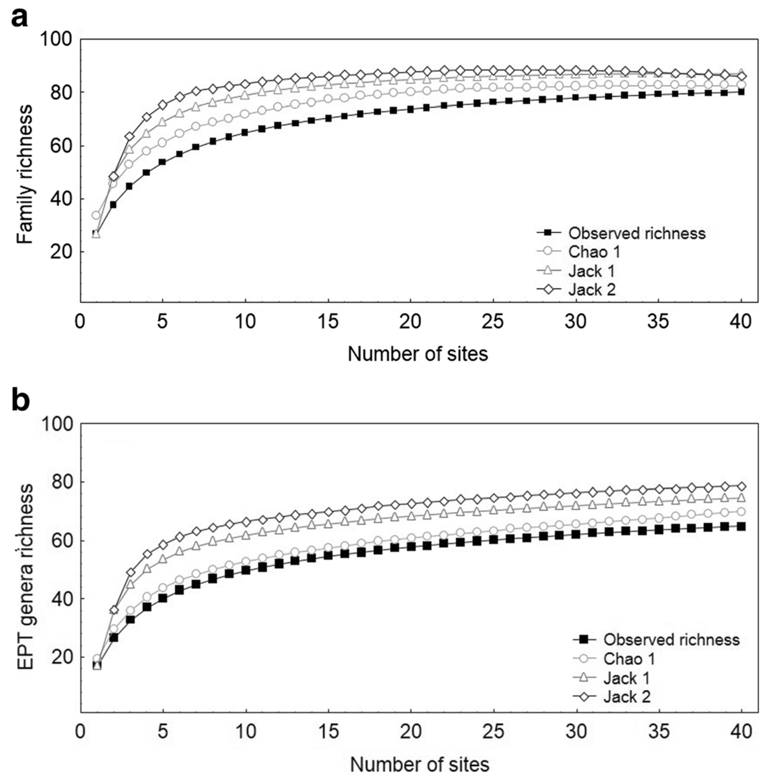

Fig. 2 Observed and estimated richness as a function of the number of sites sampled in the Upper São Francisco Basin: **a** macroinvertebrate families; **b** EPT genera

Discussion

Sampling sufficiency for estimates of taxonomic richness is an important issue in ecological studies because too little sampling greatly underestimates richness and excessive sampling is very expensive (Hughes and Peck 2008). In addition, sampling sufficiency may vary with the scale of the study (basin versus site) as well as with environmental factors influencing sampling effort.

The observed basin-scale richness curves approached stabilization and consistent estimations by Chao 1, Jack 1, and Jack 2. Although nonparametric methods of estimation are limited by their dependence on the number of rare taxa in an assemblage, they provide better estimates of true taxa richness than observed richness (Hughes et al. 2002; Melo 2004; dos Anjos and Zuanon 2007) allowing comparisons of different regions. This is particularly the case with tropical streams, which usually support many rare macroinvertebrate (Melo and Froehlich 2001; Oliveira et al. 2011).

Considering site-scale sampling effort, we did not observe curve stabilization; taxa richness increased continuously with increased numbers of subsamples for both taxonomic levels, reflecting that rare taxa continue to be added to the samples. Different stream habitats often exhibit different assemblage compositions, each one yielding its own rare species (Stout and Vandermeer 1975; Melo and Froehlich 2001). Therefore, our sampling design, collecting at many types of habitats in each site, may have contributed to the nonstabilization of the accumulation curves. Different results could have been obtained if we had focused on a single habitat type. However, multiple habitat sampling is preferable to targeting a single habitat type when the aim of the study is to obtain a comprehensive taxa list for assessing biodiversity and overall site condition (Gerth and Herlihy 2006; Hughes and Peck 2008; Buss et al. 2015). Other authors have observed the same results concerning site-scale sampling effort. Bady et al. (2005) found that ten subsamples represented only 50 % of the estimated richness in European stream sites. Li et al. (2001), studying headwater streams in Oregon (USA), also noted a constant increase in richness even after 50 subsamples were taken from each site. Sampling 11 subsamples at each of 20 random sites in seven separate Oregon and Washington rivers, Hughes et al. (2012) found that much greater sampling effort was required to estimate the true richness of riverine

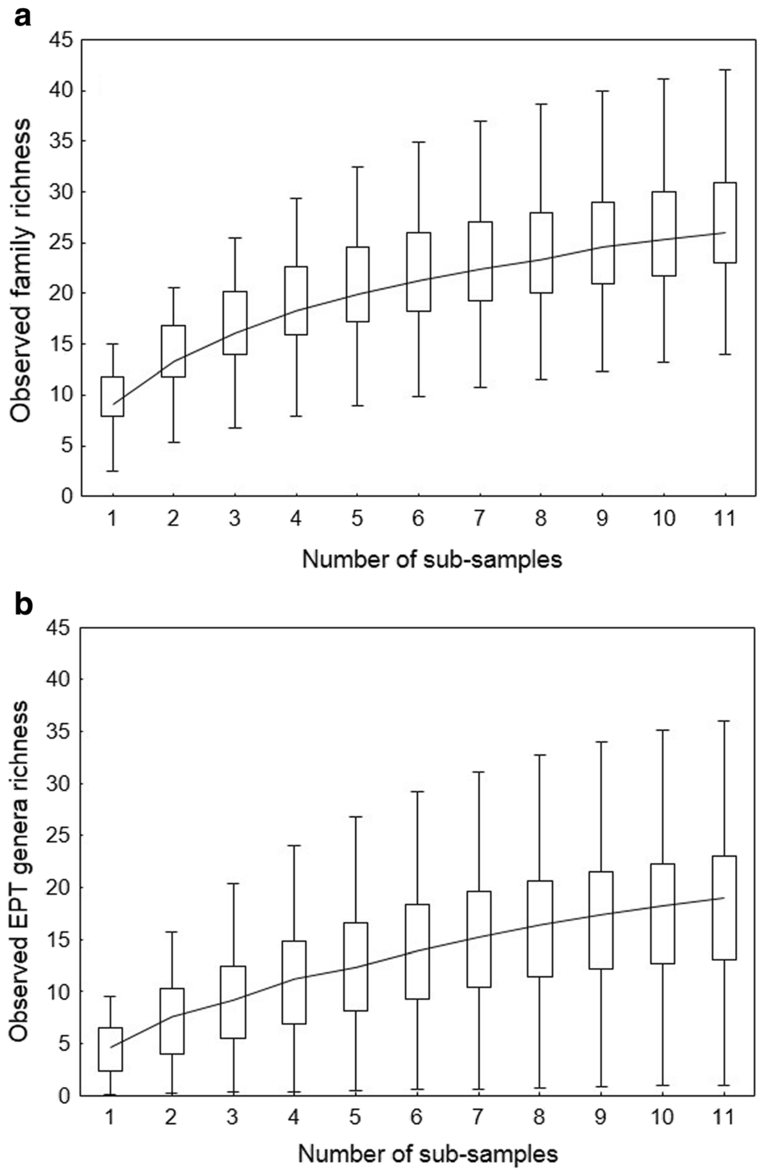

Fig. 3 Cumulative taxa richness as a function of the average number of subsamples for 39 sites in the Upper São Francisco Basin (*black line*). The *boxes* show the interquartile ranges; *whiskers* show the minimum and maximum values: **a** macroinvertebrate families; **b** EPT genera

benthic macroinvertebrate taxa. Li et al. (2014) reported that 20 subsamples were needed to reach asymptotes in sand-bottomed northeastern China streams. However, 11 subsamples per site are considered sufficient in biomonitoring studies for assessing stream ecological condition through use of multimetric indices in temperate streams (Peck et al. 2006; Hughes and Peck 2008). For the tropical region, this number is superior to the three subsamples commonly used in bioassesment studies (Moreno et al. 2010; Ferreira et al. 2011; Feio et al. 2013).

Observed taxonomic richness is highly biased toward the amount of sampling effort; however, fewer subsamples may be sufficient for estimating diversity via Simpson and Shannon-Wienner indices (Magurran 2004). In addition, fewer samples are needed to represent assemblage composition via similarity indices (Cao et al. 2001; Cao et al. 2002; Schneck and Melo 2010) and to represent ecological condition via multimetric indices (Stoddard et al. 2008; Oliveira et al. 2010; Oliveira et al. 2011; Chen et al. 2014) and via predictive models (Hawkins et al. 2000; Moya et al. 2011). Thus,

Table 2 Multiple linear regression models for habitat metrics explaining the slope of richness accumulation curves generated for macroinvertebrate families and EPT genera ($df = 4,34$)

	F value	*p* value	R^2	AIC		Beta	Std. error of beta	*p* value
					Intercept			<0.001
Families	4.97	0.003	0.369	−176.2	xfc_nat	0.337	0.148	0.030
					xbkf_w	−0.616	0.190	0.003
					bkf_wdrat	0.629	0.180	0.001
					do	0.302	0.157	0.063
					Intercept			<0.001
EPT genera	3.95	0.010	0.317	−156.9	w1h_log	−0.405	0.150	0.011
					bkf_wdrat	0.261	0.161	0.114
					xfc_brs	0.480	0.171	0.008
					do	0.339	0.162	0.044

See Table 1 for full metric names

adequate sample size will depend on the effect size of the study (Schneck and Melo 2010). Biomonitoring programs and rapid bioassessment can use small samples sizes to detect human impact, resulting also in reduced costs and laboratory time for processing the material (Lorenz et al. 2004; Vlek et al. 2006; Buss et al. 2015). However, for biology conservation studies, where a comprehensive taxonomic list is required, reduced sampling effort may increase the risk of mistakes in interpreting results (Petersen and Meier 2003; Hering et al. 2010).

Concerning taxonomic resolution, we observed greater stabilization in family taxonomic richness than in EPT genera richness at the basin scale (Fig. 2). As expected, the coarser the taxonomic resolution, the faster its stabilization relative to a finer taxonomic resolution (Gotelli and Colwell 2001). Despite this difference, the decision for a taxonomic level is usually determined by the study objectives (Jones 2008; Buss et al. 2015). Generally, the information provided by higher taxonomic levels, like family or even orders, are enough to identify environmental gradients in bioassesment and biomonitoring programs (Mao and Colwell 2005; Whittier and Van Sickle 2010; Buss and Vitorino 2010; Monk et al. 2012), and it is suggested when there are limitations in financial support, time for processing and knowledge for identification to lower levels (Buss and Vitorino 2010). However, refined taxonomic levels are preferred to distinguish subtle differences in water quality, when a high degree of confidence in conclusions is required, for conservation studies where a taxonomic list is benefited by precise identification (Lenat and Resh 2001) and when species function offers ecological insights that would be missing otherwise (Leitão et al. 2016).

Models that best explained the slopes of site-scale curves included metrics that describe habitat heterogeneity, habitat structure, anthropogenic disturbance, and water quality. Dissolved oxygen is well known as an important factor influencing the composition of freshwater communities (Dodds 2002; Connolly et al. 2004), but to our knowledge, no association with sampling effort had been done prior to our study. In our study, dissolved oxygen positively influenced the slopes of both family and EPT genera sampling curves. The greater the dissolved oxygen concentration, the greater the sampling effort needed for estimating assemblage richness. Mean bankfull width and bankfull width to depth ratio can influence the sampling effort in different ways. Those metrics describe stream size and previously have been associated with assemblage structure (Cole 2004). However, their effect on observed taxonomic richness is highly variable among studies (Clarke et al. 2008). Brush and small debris are important streambed components, providing habitat, food, and flow refuges for macroinvertebrates (Johnson and Kennedy 2003; Kaller and Kelso 2007). Those microhabitats can exhibit higher diversity and abundance of macroinvertebrates than occur in structurally simpler microhabitats (Drury and Kelso 2000). The abundance of brush and small debris influences taxonomic richness and consequently can influence sampling effort. Logging and natural cover are commonly used metrics to explain observed taxa richness (Haggerty et al. 2004; Mereta et al. 2012) and probably influence sampling effort in the same way. Ferreira et al. (2014), analyzing the same dataset, found that a different set of habitat metrics explained site EPT richness compared with those we found explaining sampling effort. Their research and ours suggest that the factors driving the sampling effort needed are relatively independent from those explaining the total observed richness of the sites. Thus, our study provides an innovative method for evaluating the relationship between sampling effort and the environmental factors influencing it.

Sampling effort evaluations are of paramount importance for obtaining high-quality data that are capable of reliably describing diversity patterns and providing useful information for theoretical advancements and ecosystem management. We showed that it is very important to consider greater basin- and site-scale sampling effort than usually expended in stream ecological surveys if one is concerned with assessing taxonomic richness. In addition, taxa accumulation curves and richness estimators are useful for assessing sampling effort adequacy. Lastly, physical habitat characteristics affect the amount of sampling effort required for a site in a different manner than they do for explaining observed taxonomic richness. Therefore, we suggest that future studies continue to explore the possible implications of habitat characteristics before establishing sampling designs, in this way maximizing the representation of species diversity in different environmental contexts.

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Capítulo 2

An Improved Macroinvertebrate Multimetric Index for the Assessment of Wadeable Streams in the Neotropical Savanna

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Original Articles

An improved macroinvertebrate multimetric index for the assessment of wadeable streams in the neotropical savanna

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ABSTRACT

Multimetric indices (MMIs) have been successfully used to assess ecological conditions in freshwater ecosystems worldwide, and provide an important management tool especially in countries where biological indicators are fostered by environmental regulations. Nonetheless, for the neotropics, the few published papers are limited to small local scales and lack standardized sampling protocols. To fill the gaps left by previous studies, we propose a stream MMI that reflects anthropogenic impacts by using macroinvertebrate assemblage metrics from a data set of 190 sites collected from four hydrologic units in the Paraná and São Francisco River Basins, southeastern Brazil. Sites were selected through use of a probabilistic survey design allowing us to infer ecological condition to the total of 9432 kilometers of wadeable streams in the target population in the four hydrologic units. We used a filtering process to determine the least- and most-disturbed sites based on their water quality, physical habitat structure, and land use. To develop the MMI, we followed a stepwise procedure to screen our initial set of biological metrics for influence of natural variation, responsiveness and discriminance to disturbances, sampling variability, and redundancy. The final MMI is the sum of 7 scaled assemblage metrics describing different aspects of macroinvertebrate assemblage characteristics: Ephemeroptera richness, % Gastropoda individuals, Shannon-Wiener diversity index, % sensitive taxa richness, % scraper individuals, temporarily attached taxa richness, and gill respiration taxa richness. The MMI clearly distinguished the least-disturbed sites from the most-disturbed sites and showed a significant negative response to anthropogenic stressors. Of the total length of wadeable streams in the study area, 38%, 35%, and 27% were classified by the MMI as being in good, fair, and poor condition, respectively. By reducing the subjectivity of site selection, rigorously selecting the set of reference sites, and following a standardized metric screening method, we developed a robust MMI to assess and monitor ecological condition in neotropical savanna streams. This improved MMI provides an effective ecological tool to guide decision makers and managers in developing and implementing improved, cost-effective environmental policies, regulations, and monitoring of those systems.

1. Introduction

High quality and abundant water resources are directly associated with the integrity of biological communities inhabiting aquatic ecosystems (Dudgeon et al., 2006). Sustainable management and use of water resources provide multiple benefits and services to humans (Grizzetti et al., 2016; Vörösmarty et al., 2010). However, despite providing essential goods, freshwater ecosystems are among the most threatened by human pressures worldwide (Dudgeon et al., 2006). The intense demand for water by constantly growing human populations and economies results in widespread degradation of freshwaters (Abell et al., 2008; Limburg et al., 2011), as a result of habitat loss, water pollution, invasive species, overharvesting, and flow modification (Abell et al., 2008; Dudgeon et al., 2006; Revenga et al., 2005). Given this scenario, assessing ecological condition of aquatic ecosystems is critical for addressing efficient management practices to protect and rehabilitate integrity and ecosystem services (Balderas et al., 2016; Revenga et al., 2005).

Some of the most recognized ecological tools to monitor and manage freshwater ecosystems are multimetric indices (MMIs). In this

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approach, a combination of metrics representing assemblage attributes (e.g., composition, structure, function) are combined into a single measure (index) capable of reflecting multiple anthropogenic disturbances (Helson and Williams, 2013; Karr, 1999). First proposed for freshwater fish assemblages (Karr, 1981) and later adapted for other assemblages and ecosystem types, the plasticity of the MMI approach is based on a robust theoretical foundation (Karr, 1981). Over the years, the methodological process for developing an MMI has experienced a series of improvements aimed at increasing its applicability (Nazeer et al., 2016). Key improvements included the definition and selection of reference sites (Elias et al., 2016; Herlihy et al., 2008; Hughes et al., 1986; Ligeiro et al., 2013b; Stoddard et al., 2006; Whittier et al., 2007), rigorous statistical metric screening (Hering et al., 2006; Stoddard et al., 2008; Whittier et al., 2007), calibration for natural variance (Cao et al., 2007; Chen et al., 2017, 2014; Moya et al., 2011; Pereira et al., 2016), continuous MMI scoring criteria (Blocksom, 2003; Hughes et al., 1998), probabilistic sampling designs (Herlihy et al., 2000; Hughes and Peck, 2008), and national applicability (Moya et al., 2011; Paulsen et al., 2008; Stoddard et al., 2008).

