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e Manejo de Vida Silvestre

Tese de doutorado

Efeito do gradiente de distúrbio antrópico sobre padrões de diversidade alfa e beta de macroinvertebrados em riachos neotropicais

Raphael Ligeiro

Belo Horizonte, julho de 2013.

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Raphael Ligeiro

Orientador:

Prof. Dr. Marcos Callisto (Departamento de Biologia Geral, ICB, UFMG)

Co-orientador brasileiro:

Prof. Dr. Adriano Sanches Melo (Departamento de Ecologia, ICB, UFG)

Co-orientador estrangeiro:

Prof. Dr. Robert Mason Hughes (Department of Fisheries and Wildlife, Oregon State University and Amnis Opes Institute)

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Dedico essa tese aos meus pais, aos meus irmãos, aos meus parentes e amigos.

"Eu vivo sempre no mundo da lua,

porque sou um cientista,

o meu papo é futurista,

é lunático"

(G. Arantes)

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RESUMO

Uma forma de se estudar a distribuição espacial da diversidade biológica em paisagens aquáticas é separá-la em um componente local (diversidade alfa), um componente de variação entre locais (diversidade beta), e um componente regional (diversidade gama). A presente tese de doutoramento avaliou os efeitos das alterações humanas sobre padrões de diversidade alfa e beta de macroinvertebrados bentônicos em duas bacias hidrográficas no Cerrado (trechos altos do Rio Araguari e do Rio São Francisco), localizadas à montante de barramentos hidrelétricos. Aplicou-se o protocolo amostral desenvolvido e utilizado pela Agência de Proteção Ambiental Norte-Americana (US-EPA). No primeiro capítulo desenvolveu-se um índice de distúrbio integrado (IDI) que utilizou informações da escala local (leito dos riachos e vegetação ripária) e da escala de bacia de drenagem dos trechos amostrados, por meio da análise dos usos do solo. Esse índice foi utilizado como base para a definição dos riachos menos e mais perturbados disponíveis, necessária para os capítulos seguintes. No segundo capítulo analisou-se a eficiência do método de subamostragem de fração fixa em caracterizar a riqueza de famílias das amostras dos pontos amostrais (diversidade alfa). Verificou-se que, independente da condição ecológica do riacho amostrado, quanto maior o número de indivíduos da amostra menor é a fração da mesma necessária para se representar a riqueza taxonômica. No terceiro capítulo avaliou-se qual metodologia de amostragem em campo e processamento em laboratório melhor diferencia riachos menos e mais perturbados com relação à composição das assembleias bentônicas e a métricas biológicas. Verificou-se que a amostragem em múltiplos habitats melhor diferenciou a composição das assembleias (dados de presença/ausência e abundância relativa) e a riqueza de famílias quando todos os indivíduos das amostras são contados. Porém, a amostragem em bancos de folhas foi mais robusta em relação ao uso de subamostragem por contagem fixa de indivíduos. O quarto capítulo avaliou a influência das alterações antrópicas sobre as diversidades alfa e beta de macroinvertebrados. Verificou-se um efeito negativo da intensidade de distúrbios (valores de IDI) na riqueza de gêneros de Ephemeroptera, Plecoptera e Trichoptera (EPT) nos riachos. Foi observado um padrão aninhado em relação ao gradiente de distúrbios; assembleias de riachos mais perturbados são subconjuntos das assembleias de riachos menos perturbados. No entanto, a diversidade beta entre os trechos mais perturbados foi maior do que entre os trechos menos perturbados. Conclui-se que a diminuição da riqueza taxonômica regional (diversidade gama) observada nos riachos mais perturbados foi causada principalmente por extinções locais de espécies, e não por meio de homogeneização biológica da paisagem aquática.

Palavras-chave: macroinvertebrados, bioindicadores, EPT, diversidade alfa, diversidade beta, Cerrado, índices de distúrbio, subamostragem, partição aditiva da diversidade.

ABSTRACT

A way to study the spatial distribution of biodiversity in riverine landscapes is to separate it in a local component (alpha diversity), a variation component (beta diversity), and a regional component (gamma diversity). This thesis evaluated the effects of human alterations on alpha and beta diversity patterns of macroinvertebrates in two river basins (High Araguari and High São Francisco river basins) in the Cerrado biome, located upstream hydroelectric reservoirs. We applied the field protocol developed and applied by the Environmental Protection Agency of the United States of America (US-EPA). In the first chapter we developed an integrated disturbance index (IDI) using data from local (in-stream and riparian vegetation) and from catchment scales, analyzing the land uses. This index was used for defining the least- and most-disturbed sites available, necessary for the development of the next chapters. In the second chapter we analyzed the efficiency of the fixed-fraction subsampling method characterizing family richness of the site samples (alpha diversity). We verified that, independent of the ecological condition of the sites, higher the number of individuals of the samples smaller the fraction necessary to represent their taxonomic richness. In the third chapter we evaluated which field sampling and laboratory processing methodologies studying macroinvertebrates better differentiated least- and most-disturbed sites according to the assemblage composition and biological metrics. We verified that sampling in many types of habitats (multihabitat sampling) better differentiated the assemblage composition (presence/absence and relative abundance data) and the family richness when counting all individuals of the samples. However, leaf packs (targeted) sampling was more robust when applying fixed-count subsampling of individuals. The fourth chapter evaluated the influence of anthropic alterations on alpha (site) and beta diversities of macroinvertebrates. We verified a negative effect of the intensity of human disturbances (IDI values) on the genera richness of Ephemeroptera, Plecoptera and Trichoptera (EPT) of the sites. It was observed a nestedness pattern along the disturbance gradient; assemblages of the more disturbed sites are subsets of the assemblages of the less disturbed sites. Still, the beta diversity among most-disturbed sites was greater than the beta diversity among least-disturbed sites. We conclude that the decrease of the regional richness (gamma diversity) observed in the most-disturbed sites was caused mainly by local extinctions of species, and not through biological homogenization of the aquatic landscape.

Key-words: macroinvertebrates, bioindicators, EPT, alpha diversity, beta diversity, Cerrado, disturbance indices, subsampling, additive partitioning of diversity.

INTRODUÇÃO

Heterogeneidade em riachos

Os ecossistemas lóticos não são ambientes homogêneos. Pelo contrário, devem ser estudados considerando-se a alta heterogeneidade que possuem, tanto em seus atributos abióticos quanto em suas comunidades biológicas (Heino *et al.*, 2004). Essa heterogeneidade pode ser observada em múltiplas escalas espaciais, da mais reduzida à mais ampla, dentro do contexto de "paisagens aquáticas" ("*riverine landscapes*", Wiens, 2002).

Frissel *et al.* (1986) definiu as diversas escalas espaciais de observação dos riachos em níveis hierárquicos que vão de microhabitats (escala mais reduzida) a bacias hidrográficas (escala mais ampla) (Figura 1). Os níveis intermediários são os habitats, os trechos e os segmentos de riachos. Os microhabitats são representados pelos diversos tipos de substratos encontrados nos leitos dos riachos. Os habitats são comumente representados pelas diversas unidades hidrogeomórficas que caracterizam o fluxo dos riachos, tais como os muitos tipos de "fluxos lentos" ("*pools”*) e "fluxos rápidos" ("*riffles*") (Allan & Castillo, 2007). Trechos e segmentos de riachos referem-se a extensões longitudinais mais extensas, sendo definidas por características das zonas ripárias e dos vales dos riachos (Gregory *et al.*, 1991). Segmentos de riachos podem facilmente ser entendidos como tributários individuais inseridos em uma rede que, em conjunto, formam as bacias hidrográficas.

Os diversos níveis espaciais formam um padrão aninhado em que os níveis superiores controlam as características dos habitats expressas nos níveis inferiores, o inverso não sendo verdadeiro (Frissel *et al.*, 1986). Assim, clima, geologia e geomorfologia definem as características de bacias de drenagem e a subsequente conformação dos segmentos e trechos de riachos. Em escala local, características dos trechos de riachos (p.ex., vegetação ripária, sinuosidade) controlam a disponibilidade de habitats e microhabitats para a biota. Todos esses níveis espaciais influenciam na estruturação das assembleias biológicas, atuando como "filtros" para o estabelecimento e para a manutenção de populações de organismos aquáticos (Poff, 1997).

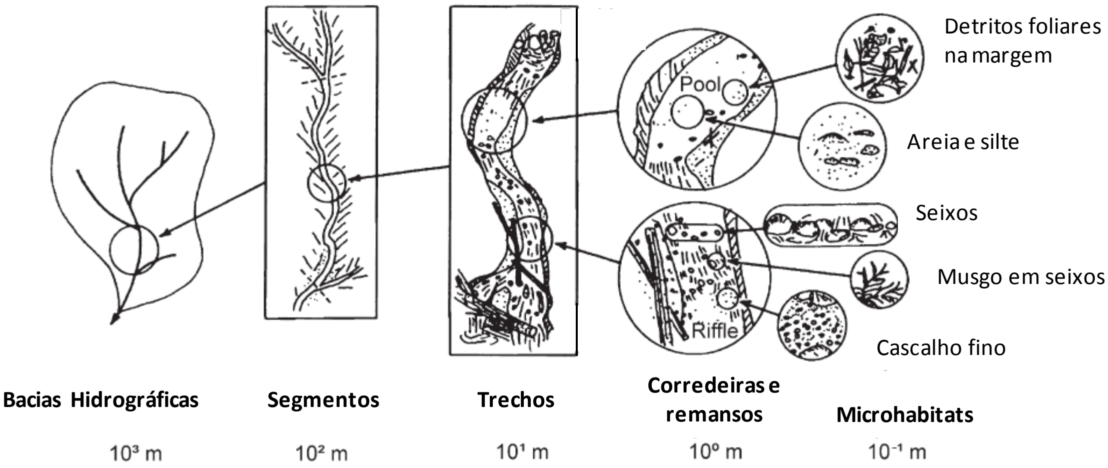

Figura 1. Esquema dos níveis espaciais hierárquicos das paisagens aquáticas. Adaptado de Frissel *et al.* (1986).

Heterogeneidade biológica

Uma forma de se estudar a distribuição da diversidade biológica nas paisagens aquáticas é separá-la em um componente local (diversidade alfa), um componente de variação entre locais (diversidade beta), e um componente regional (diversidade gama) (Veech *et al.*, 2002; Anderson *et al.*, 2011). Whittaker (1960) foi o primeiro a propor esta classificação, criando uma estrutura conceitual para estudos sobre a distribuição espacial de espécies. É importante salientar que qualquer nível hierárquico da paisagem pode ser classificado como "local" ou "regional", sem uma pré-definição específica em escala espacial. No contexto das paisagens aquáticas, o termo "regional" retrata um conjunto de "locais" de nível espacial mais reduzido, estes representando pequenas porções de microhabitats, trechos de riachos ou mesmo bacias hidrográficas inteiras (no caso da "região" ser algo ainda mais amplo).

Mensurando a diversidade alfa

As diversidades alfa e gama são frequentemente expressas por meio da riqueza taxonômica ou por meio de índices de diversidade (p.ex., índices de Simpson, Shannon-Wienner, Margalef, etc.) (Jurasinski *et al.*, 2009), embora existam muitas críticas à aplicação e à interpretação desses índices (Melo, 2008). Assim, essas duas diversidades diferem na escala avaliada mas não nas formas de mensuração, sendo ambas referidas como "diversidades de inventário" ("*inventory diversities*") (Jurasinski *et al.*, 2009).

A riqueza taxonômica é a forma mais comumente utilizada como medida da diversidade em um dado local (diversidade alfa) (Gotelli & Cowell, 2001), e tem sido empregada como base de inúmeros modelos ecológicos (McArthur & Wilson, 1967; Lande, 1996; Hubbel, 2001; Arita & Vazquez-Dominguez, 2008). Porém, apesar de definição relativamente fácil e intuitiva, sua mensuração está longe de ser algo simples (Gotelli & Cowell, 2001; Melo, 2008). O problema é que, para a maioria dos locais, o real número de *taxa* existentes é desconhecido, principalmente quando se trabalha em escalas espaciais mais amplas (trechos e segmentos de riachos) e assembleias biológicas muito diversas, como as assembleias de macroinvertebrados bentônicos. Dessa forma, faz-se necessária a padronização do número de *taxa* observados em função da área amostrada ou do número de indivíduos coletados (Gotelli & Cowell, 2001).

Hurlbert (1971) definiu duas formas básicas de mensurar a riqueza taxonômica de um local: (i) riqueza por área ("*areal richness*"), que é basicamente o número de *taxa* encontrado em uma dada área coletada em campo, e (ii) riqueza numérica ("*numerical richness*"), que é o número de *taxa* encontrados em função de um determinado número de indivíduos coletados. Dessa forma, a primeira considera a área coletada, enquanto que a segunda, o número de indivíduos coletados para calcular a riqueza taxonômica. Cada forma de mensuração pode ser útil para expressar aspectos distintos da diversidade (Gotelli & Cowell, 2001); a riqueza por área indica a "densidade taxonômica" local (Simpson, 1964), enquanto que a riqueza numérica padroniza o esforço amostral.

Na prática, em muitos projetos de pesquisa e programas de biomonitoramento, principalmente os que envolvem a coleta e o processamento de um grande número de amostras, torna-se inviável a contagem de todos os indivíduos coletados (Barbour & Gerritsen, 1996; Hughes & Peck, 2008). Nesses casos é frequente o uso de procedimentos de subamostragem, que são realizados basicamente de duas maneiras: (i) subamostragem de frações fixas ("*fixed-fraction subsampling*"), onde uma proporção fixa de cada amostra é processada, e a parte restante é descartada.

A metodologia divide cada amostra em porções (*quadrats*) de igual área e volume e então é definido o número de *quadrats* que será processado (Oliveira *et al.*, 2010). (ii) subamostragem de contagem fixa ("*fixed-count subsampling*"), onde um número fixo de indivíduos é retirado e identificado em cada amostra, e os demais são descartados (Barbour & Gerritsen, 1996).

O processamento total das amostras e identificação de todos os indivíduos pode informar tanto sobre a riqueza por área, se a área coletada for conhecida, quanto sobre a riqueza numérica, se o número de taxa observados for calculado em função do número de indivíduos. A subamostragem por contagem fixa oferece uma estimativa da riqueza numérica e a subamostragem de fração fixa destina-se principalmente a mensurar a riqueza por área.

Mensurando a diversidade beta

Ao contrário das maneiras relativamente uniformes de avaliar as diversidades alfa e gama (diversidades de inventário), a diversidade beta, sob o "guarda-chuva" da definição generalista de "variação entre locais", pode ser calculada e expressa por muitas e variadas maneiras (Anderson *et al.*, 2011). Os métodos consideram a forma multiplicativa primitiva (gama = alfa x beta) concebida por Whittaker (1960), passando pela partição aditiva (gama = alfa +beta) (Lande, 1996; Veech *et al.*, 2002), ou ainda utilizando a composição das assembléias. Como exemplo podemos citar a análise da dispersão multivariada, em que se avalia quão dissimilares são as assembléias de um dado conjunto de locais, comparando essa dissimilaridade com as observadas em outros conjuntos de locais (Anderson 2006). Há também a descrição de curvas de decaimento da similaridade entre as assembleias em relação às suas distâncias geográficas ("*distance-decay curves*") (Nekola & White, 1999).