It is desirable for MMIs to be applicable for large spatial scales (Hughes and Peck, 2008; Stoddard et al., 2008). Nonetheless, an MMI must be modified to account for regional differences (Dedieu et al., 2016; Klemm et al., 2003; Stoddard et al., 2008). In the U.S.A., specific MMIs were developed to account for well-established differences among regions (i.e. ecoregions, Omernik, 1987), subregions (Barbour and Gerritsen, 1996; Maxted et al., 2000), or aggregate ecoregions (Stoddard et al., 2008). In Europe, approaches for MMI development differ among countries and regions, considering its heterogeneous environments and political particularities (Hering et al., 2006; Mondy et al., 2012). Nonetheless, both the U.S.A. and Europe have legal statutes that support the use of biotic indicators to assess integrity at continental scales (Barbour et al., 1999; Bonada et al., 2006; Dedieu et al., 2016).

In contrast, neotropical countries lack specific legislation or guidelines for biological assessment, which is reflected by relatively few studies concerning the development and application of MMIs compared to the U.S.A. and Europe, where biotic and abiotic databases are well developed (Ruaro and Gubiani, 2013).

Despite many structural and political challenges, macroinvertebrate MMIs for neotropical regions have been successfully developed (Baptista et al., 2007; Dedieu et al., 2016; Helson and Williams, 2013; Macedo et al., 2016; Moya et al., 2011; Oliveira et al., 2011a; Pereira et al., 2016). For Brazil, there is a trend to develop macroinvertebrate MMIs for different regions (or biomes) such as the Atlantic Forest (Baptista et al., 2013, 2007; Oliveira et al., 2011a; Pereira et al., 2016; Suriano et al., 2011), Amazon (Couceiro et al., 2012), and more recently the savanna (Macedo et al., 2016). However, because they involve multiple academic institutions and lack a standardized methodology, those MMIs were developed using different methods, making it difficult to integrate information and compare results nationally (Buss et al., 2015).

The Brazilian neotropical savanna (sensu, "cerrado biome"), had an original natural cover area of approximately 2 million km^2 which has been strongly reduced as a result of pasture and monoculture expansion (Hunke et al., 2015). The second largest biome in Brazil, the savanna is considered a hotspot for biodiversity conservation strategies (Myers et al., 2000). It harbors many important large rivers and its network of headwater streams contain a large diversity of species and ecosystem services (Strassburg et al., 2017). However, stream and river ecological integrity is at risk because recent legislation has reduced the minimum required riparian buffer width (from 30 to 5–15 m, Brasil, 2012; see also Brancalion et al., 2016). Clearly there is a need to implement better ecological tools to assess stream condition (Buss et al., 2015; Moya et al., 2011).

A recent effort in the development of a preliminary macroinvertebrate MMI for savanna streams was proposed by Macedo et al. (2016), but it was developed for a single basin and based on few sites and few reference sites. As such, the index does not encompass enough variability to be applicable across the savanna biome.

To improve the development of an MMI in the neotropical savanna we: 1) extended the sampling area to four hydrologic units; 2) increased the number of least-disturbed reference sites for model development; 3) evaluated metric sampling variability by re-sampling sites; and 4) standardized the laboratory counting effort across samples. Thus, our approach embraced a greater variability and a wider range of anthropogenic impacts at multiple scales (e.g., agriculture, urbanization, nutrients, sedimentation). In that way, we not only filled gaps left by previous studies, but also provided the foundation and guidelines for developing and applying the MMI in other regions. Additionally, we used a probabilistic survey design to select the sampled sites, which allowed us to infer results to the total length of wadeable streams in the sampled area (Herlihy et al., 2000; Olsen and Peck, 2008). We also evaluated stream condition throughout each of the four different hydrologic units, and developed a regional neotropical savanna assessment. Following rigorous metric screening criteria, our objective was to develop a robust macroinvertebrate MMI for neotropical savanna streams, assess biological integrity, and relate the MMI scores to environmental disturbances.

2. Methods

2.1. Study area

The study area comprised the upstream portion of 2 important river basins in the neotropical Brazilian savanna draining into four hydropower reservoirs: Nova Ponte, Volta Grande, São Simão (Paraná River Basin) and Três Marias (São Francisco River Basin). It covers a total geographic area of 45,180 km^2 (Fig. 1). We sampled sites once in each area (hereafter: hydrologic units, sensu Ferreira et al., 2017; Firmiano et al., 2017; Seaber et al., 1987), during the dry season in 2009–2012. The dry season is preferable to other seasons for sampling because it facilitates habitat distinction, the more constant discharges reduce natural flow variability, macroinvertebrate assemblage structure is more stable, and crew safety hazards and road access difficulties are minimized (Hughes and Peck, 2008; Melo and Froehlich, 2001; Plafkin et al., 1989). We re-sampled the Nova Ponte sites in 2013 to assess interannual sampling variability within the same season (Kaufmann et al., 1999). Also, an additional set of hand-picked reference sites (see below) were sampled in preserved areas of the Nova Ponte hydrologic unit in 2014.

The regional climate in the study area is humid tropical savanna, with a well-defined dry season from May to September (Hunke et al., 2015). Average precipitation ranges from 800 to 2000 mm, and average annual temperature ranges between 18 and 28 °C (Ratter et al., 1997). The savanna vegetation consists of dispersed trees and shrubs, small palms, and grass (Quesada et al., 2008) with heterogeneous gallery forests along watercourses (Urbanetz et al., 2013). The major land uses are agricultural cash crops, charcoal production, grazing, and urbanization (Macedo et al., 2014; Ratter et al., 1997).

2.2. Survey design

Sites were selected through use of a randomized, systematic, spatially balanced sample design (Herlihy et al., 2000; Stevens and Olsen, 2004). We targeted a population of wadeable streams with access and flowing water at the time of sampling, defined as first to third order (Strahler, 1957), on 1:100,000 scale maps, located within an area 35 linear km upstream from the limits of the reservoirs. A random set of primary and alternate sites were selected to account for the fact that a number of primary random sites were non-target (e.g., dry, non-wadeable, inaccessible, access denied).

A probability survey like ours usually comprises sites across a wide

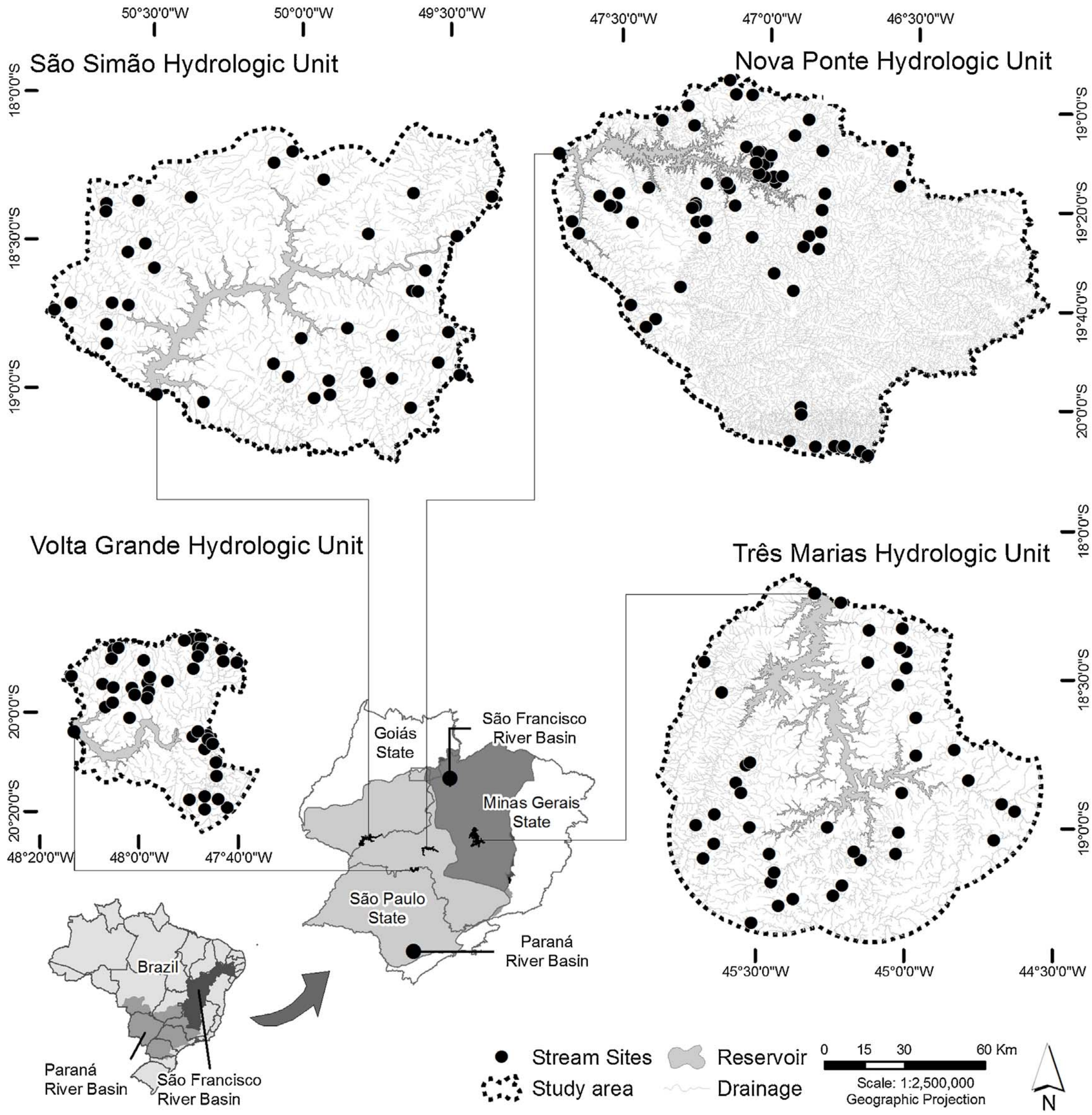

Fig 1. Distribution of sampled stream sites among four hydrologic units: Nova Ponte, Três Marias, Volta Grande, and São Simão.

range of intermediate disturbance condition, but is expected to have fewer sites in minimally disturbed or highly disturbed conditions (Herlihy et al., 2008; Stoddard et al., 2006). To guarantee a clearer disturbance gradient, we additionally hand-picked a number of sites likely to be in minimally disturbed condition (distant from urban areas, natural vegetation cover, no upstream dams or pollution sources), as well as a set of urban sites with highly altered physical and chemical conditions in each of the four hydrologic units, resulting in a total of 143 target random sites and 16 hand picked sites (~40 sites in each hydrologic unit). Because reference condition is a key component in developing a MMI, we also sampled an additional 31 hand-picked sites near or within protected areas in the Nova Ponte Basin (see Martins et al., 2017).

Each random sample site has a weight, calculated as the inverse of its selection probability, indicative of the length of stream it represents in the target population. These site weights were used to make estimates of regional condition from site data. Hand-picked sites have a weight of zero and are not used in estimating regional condition. Based on our sampling, there is an estimated 9432 km of target streams in the study area: 4515 km in Nova Ponte, 1641 km in Três Marias, 482 km in Volta Grande, and 2794 km in São Simão.

2.3. Benthic macroinvertebrate sampling

At each stream site, we set up a longitudinal sampling reach equal to 40 times the mean wetted width or a minimum of 150 m (Silva et al., 2014). Sample reaches had a mean depth of 35.4 cm (±17.1) and mean width of 3.4 m (±1.9). In each stream reach, we took six D-frame kick-net (500 μm mesh, 0.9 m^2 area) samples of the macroinvertebrate assemblage. The six samples were spaced at equal intervals along the sample reach with alternating left, center, and right cross-sectional positions, yielding a multi-habitat composite sample representative of natural patterns found in the stream reach and sensitive to environmental gradients (Gerth and Herlihy, 2006; Hughes and Peck, 2008; Li et al., 2014). Focusing on specific (target) or rare habitats can influence and overweight the final composite sample. Previous papers have found that for bioassessment purposes a systematic design is recommended (Gerth and Herlihy, 2006). The samples were fixed with 10% formalin, and taken to the laboratory. Macroinvertebrate samples were sorted and identified through use of a stereomicroscope (100× magnification) and taxonomic keys (Costa et al., 2006; Fernández and Domínguez, 2001; Merritt et al., 2008; Mugnai et al., 2010). All invertebrates were identified to the family level, except for non-insects, which were identified to either order or class levels (e.g., Oligochaeta, Bivalvia, Decapoda, Hirudinea).

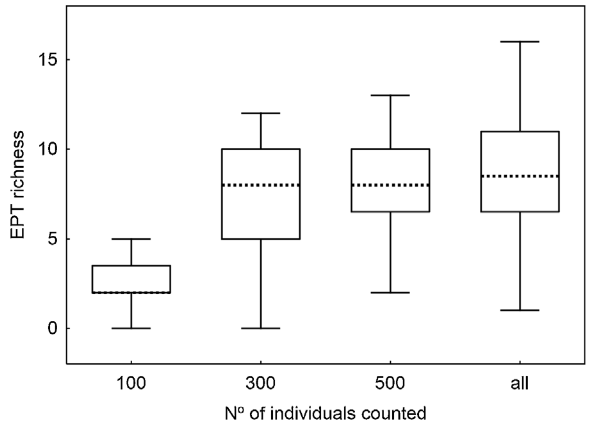

Fig. 2. Ephemeroptera, Plecoptera and Trichoptera (EPT) richness for fixed-counts of 100, 300, 500, and all individuals collected. Boxes represent the 25th and 75th percentiles, dotted lines within the boxes are medians, and whiskers are non-outlier ranges.

As taxa richness depends on the number of organisms counted, we wanted to standardize results to a fixed number of individuals in each sample (Larsen and Herlihy, 1998). We also sought to recommend a reliable number of individuals that can be used in future studies to save costs and processing time (Ligeiro et al., 2013a; Oliveira et al., 2011b). To test this, we calculated Ephemeroptera, Plecoptera and Trichoptera (EPT) richness using the full sample and fixed counts of 100, 300 and 500 individuals (Larsen and Herlihy, 1998) by just randomly picking the desired number of individuals from each sample. As expected, the total number of EPT taxa increased with increased number of individuals counted. By one-way ANOVA, counts of 300, 500, or all individuals collected did not differ significantly, but did differ from counts of 100 individuals (Fig. 2). Thus, 300 individuals counted was determined to be an appropriate sample size to assess ecological condition in savanna streams, which also limits costs and processing time without compromising ecological information. Other authors also have recommended counting 300 individuals for bioassessment purposes (Boonsoong et al., 2009; Larsen and Herlihy, 1998), although more samples and sample counts are recommended for accurate assemblage-structure comparisons (Li et al., 2014; Silva et al., 2016). Hereafter, all analyses were performed with the data set of 300 individuals counted or the entire sample when there were fewer than 300 individuals collected (less than 30% of samples).

2.4. Physical and chemical habitat measures

In each sample reach we recorded quantitative measures of physical habitat following Peck et al. (2006). Those measures describe stream channel morphology (e.g., slope, sinuosity, depth, wetted and bankfull widths, incision, bank angle), habitat features (substrate size, flow types, amount of wood in the channel), riparian structure (canopy cover, vegetation type), and human alterations in riparian zones (e.g., presence of buildings, pasture, crops, roads, trash). Following Kaufmann et al. (1999), we calculated metrics and indices combining those field measurements into a single value. For example, the riparian disturbance index (RDI) combines the various types of anthropogenic disturbance observations weighted by their proximity to the streambed. Similarly, relative bed stability is an anthropogenic sedimentation index calculated from the mean particle size measured in the field compared with the potential particle size in an undisturbed stream with the same stream power. More details on metric calculation are available in Kaufmann et al. (1999).

Water temperature, electrical conductivity, total dissolved solids, turbidity, and pH, were measured at each stream reach by use of portable equipment (YSI Model 650). A water sample was collected and transported to the laboratory for determining dissolved oxygen, total nitrogen, and total phosphorus (APHA, 1998).