Jurasinski *et al.* (2009) propuseram a divisão do grande número de análises de diversidade beta em duas abordagens principais:

1) **Diversidade de diferenciação** ("*differentiation diversity*"), que considera todas as análises que utilizam a composição das assembleias (abundância relativa ou presença/ausência), frequentemente utilizando medidas de dissimilaridade (índices ou distâncias). Nessa abordagem as amostras são geralmente organizadas na forma de matrizes ("*resemblances*") de similaridade ou dissimilaridade.

2) **Diversidade proporcional** ("*proportional diversity*"), que aborda a descrição da distribuição das espécies (em termos da riqueza taxonômica ou outros índices de diversidade) ao longo de uma ou mais escalas espaciais ou temporais. Dessa abordagem fazem parte as partições multiplicativas e aditivas da diversidade, aplicadas a uma ou mais escalas de estudo. Com efeito, são as medidas das diversidades observadas nas diferentes escalas de estudo, e não a composição das assembleias em si, as "matérias-primas" para o cálculo da diversidade beta.

Moreno & Rodríguez (2010) e Anderson *et al.* (2011) propõem uma subdivisão adicional às análises de diversidade de diferenciação, separando-as em: (i) análises de variação, quando a diferenciação das assembleias é realizada entre grupos de locais, e (ii) análises de substituição ("*turnover*"), quando a diferenciação das assembleias é avaliada ao longo de gradientes contínuos (ambientais, geográficos, de degradação, etc).

Segundo Legendre *et al.* (2005), o estudo da diversidade beta em suas variadas formas é de essencial importância para o entendimento do funcionamento de ecossistemas, para a conservação de biodiversidade e para o manejo de paisagens. Atualmente, todos esses tópicos são de grande importância no estudo de riachos, tendo em vista a crescente ameaça imposta à integridade desses ecossistemas pelas múltiplas atividades humanas.

Distúrbios antrópicos em riachos

Os ecossistemas lóticos, incluindo os riachos, estão atualmente entre os ecossistemas mais ameaçados do mundo (Dudgeon *et al.*, 2006), especialmente em países em desenvolvimento em regiões tropicais (Boyero & Bailey, 2001). A perda de biodiversidade em riachos, incluindo a diversidade de organismos bentônicos, ocorre atualmente em ritmo alarmante (Allan & Castillo, 2007). As principais ameaças são; poluição das águas, modificações no fluxo, destruição e degradação de habitats e invasão por espécies exóticas (Dudgeon *et al.*, 2006). No conjunto, essas alterações levam normalmente à simplificação dos habitats aquáticos, resultando na morte de organismos sensíveis e na diminuição da diversidade de assembleias biológicas (Karr, 1991; 1999).

No Brasil todos esses problemas têm afetado a integridade ecológica dos corpos d'água. Poluição da água, modificações no leito e morfologia de canais de riachos, degradação e destruição de habitats são marcantes principalmente em áreas próximas a centros urbanos e industriais (Moreno & Callisto, 2006; Callisto & Moreno, 2008). Em áreas rurais, a modificação da paisagem gerada por atividades agropastoris também ameaça de sobremaneira os ecossistemas lóticos, em especial aqueles com vegetação ripária desflorestada (Allan, 2004). Entre as principais alterações geradas por essas atividades incluem-se a contaminação da água por agrotóxicos, o enriquecimento por nutrientes provindos de adubos químicos (principalmente nitrogênio e fósforo, Woodward *et al.*, 2012) e o aporte de sedimentos finos para o leito de riachos (Malmqvist & Rundle, 2002). Na área de estudo contemplada nessa tese, que inclui a região Central de Minas Gerais e o Triângulo Mineiro, grande parte dos trechos de riachos encontram-se sob efeito de atividades agropastoris e, em menor intensidade, sob influências urbanas (pequenas cidades e povoamentos, estradas pavimentadas).

Estudando os processos de degradação de riachos

Para abordar eficazmente os processos de degradação que ocorrem em riachos é necessário avaliar as relações entre atividades antrópicas, alterações ambientais e impactos na biota (Karr, 1991). A influência de atividades humanas em ecossistemas aquáticos, ou em suas bacias de drenagem, pode ser caracterizada como "alterações", "pressões", "perturbações", ou "estressores", termos largamente utilizados como sinônimos. Um ambiente exposto a estressores está em situação "alterada" ou "perturbada". Quando essas perturbações desestabilizam o ecossistema de seu estado original, modificando seus padrões e processos ecológicos, diz-se então que o ambiente foi "impactado" (Norris & Thoms, 1999).

É de grande utilidade separar conceitualmente os termos "perturbado" e "impactado", visto que nem todas as alterações observadas nos ambientes aquáticos resultam em impactos nos processos ecológicos ou nas assembleias biológicas. Em situação impactada a "saúde" dos ecossistemas (*sensu* Norris & Thoms, 1999) pode ser comprometida. Ecossistemas impactados não oferecem os mesmos bens e serviços ecológicos ao homem e à biodiversidade em geral (*sensu* Costanza & Folke, 1997). Alterações de habitats físicos e condições químicas potencialmente podem levar à extinção local de espécies ou a alterações das dinâmicas populacionais e de assembleias de diversos grupos de organismos aquáticos, incluindo os macroinvertebrados bentônicos (Allan, 2004).

Efeitos de distúrbios antrópicos em assembleias de macroinvertebrados

Os impactos gerados por atividades humanas modificam processos geomórficos que mantém a paisagem aquática e sua biota (p.ex., regime natural de fluxo, integridade da vegetação ripária, estabilidade da margem e do leito). Em geral os ecossistemas lóticos são alterados em diversas escalas espaciais, levando à simplificação dos corpos d’água (Allan, 2004; Murphy & Davy-Bowker, 2005; Ciesielka & Bailey, 2007). Considera-se que a heterogeneidade de habitats é determinante para a manutenção da diversidade de macroinvertebrados bentônicos (Vinson & Hawkins, 1998). Como consequência, os ecossistemas impactados tendem a tornar-se não só mais pobres localmente, mas também espacialmente mais homogêneos (Rahel, 2002; Allan, 2004). Com isso, espera-se que os componentes alfa, beta e gama da diversidade sejam afetados por atividades humanas à medida em que os ecossistemas perdem suas condições originais (ou “naturais”) e tornam-se cada vez mais alterados (Maloney *et al.*, 2011).

A ecologia de riachos, particularmente os estudos com macroinvertebrados bentônicos, tradicionalmente têm focado em relações ecológicas de habitats e a diversidade alfa, principalmente nos níveis espaciais mais reduzidos, tais como microhabitats, habitats e trechos de riachos (Clarke *et al*., 2008). Os estudos envolvendo a variação da diversidade entre locais tem sido pouco estudado. Em sua maior parte, há mensurações e análises da diversidade beta em ambientes preservados ou pouco perturbados (p.ex., Stendera & Jonhson, 2005; Ligeiro *et al*., 2010; Hepp *et al.*, 2012; Hepp & Melo, 2013). As relações entre a diversidade alfa e a intensidade e natureza de alterações antrópicas são bem relatadas na literatura (Karr 1991, 1999). Porém, poucos ainda são os trabalhos que analisam efeitos de distúrbios sobre a diversidade beta (p.ex., Maloney *et al*., 2011).

CONTEXTUALIZAÇÃO DA TESE

Esta tese de doutorado foi realizada dentro de um amplo projeto financiado pelo PROGRAMA PEIXE VIVO da Companhia Energética de Minas Gerais S.A. (CEMIG) intitulado "*DESENVOLVIMENTO DE ÍNDICES DE INTEGRIDADE BIÓTICA PARA AVALIAÇÃO DE QUALIDADE AMBIENTAL E SUBSÍDIO PARA A RESTAURAÇÃO DE HABITATS EM ÁREAS DE SOLTURA DE ALEVINOS*". Este projeto teve início em 2009 e continua em andamento. Ele prevê ao seu final (2013) coletas nas bacias hidrográficas de 4 empreendimentos hidrelétricos da CEMIG localizados no estado de Minas Gerais (reservatórios de Nova Ponte, Três Marias, Volta Grande e São Simão). Foram realizadas campanhas e desenhos amostrais separados para os riachos tributários dos reservatórios e para os pontos localizados dentro dos reservatórios. O projeto conta com a participação em rede de diversas 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 estrangeiras (Oregon State University - OSU, US-Environmental Protection Agency).

O objetivo principal do projeto foi traduzir, aplicar e validar metodologias de avaliação de qualidade ambiental desenvolvidas e utilizadas pela Agência de Proteção Ambiental Norte-Americana (US-EPA), adaptando-as às necessidades e à realidade dos corpos hídricos do Brasil, em particular os ambientes do Cerrado de Minas Gerais. Espera-se com isso desenvolver Índices de Integridade Biótica ("*Index of Biological Integrity*" - IBI) utilizando as assembleias de macroinvertebrados e de peixes.

Essa tese utilizou dados das campanhas amostrais realizadas em 2009 e 2010 nos riachos tributários das bacias dos reservatórios de Nova Ponte e Três Marias, respectivamente. Foram descritos os gradientes de distúrbios nos riachos, sendo esses relacionados com os padrões de diversidade alfa e beta de assembleias de macroinvertebrados bentônicos.

Foram escritos quatro capítulos. Os dois primeiros já foram publicados (estão no formato de artigos), e os outros dois estão no formato de manuscritos prontos para serem submetidos. No primeiro capítulo um índice de distúrbio foi proposto e calculado para cada riacho estudado, e assim um gradiente de distúrbio foi definido em cada bacia. Este gradiente serviu como base para as análises realizadas nos capítulos seguintes. O segundo capítulo analisou a confiabilidade de um método de subamostragem, utilizando para isso amostras coletadas em riachos em diferentes condições ecológicas. No terceiro capítulo foi analisada a eficiência de diferentes métodos de amostragem em campo e de processamento de amostras de macroinvertebrados em laboratório na avaliação de distúrbios antrópicos. Por fim, no quarto capítulo foram avaliados os efeitos de distúrbios antrópicos sobre os padrões de diversidade alfa e beta de assembleias de macroinvertebrados.

Parte dessa tese foi realizada nos Estados Unidos, durante permanência como bolsista de doutorado sanduíche CNPq estudando na Oregon State University e na Agência de Proteção Ambiental norte-americana (EPA Western Ecology Division, Corvallis, Oregon) entre maio/2011 e maio/2012. Durante esse período o Dr. Robert M. Hughes (OSU/US-EPA) atuou como co-orientador estrangeiro.

PERGUNTAS, HIPÓTESES E OBJETIVOS

Esta tese de doutorado baseia-se nas seguintes perguntas e hipóteses, e é guiada pelos seguintes objetivos:

Perguntas e hipóteses (por Capítulo)

Capítulo 1: *“Defining quantitative stream disturbance gradients and the additive role of habitat variation to explain macroinvertebrate taxa richness”*

Pergunta 1:

Em quais escalas espaciais os distúrbios antrópicos afetam a diversidade alfa de assembleias de macroinvertebrados?

Hipótese 1:

Distúrbios antrópicos atuando tanto em escala local (leito dos riachos e zona ripária) quanto em escala regional (bacia de drenagem) afetam negativamente a riqueza taxonômica de organismos das ordens Ephemeroptera, Plecoptera e Trichoptera (EPT) em riachos.

Pergunta 2:

A influência relativa de distúrbios antrópicos e habitats físicos sobre a determinação da diversidade alfa (riqueza taxonômica) de assembleias de macroinvertebrados varia com a força do gradiente de distúrbio em um conjunto de riachos?

Hipótese 2:

Quanto maior a força do gradiente de distúrbio (quanto maior a diferença de condição de distúrbio entre os riachos menos e mais perturbados comparados), menor será a influência dos habitats físicos na determinação da diversidade alfa de assembleias de macroinvertebrados.

Capítulo 2: "*The problem of using fixed-area subsampling methods to estimate macroinvertebrate richness: a case study with Neotropical stream data*"

Pergunta 3:

A condição ecológica de riachos influencia a mensuração da riqueza taxonômica das amostras (diversidade alfa) em procedimentos de subamostragem por fração fixa?

Hipótese 3:

A dificuldade em representar a riqueza taxonômica de uma amostra qualquer depende mais de características particulares de cada amostra (ex., número de indivíduos, equitabilidade) do que da condição ecológica dos riachos em que foram coletadas.

Capítulo 3: "*Macroinvertebrate responsiveness to anthropogenic disturbances in streams varies with field and laboratory methodologies*"

Pergunta 4:

Qual método de amostragem em campo que gera a melhor resposta de assembleias de macroinvertebrados aos distúrbios antrópicos?

Hipótese 4:

Por meio da realização de amostragens em substratos padronizados (bancos de folhas) obtém-se uma melhor resposta de assembleias de macroinvertebrados ao gradiente de distúrbio, uma vez que esse método de amostragem pode diminuir os efeitos da variabilidade natural dos habitats entre os diferentes riachos comparados.

Pergunta 5:

Qual método de processamento das amostras em laboratório que gera a melhor resposta de assembleias de macroinvertebrados aos distúrbios antrópicos?

Hipótese 5:

Por meio do processamento de todos os indivíduos das amostras obtém-se uma melhor resposta de assembleias de macroinvertebrados ao gradiente de distúrbio, uma vez que esse processamento maximiza as diferenças de riqueza taxonômica resultantes da contagem de espécies raras.

Capítulo 4: “*Anthropogenic disturbances alter alpha and beta diversity patterns of macroinvertebrate assemblages in tropical headwater streams*“

Pergunta 6:

A diversidade alfa de assembleias de macroinvertebrados é afetada pela intensidade de distúrbios antrópicos em riachos?

Hipótese 6:

A diversidade alfa (riqueza taxonômica) de assembleias de macroinvertebrados diminui com o aumento da intensidade de distúrbios antrópicos em riachos.

Pergunta 7:

As assembleias de macroinvertebrados de riachos mais alterados são subconjuntos das assembleias de riachos menos alterados?

Hipótese 7:

As assembleias de macroinvertebrados apresentam um padrão aninhado em relação ao gradiente de distúrbio antrópico, assembleias de riachos mais perturbados sendo subconjuntos das assembleias de riachos menos perturbados.

Pergunta 8:

Os distúrbios antrópicos homogeneízam as composições de assembleias de macroinvertebrados em riachos?

Hipótese 8:

Riachos mais perturbados apresentam menor diversidade beta do que riachos preservados.

Objetivo Geral da tese

Avaliar como os componentes alfa (local) e beta (variação entre locais) da distribuição espacial de assembleias de macroinvertebrados são alterados devido a perturbações antrópicas atuando nos riachos e em suas bacias de drenagem.

Objetivos Específicos (por Capítulo)

Capítulo 1: *“Defining quantitative stream disturbance gradients and the additive role of habitat variation to explain macroinvertebrate taxa richness”*

- Definir um gradiente de distúrbio ambiental para os riachos em cada bacia hidrográfica estudada.

- Analisar como a diversidade alfa de macroinvertebrados em riachos (em termos de suas riquezas taxonômicas) se comporta em relação ao índice de distúrbio proposto.
- Avaliar a contribuição dos habitats físicos não relacionados às alterações antrópicas para explicar a variação da riqueza taxonômica de riachos.

Capítulo 2: "*The problem of using fixed-area subsampling methods to estimate macroinvertebrate richness: a case study with Neotropical stream data*"

- Avaliar a eficiência de um método comumente aplicado em laboratório (método da fração fixa) na subamostragem de macroinvertebrados em estudos de riachos.
- Verificar como riachos em diferentes condições ambientais (como definido pelo gradiente de distúrbio estabelecido no Capítulo 1) se comportam em relação ao uso da metodologia de subamostragem.

Capítulo 3: "*Macroinvertebrate responsiveness to anthropogenic disturbances in streams varies with field and laboratory methodologies*"

- Avaliar o tipo de amostragem ("reach wide" ou "target") que melhor responde ao gradiente de distúrbio ambiental (utilizando o índice descrito no Capítulo 1), considerando a composição de assembleias e métricas biológicas (riqueza taxonômica e % indivíduos EPT).

- Avaliar o tipo de processamento de amostras (contagem total ou subamostragem de indivíduos) que melhor responde ao índice de distúrbio ambiental, também considerando composição de assembleias e métricas biológicas.

Capítulo 4: "*Anthropogenic disturbances alter alpha and beta diversity patterns of macroinvertebrate assemblages in tropical headwater streams*"

- Avaliar o aninhamento das assembleias de macroinvertebrados em relação ao gradiente de distúrbio ambiental (Capítulo 1) .
- Avaliar a diversidade beta entre assembleias de riachos preservados e entre assembleias de riachos alterados por atividades antrópicas, por meio de análises da dispersão multivariada e da partição aditiva da diversidade.

ÁREA DE ESTUDOS E RESUMO DO PROCEDIMENTO AMOSTRAL GERAL

Foram estudados 40 trechos de riachos na bacia hidrográfica que drena o reservatório de Nova Ponte (Alto Rio Araguari), e outros 40 na bacia do reservatório de Três Marias (Alto Rio São Francisco) (Figura 2). O estudo de 40 riachos em cada bacia representa um equilíbrio entre as possibilidades financeiras e logísticas do projeto e o número de réplicas necessário para a realização dos estudos.

A bacia do Alto Rio Araguari está localizada na mesorregião do Triângulo Mineiro, abrangendo os municípios de Araxá, Ituiutaba, Nova Ponte e Patrocínio. A paisagem é intensamente modificada na maior parte de sua extensão, sendo a agricultura mecanizada e irrigada o uso do solo predominante (IBGE, 2012). Entre as principais culturas destacam-se o café, a cana, a soja e o milho. A bacia do Alto Rio São Francisco está localizada na mesorregião Central Mineira, abrangendo os municípios de Três Marias, Abaeté, Pompéu e Morada Nova de Minas. A agricultura mecanizada é menos intensa nessa região, nela predominando a agricultura familiar e a criação de gado de modo extensivo (IBGE, 2012).

Ambas as bacias estão inseridas no bioma Cerrado, um dos "*hotspots*" de biodiversidade definidos por Myers *et al.* (2000), possuindo muitas espécies endêmicas de flora e fauna. O clima do Cerrado é marcado por duas estações climáticas bem definidas; um período de seca que vai de abril a setembro e outro chuvoso, que vai de outubro a março. A precipitação média anual varia entre 1200-1800mm. Embora seja o segundo maior bioma brasileiro, ocupando originalmente 20% do território nacional, o Cerrado vem sendo progressivamente alterado e destruído (Wantzen *et al.*, 2006; Diniz-Filho *et al.*, 2009). Esse processo acelerou-se a partir da segunda metade do século XX em função do avanço da malha urbana e da implantação de empreendimentos agropastoris, grande parte deles destinado ao mercado externo.

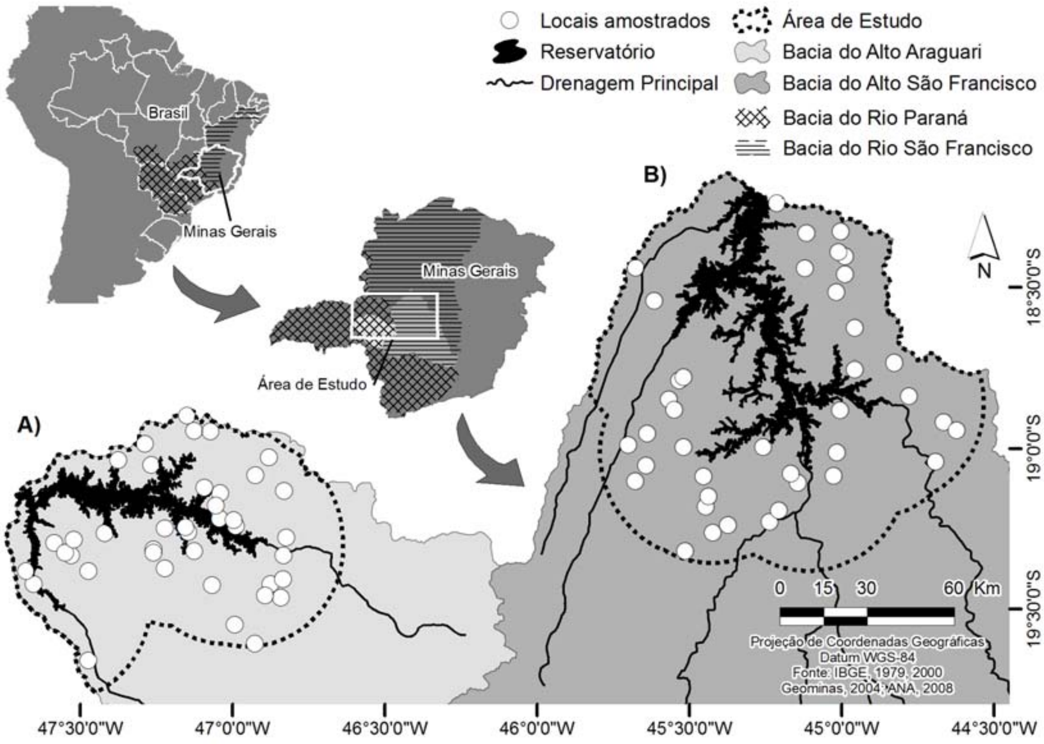

Figura 2. Localização das bacias hidrográficas estudadas e dos riachos amostrados. A) Bacia do Alto Rio Araguari (reservatório de Nova Ponte), e B) Bacia do Alto Rio São Francisco (reservatório de Três Marias).

Em cada bacia definiu-se como a região potencial de amostragem ("*buffer*") a área de 35km no entorno dos reservatórios. A seleção dos riachos foi realizada aleatoriamente seguindo um modelo computadorizado que garante uma distribuição espacialmente balanceada dos pontos amostrais (Olsen & Peck, 2008). As coletas foram realizadas nos meses de setembro de 2009 (Alto Araguari) e setembro de 2010 (Alto São Francisco), durante o período de seca no Cerrado brasileiro. Em cada trecho foi aplicado um protocolo de avaliação de habitats desenvolvido e utilizado pela Agência de Proteção Ambiental Norte-Americana (US-EPA) (Peck *et al*., 2006).

O comprimento longitudinal de cada trecho amostrado foi definido como a média da largura molhada no ponto de chegada do trecho multiplicada por 40m, tendo um comprimento mínimo de 150m. Dentro de cada trecho foram definidos 11 transectos transversais equidistantes, totalizando 10 seções (espaço entre transectos) em cada trecho (Figura 3). Em cada transecto e ao longo das seções foram sistematicamente avaliadas diversas características do leito dos riachos (p.ex., largura molhada, largura e altura do leito sazonal, profundidade, tipo de substrato, tipo de fluxo, etc.) e da vegetação ripária (p.ex., alterações humanas, cobertura vegetal, etc.). Esse protocolo tem como objetivo descrever da forma mais detalhada possível as características dos habitats físicos dos riachos.

Seguindo o mesmo protocolo, as amostragens de macroinvertebrados em cada trecho foram realizadas utilizando um amostrador "*kick net*" (30cm abertura, 500µm tamanho de abertura de malha). Esse amostrador é também conhecido como "*D-net*", em função de seu formato semelhante à letra "D". Foram realizados dois tipos de amostragens:

(i) Na amostragem "*reach wide*", onze sub-amostras (0,09m^2 cada) foram coletadas por trecho de riacho, formando uma única amostra composta por trecho. Este método possibilita a amostragem de vários tipos de habitats e microhabitats existentes no trecho, podendo assim ser chamada de "amostragem multi-habitat".

(ii) A amostragem do tipo "*target*" foi direcionada para o microhabitat do tipo folhiço (formado por depósitos de bancos de folhas no leito dos riachos). Foram coletadas 8 sub-amostras de folhiço por trecho de riacho, também formando uma única amostra composta por trecho (segundo Peck *et al*., 2006).

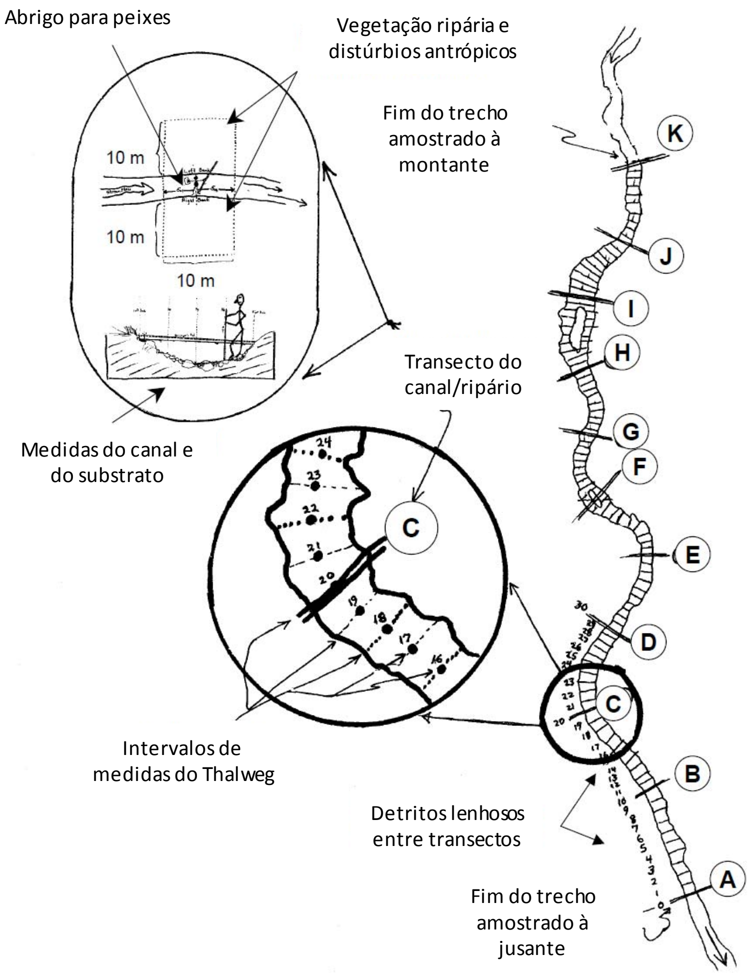

Figura 3. Desenho esquemático da metodologia amostral aplicada em campo (figura adaptada de Kaufmann *et al*., 1999).

Em laboratório os organismos coletados foram triados em bandejas e identificados em microscópio estereoscópico (aumento 32x) ao nível de família.

Os taxa EPT, organismos considerados como eficientes bioindicadores de boa qualidade de água, foram identificados ao nível de gênero. Todas as identificações foram realizadas com o auxílio de chaves taxonômicas (Pérez, 1988; Fernández & Domíngues, 2001; Costa *et al*., 2006). No Anexo I estão representadas as famílias de macroinvertebrados e no Anexo II estão representados os gêneros de EPT amostrados nas duas bacias estudadas.

Além da avaliação dos habitats aquáticos ao nível local, foi realizada também uma avaliação dos tipos e proporções dos usos do solo nas bacias de drenagem de cada trecho (p.ex., % de área utilizada para a plantação. % de pasto, % ocupação urbana e % vegetação natural). Essa avaliação foi realizada por interpretação conjunta de imagens de satélites (LANDSAT) e imagens multiespectrais de alta resolução (Camara *et al*., 1996).

Capítulo 1

Defining quantitative stream disturbance gradients and the additive role of habitat variation to explain macroinvertebrate taxa richness

Raphael Ligeiro [a], Robert M. Hughes [b], Philip R. Kaufmann [c], Diego R. Macedo [d], Kele R. Firmiano [a], Wander Ferreira [a], Déborah Oliveira [a], Adriano S. Melo [e] and Marcos Callisto [a]

[a] Universidade Federal de Minas Gerais, Instituto de Ciências Biológicas, Departamento de Biologia Geral, Laboratório de Ecologia de Bentos, Av. Antônio Carlos, 6627, CP 486, CEP 30161-970, Belo Horizonte, Minas Gerais, Brazil.

[b] Oregon State University and Amnis Opes Institute, Department of Fisheries & Wildlife, Nash Hall, 97331-4501, Corvallis, Oregon, USA.

[c] U.S. Environmental Protection Agency, Office of Research & Development, National Health & Environmental Effects Lab., Western Ecology Division, 200 SW 35 Street, 97333, Corvallis, Oregon, USA.

[d] Instituto Brasileiro de Geografia e Estatística, Rua Oliveira, 523, CEP 30310-150, Belo Horizonte, Minas Gerais, Brazil.

[e] Universidade Federal de Goiás, Instituto de Ciências Biológicas, Departamento de Ecologia, Campus II, Samambaia, CP 131, CEP 74001-970, Goiânia, Goiás, Brazil.