2.5. Land use and cover

We determined the main land uses and cover in the catchment of each site through manual interpretation of fine resolution images from Google Earth (Google, 2016) and multispectral images from the Landsat satellite (see Macedo et al., 2014). Our evaluation resulted in four vegetation cover physiognomies (forested savanna, gramineous-woody savanna, park savanna, and palm swamp), being grouped into a single metric of natural cover and three anthropogenic land uses (urban, agriculture, and pasture). Following Ligeiro et al. (2013b) we calculated the integrated disturbance index (IDI), a combination of site- and catchment −scale measurements of anthropogenic pressures. The IDI is calculated by first measuring the riparian disturbance index (RDI; described above) and the catchment disturbance index (CDI). The latter is calculated by summing the% land uses, weighted by the potential of degradation that each one has in the aquatic ecosystem (CDI = 4 x % urban + 2 x % agriculture + % pasture). The IDI is the Euclidian distance between the site and the origin of the disturbance plane formed by the RDI and CDI. Therefore, the higher the IDI, the greater the disturbance on both scales. We also calculated other commonly used metrics to characterize anthropogenic disturbance, like population size, household and road densities, and distance from roads and cities (OpenStreetMaps®).

2.6. Site disturbance classification

We used a filtering procedure to identify least- and most-disturbed sites (Herlihy et al., 2008; Waite et al., 2000). The method is based on filtering all sites to previously established thresholds for physical habitat metrics, water-quality parameters, and land use. Because we were interested in achieving a minimum number of least-disturbed sites in each hydrologic unit, we developed specific thresholds for our nine parameters in each one (Table 1). If a site failed any one of the filters in Table 1 it was not considered to be least-disturbed. In a similar way, we defined most-disturbed sites using a similar filtering process. Any site that had any urban land use in the catchment, a riparian disturbance index > 2, or extreme values for water parameters (dissolved oxygen < 4.0 mg/L; total nitrogen > 0.2 mg/L or total phosphorus > 0.1 mg/L) was considered to be most-disturbed. Sites that did not match the least- or most-disturbed categories were classified as intermediate.

Table 1
Criteria for physical habitat structure, water quality, and land use for identifying least-disturbed sites. Sites that did not meet all criteria were excluded from the least-disturbed set. NP = Nova Ponte, TM = Três Marias, VG = Volta Grande, SS = São Simão. – Indicates the absence of the criterion in a hydrologic unit.

	Filter criteria	NP	TM	VG	SS
Physical Habitat	Riparian disturbance index	< 1	< 1	< 1	< 1
	% fine substrate	< 20	< 40	< 40	< 40
Water Quality	Dissolved oxygen (mg/L)	> 6.0	> 6.0	> 6.0	> 6.0
	pH	> 6; < 9	> 6; < 9	> 6; < 9	> 6; < 9
	Turbidity (NTU)	< 10	< 10	< 10	< 10
	Total nitrogen (mg/L)	< 0.2	< 0.2	< 0.2	< 0.2
	Total phosphorus (mg/L)	< 0.03	< 0.03	< 0.03	< 0.03
Land Use	% natural cover	> 40	> 40	–	–
	% urban	0	0	0	0

2.7. MMI development

2.7.1. Candidate metrics

We proposed *a priori* 114 metrics belonging to seven categories that describe aspects of taxonomic richness, taxonomic composition, diversity and dominance, tolerance, feeding group, mobility, and respiration. Those metrics were expected to have the potential to respond to anthropogenic impacts on macroinvertebrate assemblages and discriminate least- from most-disturbed sites (Karr and Chu, 1998; Tomanova et al., 2008).

Taxonomic richness is a common biodiversity measure and is defined as the number of taxa in a known area (Gotelli and Colwell, 2001). We calculated taxonomic richness at the family level representing the total assemblage (total taxonomic richness) and by subgroups of the macroinvertebrate assemblage (e.g., Ephemeroptera richness, EPT richness). Taxonomic composition was also expressed in terms of relative abundance of selected groups in terms of both percent of individuals (number of individuals in group/total number) and percent of total taxa richness (subgroup richness/total taxa richness). Groups consisted of both families and orders (e.g., % Diptera, % Chironomidae) or combined groups (e.g., % Chironomidae plus Oligochaeta, % EPT) in different taxonomic levels. Diversity and dominance metrics were calculated through use of popular indices (e.g., Shannon-Wiener Diversity Index, Simpson Diversity Index, and Margalef Diversity Index) and individual dominance measures (e.g., % of individuals in top 2 dominant taxa).

The tolerance metrics were based on taxa sensitivity to organic pollution, where we assigned values ranging from 1 (most tolerant) to 10 (most sensitive) for each taxon following the scores proposed by Junqueira et al. (2000) and additional sources when the information was not available for a specific taxon. We calculated individual metrics (e.g., % of sensitive taxa, super-tolerant taxa richness) and biological indices adapted to Brazil, such as the Biological Monitoring Working Party (BMWP, Junqueira et al., 2000), which is a sum of taxa tolerance scores in each site.

Metrics for richness, % of individuals, and% of taxa richness were also calculated for feeding groups, mobility, and respiration autecology following the taxa classifications of Tomanova et al. (2008) for neotropical streams and by additional sources in cases where the information was lacking. For each functional attribute (e.g. feeding group), we used fuzzy-coding to assign scores, based on the taxa affinity for each category (e.g. predators, collector-gatherer, scrapers), ranging from 0 (no affinity) to 3 (strong affinity). The advantage of this approach is that it accounts for the various types and levels of information available, the plasticity of a taxon, and its different life cycle stages (Chevenet et al., 1994). The fuzzy code scores were expressed as proportions in each category and the final percentage metrics were obtained by multiplying the proportion by the abundance of individuals. To obtain the richness and percent richness metrics we only considered the presence or absence of a category independently of the score (see Supplementary Material Table S1).

2.7.2. Metric screening

To increase comparability with studies from other continents, we followed the same metric screening steps used in other MMI development studies (Hering et al., 2006; Stoddard et al., 2008). Metrics that failed any of the set of screening criteria were removed from consideration in the final MMI. Initially, we performed a range test to eliminate metrics with very low variability (richness metrics with range < 5 and percentage metrics with range < 10% were dropped).

In the second screen, we assessed the influence of natural environmental variability on macroinvertebrate metrics. To do that, we tested the relationship of macroinvertebrate biological metrics to GIS-extracted environmental variables of altitude, elevation range, catchment slope, and catchment area obtained from Shuttle Radar Topographic Mission – SRTM (3 arc seconds; USGS, 2005) and catchment total annual rainfall (ANA, 2014) (see also Chen et al., 2014; Macedo et al., 2016; Pereira et al., 2016). We used multiple linear regression models (forward-stepwise) with our biological metrics dataset from least-disturbed sites against the predictor environmental variables, normalized by $\log_{10}$ when necessary and checked by Kolmogorov-Smirnov tests. For metrics where we obtained a significant ($p < 0.001$) relationship and the correlation coefficient (R^2) was greater than 0.3, we derived a natural gradient corrected metric by replacing the original metric with the residual of the metric based on the regression equation with the natural variable(s).

We screened our set of metrics for responsiveness by calculating *t*-tests comparing mean metric values of least- versus most-disturbed sites. We also measured the discriminance effect by calculating the quartile overlap (hereafter delta) obtained by subtracting the 25th percentile of least-disturbed sites from the 75th percentile of most-disturbed sites. We excluded metrics with t-values less than 3 and or deltas with interquartiles overlapping medians.

For the last screen, we quantified the stability of each metric to sampling variability by comparing the variance among sites (signal, S) to the variance between re-visits at the same sites (noise, N) (Kaufmann et al., 1999). The higher the signal-to-noise (S:N) ratio, the more stable the metric (Herlihy et al., 2008; Stoddard et al., 2008). Thresholds to eliminate metrics based on the S:N have varied among different studies with different indicator assemblages. Because our re-sampling visit occurred 4 years after the first sampling, we adopted a somewhat conservative approach, where we kept metrics with S:N > 1 or the highest possible values for categories of metrics in which we did not obtained S:N values > 1 (see Supplementary Material Table S2).

2.8. MMI calculation and selection

All metrics that passed the screening described above were considered for the MMI. Metrics were then distributed in the 7 categories that represented different structural and functional attributes of macroinvertebrate assemblages: taxonomic richness, taxonomic composition, diversity and dominance, tolerance, feeding habit, respiration, and mobility.

We used the continuous metric scoring method to calculate the MMI because of its better responsiveness and lower variability, also avoiding the subjectivity of a discrete scoring method (Blocksom, 2003; Hughes et al., 1998; Stoddard et al., 2008). Metrics were standardized to a 0–10 scale by interpolating metrics between floor and ceiling values. We assumed equal importance of metrics considering that previous studies did not find improved MMI performance by weighting metrics (Bellenger and Herlihy, 2010; Chen et al., 2017). For metrics that responded negatively to disturbance, we set the 95th percentile of the reference values as the ceiling and the 5th percentile of all sample values as the floor. In an opposite way, metrics that responded positively to disturbance received the 5th percentile of the reference values as the floor and the 95th percentile of all sites as the ceiling. This procedure is summarized below:

$$Positive\quad metrics = 10^{*}\left(\frac{metric - floor}{ceiling - floor}\right)$$

$$Negative\quad metrics\text{: } 10^{*}\left[1 - \left(\frac{metric - floor}{ceiling - floor}\right)\right]$$

Metric scores below/above the floor/ceiling were set to 0 or 10, respectively. We obtained the final MMI value by summing the seven metric 0–10 scaled values and standardizing to a 0–100 scale by multiplying by 100/70.

We ran an all subsets procedure to assemble all possible combinations of an MMI with 7 metrics (one from each category). For each possible MMI model combination, we obtained the S:N, *t*-test, delta, and maximum correlation among the 7 metrics.

The choice of the best MMI from the all subsets results was made first by screening out candidate MMIs that had S:N values > 2 and a correlation coefficient between any two metrics in the MMI < 0.7. The remaining candidate MMIs after this screen were evaluated for both delta and t-value. We picked the top 8 candidate MMIs with the highest delta and t-values. We regressed those MMIs against a series of anthropogenic stressors, and choose as the final MMI the one with the highest correlation coefficient to the greatest number of stressors.

2.9. MMI condition classification and stressors association

To evaluate the biological condition of savanna streams we established three categories representing different ecological quality levels. Thresholds for each class were obtained from the distribution of scores in the set of least-disturbed sites. Sites with an MMI score lower than the 5th percentile of the least-disturbed distribution were classified as "poor", scores between the 5th and 25th percentile were classified as "fair", and those higher than the 25th percentile were classified as "good". We inferred those results to the total target stream length in each hydrologic unit and for the entire target region.

We were also interested in identifying a stressor-response model that integrated multiple anthropogenic disturbances and best explained the distribution of MMI scores. To do that, we performed an all-subsets multiple regression approach with the MMI as a response variable and a list of candidate anthropogenic disturbances as predictive variables. When necessary, we log-transformed and checked normality (Kolmogorov-Smirnov tests) of the explanatory metrics and excluded redundant metrics with a Pearson correlation coefficient > 0.7. To choose the best model we used the corrected Akaike Information Criterion (AICc) weighted by importance of variables, which meant that we considered not only a non-overfitting model but also took into account the frequency in which the metric was present in all possible models (Burnham and Anderson, 2004; Sifneos et al., 2010). We additionally inspected for breakpoints in AICc weighted variable importance values to help determine variables to be included in the final regression model. All analyses were performed using SAS statistical software (SAS Institute, version 8.0, Cary, North Carolina) and Statistica software (StatSoft Inc., version 8.0).

3. Results

3.1. Biological data

We collected a total of 89 taxa, 65 in Nova Ponte, 63 in Três Marias, and 59 in both Volta Grande and São Simão. Diptera (56%), Ephemeroptera (15%), and Coleoptera (11%) were the most abundant groups, represented respectively at the family level, by the Chironomidae (46%), Baetidae (7%), and Elmidae (10%).

3.2. Site disturbance classification

After filtering all the sites using the criteria in Table 1, we identified a total of 53 least-disturbed sites, 95 intermediate sites and 42 most-disturbed sites. Of the total number of least-disturbed sites, 30 were hand-picked in Nova Ponte. Those sites were within or near protected areas in the Serra da Canastra National Park and Serra do Salitre region, whereas the others were distributed throughout the study area. Therefore, we adopted the "least-disturbed" term for regions where "minimally disturbed" sites did not exist because of intensive human exploitation of the land (Stoddard et al., 2006; Whittier et al., 2007). Although we recognize the lack of some important filters in our definition of least-disturbed (e.g., presence of contaminants), we followed rigorous criteria with 9 disturbance factors to obtain the set of least-disturbed sites (Table 1). Flexibility in some thresholds also allowed a minimum number of least-disturbed sites for each hydrologic unit and ensured that our MMI was representative of all four hydrologic units

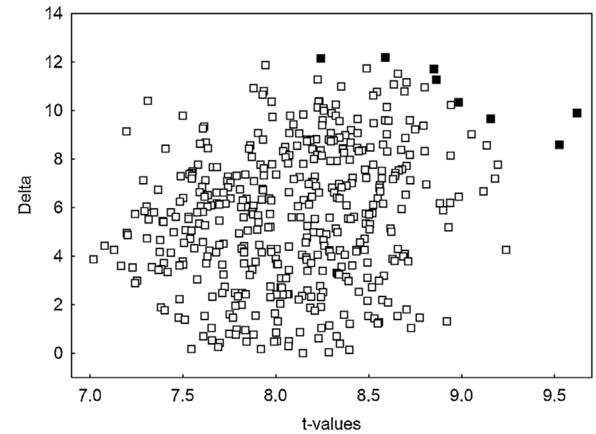

Fig. 3. All subsets results of MMI delta (disturbance interquartile overlap) and t-values for distinguishing least- from most-disturbed sites. Only models with an S:N > 2, maximum correlation < 0.7, and delta > 0 are shown. Dark rectangles represent the MMIs with the best balance between both values that were chosen for final consideration.

and could be extended to the neotropical savanna.

3.3. Metric selection and index development

We reduced our initial set of 114 metrics down to 35 by excluding 11 metrics that showed no variability, 28 metrics that failed to distinguish least- from most-disturbed sites in the responsiveness test (t-value < 3), 19 that failed the delta discriminance evaluation, and 21 metrics with low signal to noise (S:N < 1). We only found 3 metrics correlated with natural environmental variables: % of Diptera individuals (catchment area, altitude, catchment slope), BMWP index (altitude, catchment slope), and % of gilled individuals (catchment rainfall, catchment elevation range, catchment slope), which we adjusted based on the residuals of the models.

The final set of 35 metrics that were candidates for the MMI included 6 taxonomic richness metrics, 7 taxonomic composition metrics, 3 diversity and dominance metrics, 8 tolerance metrics, 5 feeding group metrics, 2 mobility metrics, and 4 respiration metrics. All possible combinations of metrics that included one from each group yielded 40,320 different MMI models. We picked eight models with the highest balance of delta and t values (Fig. 3), and chose the one with the best response to anthropogenic disturbances (Table 2). The final MMI metrics were Ephemeroptera richness, % Gastropoda individuals, Shannon-Wiener diversity index, % sensitive taxa richness, % scraper individuals, temporarily attached taxa richness, and gill respiration taxa richness.

The final MMI scores clearly separated the 25th percentile of least-disturbed sites from the 75th percentile of most-disturbed sites (Fig. 4). When we examined the MMI by hydrologic unit, we observed an interquartile overlap only for the Volta Grande hydrologic unit (Fig. 5), in which the least-disturbed sites were more disturbed by anthropogenic stressors than in the other hydrologic units.

3.4. Assessment of ecological status

The final MMI scores were strongly associated with anthropogenic disturbances ($r^2 = 0.41$) and the stressor model that best explained the MMI included the integrated disturbance index (IDI) score, % fines, log relative bed stability, total nitrogen, % urban land use, and distance from road (Table 3). The IDI was the metric that contributed most to explaining MMI scores and was present in all considered models (AICc weighted importance value of 1.0).

Because of our probabilistic survey design, we were able to infer our results to the total target stream length in the sampled area to assess ecological status (Fig. 6). The Nova Ponte hydrologic unit had the

Table 2
Pearson correlation coefficients between the final MMI and anthropogenic stressors.

Anthropogenic stressors	Correlation coefficient (r)	
IDI – Integrated Disturbance Index[a]	−0.52	**
Land use and cover		
% anthropogenic land use	−0.38	**
% urban	−0.36	**
% pasture	−0.09	n.s.
% agriculture	−0.13	n.s.
Catchment road density	−0.31	**
Distance from road	0.18	*
Distance from cities	0.11	n.s.
Physical habitat		
Riparian disturbance index[b]	−0.43	**
Mean embeddedness	−0.31	**
Log relative bed stability	0.45	**
Mean woody riparian vegetation	0.23	*
% fine sediment	−0.46	**
Chemical habitat		
Dissolved oxygen	−0.10	n.s.
Turbidity	−0.32	**
Conductivity	−0.29	**
Total dissolved solids	−0.22	*
Total nitrogen	−0.36	**

*$p < 0.01$, **$p < 0.0001$, n.s. not significant.
[a] Ligeiro et al. (2013b).
[b] Kaufmann et al. (1999).