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Original Articles

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Raphael Ligeiro[a,*], Robert M. Hughes[b], Philip R. Kaufmann[c], Diego R. Macedo[a,d], Kele R. Firmiano[a], Wander R. Ferreira[a], Déborah Oliveira[a], Adriano S. Melo[e], Marcos Callisto[a]

[a] Laboratório de Ecologia de Bentos, Departamento de Biologia Geral, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, Av. Antônio Carlos 6627, CP 486, CEP 30161-970 Belo Horizonte, Minas Gerais, Brazil
[b] Department of Fisheries & Wildlife, Oregon State University and Amnis Opes Institute, Nash Hall, 97331-4501 Corvallis, OR, USA
[c] U.S. Environmental Protection Agency, Office of Research & Development, National Health & Environmental Effects Lab., Western Ecology Division, 200 SW 35 Street, 97333 Corvallis, OR, USA
[d] Instituto Brasileiro de Geografia e Estatística, Rua Oliveira 523, CEP 30310-150 Belo Horizonte, Minas Gerais, Brazil
[e] Departamento de Ecologia, Instituto de Ciências Biológicas, Universidade Federal de Goiás, Campus II, Samambaia CP 131, CEP 74001-970 Goiânia, Goiás, Brazil

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ABSTRACT

Most studies dealing with the use of ecological indicators and other applied ecological research rely on some definition or concept of what constitutes least-, intermediate- and most-disturbed condition. Currently, most rigorous methodologies designed to define those conditions are suited to large spatial extents (nations, ecoregions) and many sites (hundreds to thousands). The objective of this study was to describe a methodology to quantitatively define a disturbance gradient for 40 sites in each of two small southeastern Brazil river basins. The assessment of anthropogenic disturbance experienced by each site was based solely on measurements strictly related to the intensity and extent of anthropogenic pressures. We calculated two indices: one concerned site-scale pressures and the other catchment-scale pressures. We combined those two indices into a single integrated disturbance index (IDI) because disturbances operating at both scales affect stream biota. The local- and catchment-scale disturbance indices were weakly correlated in the two basins ($r = 0.21$ and 0.35) and both significantly ($p < 0.05$) reduced site EPT (insect orders Ephemeroptera, Plecoptera, Trichoptera) richness. The IDI also performed well in explaining EPT richness in the basin that presented the stronger disturbance gradient ($R^2 = 0.39$, $p < 0.001$). Natural habitat variability was assessed as a second source of variation in EPT richness. Stream size and microhabitats were the key habitat characteristics not related to disturbances that enhanced the explanation of EPT richness over that attributed to the IDI. In both basins the IDI plus habitat metrics together explained around 50% of EPT richness variation. In the basin with the weaker disturbance gradient, natural habitat explained more variation in EPT richness than did the IDI, a result that has implications for biomonitoring studies. We conclude that quantitatively defined disturbance gradients offer a reliable and comprehensive characterization of anthropogenic pressure that integrates data from different spatial scales.

1. Introduction

The development and maintenance of human societies rely on the conservation of freshwater resources and of the ecological services that streams and rivers provide (Karr, 1999). Monitoring the “ecosystem health” of streams (*sensu* Norris and Thoms, 1999) is a fundamental step for conscious and effective management of catchments (Boulton, 1999). Currently, biomonitoring is considered one of the most efficient ways to assess stream condition (Marchant et al., 2006). Macroinvertebrate assemblages are responsive to environmental condition and thus integrate physical, chemical and biological aspects of ecosystems. Accordingly, they are considered good biological indicators of stream ecological condition (Karr and Chu, 1999; Bonada et al., 2006; Hughes and Peck, 2008) and are extensively used in multimetric indices (MMIs) for such purposes (Reynoldson et al., 1997; Barbour et al., 1999; Klemm et al., 2003; Hering et al., 2006; Whittier et al., 2007a). The EPT assemblages (insect orders Ephemeroptera, Plecoptera and

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Abbreviations: LDI, local disturbance index; CDI, catchment disturbance index; IDI, integrated disturbance index.

* Corresponding author. Tel.: +55 31 3409 4597; fax: +55 31 3409 2569.
E-mail addresses: ligeirobio@gmail.com (R. Ligeiro), Hughes.Bob@epamail.epa.gov (R.M. Hughes), Kaufmann.Phil@epamail.epa.gov (P.R. Kaufmann), rodriguesmacedo@gmail.com (D.R. Macedo), kelerocha@gmail.com (K.R. Firmiano), ferreirawr@gmail.com (W.R. Ferreira), deborah.ufmg@gmail.com (D. Oliveira), asm.adrimelo@gmail.com (A.S. Melo), mcallisto13@gmail.com (M. Callisto).

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Trichoptera), particularly, have proven effective ecological indicators of human disturbances (Rosenberg and Resh, 1993; Stoddard et al., 2008).

A goal of many biomonitoring approaches is to report how test sites deviate from the "undisturbed" (natural) condition in terms of the structure and/or composition of the assemblages they support. This is typically accomplished by designating "reference sites", that is, sites minimally affected by human activities and whose biological, physical and chemical features serve as reference condition for natural levels of patterns and processes (Hughes et al., 1986; Stoddard et al., 2006; Hawkins et al., 2010). A set of reference sites should be specific for a particular typology (e.g., altitude, stream size, and predominant substrate) and geographic domain (biome and ecoregion) because these are important natural drivers of stream characteristics, including their biota (Hughes et al., 1986, 1990; Gerritsen et al., 2000; Waite et al., 2000; Sánchez-Montoya et al., 2007). This framework has been established as the "reference condition approach" (RCA) (Bailey et al., 2004). In most cases it is not practical to seek sites that have truly undisturbed/minimally disturbed conditions because (1) human modifications are widespread in most landscapes worldwide, and (2) many places have been modified for hundreds (or even thousands) of years (Stoddard et al., 2006; Whittier et al., 2007b; Herlihy et al., 2008). Instead, sites in least-disturbed condition, i.e., the best set of sites available in a continuous gradient of disturbance, are typically used to represent "reference" conditions (Reynoldson et al., 1997; Stoddard et al., 2006; Yates and Bailey, 2010).

It is explicitly stated in the RCA that the reference condition should be chosen based strictly on criteria concerning the minimal exposure of the sites to human disturbances (Bailey et al., 2004). Although human disturbances affect stream biological and habitat attributes (Maddock, 1999), reference site selection should not be based on either because it is difficult to distinguish between effects from human disturbance and natural variation (Dovciak and Perry, 2002; Moreno et al., 2006). In fact, a key aspect of the RCA is that natural variability is intrinsic in ecosystems and that this variability must be accounted for by using models to understand the effects of human disturbance on assemblage structure of fish (Oberdorff et al., 2002; Tejerina-Garro et al., 2006; Pont et al., 2006, 2009) and macroinvertebrates (Clarke et al., 2003; Bailey et al., 2004; Hawkins et al., 2010; Moya et al., 2011).

A multitude of stressors have been identified and used as criteria for determining reference sites. As geographic information system (GIS) technology has become operationally simpler and widely available (King et al., 2005), disturbances identified at the catchment scale have been used for defining potential reference areas (Collier et al., 2007; Wang et al., 2008). However, human modifications acting at both large (catchment) and local (stream channel and riparian zone) scales should be investigated because pressures or stressors operating at both scales can impair the stream biota (Bryce et al., 1999; Whittier et al., 2007b; Hughes et al., 2010).

Increasingly, methods for defining and selecting reference sites are applied to large spatial extents (whole ecoregions, states, and countries), commonly involving hundreds or thousands of sites. The Environmental Protection Agency of the United States of America (US-EPA), in its national Wadeable Stream Assessment (WSA) program, screened a series of physical habitat and water quality data, setting thresholds for the selection of least-disturbed sites in different ecoregions (Herlihy et al., 2008). The same "filtering" approach was employed in regional assessments made by the same agency (Klemm et al., 2003; Whittier et al., 2007b). In a similar approach, a large set of criteria of human disturbances operating at both local and regional spatial scales were used to select least- and most-disturbed sites on European streams (Nijboer et al., 2004; Pont et al., 2006; Sánchez-Montoya et al., 2009).

However, methodologies employed at large spatial extents may be inappropriate for studies dealing with more restricted spatial extents and far fewer sites. First, for most ecosystems located in less studied regions of the world, such as in tropical developing countries, there is no reliable information about the physical and chemical thresholds that indicate substantial disturbance (Boyero et al., 2009). Second, the application of rigid filters to a small number of sites is likely to select too few sites, or none at all. Even in Europe, when hundreds of sites from 4 countries were analyzed, for many stream types it was not possible to find any single site that fulfilled all the criteria proposed for European reference conditions (Nijboer et al., 2004). Nevertheless, many monitoring initiatives are applied at more restricted geographic areas (small to medium-sized basins or sub-basins) and far fewer sites (dozens at best) (Baptista et al., 2007; Moreno et al., 2009; Oliveira et al., 2011; Suriano et al., 2011). To our knowledge, no systematic methodology has been proposed to clearly define disturbance conditions in those situations.

When working with few sites, instead of trying to allocate sites into 'boxed' categories from the onset of the project (e.g., least-, intermediate-, and most-disturbed sites), the use of a continuous disturbance gradient can be more advantageous for classifying the sites included in the study, enabling the definition a posteriori of the least-disturbed sites and the most-disturbed sites. This contrast is necessary for the development of MMIs (e.g., Stoddard et al., 2008; Oliveira et al., 2011). For instance, predictive models are first concerned with describing assemblage composition in reference conditions (Reynoldson et al., 1995), i.e., the "good tail" of a disturbance gradient. In addition to biomonitoring studies, any applied ecological research concerned with changes in patterns and processes associated with the intensity of human modifications will benefit from the use of a disturbance gradient.

In this study we present a methodology to quantitatively define disturbance gradients in two basins sampled with a relatively small number of sites (40 each), each basin including a range of sites from relatively undisturbed to greatly altered. To this end, we worked with two hypotheses. (1) Disturbances taking place at both local (stream sites) and catchment spatial scales reduce the EPT assemblage richness of the sites. (2) The proportion of variation in EPT richness associated with natural variability among site habitats will be greater in the basin with the weaker anthropogenic disturbance gradient.

2. Methods

2.1. Study area

We sampled streams in two basins of the Cerrado biome in the state of Minas Gerais, southeastern Brazil: Upper Araguari basin (in the Paraná river basin) and the Upper São Francisco basin (in the São Francisco river basin) (Fig. 1). Both study areas were demarcated upstream of the first big reservoir of each basin (Nova Ponte and Três Marias reservoirs, respectively). The Cerrado is the second-most extensive biome of the Neotropics (Wantzen, 2003), originally covering 20% of Brazilian territory, and one of the terrestrial biodiversity "hotspots" of the planet (Myers et al., 2000). It is also one of the most threatened due to ever-expanding pasture and agricultural activities (Wantzen et al., 2006). The Cerrado climate has two well defined annual seasons: a dry season from October to March, and a wet season from April to September, with 1200–1800 mm of precipitation per year. The vegetation is typically savannah-like, with denser forest formations along water courses and wet areas.

Most people living in the study areas dwell on farms and in small towns (up to 20,000 inhabitants), although a few small cities (up to 80,000 inhabitants) are present. The Upper Araguari has a well developed system of irrigated agriculture, encompassing mainly

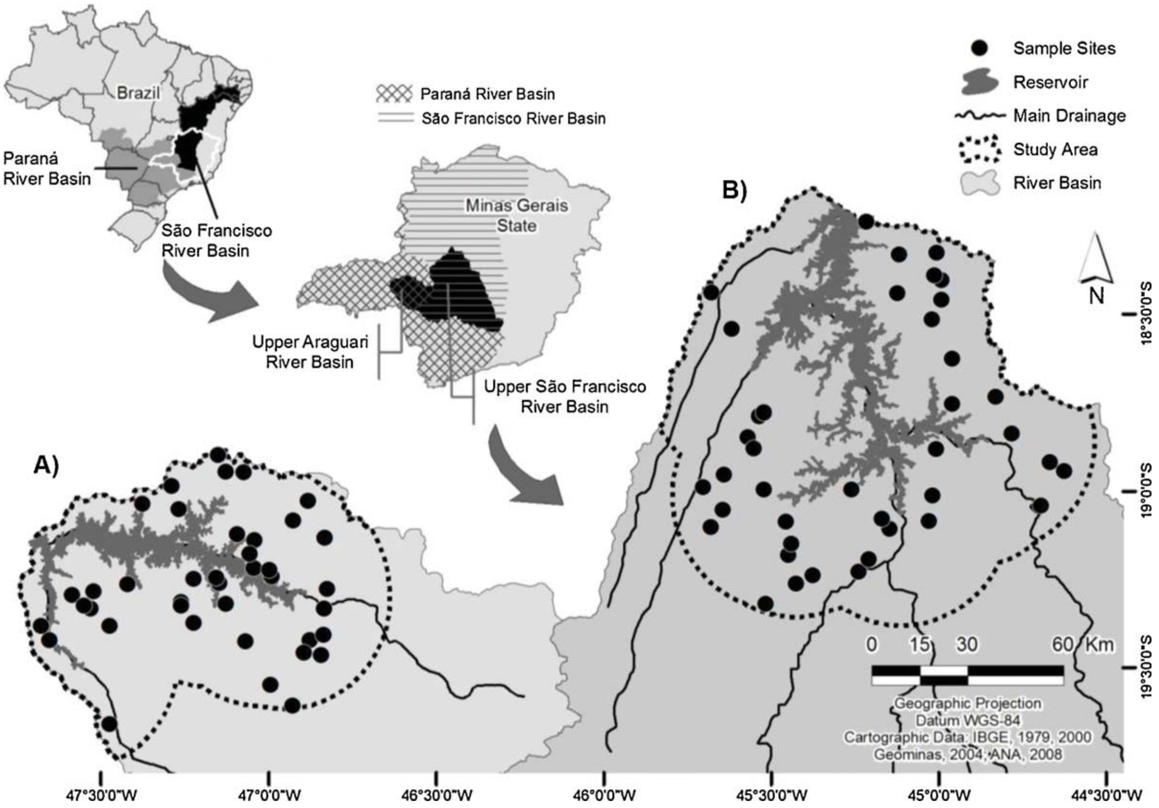

Fig. 1. Location of the basins and stream sites sampled. (A) Upper Araguari basin and (B) Upper São Francisco basin.

soy, coffee, corn, and sugar cane culture. Irrigated agriculture is less common in the Upper São Francisco, where pasture and small family farms predominate.

2.2. *Site selection*

Forty "wadeable" stream sites (that can be traversed by a person wading) ranging from 1st to 3rd order (*sensu* Strahler, 1957) were selected on 1:100,000 scale maps in each basin and sampled during the dry season. The site selection was performed through a probability-based design as described in Olsen and Peck (2008), the same procedure used by the US-EPA in the Environmental Monitoring and Assessment Program Western Pilot Study (EMAP-West, Stoddard et al., 2005) and its national Wadeable Stream Assessment (WSA, Paulsen et al., 2008). In this approach, a master sample frame (MS) is first established using a digitized drainage system map (1:100,000 scale), and then the sample sites are selected via a hierarchical, spatially weighted criteria (Stevens and Olsen, 2003). This procedure assures a balanced selection of sites across the range of stream orders and geographic location. The Upper Araguari sites were sampled in September 2009 and the Upper São Francisco sites were sampled in August/September 2010.