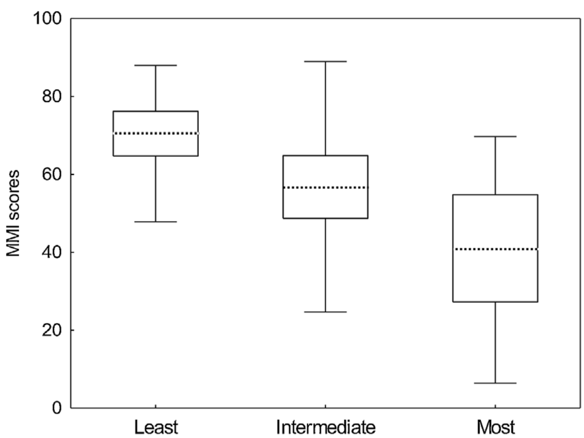

Fig. 4. Regional assessment for disturbance categories (ANOVA test, $F_{(2,187)} = 51.6$, $p < 0.0001$). Boxes represent the 25th and 75th percentiles, dotted lines within the boxes are medians, and whiskers are non-outlier ranges.

highest percent of stream length in good condition (over 50%), whereas Volta Grande had the highest percent of stream length in poor condition (35%). Our overall regional bioassessment estimated that 38% of the stream length sampled (3594 km) was in good condition, 35% (3275 km) in fair condition, and 27% (2546 km) in poor condition.

4. Discussion

4.1. Reference site and metric selection

We developed a macroinvertebrate MMI based on 7 metrics to assess the ecological condition of wadeable streams in the Brazilian neotropical savanna. The use of different hydrologic units allowed us to account for different sources of anthropogenic disturbances and natural environmental conditions. Therefore, the index should be applicable in other neotropical savanna streams and perhaps savanna globally. In addition, the methodological development of the MMI followed rigorous criteria, facilitating comparisons with other studies.

The building of an MMI is a stepwise process that begins with the

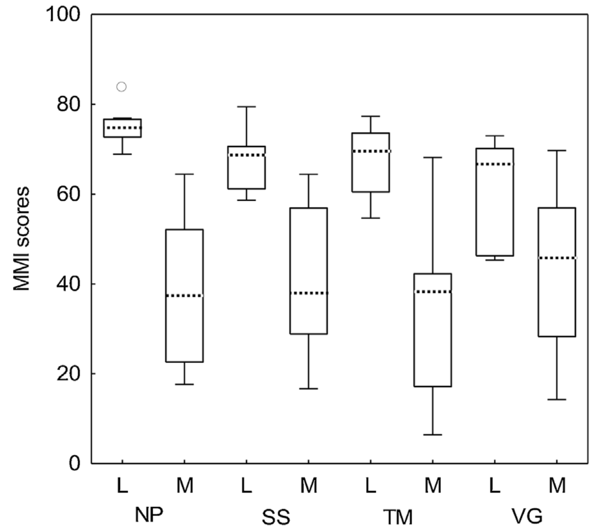

Fig. 5. MMI scores for disturbance categories in each of the four hydrologic units. Boxes represent the 25th and 75th percentiles, dotted lines within the boxes are medians, whiskers are non-outlier ranges, and outlier denoted by open circle. L = least-disturbed, M = most-disturbed, NP = Nova Ponte, SS = São Simão, TM = Três Marias, and VG = Volta Grande.

Table 3
Multiple linear regression model of predicted anthropogenic stressors explaining the final MMI ($F = 22.4_{6,181}$; AICc = 1500; adjusted $R^2 = 0.41$; $p < 0.0001$). Beta is the regression coefficient and Std. Err. is the standard error of the regression coefficient. The AICwi column indicates the stressor variable weighted by its AIC importance.

	AICwi	Beta	Std.Err.	*p* value
Intercept	1.00	73.4	2.83	< 0.0001
Integrated Disturbance Index (IDI)	1.00	−16.3	3.69	< 0.0001
% fines	0.99	−0.127	0.0543	0.021
Log relative bed stability	0.88	2.27	1.106	0.041
Total nitrogen (mg/L) (log x + 1)	0.79	−135	67.1	0.046
% urban	0.76	−0.217	0.112	0.054
Distance from road (km) (log x + 1)	0.71	0.000409	0.000231	0.079

definition of reference sites. An MMI is expected to be able to distinguish disturbed sites from a reference natural condition by means of the biological assemblage responses (Hughes et al., 1986; Ligeiro et al., 2013b; Stoddard et al., 2006). Because of this, the reference condition should describe sites minimally affected by anthropogenic activities and where physical, chemical, landscape, and biological features represent natural patterns and processes (reference) condition across a region (Ligeiro et al., 2013b; Stoddard et al., 2006; Whittier et al., 2007). However, minimally disturbed sites are rarely found in regions where human activities are long-term and widespread (Whittier et al., 2007). This is especially the case for the neotropical savanna, where agriculture and pasture activities are in continuous expansion (Hunke et al., 2015). Our selected reference sites were filtered for well defined criteria of water quality parameters, land use, and riparian disturbance and can be considered as least-disturbed sites (Herlihy et al., 2008; Whittier et al., 2007). Specific hydrologic units had their thresholds relaxed to allow the inclusion of sites in the best available condition for that unit. Still, in general, our set of least-disturbed sites described streams with low nutrient concentrations, minimal riparian disturbance, and no urbanization in the catchment. Although not representing an optimal scenario of natural condition (a true minimally-disturbed reference condition, sensu Stoddard et al., 2006), the proposed MMI succeeded in distinguishing least- from most-disturbed sites.

The proposed MMI based on 7 macroinvertebrate assemblage metrics represents different structural and functional assemblage attributes. This approach accounts for the various dimensions of biological systems, which facilitates its ability to reflect human disturbances in

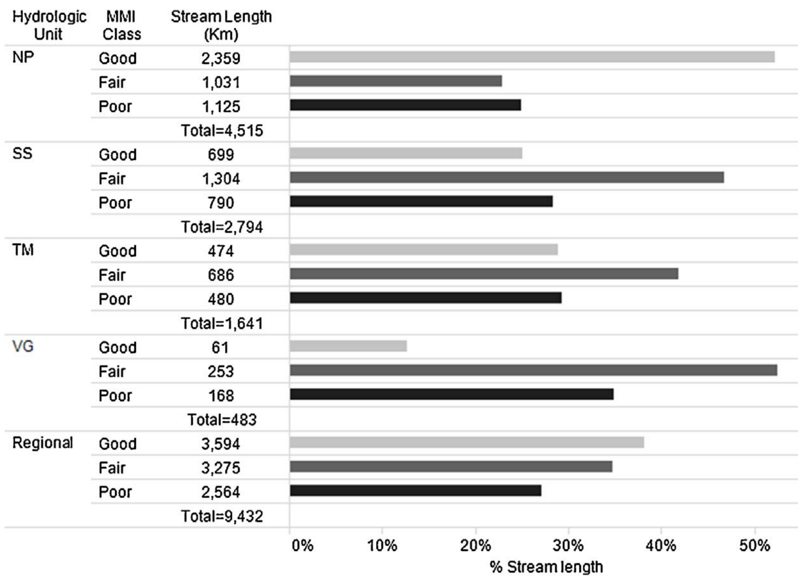

Fig. 6. Total and percent of stream length in MMI classes of biological condition (good, fair, and poor) in each hydrologic unit and for the regional assessment. NP = Nova Ponte, SS = São Simão, TM = Três Marias, and VG = Volta Grande.

aquatic ecosystems (Karr and Chu, 1998). The representativeness of several biological aspects in an integrated MMI is appropriate and recommended by many authors (Hering et al., 2006; Huang et al., 2015; Karr and Chu, 1998; Stoddard et al., 2008).

Considering the metrics included in our MMI, the number of Ephemeroptera families is a common richness metric also used in other MMIs (Bellucci et al., 2013; Mereta et al., 2013). This macroinvertebrate order has long been recognized as an important indicator of biological health because of its sensitivity to disturbance (Arimoro and Muller, 2010; Bauernfeind and Moog, 2000; Pond, 2010; Siegloch et al., 2014). Firmiano et al. (2017) found a clear decrease in specific Ephemeroptera taxa to multiple anthropogenic disturbances. Although some Ephemeroptera taxa are tolerant (e.g., some Baetidae and Caenidae), which can lead to a variable response to disturbance (Pereira et al., 2016), that seems more reflected in composition metrics than richness metrics. Percent Gastropoda individuals was the metric representing the composition category, increasing in abundance with increased disturbance. Those organisms are commonly associated with the increase in organic matter accompanying eutrophication processes (Verdonschot et al., 2012).

The Shannon-Wiener diversity index is a common diversity and dominance metric in bioassessment studies, including many indices developed for the neotropical region (Dedieu et al., 2016; Helson and Williams, 2013; Suriano et al., 2011; Touron-Poncet et al., 2014). It differs from taxonomic richness by including both a taxa richness component and an evenness component, and in our case it was not correlated with richness of Ephemeroptera families.

For the tolerance category, we selected the percent of taxa richness with pollutant tolerance values ≥ 7 (on a scale where 10 is the most sensitive taxa to pollution and 0 is the most tolerant). This category is especially important because, as opposed to diversity or richness metrics, it accounts for the specific responses of the different taxa to disturbance (Gabriels et al., 2010; Hilsenhoff, 1988; Whittier and Van Sickle, 2010).

The use of functional attributes of assemblage composition is also recommended for the development of MMIs (Moya et al., 2011; Saito et al., 2015), because of their ability to detect anthropogenic disturbances independently of taxonomic composition (Tomanova and Usseglio-Polatera, 2007). We evaluated 3 functional attributes to improve the robustness of our MMI approach. The richness of taxa that use gills for respiration is particularly sensitive to environmental stress because of the permeability of gills and their relation to dissolved oxygen concentrations (Chapman et al., 2004; Dolédec et al., 2006; Saito et al., 2015). Temporarily attached taxa richness was the metric selected in the mobility category; those organism often have adaptations (hooks, suction structures or fixed cases) to heterogeneous habitats, characterized by fast flows (Lamouroux et al., 2004). Such organisms are sensitive to the amounts of sand and fine sediments suspended in the water column and deposited on the streambed, which are typical products of excess catchment and streambank erosion (Bryce et al., 2010). Finally, in the feeding group category, % scraper individuals responded negatively to impairment. That metric is commonly associated with sediment and nutrient inputs to the streambed (Larsen et al., 2011).

Differences in the final selected metrics for the MMI compared with other neotropical studies can be attributed to biogeographic differences, anthropogenic impact levels and types, and differences in the methodological development of the MMI (Dedieu et al., 2016). Another important differences is the taxonomic resolution that the different studies used (Dedieu et al., 2016). A MMI can benefit from refined taxonomic resolution because of the greater sensitivity of certain genera (or species) in detecting multiple stressors and better discriminating differences in biological condition (Lakew and Moog, 2015; Touron-Poncet et al., 2014). Nonetheless, we developed an MMI at the family taxonomic level that can be reproduced at lower cost, less laboratory time, and by non-experts (Hilsenhoff, 1988; Ríos-Touma et al., 2014; Suriano et al., 2011) without compromising excellent index performance.

As highlighted by Macedo et al. (2016), who developed a preliminary MMI for one hydrologic unit in the neotropical savanna, further improvements were needed to select metrics. Indeed, evaluating metric stability (S:N test) helped us to identify metrics that varied among sites because of differences in stream condition rather than by sampling variation within a site (Chen et al., 2014; Stoddard et al., 2008). From the 4 metrics selected by Macedo et al. (2016) to build their best performing MMI, 3 of them failed in our responsiveness or discriminance test and had low signal-to-noise values in the screening step. Because of that, their index may perform well in the context it was developed but certainly would perform below expectations when applied elsewhere.

Finally, another important step in developing MMIs involves correctly distinguishing the effect of natural variation from covarying anthropogenic pressures. Recent papers suggested that correcting for the effect of natural variation on biological metrics increased MMI responsiveness and sensitivity to impairment, although responding similarly when compared with unadjusted models (Carvalho et al., 2017; Chen et al., 2014; Macedo et al., 2016; Pereira et al., 2016). Macedo et al. (2016) found that the adjusted MMI for savanna streams in a small geographic area (~7500 km^2) performed better when compared with unadjusted models, and recommended adjustments for future studies especially in larger geographic areas, as we did in this study (~45,000 km^2). Nonetheless, although we corrected 3 metrics, none of them were retained in the final MMI. Besides, our final MMI was not related to any of the natural gradients we assessed ($r^2 < 0.06$), and was more strongly related to anthropogenic stressors. Because natural variation is important in larger geographic areas and can affect biological assemblages differently (Stoddard et al., 2008), we recommend that future studies evaluate that variation to avoid biased assessments and erroneous inferences (Chen et al., 2017, 2014).

4.2. Overall MMI assessment

The multiple linear regression model explained 41% of the variation in MMI scores through the combination of six explanatory variables describing land use, water parameters, and physical habitat structure. The Integrated Disturbance Index (IDI; Ligeiro et al., 2013b) was the explanatory variable that most contributed to the model explanation. That index aggregates in a single measure information about anthropogenic disturbance at both local and catchment scales (Ligeiro et al., 2013b). The IDI was used as an objective method to define least- and most-disturbed sites in other studies concerning the development of multimetric indices in the neotropics (Chen et al., 2017; Macedo et al., 2016; Terra et al., 2013) because of its ability to summarize multiple anthropogenic disturbances independently of biological measures (Ligeiro et al., 2013b). Other authors found the IDI an important explanatory variable for macroinvertebrate richness in streams (Firmiano et al., 2017) and reservoirs (Martins et al., 2015), reinforcing the ability of the IDI to reflect biological condition and corroborating our findings. Two sediment related variables were also in the model: log relative bed stability and% fine sediments. Although based on different concepts, both variables have been reported as strongly affecting macroinvertebrate assemblage composition, structure, and function (Bryce et al., 2010; Sutherland et al., 2012).

Nutrient enrichment of aquatic ecosystems is the main cause of water quality impairment worldwide (Woodward et al., 2012). Previous studies have demonstrated the association of watershed land use with the concentration of nutrients in aquatic ecosystems (Herlihy et al., 1998). Excess nutrients in water bodies result in a cascade of effects involving excessive primary production, habitat degradation, altered food sources, higher turbidity, and fish kills, among others (Herlihy and Sifneos, 2008; Wang et al., 2007). Although nutrient effects on aquatic communities can be variable (Heino et al., 2003), other authors have addressed the negative impacts of increased nitrogen on macroinvertebrate structure, corroborating our findings (USEPA, 2016; Wang et al., 2007; Yuan, 2010).

Both the urbanization and distance from road explanatory variables reflect changes to the natural land cover of the region, which can have substantial impacts on stream ecosystems (Roy et al., 2003; Walsh et al., 2007; Wang et al., 2012). Urbanization and roads affect the biota via multiple direct and indirect pathways by increasing impervious surface area, altering hydrology and sediment transportation, modifying channel morphology, increasing pollutant loads, and creating migration barriers (Hughes and Dunham, 2014; Leitão et al., 2017; Sterling et al., 2016).

Differences found in ecological conditions among the four hydrologic units can be associated with the different degrees and types of human impacts and the quality of the selected reference (or least-disturbed) sites (Hughes et al., 1986; Pont et al., 2009). These differences explain why Volta Grande showed an interquartile overlap between least- and most-disturbed sites (Fig. 5), had the highest percentage of stream length in poor condition, and had the lowest percentage of stream length in good condition (Fig. 6). Volta Grande is dominated by row crop agriculture (mean ~70% in site catchments) and it has the most intense urban use compared with Nova Ponte, São Simão, and Três Marias (Callisto et al., 2014; Ferreira et al., 2017), so it is likely that Volta Grande lacks streams with biological integrity. That said, our MMI scoring was subject to the varying thresholds used in each hydrologic unit to classify least-disturbed condition, and interpretations of results must account for that. MMI assessments based on a hydrologic unit perspective facilitate focusing on more local management practices. For example, Nova Ponte had the highest percentage of stream length in good condition of the four hydrologic units, which should guide efforts toward catchment protection. However, Volta Grande management efforts should focus on catchment rehabilitation and mitigation of human impacts.

Overall, our regional bioassessment estimated that 27% of the stream length was in poor condition. Jim & nez-Valencia et al. (2014) estimated that 62% of the stream length in the Guapiaçu-Macacu Basin (Rio de Janeiro state) was in poor condition. The major stressors there were site habitat degradation and riparian and catchment deforestation. In the conterminous U.S.A., Paulsen et al. (2008) reported that 28%, 52%, and 40% of stream length was in poor condition in the West, Eastern Highlands, and Plains and Lowlands aggregated ecoregions, respectively. They did not assess catchment disturbance, but determined that excess nutrients and fine streambed sediments were the most important stressors in all three regions.

4.3. Future perspectives

The MMI we developed accounted for many shortcomings in previous studies, allowing us to improve current methodologies in developing a cost-effective biological tool to assess stream condition. Our methodology is being effectively applied in another important Brazilian hydrographic basin assessment (the Pandeiros River Basin; FAPEMIG, 2015). Nonetheless, in Brazil, as in most South American countries, the lack of legislation for biological assessments hinders the application and development of MMIs. Minas Gerais state is an exception because it has a regulation recommending the use of biological indicators in the assessment of aquatic ecosystems (COPAM/CERH-MG/2008). Our MMI application not only demonstrates development of another MMI, it is also demonstrates how a state-wide and national biological assessment could be implemented in a cost-effective manner.

5. Summary and conclusions

We successfully developed a macroinvertebrate-based MMI capable of distinguishing least- from most-disturbed streams. The MMI responded to a variety of anthropogenic stressors describing land use, water quality, and physical habitat structure. Our MMI is an improvement over the preliminary MMI of Macedo et al. (2016) for several reasons. Compared with Macedo et al. (2016), we sampled 4 hydrologic units versus 1, 190 sites versus 40, 31 additional least-disturbed reference sites, and 40 revisit sites. In addition, we selected reference sites by a filtering process based largely on site abiotic conditions, evaluated 114 metrics versus 80, added a signal-noise screen of the candidate metrics, selected 7 versus 4 final metrics, and examined 8 candidate MMIs versus 4. Those differences resulted in our ability to conduct both regional and hydrologic unit assessments and develop a more rigorous MMI with only 1 metric in common with Macedo et al. (2016). Finally, we inferred our results from approximately 22 km of studied stream reaches to the whole population of over 9000 km of wadeable streams in the sample region.

To guarantee wide applicability of the MMI, we followed a probabilistic sampling design to select sites where we applied a standardized field sampling protocol (US-EPA, Peck et al., 2006) and established rigorous criteria for defining reference sites (Herlihy et al., 2008) and screening metrics (Hering et al., 2006; Stoddard et al., 2008). This resulted in a more robust and accurate tool for assessing ecological condition of neotropical savanna streams. We believe that our index can be applied in the four studied hydrologic units as well as in all neotropical savanna streams. Furthermore, our approach is especially important to hydropower companies and environmental managers, because our study area comprises the catchments of four important hydroelectric dams in the states of Minas Gerais, São Paulo, and Goiás. Therefore, our MMI can be used for assessing the effects of management practices, land uses, and mitigation projects in those states (Macedo et al., 2016).

The constant threats to the savanna biome, its hydropower potential, and its high biological diversity, make effective conservation practices and sustainable management urgent (Callisto et al., 2014; Loyola and Bini, 2015). Our improved MMI is intended to support decision makers and scientists interested in: 1) assessing and diagnosing the stream-length condition of the entire neotropical savanna, 2) detecting potential areas for focused management and conservation practices, 3) identifying the major stressors altering biological condition, and 4) providing an ecological foundation for managing river basins, including those influenced by hydropower plants. In other words, we believe that our results provide a foundation for developing improved legal policies and monitoring programs for improving and assessing water resources comparable to those existing in the U.S.A., Europe, and Australia (Clean Water Act, Water Framework Directive, and Sustainable Rivers Audit, respectively).

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ecolind.2017.06.017.

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Table S1: Taxa list, tolerance values (1-10) and fuzzy-code affinity ranges (0-3) for functional categories. cg = collector-gatherers, sh = shredders, sc = scrapers, cf = collectors-filters, pr = predators; sw = swimmers, cr = crawlers, bu = burrowers, ta = temporary attached, sk = skaters; tg = tegumentary, gi = gills, ae = aerial, and ref = references used for classification.

Taxa list	Tolerance		Functional Feeding Group						Mobility						Respiration			
	score	ref	cg	sh	sc	cf	pr	ref	sw	cr	bu	ta	sk	ref	tg	gi	ae	ref
Arthropoda																		
Coleoptera																		
Curculionidae	4	1	0	3	0	0	0	7	0	3	0	0	0	7	0	0	3	7
Dryopidae	5	1	0	3	2	0	0	3, 7	0	3	3	0	0	7	0	0	3	7, 9
Dytiscidae	4	5	0	0	0	0	3	3, 7, 6	3	3	0	0	0	7	1	0	3	7, 9
Elmidae	5	5	3	2	1	0	0	13, 3, 7	0	3	1	0	0	7,13	1	3	3	13
Gyrinidae	5	5	0	0	0	0	3	3, 7, 11	2	3	0	0	0	7	1	3	3	7
Hydrophilidae	5	5	2	2	0	0	3	13, 3, 7, 6	3	3	1	0	0	7,13	1	1	3	7,13
Hydroscaphidae	10	5	0	0	3	0	0	7	0	3	0	0	0	7	0	0	3	7
Lutrochidae	6	8	0	3	0	0	0	7	0	3	0	0	0	7	0	3	3	7
Noteridae	4	8	0	0	0	0	3	3	3	1	3	0	0	7	0	0	3	7
Psephenidae	8	5	0	0	3	0	0	3, 7	0	3	0	0	0	13	1	3	0	7, 9, 13
Ptilodactylidae	10	8	0	3	3	0	0	14, 7	0	3	3	0	0	14	0	3	0	7, 14
Scirtidae	7	8	2	2	2	0	0	7	0	3	0	0	0	7	0	0	3	7
Staphylinidae	6	8	2	2	1	0	2	14, 3, 7	0	3	1	0	0	7,14	1	0	0	14
Diptera																		
Ceratopogonidae	4	5	1	0	1	0	3	13, 3, 7, 11	0	3	3	0	0	13	3	0	0	13
Chaoboridae	4	6	0	0	0	0	3	7, 11, 6	2	3	0	0	0	7	3	0	0	7
Chironomidae	2	5	1	3	1	1	0	13, 3, 7, 6	1	2	2	0	0	13	3	2	0	9,13
Culicidae	1	5	2	0	0	2	0	3, 7, 6	3	1	0	0	0	7	0	0	3	7
Dixidae	6	5	3	0	0	2	0	14, 7, 6	3	2	0	0	0	7	0	0	3	7,13
Dolichopodidae	4	5	1	0	0	0	3	14, 3, 7	0	3	2	0	0	14, 7	0	0	3	7,13
Empididae	4	5	1	0	0	0	3	13, 3, 7	0	3	3	0	0	13, 7	2	0	1	9,13
Ephydridae	2	5	3	3	3	0	1	14, 7, 6	0	2	3	0	0	14, 7	3	0	3	7,9,13
Muscidae	2	8	0	0	0	0	3	7	0	3	0	0	0	7	0	0	3	
Phoridae			3	0	0	0	0	7	0	0	3	0	0	7	0	0	3	7
Psychodidae	2	5	3	1	1	0	0	13, 3, 7, 6	1	0	3	0	0	13, 7	0	0	3	7,13
Scatopsidae			0	0	0	0	0		0	0	0	0	0		0	0	3	
Simuliidae	5	5	0	0	1	3	0	13, 3, 7	0	2	0	3	0	13	3	1	1	7,9,13
Stratiomyidae	2	5	3	0	0	0	0	3, 7, 6	1	3	2	0	0	13, 7	0	0	3	7,13
Syrphidae	2	5	3	2	0	0	0	3, 7	0	0	3	0	0	7	0	0	3	7
Tabanidae	3	5	0	0	0	0	3	3, 7	0	3	3	0	0	7	0	0	3	7
Tipulidae	5	5	2	2	1	0	2	13, 3, 7	0	1	2	0	0	13, 7	2	2	3	7,9,13
Ephemeroptera																		
Baetidae	5	5	3	1	2	0	0	13, 3, 7, 2, 6	3	3	0	0	0	13, 7	1	3	0	9,13
Caenidae	4	1	3	0	2	0	0	13	0	3	1	0	0	13, 7	1	3	0	9,13
Ephemeridae	10	1	3	0	0	0	0	3	0	0	3	0	0	7	1	3	0	9
Leptohyphidae	8	5	3	0	1	0	0	3, 7, 2	1	3	1	0	0	10, 13	1	3	0	13
Leptophlebiidae	10	5	3	0	2	1	0	13, 3, 7, 2	3	3	0	0	0	7, 9, 10, 13	1	3	0	9,13
Polymitarcyidae	5	1	3	0	0	1	0	3,7	0	0	3	0	0	7	1	3	0	9
Hemiptera																		
Belostomatidae	5	5	0	0	0	0	3	3, 4, 6	2	3	0	0	0	7	0	0	3	9
Corixidae	5	5	0	0	1	0	3	14, 3, 7, 6	3	0	0	0	0	7, 14	1	0	3	14
Gerridae	5	5	0	0	0	0	3	4, 6	0	0	0	0	3	7, 9	0	0	3	9
Helotrephidae			0	0	0	0	3	4	0	0	0	0	0		0	0	3	7
Mesoveliidae	5	5	0	0	0	0	3	4	0	3	0	0	3	7	0	0	3	
Naucoridae	5	5	0	0	0	0	3	13, 3, 4	3	1	0	0	0	7, 13	0	0	3	7,13
Notonectidae	3	1	0	0	0	0	3	4, 6	3	0	0	0	0	7	0	0	3	7

Taxa list	Tolerance		Functional Feeding Group						Mobility						Respiration			
	score	ref	cg	sh	sc	cf	pr	ref	sw	cr	bu	ta	sk	ref	tg	gi	ae	ref
Pleidae	3	1	0	0	0	0	3	3, 4	3	2	0	0	0	7	0	0	3	7
Saldidae	8	8	0	0	0	0	3	4	0	3	0	0	0	7	0	0	3	
Veliidae	7	5	0	0	0	0	3	4	0	0	0	0	3	7, 9	0	0	3	9
Lepidoptera																		
Pyralidae	8	5	0	2	2	0	0	3	1	3	2	3	0	13	0	3	0	13
Megaloptera																		
Corydalidae	4	5	0	0	0	0	3	3, 4	1	3	0	0	0	13	0	3	0	13
Sialidae	6	8	0	0	0	0	3	3, 4	1	2	3	0	0	7, 9, 13	1	3	0	13
Odonata																		
Aeshnidae	8	5	0	0	0	0	3	3, 7, 11, 6	0	3	0	0	0	7, 9	0	3	0	7,9
Calopterygidae	8	5	0	0	0	0	3	3, 7	0	3	0	0	0	7, 9	0	3	0	7
Coenagrionidae	7	5	0	0	0	0	3	13, 3, 7, 6	1	3	0	0	0	7, 9	1	3	0	13
Corduliidae	8	1	0	0	0	0	3	3, 7, 11, 6	0	3	0	0	0	7, 9	0	3	0	7,9
Dicteriadidae			0	0	0	0	3	3, 4	0	3	0	0	0	7	1	3	0	
Gomphidae	5	5	0	0	0	0	3	13, 3, 7, 11	0	2	3	0	0	13	1	3	0	7,9,13
Libellulidae	8	5	0	0	0	0	3	13, 3, 7, 6	0	3	2	0	0	7, 9, 13	1	3	0	7,9,13
Megapodagrionidae	6	8	0	0	0	0	3	3, 7	0	0	0	0	0		0	3	0	
Perilestidae			0	0	0	0	3	3, 7	0	3	0	0	0	7	1	3	0	
Polythoridae	10	11	0	0	0	0	3	3, 7	1	3	0	0	0	14	0	3	0	9,14
Plecoptera																		
Gripopterygidae	10	5	1	3	1	0	0	3, 4	0	3	0	0	0		0	3	0	9
Perlidae	8	5	1	1	0	0	3	13, 3, 7, 4	1	3	0	0	0	13	0	3	0	13
Trichoptera																		
Calamoceratidae	10	8	1	3	1	0	0	13, 3, 7	0	3	0	0	0	13	2	3	0	9,13
Ecnomidae	7	1	0	0	0	3	0	14	0	3	0	0	0	7	3	0	0	
Glossosomatidae	7	5	0	0	3	0	0	3, 7	0	3	0	3	0	13	3	2	0	9,13
Helicopsychidae	10	5	0	0	3	0	0	3, 7	0	3	0	3	0	13	3	2	0	9,13
Hydrobiosidae	7	5	0	1	0	0	3	13, 3, 4	0	3	0	0	0	7, 10	3	0	0	
Hydropsychidae	6	5	0	1	0	3	0	13, 3, 7	1	3	0	2	0	13	1	3	0	13
Hydroptilidae	7	5	1	1	3	0	3	13, 3, 7	0	3	0	2	0	13	3	1	0	13
Leptoceridae	7	5	2	3	1	2	1	13, 3, 7	1	3	0	0	0	13	2	3	0	13
Odontoceridae	10	5	3	1	3	0	1	13, 3, 7	0	3	0	0	0	13	3	2	0	13
Philopotamidae	8	5	0	1	0	3	0	13, 3, 7, 11	0	3	0	3	0	13	3	0	0	13
Polycentropodidae	7	5	0	0	0	3	1	3, 7	0	3	0	3	0	13	3	0	0	13
Sericostomatidae	10	1	0	3	0	0	0	3, 7	0	3	0	0	0	7, 9, 10	0	3	0	9
Xyphocentronidae	9	5	3	0	0	0	0	13, 7	0	0	0	0	0		0	0	0	
Collembola	6	6	3	0	0	0	0	6	0	2	0	0	3	7	3	0	0	7
Crustacea																		
Decapoda	3	6	0	3	0	0	0	3, 6	0	0	0	0	0		0	0	0	
Ostracoda	3	1	3	0	0	0	0	3, 6	0	0	0	0	0		0	0	0	
Arachnida																		
Hydracarina	4	1	0	0	0	0	3	14, 3, 6	1	3	0	0	0	13	3	0	1	13
Mollusca																		
Bivalvia	3	1	0	0	0	2	0	13, 3, 6	0	0	3	0	0	13	1	3	0	13
Gastropoda																		
Ampullaridae	9	8	0	0	2	0	0	13,3	0	0	3	0	0	13	1	3	0	13
Ancylidae	6	5	0	0	2	0	0	13,3	0	0	3	0	0	13	1	3	0	13
Hydrobiidae	3	1	0	0	2	0	0	13,3	0	0	3	0	0	13	1	3	0	13
Physidae	3	5	0	0	2	0	0	13,3	0	0	3	0	0	13	1	3	0	13
Planorbidae	3	5	0	0	2	0	0	13,3	0	0	3	0	0	13	1	3	0	13
Thiaridae	6	1	0	0	2	0	0	13,3	0	0	3	0	0	13	1	3	0	13
Annelida																		
Hirudinea	3	1	0	0	0	0	3	13, 3, 6	1	2	0	0	0	13	3	0	0	13

Taxa list	Tolerance		Functional Feeding Group						Mobility						Respiration			
	score	ref	cg	sh	sc	cf	pr	ref	sw	cr	bu	ta	sk	ref	tg	gi	ae	ref
Oligochaeta	1	5	3	0	0	0	0	13, 3, 11, 6	0	0	3	0	0	13	3	0	0	13
Nematoda	6	6	0	0	0	0	3	6	0	0	0	0	0		3	0	0	
Platyhelmintes																		
Turbellaria																		
Planariidae	5	1	1	0	0	0	3	13, 3, 7	0	3	0	0	0	13	3	0	0	13

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Table S2. Macroinvertebrate metrics tested for criteria of variability, responsiveness, discriminance, and signal to noise (S:N). Metrics checked (+) for all criteria were considered for all subsets run procedure. Metrics that fail (-) for a criteria was not considered for further analysis. Metrics selected for the final MMI in bold.