2.3. *Site habitat measurements*

The field physical habitat was measured as described in Peck et al. (2006). The site lengths were set at 40 times their mean wetted width, and a minimum of 150 m. Given their narrow widths, most sites were 150 m long. In each site, 11 equidistant cross-sectional transects were marked, defining 10 sections of the same length.

In each transect and along the sections, a large set of measurements were recorded, including site morphology (e.g., slope, sinuosity, wetted and bankfull width, depth, and incision height), habitat characteristics (e.g., substrate size and embeddedness, flow type, and large wood), riparian structure (e.g., mid-channel and margin shading, tree and herbaceous cover density) and human disturbance in the channel and riparian zone (e.g., presence of pasture, crops, pipes, and trash). Habitat metrics were then calculated following Kaufmann et al. (1999).

The following physical and chemical characteristics of the water column were also measured in the field for each site: pH, electrical conductivity, and total dissolved solids (TDS). Water samples were collected for further analysis in the laboratory, including dissolved oxygen, turbidity, total alkalinity, total nitrogen, and total phosphorus. Those analyses were conducted following APHA (1998).

The site nutrient concentrations of both basins were extremely low and not indicative of anthropogenic sources. In the Upper Araguari, the values were 0.06 ± 0.01 mg/L (mean ± SD) for total nitrogen and 0.03 ± 0.01 mg/L for total phosphorus. The concentrations in the Upper São Francisco were 0.08 ± 0.06 mg/L for total nitrogen and 0.02 ± 0.01 mg/L for total phosphorus.

2.4. *Macroinvertebrate sampling and laboratory processing*

The biological sampling also followed the protocol of Peck et al. (2006) and Hughes and Peck (2008). Eleven sample units were taken per stream site, one per transect, generating one composite sample for each site. Each sample unit was collected through use of a D-net (30 cm mouth width, 500 μm mesh), effectively sampling 1 m^2 of stream bottom area sampled per site. The sample units were obtained by following a systematic zigzag pattern along the sites to avoid bias in habitat selection. Immediately after collection, the composite samples were placed in individual plastic buckets and preserved with 10% formalin.

In the laboratory, the macroinvertebrates were sorted by eye, and the EPT individuals were identified to genus under a 100× magnification stereoscope microscope through use of taxonomic

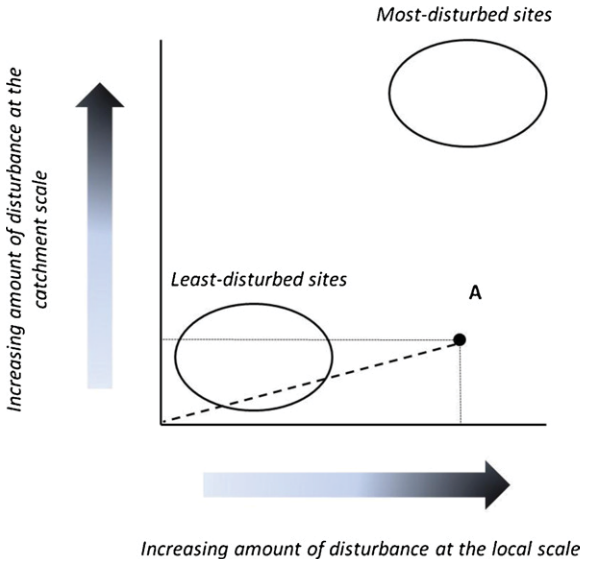

Fig. 2. Conceptual model of the disturbance plane whose axes represent the amount of disturbance observed at the local scale (in-stream and riparian zone) and at the catchment scale. The ideal least-disturbed sites would be those located closest to the origin of the axes, with few disturbances observed at both scales. The ideal most-disturbed sites would be those located in the opposite corner of the plane. A single measurement of disturbance can be the Euclidean distance (calculated through the Pythagorean theorem) between the location of the site in the plane and the origin of the axes (see the example in the figure with site "A"). For this purpose the axes values should be standardized at the same scale.

keys (Pérez, 1988; Fernández and Domínguez, 2001; Mugnai et al., 2010).

2.5. Data analyses

2.5.1. Calculation of the disturbance gradient

To describe the total exposure of the sites to human pressures, we developed two separate indices: one reflecting disturbances at the site scale and one reflecting disturbances at the catchment scale, both having their origins (0 values) representing the absence of evidence of disturbances. In each index, the higher the site value, the greater the intensity of human modifications observed for that site, i.e., the greater the deviation from the pristine condition at that spatial scale. Thus, we positioned each site in a 'disturbance bi-plane' constructed with the two disturbance indices as axes. The 'ideal' reference sites should be those lacking evidence of human modifications at both near/in-stream and catchment scales (concept of minimally disturbed condition; Stoddard et al., 2006). Typically, however, reference sites are those with the least disturbances among the sites available (concept of least-disturbed condition; Stoddard et al., 2006). Through this conceptual model, the least- and most-disturbed sites in a pool of sites can be visualized according to their positions in the disturbance plane, the least-disturbed sites being closer to the origin (lower left corner of the plane) and the most-disturbed sites being farthest from the origin (upper right corner of the plane) (Fig. 2).

For quantifying the local disturbance index (LDI) we used the metric *W1_hall*, calculated as described in Kaufmann et al. (1999), a measure commonly used in the US-EPA stream assessments. This metric summarizes the amount of evidence observed in-channel and in the riparian zone for 11 types of disturbances (buildings, channel revetment, pavement, roads, pipes, trash and landfill, parks and lawns, row crop agriculture, pasture, logging and mining) along the eleven transects demarked at the stream site. The values are weighted according to the proximity of the observation from the stream channel (Kaufmann et al., 1999).

We assessed watershed land uses for each site through use of manual image interpretation. Watersheds were extracted from the terrain model from the Shuttle Radar Topographic Mission – SRTM (USGS, 2005). We manually interpreted high resolution multispectral images in conjunction with the Landsat TM sensor using Spring software (Camara et al., 1996). The high-resolution images provided information about the shape and texture of the elements, and the Landsat images showed spectral response for different targets. Our mapping identified three human-influenced land uses (pasture, agriculture, and urban). The catchment percentages of each land use were estimated for each site.

The catchment disturbance index (CDI) was based on the human land uses in the catchments and was calculated following Rawer-Jost et al. (2004), according to the formula:

$$\text{catchment disturbance index (CDI)} = 4 \times \%\,\text{urban areas} + 2 \times \%\,\text{agricultural areas} + \%\,\text{pasture areas}$$

We evaluated the collinearity between local and catchment human disturbances in each basin through use of Pearson correlations between the LDI and the CDI values of the sites.

Because the local and the catchment disturbance indices do not share the same numerical scale, both were separately standardized to provide a similar scale in values. This transformation was necessary to reliably calculate an integrated disturbance index for each site, based on both the local and catchment indices (see below). The values of each index were divided by 75% of the maximum value that each can theoretically achieve. We did not use the maximum values of each index for these standardizations because those values are rarely achieved. Dividing by the maximum values would shrink greatly and unnecessarily the values in the standardized indices, shifting nearly all the sites very close to the origin of the disturbance plane.

The CDI values potentially range from 0 (no land use in the catchment) to 400 (entire catchment occupied by urban areas). So the values of this index were divided by 300. The LDI values (*W1_hall* metric) potentially range from 0 (no evidence of any type of disturbance in the channel or riparian zone) to 16.5 (all 11 types of disturbances observed inside the stream channel in all transects). But this theoretical upper value is highly unlikely because of spatial limitations and negative colinearities among the types of disturbance (listed above). The empirical maximum value of the *W1_hall* metric is around 7 (Kaufmann et al., 1999), so the values of this index were divided by 5.

To summarize the disturbances measured at both scales in a single index we calculated for each site an integrated disturbance index (IDI). It was measured as the Euclidian distance between the position of the site in the disturbance plane (axes standardized) to the origin of the plane (Fig. 2). This was performed through application of the Pythagorean theorem:

$$\text{integrated disturbance index (IDI)} = \left[\left(\frac{\text{LDI}}{5}\right)^2 + \left(\frac{\text{CDI}}{300}\right)^2\right]^{1/2}$$

The higher the IDI of a site, the more that site deviates from the 'origin', i.e., from the 'ideal' reference condition of no disturbance inside the stream channel, in the riparian zone, or in the catchment. Thus, we defined the disturbance gradient simply as the ascending ordination of the IDI's in a pool of sites. The steeper the disturbance gradient in a pool of sites, the greater the difference in ecological condition between the least- and most-disturbed sites in the pool.

2.5.2. EPT richness associations with the disturbance indices

To evaluate how EPT assemblages responded to the degree of human disturbances at both local and catchment scales, we

Table 1
Candidate site habitat metrics for explaining EPT richness variability in both studied basins.

Metric name	Metric code	Not significantly correlated		Not strongly correlated	
		With disturbances ($p > 0.05$)		Among each other ($r < 0.6$)	
		Upper Araguari	Upper São Francisco	Upper Araguari	Upper São Francisco
Mean width	xwidth		*		
Mean depth	xdepth	*			
Mean slope	xslope				
Mean bankfull width	XBKF_W				
Mean width × mean depth	XWXD	*	*	*	*
Mean (width/depth)	xwd_rat		*		*
Mean depth × mean slope	xdepth_xslope				
Bankfull (width/depth)	BKF_WDrat	*	*	*	*
Mean residual pool area	rp100	*			
Mean water volume/m^2	v1w_msq				
Riparian canopy (>5 m high) presence	xpcan				
Riparian canopy (>5 m high) cover	XC		*		
Total riparian cover (all vegetation layers)	xcmg	*	*	*	*
Total riparian woody cover	xcmgw				
Mean canopy density (mid-stream)	xcdenmid				
Natural cover in the stream (all)	xfc_nat		*		*
Natural cover provided by large wood	xfc_lwd	*		*	
Percentage of fast water	pct_fast	*	*	*	*
Percentage of fines (silt and clay)	pct_fn		*		
Percentage of sand + fines	pct_sfgf		*		
Percentage of cobble	pct_cb		*		*
Percentage of coarse substrate (>16 mm)	pct_bigr		*		
Log of mean substrate diameter	lsub_dmm		*		*
Mean substrate embeddedness	xembed				
Log of relative bed stability	LRBS				
pH	pH		*		*
Conductivity (μS/cm)	Cond				
Total dissolved solids (g/L)	TDS		*		*
Turbity (NTU)	Turb	*		*	
Dissolved oxygen (mg/L)	DO	*		*	
Alkalinity (mequiv./L)	Alk				

conducted multiple linear regressions between EPT richness and the standardized LDI and CDI of the sites for each basin. We also regressed EPT richness against the IDI to evaluate its performance relative to EPT richness variability.

2.5.3. *Contribution of natural variability of site habitat characteristics to explaining the variation of EPT richness*

Through the following methodology, we evaluated how much natural physical habitat variability added to the explanation of EPT richness provided by the disturbance gradient alone. The process was performed separately for each basin (Fig. 3).

We started with a set of 31 habitat metrics calculated from the raw field data (Table 1). With these metrics we aimed to represent key aspects of the habitats of the sites, such as morphology (e.g., mean wetted and bankfull width, mean depth, and mean slope), riparian condition (e.g., riparian vegetation extent and mean canopy cover), habitat heterogeneity (e.g., % fast water, % large substrates, % fine substrates, and mean substrate embeddedness) and water quality (e.g., dissolved oxygen, pH, and alkalinity). We obtained Pearson correlations between those metrics and all the disturbance descriptors we had available: the 3 land uses percentages, the 11 types of local site disturbances, the LDI, the CDI and the IDI. All metrics significantly correlated ($p < 0.05$) with any of the disturbance descriptors were disregarded for the next step of the analysis. In this way, we filtered all the habitat metrics that could be affected by human disturbances of any kind; the remaining metrics were considered as sources of natural variation in the sites. Next, a Pearson product–moment correlation matrix was calculated with all the metrics not correlated with disturbance evidence. The redundant metrics ($r > 0.6$) were removed and the choice of the metrics to be retained was based on ecological rationale.

Among the 31 initial habitat metrics, many were not significantly correlated with human disturbances (9 in the Upper Araguari and 15 in the Upper São Francisco, Table 1). In both basins, some of the remaining metrics were removed because of high colinearities ($r > 0.6$). In the Upper Araguari, mean depth and mean residual pool area were removed and mean wetted width × mean thalweg depth was kept, because we believe the latter metric best summarized the stream channel size. In the Upper São Francisco, mean wetted width was removed and mean wetted width × mean thalweg depth was kept (same reason as above) and riparian canopy cover was removed and total riparian cover, a more embracing metric, was kept. Percentage of coarse substrates (>16 mm), percentage of fines (<0.06 mm: silt and clay), percentage of sand + fines (<2.0 mm), and log of the geometric mean substrate diameter had high correlations. We chose to include log of mean substrate diameter because it best represented the predominant substrate sizes of the sites.