Categories	Description	Code	Variability Test	Responsiveness	Discriminance	S:N
Richness						
	Total taxa richness	S	+	+	+	+
	EPT family richness	EPT_S	+	+	+	+
	Ephemeroptera family richness	**EPH_S**	**+**	**+**	**+**	**+**
	Trichoptera family richness	TRI_S	+	+	+	+
	Odonata family richness	ODO_S	+	+	-	
	Lepidoptera family richness	LEP_S	-			
	Coleoptera family richness	COL_S	+	-		
	Megaloptera family richness	MEG_S	-			
	Diptera family richness	DIP_S	+	+	+	-
	Heteroptera family richness	HET_S	+	-		
	EPT richness/total taxa richness	EPT_S/S	+	+	+	+
	Ephemeroptera richness/total taxa richness	EPH_S/S	+	-		
	Plecoptera richness/total taxa richness	PLE_S/S	+	+	+	-
	Trichoptera richness/total taxa richness	TRI_S/S	+	+	+	+
	Odonata richness/total taxa richness	ODO_S/S	+	-		
	Lepidoptera richness/total taxa richness	LEP_S/S	+	+	+	-
	Coleoptera richness/total taxa richness	COL_S/S	+	-		
	Megaloptera richness/total taxa richness	MEG_S/S	-			
	Heteroptera richness/total taxa richness	HET_S/S	+	-		
	Diptera richness/total taxa richness	DIP_S/S	+	-		
Composition						
	Total Abundance	N	+	-		
	% Ephemeroptera, Trichoptera and Plecoptera individuals	%EPT	+	+	+	+
	% Ephemeroptera individuals	%EPH	+	+	+	+
	% Plecoptera individuals	%PLE	+	+	+	-
	% Trichoptera individuals	%TRI	+	+	+	+
	% Odonata individuals	%ODO	+	-		
	% Lepidoptera individuals	%LEP	-			
	% Coleoptera individuals	%COL	+	-		
	% Megaloptera individuals	%MEG	-			
	% Heteroptera individuals	%HET	+	-		
	% Diptera individuals	%DIP	+	-		
	% Oligochaeta individuals	%OLI	+	+	-	
	% Chironomidae individuals	%CHI	+	+	-	
	% Hirudinea individuals	%HIR	-			
	% Gastropoda individuals	**%GAS**	**+**	**+**	**+**	**+**
	% Nematoda individuals	% NEM	-			
	% Planariidae individuals	%PLA	-			

Categories	Description	Code	Variability Test	Responsiveness	Discriminance	S:N
	% Chironomidae plus oligochaeta individuals	%CHOL	+	+	+	+
	% Non insect individuals	%N_INS	+	+	+	+
	% Diptera minus chironomidae individuals	%DIP-CHI	+	-		
	Total abundance minus Chironomidae	N-CHI	+	+	-	
	Baetidae/Ephemeroptera individuals	BAE/EPHE	+	+	+	-
	EPT/Chironomidae individuals	EPT/CHI	+	+	+	+
	Hydropsychidae/Trichoptera individuals	HYD/TRI	+	+	-	
	Chironomidae/Diptera individuals	CHI/DIP	+	+	+	-
Tolerance						
	Biological Monitoring Working Party adapted (Sum of tolerance values)	BMWPa	+	+	+	-
	Biological Monitoring Working Party adapted/total taxa richness	BMWP/S	+	+	+	+
	Average Score per Taxon (tolerance values*taxa abundance)	ASPT	+	+	-	
	Super-tolerant taxa richness (richness of taxa with value 1 or 2)	S-TOL_S	-			
	Super-tolerant taxa richness (richness of taxa with value 1 or 2)/total taxa richness	S-TOL_S/S	+	+	-	
	% Super-tolerant taxa (% of taxa with value 1 or 2)	% S-TOL	+	+	-	
	Tolerant taxa richness (richness of taxa with value 1,2 or 3)	TOL1_S	+	-		
	Tolerant taxa richness (richness of taxa with value 1,2 or 3)/total taxa richness	TOL1_S/S	+	+	+	+
	% Tolerant taxa (% of taxa with value 1,2 or 3)	% TOL1	+	+	+	+
	Tolerant taxa richness (richness of taxa with value 1,2,3 or 4)	TOL_S	+	-		
	Tolerant taxa richness (richness of taxa with value 1,2,3 or 4)/total taxa richness	TOL_S/S	+	+	-	
	% Tolerant taxa (% of taxa with value 1,2,3 or 4)	% TOL	+	+	-	
	Super sensitive taxa richness (Richness of taxa with value 9 or 10)	S-SEN_S	+	+	+	+
	Super sensitive taxa richness (Richness of taxa with value 9 or 10)/total taxa richness	S-SEN_S/S	+	+	-	
	% Super-sensitive taxa (% of taxa with value 9 or 10)	%S-SEN	+	+	+	-
	Sensitive taxa richness (Richness of taxa with value 8, 9 or 10)	SEN1_S	+	+	+	-
	Sensitive taxa richness (Richness of taxa with value 8, 9 or 10)/total taxa richness	SEN1_S/S	+	+	+	-
	% Sensitive taxa (% of taxa with value 8, 9 or 10)	%SEN1	+	+	+	+
	Sensitive taxa richness (Richness of taxa with value of 7, 8, 9 or 10)	SEN_S	+	+	+	+
	Sensitive taxa richness (Richness of taxa with value of 7, 8, 9 or 10)/total taxa richness	**SEN_S/S**	**+**	**+**	**+**	**+**
	% Sensitive taxa (% of taxa with value 7, 8, 9 or 10)	%SEN	+	+	+	+
Diversity and dominance						
	Margalef diversity	d'	+	+	+	+
	Evenness	J'	+	+	+	-
	Shannon diversity	**H'**	**+**	**+**	**+**	**+**
	Simpson diversity	λ'	+	+	+	-
	% 1 dominant taxa	%1TAX	+	+	-	
	% 2 dominant taxa	%2TAX	+	+	+	-
	% 3 dominant taxa	%3TAX	+	+	+	+
	% 4 dominant taxa	%4TAX	+	+	-	
	% 5 dominant taxa	%5TAX	+	+	+	-
Functional Feeding Group						
	% Collector-Gatherer individuals	%CG	+	-		
	% Shredder individuals	%SH	+	-		

Categories	Description	Code	Variability Test	Responsiveness	Discriminance	S:N
	% Scraper individuals	**%SC**	**+**	**+**	**+**	**+**
	% Collector-filterer individuals	%CF	+	-		
	% Predator individuals	%PR	+	-		
	Collector-Gatherer richness	CG_S	+	+	+	-
	Shredder richness	SH_S	+	+	+	+
	Scraper richness	SC_S	+	+	+	+
	Collector-filterer richness	CF_S	+	+	+	+
	Predator richness	PR_S	+	+	-	
	Collector-Gatherer richness/total taxa richness	CG_S/S	+	-		
	Shredder richness/total taxa richness	SH_S/S	+	+	-	
	Scraper richness/total taxa richness	SC_S/S	+	+	+	+
	Collector-filterer richness/total taxa richness	CF_S/S	+	-		
	Predator richness/total taxa richness	PR_S/S	+	+	+	-
Respiration						
	% Tegumentary respiration individuals	%TEG	+	+	-	
	% Gills respiration individuals	%GILL	+	+	+	+
	% Plastron, spiracles (or aerial respiration) individuals	%PL/SP/AE	+	-		
	Tegumentary respiration richness	TEG_S	+	+	+	+
	Gill respiration richness	**GILL_S**	**+**	**+**	**+**	**+**
	Plastron, spiracles (or aerial respiration) richness	PL/SP/AE_S	+	+	-	
	Tegumentary respiration richness/total taxa richness	TEG_S/S	+	-		
	Gills respiration richness/total taxa richness	GILL_S/S	+	+	+	+
	Plastron, spiracles (or aerial respiration) richness/total taxa richness	PL/SP/AE_S/S	+	-		
Mobility						
	% swimmer individuals	%SW	+	-		
	% crawler individuals	%CR	+	+	+	-
	% burrower individuals	%BU	+	+	-	
	% temporarily attached individuals	%TA	+	-		
	% skater individuals	%SK	-			
	Swimmers richness	SW_S	+	+	+	-
	Crawlers richness	CR_S	+	+	+	-
	Burrowers richness	BU_S	+	+	-	
	Temporarily attached richness	**TA_S**	**+**	**+**	**+**	**+**
	Skaters richness	SK_S	-			
	Swimmers richness/total taxa richness	SW_S/S	+	-		
	Crawlers richness/total taxa richness	CR_S/S	+	+	+	-
	Burrowers richness/total taxa richness	BU_S/S	+	+	+	-
	Temporarily attached richness/total taxa richness	TA_S/S	+	+	+	+
	Skaters richness/total taxa richness	SK_S/S	+	-		

Capítulo 3

Assessing the Extent and Relative Risk of Aquatic Stressors on Stream Macroinvertebrate Assemblages in the Neotropical Savanna

Abstract

Freshwater ecosystems are among the most threatened by human activities, influencing loses of biodiversity. To efficiently address management practices to conserve and restore those ecosystems it is important to correctly identify and quantify the severity and magnitude of anthropogenic stressors degrading freshwater biota. In this study we assessed seven stressors describing poor water quality, physical habitat alteration, and land use by means of the relative risk (RR) and relative extent (RE) approach. The RR measures the co-occurrence probability of high stressor condition and poor biological condition. The RE measures the proportion of stream length in the region in high stressor condition. To obtain accurate estimations of RR and RE we used a probabilistic survey design to select a representative sample of perennial, wadeable and accessible streams within four hydrologic units in the neotropical savanna. Results were evaluated at two spatial scales: local – within each of the four hydrologic units, and regional – all four units combined. From 143 randomly selected sites we inferred our results to a target population of 9,432 km of streams. Regionally, we found turbidity, % fines and % agriculture as key stressors associated with poor biological condition. At the local scale, we also found that % pasture and total nitrogen were key stressors to biological condition, but their extent was relatively small. By evaluating both RR and RE we conclude that reducing excess sedimentation on streambeds should be the most effective means of improving biological condition over the region. That finding should guide decision makers and land managers to better focus their efforts and resources in improving ecological condition of savanna streams.

Keywords: biological assessment, probabilistic survey design, macroinvertebrates, wadeable streams, cerrado biome, relative risk, relative extent

Introduction

Freshwater ecosystems are among the most threatened ones, facing a long history of exploitation of their resources to meet human-needs (Dudgeon, 2010; Dudgeon et al., 2006; Nieto et al., 2017; Revenga et al., 2005). Intense human pressures on these ecosystems from water pollution, sedimentation, habitat degradation, flow regulation, overfishing, and alien species invasion are among the main causes of biodiversity losses (Dudgeon, 2010; Vörösmarty et al., 2010). Because of the extents and intensities of such stressors, development of environmental assessment methods are urgently need to improve the management, conservation, and rehabilitation of aquatic resources (Buss et al., 2015; Helson and Williams, 2013; Ruaro and Gubiani, 2013). In particular, it is essential to identify and focus on managing the major stressors directly or indirectly impairing freshwater ecosystems. In addition, it is critical to employ biologically based approaches for assessing stream condition because of the ability of biological indicators to integrate and detect multiple stressors in aquatic environments (Hughes et al., 2000; Karr, 1981; Moya et al., 2011).

In the United States (US), the relative risk (RR) and relative extent (RE) approach has been used by the Environmental Protection Agency (EPA) to report on the regional and national condition of wadeable streams, boatable rivers, lakes, and wetlands (Angradi et al., 2011; Paulsen et al., 2008; USEPA, 2016a, 2016b, 2016c). The foundation of this approach is its ability to provide quantifiable associations between key stressors of concern and biological responses (Van Sickle et al., 2006; Van Sickle and Paulsen, 2008). RR describes the probability of good versus poor biological condition given the presence/absence of low versus high stressor condition. RE provides the magnitude of which the high stressor condition was found within a region. It should be noted that the RR and RE approaches are based on discrete measures (good and poor classes) rather than continuous variables. They provide risk estimates that are easily interpreted and familiar to broad audiences (Van Sickle et al., 2006; Van Sickle and Paulsen, 2008). In addition, the RR and RE approaches help decision makers focus regulation, rehabilitation, and mitigation efforts on the stressors most strongly associated with poor biological condition (Hughes et al., 2000).

Accurate estimations of RR and RE can be obtained by randomly sampling sites (Van Sickle and Paulsen, 2008). The use of probabilistic survey designs is strongly recommended for site selection in regional stream condition assessments for several reasons (Dobbie and Negus, 2013; Herlihy et al., 2000; Olsen and Peck, 2008; Stevens and Olsen, 2004): 1) This design ensures representativeness over the surveyed region (Herlihy et al., 2008, 2000), where physical, chemical and biological characteristics reflect the ecological condition (Macedo et al., 2014); 2) It is a cost-effective tool that allows confident and precise inferences to larger geographic areas with a minimum number of sites (Paulsen et al., 2008); 3) Such a design allows statistical estimations over the stream length of the entire target population of interest (Herlihy et al., 2000); 4) Finally, this randomized approach avoids biased conclusions inherent when convenience-based sampling site selection is used in ecological assessment studies (Dobbie, et al., 2008; Dobbie and Negus, 2013; Jiménez-Valencia et al., 2014).

A well-designed monitoring program provides reliable, credible, and valid inferences regarding environmental questions of concern (Dobbie and Negus, 2013; Paulsen et al., 2008). However, the practice is still neglected in Brazil and most other South American countries (Jiménez-Valencia et al., 2014; Macedo et al., 2014), where biodiversity is high (Barlow et al., 2016; Brook et al., 2006; Myers et al., 2000) and widespread environmental changes are rapidly occurring (Barlow et al., 2016; Brook et al., 2006; Hernández et al., 2016;

Vörösmarty et al., 2010). There is an urgent need to improve methods of selecting sites to achieve high quality data that support improved management practices to protect and rehabilitate streams. This is especially the case for the neotropical savanna, a highly threated biome, suffering from rapid natural cover replacement and pasture and crop expansion (Hunke et al., 2015; Ratter et al., 1997; Strassburg et al., 2017).

Therefore, the goal of our study was to 1) estimate total stream length of a target population of wadeable, perennial and accessible streams, and 2) evaluate the extent of stressors and their risk to biological condition. We used a macroinvertebrate multimetric index developed for savanna streams as a measure of biological condition (Silva et al., 2017) and stressors describing physical habitat degradation, poor water quality and land uses. We assessed results at two scales: local (within each of four hydrologic units) and regional (all four hydrologic units combined). To achieve our objectives, we used a probabilistic survey design to obtain quantifiable stream length estimations in our study area.

Methodology

Study area

We defined our sample frame as the stream network in the drainage area within 35 km upstream of the limits of four major hydropower reservoirs: Nova Ponte, Volta Grande, São Simão (Paraná River Basin) and Três Marias (São Francisco River Basin) (Fig. 1). This geographic area covered a total of 45,180 km^2, with land uses and cover characterized by agricultural cash crops, charcoal production, grazing, and urbanization (Macedo et al., 2014a). Climate is described as humid tropical savanna, with a dry season from May to September, precipitation averaging from 800 to 2000 mm, and air temperature averaging between 18 and 28°C (Ratter et al., 1997).

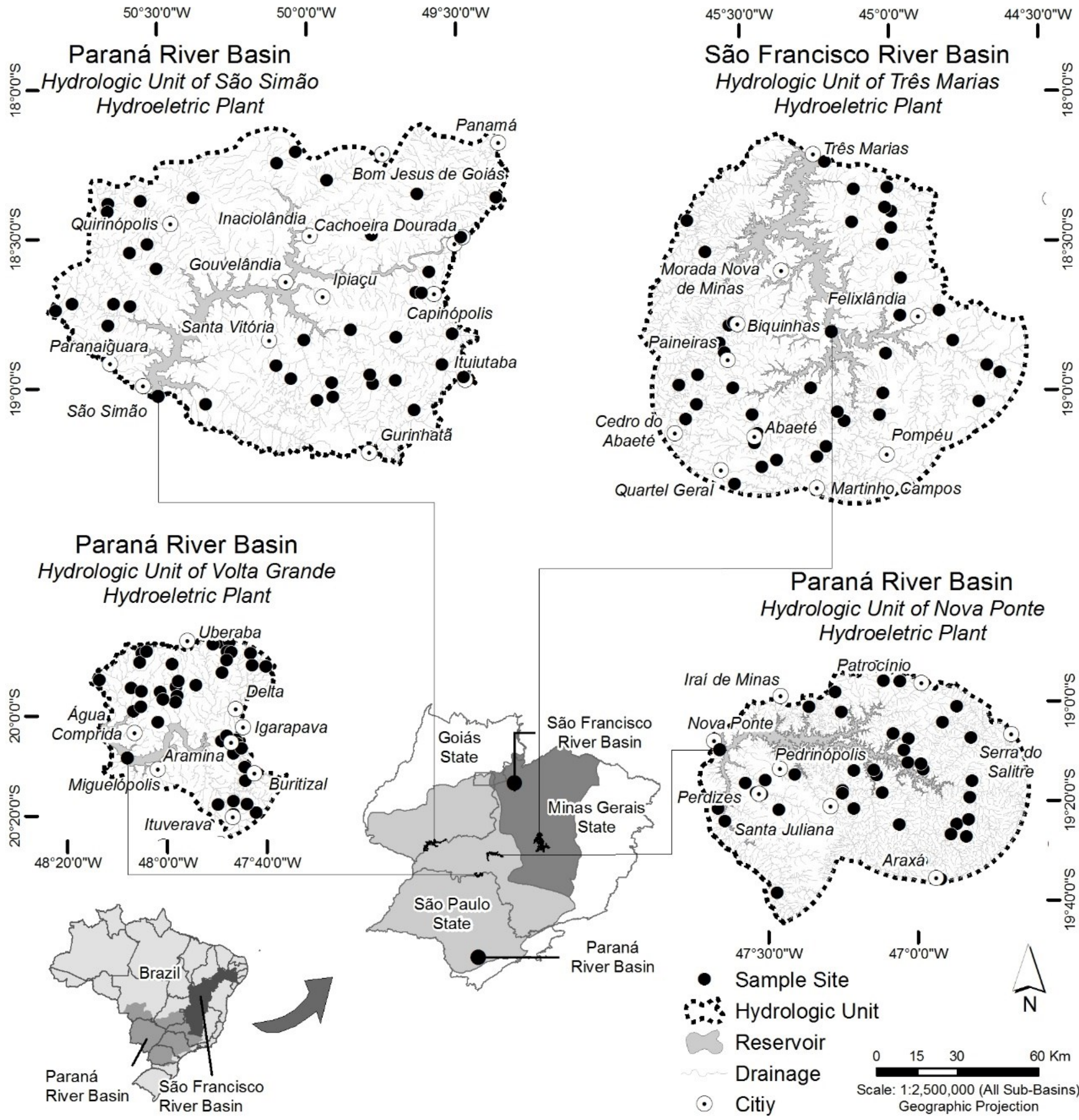

Figure 1. Locations of 143 random sites sampled in each of four hydrologic units.

Survey design and sampling sites

Sites were selected based on a probabilistic survey design in each of the four areas (hereafter hydrologic units, sensu Ferreira et al., 2017; Firmiano et al., 2017; Seaber et al., 1987). This site selection procedure is based on the one used by the US-EPA in the

Environmental Monitoring and Assessment Program for the Mid-Atlantic Highlands Streams Assessment (Herlihy et al., 2000) and refined in subsequent regional and national stream monitoring programs (Olsen and Peck, 2008; Paulsen et al., 2008; Stoddard et al., 2005). The approach consists in the establishment of a sample frame in a 1:100,000 scale digitized map where we sorted sites by means of a randomized, systematic, spatially balanced criterion (Herlihy et al., 2000; Olsen and Peck, 2008; Stevens and Olsen, 2004). This survey design allowed us to obtain an even distribution of sites over the geographic location (Herlihy et al., 2000; Stevens and Olsen, 2004) and obtain a reliable population estimation from a representative set of streams in the surveyed region.

We targeted a population of wadeable, perennial and accessible streams, of first- through -third order (*sensu* Strahler, 1957). For each hydrologic unit, we established a random set of potential sampling sites and an additional set of sites to use as substitutes where a site was non-target (e.g., dry, non-wadeable, access denied, map error). Each site received a weight proportional to the inverse of its selection probability, interpreted as the stream length it represents in the target population. These site weights were then used to estimate stream condition extents and relative risk.

A field reconnaissance was necessary to confirm the set of sampling (or target) sites, account for situations where sites were not accessed for any reason (non-target sites), and to optimize field crew time (Macedo et al., 2014a). Sampling occurred during the dry season in each hydrologic unit in subsequent years from 2009 to 2012. In each stream site, a sampling reach of 40 mean wetted channel widths or a minimum of 150 m was established. Macroinvertebrate assemblages were sampled with a kick-net sampler (500 µm mesh, 0.9 m^2 area). Samples were taken to the laboratory where the individuals were sorted and identified through use of a stereomicroscope (100× magnification) and taxonomic keys (Costa et al., 2006; Fernández and Domínguez, 2001; Merritt et al., 2008; Mugnai et al., 2010). At the stream reach we obtained quantitative measures of the physical habitat structure following Peck et al. (2006). Metrics describing channel morphology, habitat features, riparian structure, and human alterations in the riparian zone were calculated following (Kaufmann et al., 1999). Water temperature, electrical conductivity, total dissolved solids, turbidity, and pH were measured at each stream reach by use of portable equipment (YSI Model 650). A water sample was collected and transported to the laboratory for determining dissolved oxygen, total nitrogen and total phosphorus (APHA, 1998). We characterized the different land uses (% natural cover, % agriculture, % pasture, % urban area) in the catchment of each site via satellite images provided by Landsat TM sensor and Google Earth fine resolution imageries (Google, 2016; Macedo et al., 2014).

Establishment of stressor and biological indicator thresholds

We used the macroinvertebrate multimetric index (MMI) developed by Silva et al. (2017) as a measure of stream biological condition. This MMI is composed of 7 metrics describing macroinvertebrate composition, richness, tolerance, diversity, feeding groups, mobility, and respiration type. Condition class thresholds were established based on the distribution of MMI scores in the least-disturbed sites. MMI scores lower than the 5th percentile of the least-disturbed distribution were classified as “poor”, scores between the 5th and 25th percentile were classified as “fair”, and those higher than the 25th percentile were classified as “good” (Silva et al., 2017). The classes were used as thresholds to indicate quality boundaries for further analysis.

From a list of potential stressors indicating physical habitat alteration, water quality and land uses disturbances we selected: dissolved oxygen, turbidity, total nitrogen, % of fine sediments, riparian disturbance index, % agriculture and % pasture for RE and RR analysis. Thresholds for water quality parameters of dissolved oxygen and turbidity were based on Brazilian legislation (CONAMA 357, 2005), whereas for total nitrogen we used a more restricted value. Thresholds for physical habitat structure are based on regional distributions of observed values at least-disturbed sites (Silva et al., 2017). Using a similar approach as for biological condition, we defined sites with % of fines greater than the 75th percentile of least-disturbed sites distribution as in poor condition and sites with % of fines below the 75th percentile as good condition (Van Sickle et al., 2006; Van Sickle and Paulsen, 2008). For the riparian disturbance index (RDI), a measure that combines the various types of human disturbance observations weighted by their proximity to the streambed, we used the same threshold value that Silva et al. (2017) used to screen least-disturbed sites is savanna streams, where sites with RDI < 1 were classified as good and RD1 > 1 were classified as poor. We quantified % of agriculture and pasture in the catchment area upstream of each site and defined thresholds based on Silva et al. (2017), considering that each hydrological unit is strongly dominated by agriculture or pasture. Condition classes and thresholds are summarized in Table 1.

Table 1. Thresholds of condition classes for macroinvertebrate MMI and stressor indicators.

Variable	Thresholds		Source
	Good/Low	Poor/High	
Biological Condition			
MMI	>65	<47	25th/5th percentile of reference sites (Silva et al. 2017)
Stressors Condition			
Land use			
%Agriculture	<60	≥60	Silva et al. 2017
%Pasture	<60	≥60	Silva et al. 2017
Physical habitat			
Riparian Disturbance Index	≤1	>1	Silva et al. 2017
% fines (silt/clay; < 0.6 mm)	≤40	>40	75th percentile
Water quality			
DO (mg/L)	≥6	<6	CONAMA 2005
Turbidity (NTU)	≤20	>20	CONAMA 2005
N (mg/L)	≤0.2	>0.2	Silva et al. 2017

Assessing relative risk and extent

We used the relative risk approach (RR) to evaluate the severity of seven stressors in affecting biological condition (measured by the MMI) and the relative extent (RE) to evaluate the magnitude of the stressors (Van Sickle et al., 2006; Van Sickle and Paulsen, 2008). We obtained the RR by means of a contingency table whereby we addressed all possible situations of having good or poor macroinvertebrate MMI condition given high or low stressor condition. We excluded the MMI “fair” condition category to obtain a 2 x 2 table. Instead of using number of sites counted in each situation, we used the estimated stream length in each category based on the site survey design weights.

The RR is a conditional probability representing the likelihood that poor MMI scores are associated with high stressor scores and is calculated as follows:

$$RR = \frac{\mathrm{Pr\ (MMIp|Sh)}}{\mathrm{Pr\ (MMIp|Sl)}}$$

where the numerator is the probability of finding poor biological conditions (MMIp) given high stressor scores (Sh) and the denominator is the probability of finding poor biological condition given low stressor scores (Sl) (Van Sickle et al., 2006; Van Sickle and Paulsen, 2008).

In this formulation, a RR equal to 1 denotes the absence of association between the biological indicator and the stressor, i.e., poor MMI scores are equally likely to occur with both high and low stressor scores (Van Sickle et al., 2006). For a RR greater than 1, we interpret the value as how many times more likely poor MMI condition would occur given high stressor conditions relative to low stressor condition. We calculated 95% confidence intervals for RR estimations using the method of Van Sickle et al. (2008), and considered the RR to be significant when the lower 95% confidence interval was > 1.

Whereas the RR measures the severity of a stressor, the RE is a measure of its magnitude (Angradi et al., 2009; Van Sickle and Paulsen, 2008). The RE represents the length and proportion of streams with high stressor scores within a given study area. It is obtained as a sum of sampling weights of sites found with high stressor scores divided by the sum of all site weights (expressed in %). Combined, RR and RE provide an excellent overview of the stressor's dimensions and its association with biological condition (Van Sickle et al., 2006; Van Sickle and Paulsen, 2008). We also calculated the Pearson correlations between all stressors to assess stressor relationships and evaluate stressor independence RR, RE, and confidence intervals were obtained using the SPSURVEY package for R.

Results

We estimated a total map length of 24,417 kilometers of streams belonging to first to third order in the sample frame. From this, 9,432 kilometers of streams (or 39% of the total frame length) were part of the target length, i.e., sampled sites, defined as perennial, accessible, wadeable, and with flowing water (Table 2). The difference was a result of a number of non-target sites that weren't sampled because they did not meet target criteria. The main reason for dropping a site was lack of water, which accounted for 32% of the total frame length. The impossibility of access accounted for 17% of the total frame length. Non wadeable streams accounted for 12% of the map frame length, map errors (where a stream was not found based on the geographic coordinates) were rare and accounted for only 0.7% of the mapped length. A total of 143 probabilistic sites were sampled and 176 sites were dropped. Detailed results on target and frame stream length for each hydrologic unit are shown in Table 2.

Table 2. Estimated extent (km) of target stream sites for the regional assessment and for each hydrologic unit: Três Marias (TM), Volta Grande (VG), São Simão (SS), and Nova Ponte (NP), parenthesis indicate the number of probability sites in each hydrologic unit. Numbers in bold are the percents of frame length.

			Non-target length			
Scale	Frame Length (km)	Target Length (km)	Dry (km)	Non-wadeable (km)	No access (km)	Map error (km)
Hydrologic Unit						
TM (36)	5989	1640.5	2445.4	923.2	979.5	0.0
		27.4%	**40.8%**	**15.4%**	**16.4%**	**0.0%**
VG (38)	1528	516.3	163.8	457.2	234.7	156.1
		33.8%	**10.7%**	**29.9%**	**15.4%**	**10.2%**
SS (37)	5705	2794.0	1316.6	1230.8	341.4	22.6
		49.0%	**23.1%**	**21.6%**	**6.0%**	**0.4%**
NP (32)	11195	4514.8	3877.5	222.0	2581.2	0.0
		40.3%	**34.6%**	**2.0%**	**23.1%**	**0.0%**
Regional (143)	24417	9465.7	7803.2	2833.2	4136.8	178.7
		38.8%	**32.0%**	**11.6%**	**16.9%**	**0.7%**

A correlations matrix suggested little confounding effects between pairs of stressors, with estimated product-moment <0.6 (Table 3), allowing us to interpret associations of stressors and biological condition independently.

Table 3. Pearson cCorrelation coefficients among stressors in the study region.

	Riparian Disturbance Index	% Fines (silt+clay)	Turbidity (NTU)	Nitrogen (mg/L)	DO (mg/L)	% Pasture	% Agriculture
Riparian Disturbance Index	—						
% Fines (silt+clay)	0.28	—					
Turbidity (NTU)	0.18	0.28	—				
Nitrogen (mg/L)	0.25	0.26	0.35	—			
DO (mg/L)	-0.15	-0.15	-0.21	-0.01	—		
% Pasture	0.14	0.26	0.07	-0.03	-0.13	—	
% Agriculture	0.12	0.04	0.01	0.25	0.15	-0.52	—

For the regional assessment, we found that turbidity, % fines, and % agriculture were the only stressors significantly associated with increased risk for poor macroinvertebrate MMI condition with relative risk values of 1.8-2. In terms of extent, agriculture was the most widespread stressor in the region (over 40% of stream length exceeded the agriculture threshold) followed by fine sediment (18% of total stream length). High turbidity was found in less than 6% of stream length. Although high riparian disturbance occurred in almost 55% of the stream length it did not significantly represent a risk for biological condition (Fig. 2).

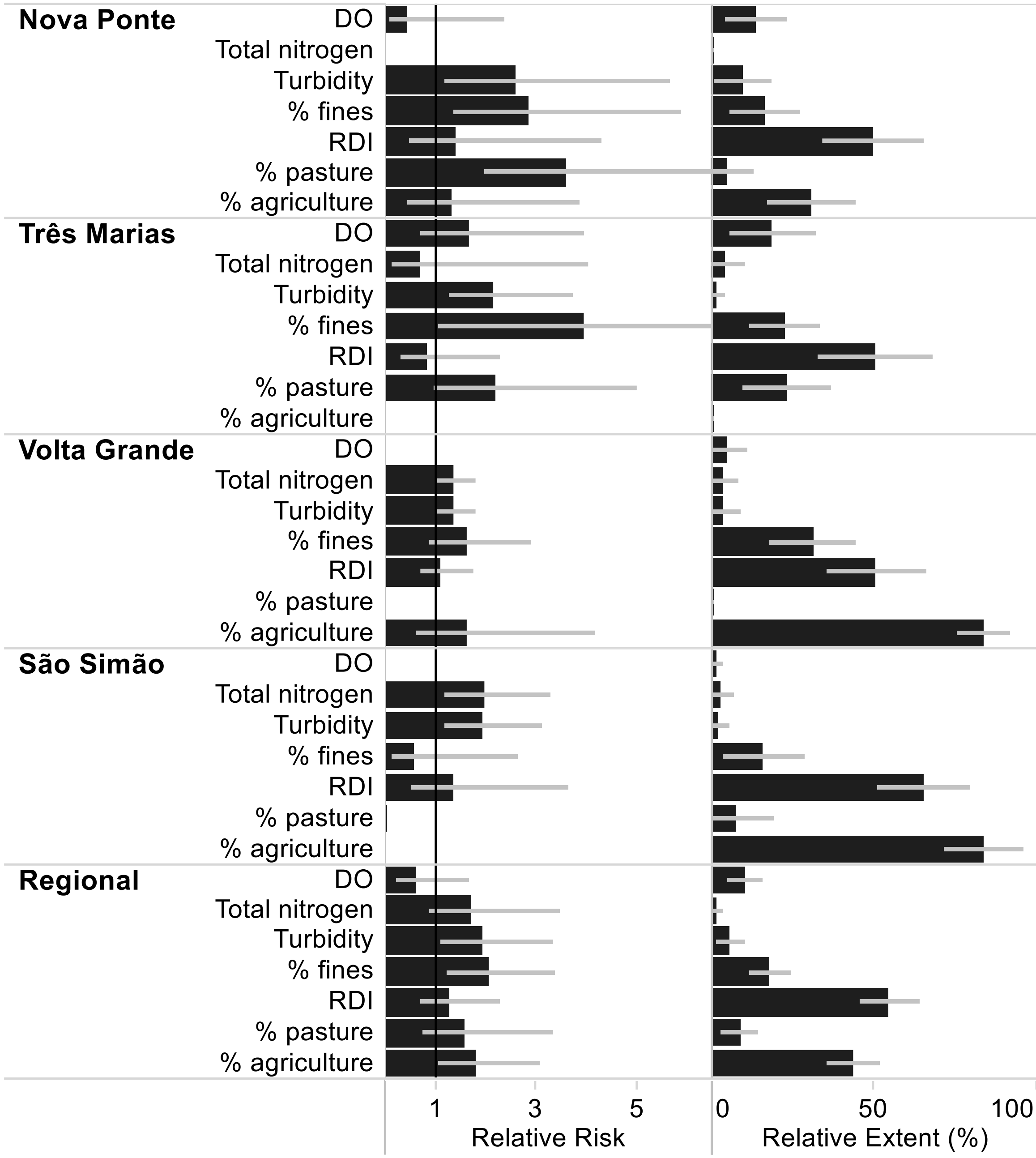

Figure 2. Estimated relative risk (RR) for the MMI condition given seven stressors and their relative extents (RE) in high levels of stress for each hydrologic unit and for the regional assessment (represented as % of total target stream length). Lines represents 95% confidence boundaries. RR below 1 indicate the absence of association. (DO = dissolved oxygen; RDI = Riparian Disturbance Index).

RR and RE estimations varied among the hydrologic units. Turbidity, % fines, and % pasture were the stressors representing risk to biological condition in NP and TM. In NP, the risk of finding poor MMI condition given high pasture (i.e., high stressor condition) was 3.6 times more likely to occur compared to a situation where pasture did not exceed the threshold (i.e., low stressor condition). Nonetheless, high percent of pasture only occurred in less than 5% of the total stream length. In TM, streams with an excess of fine sediment or pasture were 2 to 4 times more likely to have poor MMI condition than streams without. Those stressors were each present in ~23 % of the TM stream length. In both NP and TM, although turbidity indicates a risk, it rarely occurred (< 10% of total stream length). In SS and VG, excess nitrogen and turbidity represented a risk (1.3-2), but with very low occurrence (around 3% of the total stream length). We observed that low dissolved oxygen did not have a significant relative risk in any hydrologic unit or in the whole regional assessment. Although we considered pH and total phosphorus as potential stressors to biological condition, very few or no sites had high stressor levels so it was not possible to calculate relative risks for them.