We used the reduced set of habitat metrics to perform a hierarchical multiple regression, forcing the entrance of the integrated disturbance index (IDI) in the first block and allowing, in the second block, a best-subsets multiple regression procedure search for the combinations of habitat metrics that best explained the remaining variability in EPT richness. The R^2 values were considered as criteria for the selection of the best models. We restricted the number of predictor variables in the final models to a total of 4 (10% of 40 sites) to avoid model over-fitting (Harrell, 2001; Tabachnick and Fidell, 2007). Thus, in addition to the IDI in the first block, three habitat metrics were allowed to enter in the second block. Hierarchical multiple regression is an efficient way to isolate the contribution of some factor in a regression model because residual regressions can lead to biased estimations of the parameters of the models (see Freckleton,

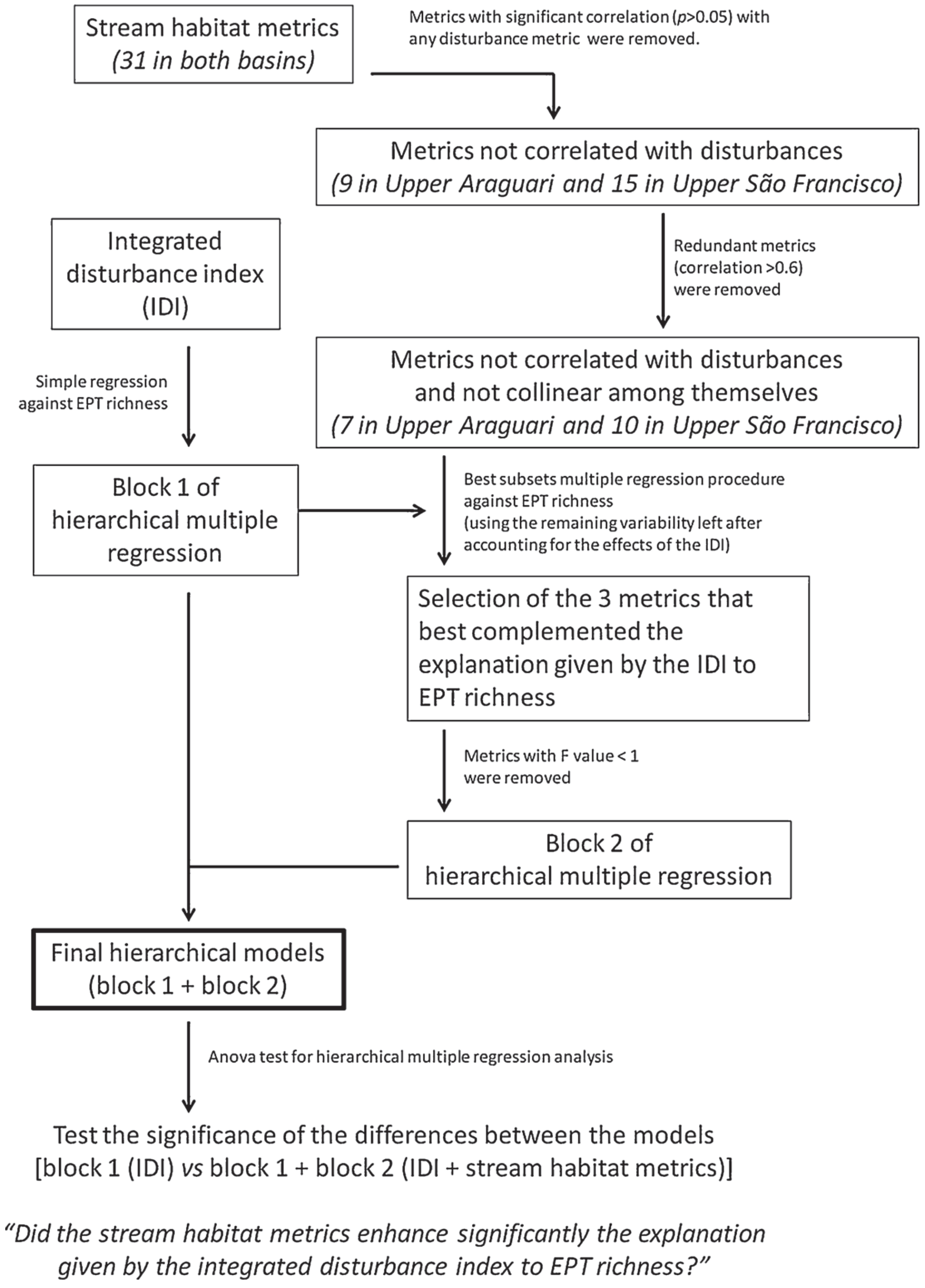

Fig. 3. Summary of the methodological design used to test statistically how site habitat metrics not subjected to human disturbances enhanced the explanation given by the integrated disturbance index (IDI) to EPT richness in each basin.

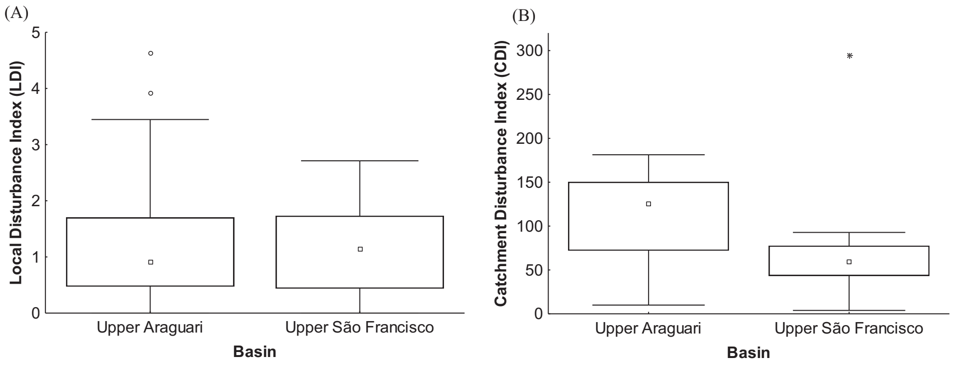

Fig. 4. Distribution (medians and quartiles) of the values of the (A) local disturbance index and of the (B) catchment disturbance index in each studied basin.

2002). Only predictor variables with individual *F*-values > 1 were allowed in the final models. The statistical significance of the hierarchical multiple regressions (block 1 vs block 1 + block 2) were tested through analysis of variance (ANOVA's). In this way we tested whether the habitat metrics contributed significantly to the explanation of EPT richness derived from the IDI for each basin.

3. Results

3.1. *Local and catchment disturbance indices and the disturbance plane*

The two basins had similar patterns in most LDI values (Fig. 4A), although the Upper Araguari basin had a few higher values, resulting from urban sites. On the other hand, the patterns of CDI values varied considerably between the basins, the Upper Araguari had higher CDI values than the Upper São Francisco (Fig. 4B). In the Upper São Francisco, only one site had a CDI value >100. The differing patterns are explained by the land use patterns in both basins (Fig. 5A–C). In the Upper Araguari we observed a higher proportion of agriculture in the catchments, whereas in the Upper São Francisco pasture predominated. Proportions of urban areas were low in both basins, most catchments having none. The Pearson correlations between the LDI and CDI scores were weak ($r = 0.21$ in the Upper Araguari and $r = 0.35$ in the Upper São Francisco).

In both basins few sites were located close to the origin on the disturbance plane (Fig. 6), but because of higher CDI values, more Upper Araguari sites were located farther from the origin. This distribution pattern is summarized by the different slopes of the disturbance gradients of the basins, showing the IDI values in ascending order (Fig. 7). In the Upper Araguari we observed a much wider range in site IDI values (i.e., more sites nearer and farther from the origin), indicating a much stronger disturbance gradient in that basin.

3.2. *Description of the EPT assemblages*

A total of 5463 EPT individuals (61 genera) were identified in Upper Araguari sites, and 15,133 EPT individuals (65 genera) were identified in Upper São Francisco sites. In both basins Ephemeroptera comprised the majority of the EPT genera (30 in the Upper Araguari and 35 in the Upper São Francisco) and number of organisms (3291 in the Upper Araguari and 12,529 in the Upper São Francisco). In the Upper Araguari, the most abundant genera were *Smicridea* (Trichoptera), and the Ephemeroptera *Thraulodes*, *Traverhyphes* and *Tricorythopsis*. Those four genera represented 43% of the EPT individuals collected in the Upper Araguari. In the Upper São Francisco, the most abundant genera were *Callibaetis*, *Cloeodes*, *Americabaetis*, *Caenis* and *Traverhyphes*, all Ephemeroptera. Those five genera represented 54% of the EPT individuals collected in the Upper São Francisco. Around 25% of the taxa identified in the Upper Araguari, and 20% of the taxa identified in the Upper São Francisco, can be considered rare taxa, with just 5 or fewer individuals identified across all sites of each basin.

3.3. *EPT richness versus disturbance indices*

The variation of EPT richness explained by the LDI and CDI together was much higher in the Upper Araguari ($R^2 = 0.40$) than in the Upper São Francisco ($R^2 = 0.18$) (Table 2). In both basins, EPT richness was significantly related to the CDI, but only in the Upper Araguari did the LDI contribute significantly to explain EPT richness variation (Table 2). The slope between LDI and EPT richness in the Upper São Francisco approached zero (Table 2). As expected, all significant relationships were negative. In the Upper Araguari, the IDI explained a moderate amount of EPT richness (Simple linear regression, $R^2 = 0.39$, $F_{(1,38)} = 24.6$, $p < 0.001$; Fig. 8A), nearly the same as the combined explanations given by the LDI and CDI in the multiple regression. In the Upper São Francisco, the IDI explained poorly, but significantly, EPT richness variation (simple linear regression, $R^2 = 0.11$, $F_{(1,38)} = 4.55$, $p = 0.039$; Fig. 8B).

Table 2
Multiple regression results for each basin with EPT richness as the response variable and the local disturbance index (LDI) and the catchment disturbance index (CDI) as predictor variables.

	F-Value (2,37)	*p*-Value	*R*-Square		Beta	Std. err. of beta	*t*(37)	*p*-Value
Upper Araguari	12.5	<0.001	0.403	Intercept			9.806	<0.001
				CDI	−0.450	0.130	−3.467	0.001
				LDI	−0.364	0.130	−2.802	0.008
Upper São Francisco	4.005	0.027	0.178	Intercept			8.820	<0.001
				CDI	−0.424	0.159	−2.671	0.011
				LDI	0.007	0.159	0.043	0.966

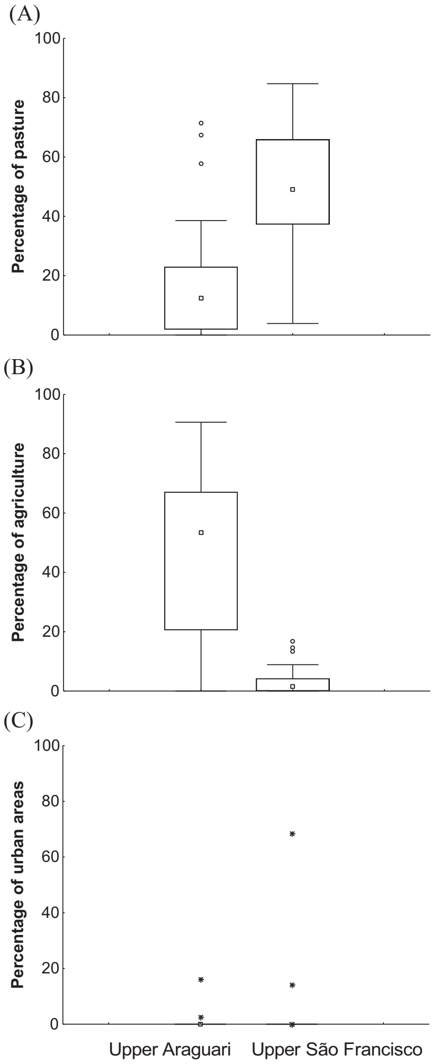

Fig. 5. Distribution (medians and quartiles) of the percentages of (A) pasture, (B) row crop agricultural and (C) urban areas in the catchments of the sites sampled in each basin.

3.4. Contribution of natural variability of habitat characteristics in explaining EPT richness

The hierarchical regressions informed how the explanations (R^2 values) given by the IDIs to EPT richness variations were increased by the addition of habitat metrics not related to human disturbances. In the Upper Araguari, the increment was low and just marginally significant (Table 3). In that basin, the R^2 value increased from 0.39 to 0.49, an increase of 0.1. On the other hand, in the Upper São Francisco the increment was much greater, the R^2 value rising from 0.11 to 0.50, an increment of 0.39. The amount of explanation given by the combined models (IDI + habitat metrics not correlated with disturbance) were similar in both basins, with R^2 values around 0.5, meaning that the final models explained only about half the variation.

The combined models generated from best-subsets multiple regressions had, in addition to the IDI, 2 habitat metrics in the Upper Araguari and 3 habitat metrics in the Upper São Francisco (all with *F*-values > 1, Table 3). In both basins, a site size metric (mean width × mean depth) was important in explaining EPT richness variation. In the Upper Araguari, another morphologic metric (bankfull width/depth) was incorporated in the model, whereas in the Upper São Francisco, microhabitat metrics (percent fast flows and log of mean substrate diameter) were included.

4. Discussion

4.1. Premises for comparisons between sites

It has been long recognized that some geographic (e.g., ecoregions) and non-geographic features (e.g., typologies) of stream sites exercise a strong influence on the composition and structure of their macroinvertebrate assemblages (Hughes, 1985, 1995; Gerritsen et al., 2000). Accordingly, it is important for the assigned reference sites and the test sites of a study to share these key biological drivers, allowing reliable comparisons between them (Herlihy et al., 2008). In the words of Gerritsen et al. (2000) it is important to "put like with like".

Gradual changes in the habitat template, in the available food resources, and in the biological assemblages naturally occur along the longitudinal gradient of lotic ecosystems (from spring to mouth), resulting mainly from downstream changes in their morphological dimensions, catchment areas and discharges (Vannote et al., 1980; Poole, 2002; Hughes et al., 2011). We reduced such sources of variation by selecting streams with similar morphological dimensions. All sites can be classified as small streams, close to the headwaters.

There is no geographic classification formally designed for Brazil that is comparable in detail to the ecoregion classifications of the USA (Omernik, 1995) or Europe (e.g., Gustafsson and Ahlén, 1996). However, the basins studied are in the same biome

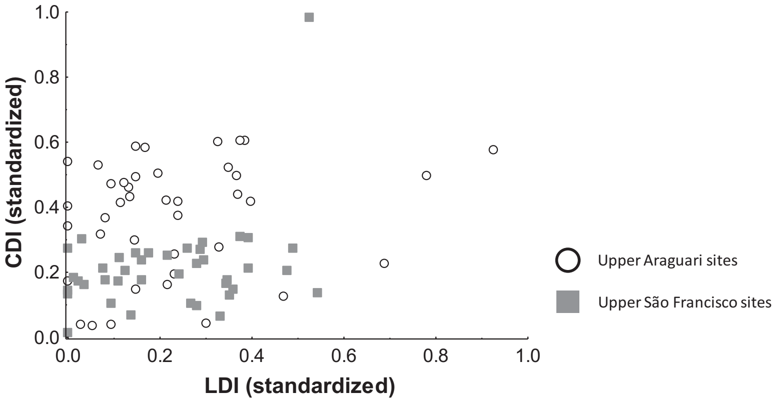

Fig. 6. Distribution of the sites of each basin in the disturbance plane, with Upper Araguari sites represented by open circles (○) and Upper São Francisco sites represented by filled boxes (■). The axes of the local disturbance index (LDI) and the catchment disturbance index (CDI) were standardized at the same scale (relative positions of the sites on each axis, and in the plane, were retained).

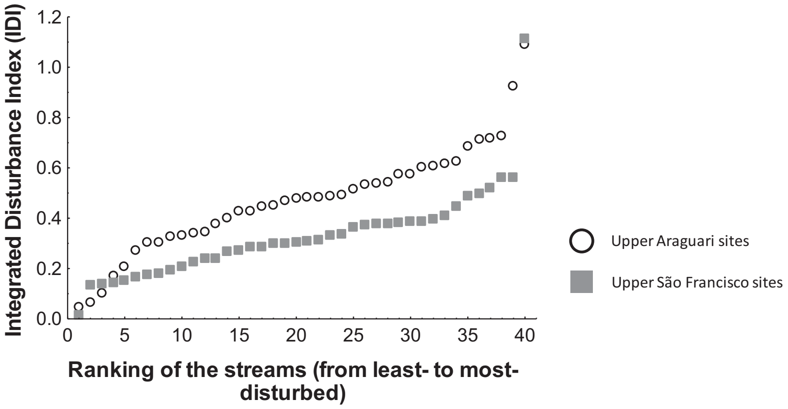

Fig. 7. Disturbance gradients in both basins, represented by ascending site values of the integrated disturbance index (IDI). Upper Araguari sites are represented by open circles (○) and Upper São Francisco sites are represented by filled boxes (■).