Considering the MMI weighted distributions, we observed NP as the hydrologic unit in better ecological condition (Fig. 3). Total nitrogen that posed a risk only in VG and SS also showed similar means in both hydrologic units. Similar means were also found for turbidity among the four hydrologic units. Concerning land uses, we found TM as the hydrologic unit most influenced by pasture, whereas the others had highest means of agriculture land uses. Detailed weighted distributions of MMI and stressors scores are shown in Figure 3.

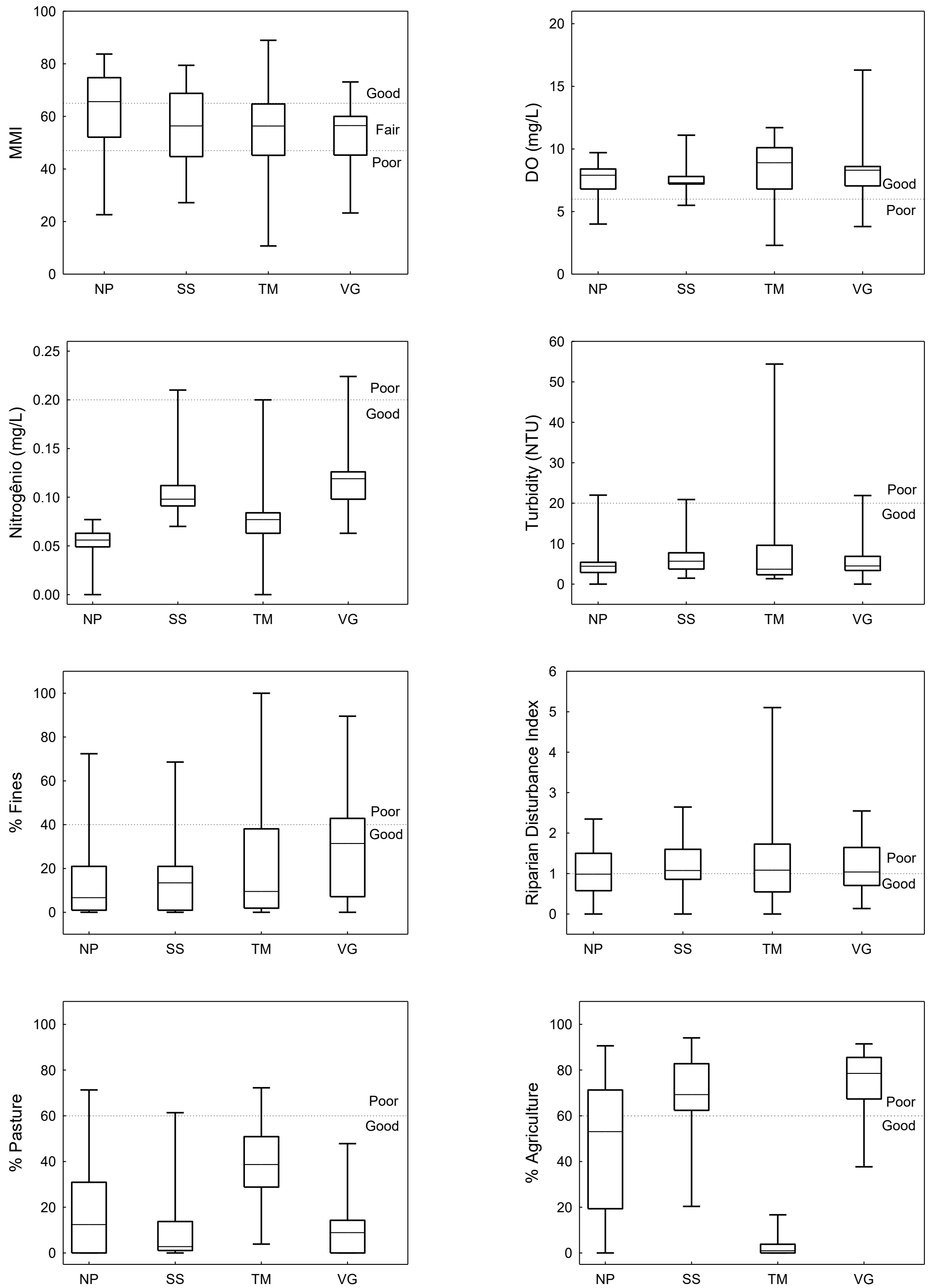

Figure 3. Weighted box-plots for the MMI and seven selected stressors for each hydrologic unit. Boxes represent the 25th and 75th percentiles, whiskers are minimum and maximum ranges, and lines within boxes are medians. Dotted lines represent threshold limits for condition classes.

Discussion

Although providing benefits in ecological assessments, the use of probabilistic survey designs in estimating stream lengths are in their early stages in the neotropics (Carvalho et al., 2017; Jiménez-Valencia et al., 2014; Macedo et al., 2014). Jiménez-Valencia (2014) conducted a risk assessment of tropical rainforest streams in a small Brazilian river basin and reinforced the importance to employ more probability-based surveys to improve the quality of regional assessments in tropical regions experiencing rapid land use conversion and dam construction. Under this perspective, our study represents a first attempt to use a probabilistic design to rigorously assess the relative risk and extent of stressors of biological condition in neotropical savanna streams.

We were able to infer results to a target population of wadeable, accessible and perennial streams of 9,432 km representing 39% of the total frame length depicted on maps. A reconnaissance field trip prior to the sampling helped us properly account for non-target situations (61% of frame length). Regionally, map errors represented only 0.7% of the sample frame, mainly occurring in VG where the total length of target streams is relatively small. However, dry streams accounted for 32% of the sample frame, especially in first order streams. This situation is recurrent in other studies that normally adjust probability inclusions to account for the high % of expected dry first order streams (Herlihy et al., 2000; Jiménez-Valencia et al., 2014). Climate change (Marengo et al., 2012) and the commonly severe droughts experienced in the Brazilian savanna (Oliveira et al., 2014), can also contribute to a high% of dry stream length. Other reason can be the quality of Brazilian 1:100,000 topographic maps, because they were built with 1960's aerial photographs and through two methodological approaches by IBGE (Brazilian Statistic and Geographic Institute) and DSG

(Brazilian Army Geographic Division), resulting in an heterogeneity hydrographic map (Guimarães et al., 2008). Although sampling in the wet season would result in a higher % of streams with flowing water, it would also threaten crew safety, reduce site access, and increase sampling variability (Hughes and Peck, 2008).

Two other conditions produced non-target streams. The non-wadeable streams in the regional sample frame (12%) were mainly identified as marshes, very deep streams or small impoundments, not representing our target criteria. A first contact with land owners secured permission for sampling in most situations, however, a number of sites were inaccessible mainly due to locked gates, and physical barriers (e.g. canyon streams) or absence of tracks or roads. Olsen and Peck, (2008) reported 11.5% of the frame length not sampled due to access denials in a probability survey of the conterminous US. Lesser (2001) also reported a high % of denials in wetland assessments and suggested adjustments to reduce bias. Genet and Olsen (2008), assessing wetlands in Minnesota, USA, reported a successful access to private properties by making personal contacts with land owners. In general, they all agreed that a first contact (mailing, in person) helps reducing this problem providing better estimations. That reinforces the importance of reconnaissance prior to sampling, which adds costs and time, but increases chances of successful access (Olsen and Peck, 2008). Besides that, another reason for the success of our access to private property is related to the research character of an academic institution, being totally dissociated from federal or state governmental demands, which could represent an obstacle to access permission.

Identifying stressors that can properly represent a risk and their magnitudes is strongly desirable for assessment and management purposes. In our study, excess fine sediments posed the greatest risk to biological condition with a considerable extent, not only at the regional scale but also within specific hydrologic units. Paulsen et al. (2008) and Van Sickle et al. (2006) also found fine sediments a stressor of major concern for macroinvertebrates for both regional and national stream assessments in the US. Although finding a high percentage of stream length with excess sediment in the neotropical Atlantic Forest, Jiménez-Valencia et al. (2014) did not find significant risk to the biota (lower 95% confidence boundary <1), likely because their mountain streams have steep slopes. Excess fine sediments in streambeds are regarded as one of the most important threats to ecological integrity in lotic ecosystems (Bryce et al., 2010; Buendia et al., 2013; Wood et al., 2016; Wood and Armitage, 1997). It alters habitat availability and suitability for aquatic biota (Buendia et al., 2013), directly affecting macroinvertebrate assemblage structure, composition and function (Mathers and Wood, 2016; Wood and Armitage, 1997).

Although agriculture land uses are spread out over most of the hydrologic units, it only showed significant risk as a stressor in the whole regional assessment (RR=1.8). Agriculture, as well as other forms of land use, provide an indirect measure of one or more stressors affecting biological condition, other than the direct measures generally preferred in the relative risk approach (Paulsen et al., 2008). Nonetheless, we were interested in obtaining a general overview of its association with poor biological condition because others have found it significantly related to degraded biological condition (Allan, 2004; Hughes et al., 2006; Macedo et al., 2014)

High turbidity was a good indicator (high relative risk) of poor biological condition in the regional assessment and in all hydrologic units, but only occurred in a small proportion of streams lengths. That was similar to the high nitrogen concentrations found in VG and SS, which represented a high risk to biological condition but occurred in low extents. Both of indicators are proxy to anthropogenic activities pressures, like sedimentation and water eutrophication.

The combined RR and RE approach provides guidance for management practices. Regional efforts toward reducing excess sediment inputs to streams would greatly improve biological condition based on both the severity and magnitude of the stressor. On the other hand, high turbidity and nitrogen were two high risk stressors that were relatively uncommon throughout the region. They may be best managed at a more local focused scale, rather than through a regional effort. Given the continued growth of agricultural land use in Brazil and throughout the tropics, the results found for the risk of high levels of agricultural land use should be seen as an important alert for future studies to identify its effects on stream biota and to mitigate its impacts. Others have also determined that extensive row crop agriculture is detrimental to stream biota (Allan, 2004a; Wang et al., 2006).

Van Sickle et al. (2006) attempted to correctly interpret RR and RE estimations in the assessment of stream condition. One important concern is to keep in mind that "high" and "low" condition classes are based on established thresholds for the different stressors and the obtained estimations of RR and RE must necessarily be constrained to them. Water parameter thresholds were based on Brazilian Federal Legislation (CONAMA 357/2005), but do not account for natural variability (Jiménez-Valencia et al., 2014) or intensity of human activities across different regions (Paulsen et al., 2008). Firmiano et al. (2017) reported that the legislation thresholds are much higher than the turnover thresholds of several mayfly genera, suggesting that the legislation should set benchmarks considering biological information to avoid the loss of sensitive taxa. Future studies should consider the inclusion of other water parameters that we did not consider because of their lack of responses in our study. Dissolved oxygen did not represent a risk to biological condition and phosphorus and pH were omitted because in general they did not exceed thresholds. However, they are likely important stressors in mining (Daniel et al., 2015; Hughes et al., 2016), urban (Pompeu et al., 2005; Yeakley, 2014), or intensively farmed (Allan, 2004b; Paulsen et al., 2008) regions.

Summary and conclusions

The probabilistic survey design allowed us to estimate the total stream length of a target population of wadeable, accessible and perennial streams and account for non-target situations. This design provided a consistent base from which we estimated relative risks and extents to assess ecological condition in neotropical savanna streams at both hydrologic unit and regional perspectives. This approach facilitates 1) identification of the major stressors associated with poor biological condition; 2) evaluation of the magnitude of the stressors, and 3) provision of guidelines to improve stream condition by focusing management efforts on specific targets when a stressor poses a risk but is not widespread or at large scales when stressors represent regional risks.

Overall, our results should assist decision makers and managers interested in improving the ecological integrity of savanna streams. In particular, we recommend mitigating the sources of excessive sediments, which could lead to marked improvements in the biological condition of neotropical savanna streams and reduce the erosion and deterioration of agricultural soils. Future studies in the neotropical region would benefit from: 1) the use of probabilistic designs for unbiased site selection; 2) the establishment of thresholds representative of the study region, and 3) the assessment of other potential stressors associated with poor biological condition.

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Conclusões

Esta tese buscou avaliar diversos aspectos relacionados à avaliação de condições ecológicas em riachos no cerrado utilizando assembleias de macroinvertebrados bentônicos como indicadores. Para tanto: 1) utilizei uma forma inovadora de avaliar o esforço amostral da riqueza taxonômica e suas relações com o habitat físico, 2) aprimorei o desenvolvimento de índices de integridade biótica no cerrado e 3) fiz uma primeira avaliação utilizando a abordagem de risco relativo e extensão de estressores associados ao MMI, através de um desenho amostral probabilístico. Desta forma, busquei preencher as lacunas do conhecimento quanto à condição ecológica de riachos localizados em uma área de tamanha importância como é o cerrado.

Atendendo aos objetivos propostos nesta tese, viu-se que avaliações do esforço amostral da riqueza taxonômica de macroinvertebrados bentônicos variam quanto à escala de estudo (local e de bacia) e quanto à resolução taxonômica (famílias de macroinvertebrados e gêneros de EPT). Em escala de bacia, 39 riachos foram suficientes para estimar a riqueza de famílias de macroinvertebrados e gêneros de EPT. No entanto, em escala local, a média das curvas de acumulação de *taxa* mostraram um contínuo crescimento com o aumento no número de amostras, ou seja, não foi observada estabilização nas curvas de riqueza. Além disso, características do habitat físico explicaram um percentual significativo do esforço amostral necessário para caracterização da riqueza de macroinvertebrados bentônicos (Capítulo 1).

Através do desenvolvimento de um índice multimétrico foi possível avaliar a integridade ecológica de riachos no cerrado neotropical. O MMI desenvolvido distinguiu corretamente sítios impactados de sítios de referência e mostrou resposta significativa a estressores antropogênicos que descrevem características físicas do habitat, uso do solo e qualidade de água. O desenho amostral permitiu extrapolar os resultados para a extensão total de riachos em cada unidade hidrológica e para avaliação regional. O desenvolvimento do MMI seguiu metodologia padronizada, aprimorou versões anteriores de índices multimétricos, possibilitando sua aplicação para todo o cerrado. (Capítulo 2).

Através de um desenho amostral probabilístico foi possível estimar a extensão de riachos perenes e acessíveis. A abordagem de risco relativo (RR) e extensão de estressores (RE) permitiu identificar turbidez, % de sedimento fino e % de agricultura como os principais estressores associados a piores condições biológicas. De maneira geral, avaliando juntos o RR e o RE, viu-se que mitigar o impacto do excesso de sedimentos no leito dos riachos pode melhorar consideravelmente a condição biológica da região (Capítulo 3).

Finalmente, os produtos desta tese visam dar suporte a ações eficientes de manejo através de: 1) delineamento amostral; 2) avaliação e diagnóstico de condições ecológicas em riachos no cerrado, 3) identificação de estressores antrópicos e de áreas potenciais para práticas de manejo e conservação, e 4) gestão de bacias com foco em áreas sob influência de hidrelétricas ou outros usos antrópicos que potencialmente podem trazer riscos à integridade biológica.

Perspectivas Futuras

Os resultados desta tese evidenciam a importância do desenvolvimento de ferramentas de avaliação de condições ecológicas no cerrado, um bioma único e seriamente ameaçado por atividades antrópicas. Para futuros desdobramentos sugiro:

1. Explorar as implicações da escala de estudo (local, regional, nacional), da resolução taxonômica (famílias, gêneros, espécies) e de características do habitat físico (substratos, fluxos, estrutura física, vegetação ripária, etc) para subsidiar o planejamento de amostragens em Programas de Monitoramento Ambiental no cerrado brasileiro.

2. À Companhia Energética de Minas Gerais, com incentivos da ANEEL, aplicar o MMI desenvolvido nesta tese como ferramenta prática e eficiente de diagnóstico de condições ecológicas em unidades hidrológicas de outros empreendimentos hidrelétricos no cerrado.

3. Aos órgãos de gestão ambiental do Estado de Minas Gerais (IGAM, IEF, SEMAD, etc), aplicar o MMI aqui proposto em outras bacias hidrográficas no cerrado, visando diagnosticar a qualidade ambiental de riachos de cabeceira sob influência de

atividades de mineração, siderurgia, petróleo e qualquer outra área cujo uso antrópico possa significar um risco à integridade biótica

4. De maneira similar à realidade ambiental nos EUA e União Europeia, recomendo à Agência Nacional de Águas a implementação de um programa de biomonitoramento levando em conta as etapas metodológicas aqui propostas para o desenvolvimento de MMIs nos biomas brasileiros visando a gestão de bacias hidrográficas em escala nacional.

5. Considerar o uso de protocolos padronizados de amostragem e uso de desenho amostral probabilístico na seleção de sítios amostrais na condução de estudos de avaliação de condição ecológica em riachos de cabeceira, dando suporte para ações de conservação e manejo de recursos hídricos e sua biodiversidade, no Brasil.

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