(Cerrado) and in the same general terrestrial and aquatic ecoregions outlined by Olson et al. (2001), meaning that the sites share similar climatic, edaphic, vegetation, geological and biogeographic conditions (Olson et al., 2001; Wantzen, 2003). Moreover, the basins were analyzed separately, and their individual areas are much smaller than those of the US level IV ecoregions, the most detailed level of their classification. Thus, although lacking an official detailed classification, we consider all the sites in the same ecoregion.

4.2. *The role of the disturbances measured at local and catchment spatial scales*

As stated in the classical view of stream impairment, human disturbances operating at multiple scales can alter patterns and processes of the natural habitat, ultimately leading to modifications or impairment of biological assemblages (Karr, 1999; Norris and Thoms, 1999; Bryce et al., 1999; Feld and Hering, 2007). However, the exact mechanistic pathways among the origins of impairment, the habitat modifications, and the biological responses are not well known in most cases (Bedford and Preston, 1988; Karr, 1991). For this reason, rather than searching for all the individual sources of impairment, it is important to develop a group of disturbance metrics that can serve as general indicators of the total pressure to which an ecosystem may be subjected (Boulton, 1999).

Disturbances in the channel or riparian zone can impair the habitats and the biota (Bryce et al., 1999; Death and Joy, 2004; Kaufmann and Hughes, 2006). Because catchments drive the stream features in almost every aspect (Hynes, 1975; Wiens, 2002), human land uses are also usually linked with the ecological condition of streams (Bryce et al., 1999; Allan, 2004; Wang et al., 2008). Non-point sources in catchments commonly contribute excess sediments, nutrients and pollutants to streams and rivers (Allan and Castillo, 2007; Allan, 2004). Human activities in the catchment also influence the condition of stream riparian zones (Van Sickle et al., 2004; Sponseller et al., 2001; Miserendino et al., 2011). The ordering of "disturbance potential" used in this study (urban areas having more weight than row crop agriculture, which in turn has more weight than pasture), as well as the use of the whole catchment area as the "buffer" to estimate catchment human pressures, are corroborated by many previous studies (Sponseller et al., 2001; Mebane et al., 2003; Wang et al., 2008; Gucker et al., 2009; Trautwein et al., 2011). In our study, disturbances measured at local and catchment spatial scales both reduced EPT richness, corroborating our first hypothesis. In agreement with Kail et al. (2012), catchment disturbances had a greater effect than local disturbances in these basins. The latter were not even significantly related to macroinvertebrate richness in the Upper São Francisco sites.

Local disturbance was not correlated with catchment disturbance. This lack of association means that catchment land uses were not driving near or in-stream modifications, and what is observed at one scale can differ from what is observed at the other. For instance, in our study we observed catchments highly dominated by row crop agriculture but with undisturbed riparian vegetation and stream channels. Conversely, we also had catchments with mostly natural land cover but stream channels altered by livestock. Scenarios like these are likely to happen elsewhere (Nijboer et al., 2004). Consequently, relying on just one scale to describe the level of human pressure at a site can lead to misleading interpretations of biological responses (Bryce et al., 1999; Feld and Hering, 2007).

Table 3
Hierarchical multiple regression results contrasting the significance of the differences between the regression models in each basin. The first models (block 1) consisted of simple regressions with EPT richness as the response variable and the integrated disturbance index (IDI) as the predictor variable. The second models (block 1 + block 2) included as predictor variables the habitat metrics selected by the best subsets procedure as those which, together with the IDI, better explained EPT richness. Habitat metric codes are defined in Table 1.

Basin		*F*-Value	*p*-Value	*R*-Square	Metrics' mean beta values				ANOVA test for hierarchical regression analysis [block 1 vs (block 1 + block 2)]	
									F-Value	*p*-Value
Upper Araguari	Model 1	24.6	<0.001	0.393	IDI −0.627				3.194	0.053
	Model 2	11.28	<0.001	0.484	IDI −0.523	XWXD 0.227	BKF_WDrat 0.299			
Upper São Francisco	Model 1	4.548	0.04	0.107	IDI −0.327				9.144	<0.001
	Model 2	8.726	<0.001	0.499	IDI −0.134	XWXD 0.245	pct_fast 0.414	lsub_dmm 0.276		

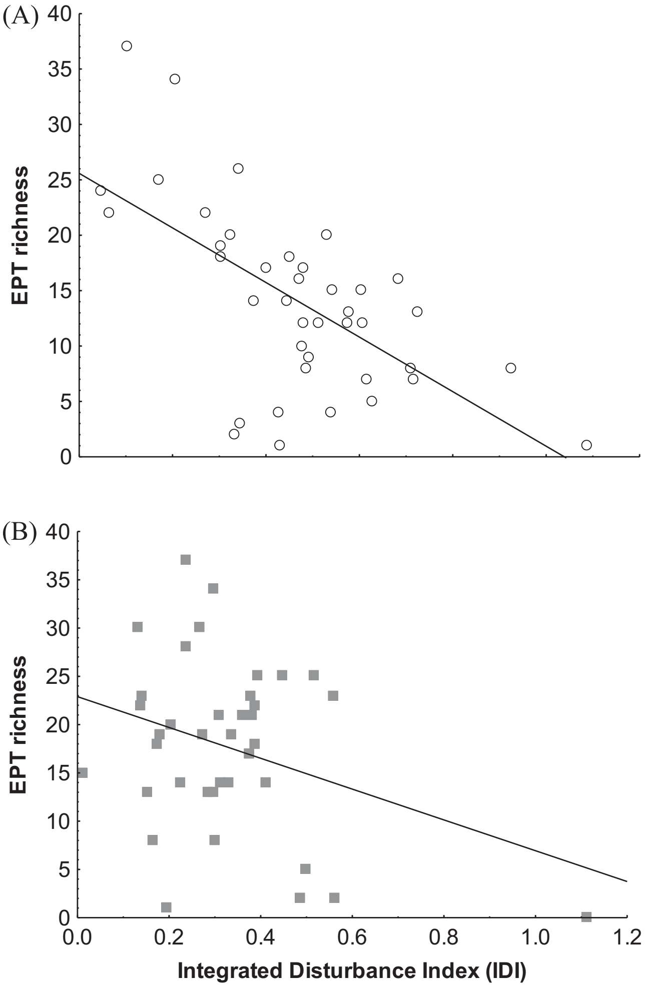

Fig. 8. Linear regressions between the integrated disturbance index (IDI) values and the EPT richness of the sites of (A) the Upper Araguari basin, represented by open circles (○), and (B) the Upper São Francisco basin, represented by filled boxes (■).

The integrated disturbance index (IDI) proved to be a useful and accurate univariate descriptor of the totality of disturbances measured at different spatial scales. It explained the variability in EPT richness better than separate local and catchment indices, and almost as well as when those two indices were separately included in multiple regression. The existence of a single index to summarize the overall ecological condition, although never perfect, is a quick and practical way to describe the condition of individual sites and the relative condition of a site in comparison to others (Bryce et al., 1999; Wang et al., 2008). This is necessary to set disturbance thresholds and to present to society and stakeholders an objective and simple measurement of site conditions (Hughes and Peck, 2008). The range and distribution of IDI values across a representative pool of sites can indicate the strength of the disturbance gradient in a region. The greater the range and evenness of the distribution of sites across that range, the greater the strength of the disturbance gradient (shown in the ascending ordinations of Fig. 7), and the greater the expected differences in ecological condition between the least- and the most-disturbed sites.

4.3. *The role of natural habitat variation*

The importance of natural stream habitat variation has been long recognized in stream ecology (Karr and Dudley, 1981; Allan and Castillo, 2007). Metrics related to hydromorphology (percentage of fast flows, mean wetted width × mean thalweg depth, bankfull width/depth, log of geometric mean substrate diameter), which were not related to human disturbances in these basins, helped explain EPT richness variability, apart from the effects that could be attributed solely to human influences. Those factors are commonly reported as important for structuring stream macroinvertebrate assemblages (Schmera and Erős, 2004; Brooks et al., 2005; LeCraw and Mackereth, 2010). Consistent with our second hypothesis, the relative and absolute contribution of the natural habitat was much more pronounced in the Upper São Francisco basin, which had a weaker disturbance gradient.

One conclusion emerging from our results is that if the anthropogenic disturbance gradient is not strong, the deleterious effect of human activities on assemblage richness will be mostly eclipsed by variation associated with stream habitat natural variability. In other words, the disturbance "signal" will be buried by habitat variation "noise" (Parsons and Norris, 1996; Gerth and Herlihy, 2006). As can be observed in the Upper São Francisco Basin (Fig. 8B), sites that were slightly more perturbed frequently had higher EPT richness than others that were slightly less perturbed. Many of these divergences in relation to what would be expected from the disturbance-only model were probably driven by differences in stream hydromorphology. In the Upper Araguari basin, which had a stronger disturbance gradient, those situations also occurred, but less frequently (Fig. 8A). A second conclusion is that the effort to control broad-scale drivers of biological assemblages through use of ecoregions and stream typologies does not eliminate the necessity to account for local habitat variability when comparing sites (Hughes et al., 1986; Waite et al., 2000; Pinto et al., 2009). Although we aimed to standardize the stream sizes, a size metric (mean width × mean depth) still explained significant differences in EPT richness. In addition, even neighboring sites may have highly dissimilar habitats and biological assemblages (Downes et al., 2000; Finn and Poff, 2005; Ligeiro et al., 2010), so that ecoregion standardization also is not enough.

The amount of EPT richness variability explained was similar in both basins (around 50%). This value can be considered high, given: (1) the intrinsic complexity and unpredictability of stream ecosystems and the difficulty of obtaining good models of them (Harris and Heathwaite, 2011), (2) the sources of variation not accounted for in this study, such as legacy effects (Allan, 2004) and conditions at upstream reaches (Kail and Hering, 2009) or at neighboring sites (Sanderson et al., 2005), and (3) the intrinsic unpredictability ("noise") related to seasonal and sampling variability (Kaufmann et al., 1999; Kaufmann and Hughes, 2006). We emphasize that the stream habitat contribution to richness explanation was analyzed in a very conservative way. To reliably determine the degree that natural habitat variability can add explanation at varying levels of disturbance strength, we dealt only with the habitat metrics not significantly correlated with any of the disturbance measurements we had available. In this regard, we even discarded metrics significantly but weakly correlated to disturbance (e.g., $r < 0.4$). Thus, we believe that habitat variability has a greater role in structuring macroinvertebrate assemblages than shown in our results, because those rejected habitat metrics that were related to human disturbances were also driven by natural variability to some degree (King et al., 2005).

4.4. *Importance of the construction of a disturbance gradient*

The explicit, quantitative determination of a disturbance gradient is more advantageous than a set of disturbance categories because distinct separations in ecological conditions should be rare in any group of sites (Whittier et al., 2007b; Herlihy et al., 2008). This is true for all sites we call reference, least-disturbed, most-disturbed, or impaired. Depending on the intensity and extent of human influences in the landscape, sometimes it is necessary to relax the stringency of the acceptance thresholds in order to find least-disturbed conditions (Stoddard et al., 2006; Whittier et al., 2007b; Herlihy et al., 2008). So it is important to recognize the relativity of terms like “least”, or “most”, when describing ecological condition (Stoddard et al., 2006). Absolute, “boxed” designations, although comfortable and operationally easier to handle, can lead to misunderstandings or erroneous comparisons among studies simply because the true ecological conditions of the sites along the disturbance gradient continuum were not explicitly stated.

Often the designations of reference and most-disturbed sites are made prior to sampling (Bailey et al., 2004). GIS data and techniques have been widely applied when screening for reference sites (Collier et al., 2007; Yates and Bailey, 2010) and field reconnaissance is strongly recommended (Hughes et al., 1986; Yates and Bailey, 2010). Yet, even in those cases we encourage researchers to quantitatively re-assess the disturbance gradient after field sampling to check the validity of any previous classifications and the exact quantitative difference in the conditions between the “reference” and “test” sites.

4.5. The benefits, scope and further possibilities of the proposed methodology

The disturbance plane conceived in this work, visually describing the intensity of human disturbances at both local and catchment scales, established an easy and intuitive way to describe the total amount of pressure at sites. The disturbance plane facilitates comparisons of site conditions in a more straightforward and specific manner, quantitatively positioning each site along a disturbance continuum, rather than assigning labels to the sites. When necessary, labels such as “minimally-”, “least-” and “most-disturbed” can be assigned to sites based on quantitative data versus subjective decisions. Objective criteria and quantitative approaches to select reference sites have been proven more efficient for selecting the “best” sites (Whittier et al., 2007b), and the same may be true for selecting the “worst” ones.

Because only direct observations of human activities were used to describe anthropogenic pressure, further characterization of the chemical and physical habitat of the least- and most-disturbed sites can be made without incurring any conceptual circularity. As addressed before, metrics like dissolved nutrient concentrations, riparian cover and sediment sizes, although commonly associated with human modifications, are also subject to natural variability (King et al., 2005; Miserendino et al., 2011). For example, in this study no land use measurement or local modification was correlated with nutrient concentrations (total phosphorous and total nitrogen). Low nutrient concentrations are common in Cerrado streams because of naturally oligotrophic soils (Wantzen, 2003). In the Upper São Francisco, no evidence of disturbance was correlated with substrate sizes and riparian vegetation cover (Table 1). So, in accord with Bailey et al. (2004), natural patterns, not researchers' opinions, should be used to characterize reference condition attributes.

The proposed methodology was well suited for describing the disturbance gradient of the 40 sites we studied in each basin. When necessary, sites from different regions can be incorporated in the same disturbance plane (as shown in Fig. 6). We believe that this methodology is also applicable to larger datasets, although further research is needed to confirm this assumption and to compare outputs generated through other approaches.

Depending on researcher preferences and the amount of data available, local and/or catchment disturbance indices can be calculated in different ways, perhaps using different disturbance measurements. For instance, other commonly used metrics to characterize human pressure include human population density, livestock density, number of dwellings and road density (Wang et al., 2008; Brown et al., 2009). If one desires further changes in this methodology, more disturbance axes can be added to the model, perhaps representing factors considered key stressors in particular studies (e.g., dams and toxic substances). This will generate n-dimensional disturbance polygons, rather than the bi-dimensional disturbance plane presented in this work. Although such refinements erode the simplicity and visual appeal of the model, they could improve the accuracy of the integrated disturbance quantifications of the sites (Danz et al., 2007).

In our study, the IDI was a reliable univariate measurement of site disturbance status. The IDI is also a good tool for describing the disturbance gradient strength in a pool of sites, via the range and distribution of its values. So, rather than a standardized and rigid methodology, we offer a flexible and adaptive framework for characterizing and quantifying disturbance in many situations.

5. Conclusions

We showed through our results that a reliable and comprehensive characterization of human pressures on streams relies on the use of different tools and should integrate data from different spatial scales. In our study, local and catchment disturbances were not correlated, and both independently affected site EPT assemblages. The proposed methodology quantified the human pressure on sites without resorting to naturally varying habitat metrics. We demonstrated that the strength of the disturbance gradient influenced the degree to which natural habitat variability explained EPT richness variation, a finding that has important implications for biomonitoring studies. Thus, the use of quantitative disturbance gradients is essential for efficient use of ecological indicators and we advise researchers to define quantitatively the disturbance status of their study sites. In this study we presented a framework for doing so.

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Capítulo 2

The problem of using fixed-area subsampling methods to estimate macroinvertebrate richness: a case study with Neotropical stream data

Raphael Ligeiro [a], Wander Ferreira [a], Robert M. Hughes [b] and Marcos Callisto [a]

[a] Universidade Federal de Minas Gerais, Instituto de Ciências Biológicas, Departamento de Biologia Geral, Laboratório de Ecologia de Bentos, Av. Antônio Carlos, 6627, CP 486, CEP 30161-970, Belo Horizonte, Minas Gerais, Brazil.

[b] Oregon State University and Amnis Opes Institute, Department of Fisheries & Wildlife, Nash Hall, 97331-4501, Corvallis, Oregon, USA.

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The problem of using fixed-area subsampling methods to estimate macroinvertebrate richness: a case study with Neotropical stream data

**Raphael Ligeiro · Wander Ferreira ·
Robert M. Hughes · Marcos Callisto**

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Abstract Subsampling has been widely applied in the laboratory to process freshwater macroinvertebrate samples. Currently, many governmental agencies and research groups apply the fixed-count approach, targeting a number of individuals per sample, and at the same time keeping track of the number of quadrats (fraction of the sample) processed. However, fixed-area methods are still in use. The objective of this paper was to evaluate the reliability of macroinvertebrate taxonomic richness estimates developed from processing a standard number of subsampling quadrats (i.e., fixed-area approaches). We used a dataset from 18 tropical stream sites experiencing three different levels of human disturbance (most-, intermediate-, and least-disturbed). With 12 quadrats processed (half the sample), the collection curves started to stabilize, and for more than half of the sites studied, it was possible to sample at least 80 % of the total taxonomic richness of the sample. However, we observed that the minimum number of quadrats to achieve 80 % of taxonomic richness was strongly negatively correlated with the number of individuals collected in each site: the fewer the individuals in a sample, the greater the processed proportion of that sample needed to represent it properly. Thus our results indicate that for any given areal subsampling effort (any fixed fraction of the sample), samples with different numbers of individuals will be represented differently in terms of the proportion of the total number of taxa of the whole samples, those with greater numbers being overestimated and those with fewer numbers being underestimated. Therefore, we do not recommend the use of fixed-area subsampling methods alone if the main purpose is to measure and analyze taxonomic richness; instead, we encourage researchers to use fixed-count approaches for this purpose.

Keywords Subsampling methods · Taxonomic richness · Laboratory procedures · Disturbance categories · Stream research

Introduction

Taxonomic richness is a key measurement for assessing biological assemblage diversity at many spatial scales (Gotelli and Cowell 2001), including macroinvertebrate assemblages (Melo and Froehlich 2001). Taxonomic richness is the basis for many ecological models (MacArthur and Wilson 1967; Lande 1996; Arita and Vazquez-Dominguez 2008) and a common

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R. Ligeiro (✉) · W. Ferreira · R. M. Hughes · M. Callisto
Departamento de Biologia Geral,
Universidade Federal de Minas Gerais,
Belo Horizonte, Minas Gerais, Brazil
e-mail: ligeirobio@gmail.com

R. M. Hughes
Amnis Opes Institute,
Corvallis, OR 97333, USA

R. M. Hughes
Department of Fisheries and Wildlife,
Oregon State University,
Corvallis, OR 97333, USA

component of multimetric indices used in ecosystem biomonitoring (Barbour et al. 1999; Klemm et al. 2003; Baptista et al. 2007; Stoddard et al. 2008; Suriano et al. 2011). Thus, taxonomic richness is a cornerstone of both basic and applied studies dealing with macroinvertebrate assemblages, as well as for developing conservation strategies for watersheds (Clarke et al. 2010; Richardson and Whittaker 2010). Accordingly, measuring taxonomic richness, whether expressed in terms of species, morphospecies, genera, or families, is often a key objective when processing samples of freshwater macroinvertebrate assemblages (Clarke et al. 2008).

Despite the importance of taxonomic richness in macroinvertebrate studies, representing taxonomic richness of samples is not an easy task (Vinson and Hawkins 1996). In the laboratory, processing the whole sample is often impracticable because programs and researchers have limited time, money, and personnel resources (Nichols and Norris 2006). In many studies dealing with large spatial extents and high numbers of samples, processing the whole sample is not a viable option (Hughes and Peck 2008). Taking portions of the samples, i.e., subsampling, has been widely applied to resolve this dilemma. There are two major ways to subsample: fixed-count and fixed-area methods (Barbour and Gerritsen 1996). Both can be applied to represent different sampling spatial extents, from microhabitat samples to site composite samples. In fixed-count methods, a fixed number of individuals is randomly picked and identified from the homogenized sample. In the fixed-area methods, also known as fixed fraction and proportional subsampling, the whole sample is homogenized and a fixed proportion of it is then fully processed. This is usually accomplished by spreading the sample in a divided tray and processing a certain number of “quadrats,” proportional subdivisions of the tray (Oliveira et al. 2010).

Currently, many governmental agencies and research groups apply the fixed-count approach, targeting a number of individuals per sample, and at the same time recording the number of quadrats processed, to also measure individual densities (e.g., Moulton et al. 2000). The number of individuals processed usually varies between 100 and 500, depending on the study objectives, the amount of available resources, and the agency/research group (Carter and Resh 2001). For example, the United States Environmental Protection Agency applies a combined quadrat, fixed-count (500 individuals), including a large/rare search, in its macroinvertebrate sample processing (Stoddard et al. 2008). However, we doubt whether every research group follows this approach. Fixed-area subsampling has been considered a traditional method for a long time (Barbour and Gerritsen 1996), and recently, many publications presented results on subsampling effort and metric variability for different fractions of the sample processed (e.g., King and Richardson 2002; Petkovska and Urbanic 2010; Oliveira et al. 2010). However, these authors were concerned with many other metrics in addition to taxonomic richness.

The purpose of subsampling methods is to reduce the amount of work in the laboratory and still obtain a dataset not biased by the procedure, capable of reliably representing the samples and precisely answering the research questions (Wrona et al. 1982; Barbour and Gerritsen 1996). Our objective in this paper was to evaluate the reliability of macroinvertebrate taxonomic richness estimates developed from processing a standard number of subsampling quadrats. To do so, we used a dataset from tropical stream sites experiencing three different levels of human disturbance (most-, intermediate-, and least-disturbed).

Materials and methods

Field sampling

We sampled 18 headwater stream sites located in the Araguari River Basin, Minas Gerais, southeastern Brazil, during the dry season of 2009. In this period, discharge is more constant, habitats are most distinct, and macroinvertebrate densities are usually higher (Callisto et al. 2001). Sites ranged from Strahler order 1–3 on 1:50,000 scale maps, with mean wetted channel widths ranging from 1–5 m. The altitudes varied little, ranging from 823 to 954 m. According to the disturbance level of the sites and their catchments, sites were classified a priori as least-disturbed, most-disturbed, and intermediate-disturbed. Least-disturbed sites had clear water, well-developed riparian vegetation, and high in-stream habitat complexity. Their catchments were inside well-preserved areas, some of them inside conservation units, with minor or no human habitation and land use. Most-disturbed sites were inside catchments of small urban areas and/or high agricultural land use. They had poor water quality and evidence of numerous human alterations in their channels, such as absence of riparian vegetation, presence of trash, pipes, fine sediments, and simplified habitats. Our most-disturbed sites lacked high organic impairment; rather they were characterized as having simplified hydromorphology. Intermediate-disturbed sites, although having little evidence of human alterations of their channels or riparian vegetation, were inside highly disturbed catchments, mostly by agriculture and pasture. Six sites of each disturbance level were sampled. At each site, 11 kick net samples (500 µm mesh, 30 cm mouth width) were taken systematically in a zigzag pattern along the whole site and combined, generating one composite macroinvertebrate sample per site (Peck et al. 2006; Hughes and Peck 2008). A total area of 1 m^2 was sampled in each site, and all samples were preserved in 10 % formalin and stored in tightly sealed plastic buckets.

Laboratory and subsampling procedures

In the laboratory, the samples were first washed through 500 µm sieves to remove much of the mineral substrate (mud, sand, fine gravel, etc.) and larger twigs and leaves. Sample material was then placed in a white enamel tray 36×66×7.5 cm. The tray was half filled with water and the sample was homogenized for 5 min. After that, a metal grid of the same dimensions as the tray was placed upon the sample. This metal grid consisted of 4×6 quadrats (24 in total); each quadrat measured 8.5×10.5 cm and corresponded to nearly 4.2 % of the total sample. The material of each quadrat was carefully removed and stored in plastic jars containing 70 % alcohol. All material that was more than halfway inside a quadrat was considered as part of that quadrat. The macroinvertebrates of each jar were fully sorted and identified to family through use of taxonomic keys (Pérez 1988, Fernández and Domíngues 2001; Costa et al. 2006).

Data analyses

We calculated processing effort (collection) curves for macroinvertebrate taxonomic richness for any given number of quadrats using a randomization technique (1,000 times). We used box plots to show the mean relative richness sampled in each stream along the 24 quadrats processed. We also used the slope of the collection curves to determine when the curves were starting to level off. For each stream site, the slope was calculated by dividing the mean richness predicted in a quadrat n by the mean richness predicted in the quadrat $n+1$. These values expressed the average percentage of richness that is gained by processing a subsequent quadrat. A threshold slope of 3 % was defined as indicating curve leveling. A 3 % slope is less restrictive than a 1 % slope (which occurred with few curves, even when almost all the quadrats were processed) and not as permissive as a 5 % slope (which represents a curve still increasing considerably).

We calculated the minimum number of quadrats (MNQ) for each stream to achieve 80 % of the total richness observed in its whole sample, which we deemed a satisfactory amount in terms of subsampling. The MNQ values were regressed against the number of organisms, equitability, and Shannon–Wiener and Simpson indices of the macroinvertebrate assemblages calculated for the whole samples to verify which sample characteristics influenced the MNQ observed, i.e., which characteristics were responsible for differences in the subsampling effort.

Lastly, we calculated the average number of quadrats necessary to produce 200 and 300 individuals from the stream site samples. These numbers are common goals in many subsampling protocols (e.g., Norris et al. 1995; Carter and Resh 2001; Lorenz et al. 2004). Some protocols require 500 individuals per sample (e.g., Stoddard et al. 2008), but the invertebrate densities of the sites we studied were low and many of our samples did not yield 500 individuals.

Results

We collected 11,994 macroinvertebrate individuals and 66 families from the 18 sites. The Insecta comprised the majority of both individuals (96 %) and families (89 %). The relative abundance of taxa followed the common pattern of stream invertebrate assemblages, with few very abundant taxa and many rare ones. The six most abundant families were, in decreasing order: Chironomidae, Elmidae, Simuliidae, Leptophlebiidae, Leptohyphidae, and Baetidae. Each family was represented by >500 individuals, and together, they included 78 % of all individuals collected in the study. On the other hand, almost half of the taxa collected (31 families) can be considered rare; each family being represented by <0.1 % of all individuals collected.

The majority of the families of the insect orders Diptera (Dixidae, Dolichopodidae, Psychodidae, Phoridae, Muscidae, Syrphidae, Tabanidae, and Stratiomyidae), Heteroptera (Belostomatidae, Gerridae, Veliidae, Mesoveliidae, and Notonectidae), and Coleoptera (Curculionidae, Gyrinidae, Dryopidae, Lutrochidae, Noteridae, Scirtidae, and Ptilodactylidae) were rare.

Biotic metric values, based on entire samples, differed among sites with different disturbance levels (Table 1). Both least-disturbed and intermediate-disturbed sites had higher median values than most-disturbed sites for taxonomic richness, number of organisms, equitability, and Shannon–Wiener and Simpson indices. Median metric values and ranges for least-disturbed and intermediate-disturbed sites were similar, with least-disturbed values being slightly higher in most cases.

The box plots generated from the mean values of subsampling effort for all 18 stream sites show that the curves did not reach an asymptote (Fig. 1a). Some proportion of the richness sampled is gained until the last quadrat (the 24th) is processed, although the gain ratio decreases greatly after the 12th quadrat is processed. We observed an average slope of 3 % or less with 12 quadrats processed (half the sample), for more than half the sites studied (Fig. 1b).

With 12 quadrats being processed, it was also possible to gather at least 80 % of the total richness of the whole samples for at least half of the sites studied. However, we observed from the regressions that the minimum number of quadrats to achieve 80 % of taxonomic richness were strongly negatively correlated with the number of individuals collected in each stream (linear regression, adjusted $R^2=0.58$, $F=24.53$, $p<0.001$, Fig. 2a). The other metrics of the whole site samples (equitability, Shannon–Wiener and Simpson indices) were not correlated significantly with the subsampling effort (p value >0.05 in all cases, Fig. 2b–d).

There was considerable variation in the mean number of quadrats processed needed to achieve a predetermined number of 200 and 300 individuals (Fig. 3). On average, from 3 to 24 quadrats were needed to yield 200 individuals and from 5 to 24 quadrats were needed to yield 300 individuals. Five sites did not yield 300 individuals even when their entire samples were processed. A mean of four more quadrats were needed to produce 300 individuals versus 200 individuals. In sites with high invertebrate densities, an average of two to three quadrats more were needed, and in sites with the lowest invertebrate densities, four to eight more quadrats were needed (Fig. 3). Most-disturbed sites tended to require more quadrats, which were associated with their lower organism densities compared with the intermediate- and least-disturbed sites. On the other hand, the intermediate- and least-disturbed sites included sites with low and high organism densities, and the number of quadrats varied accordingly (Fig. 3).

Discussion

Some authors advocate towards processing entire samples in the laboratory (e.g., Courtemanch 1996; Doberstein et al. 2000). Their argument is that this would be the only way to record all the rare species collected, which represent a major part of macroinvertebrate biodiversity. However, it has been long recognized that subsampling procedures are necessary to complete most studies dealing with large spatial extents and many samples, in this way saving money

Table 1 Median values and ranges (in parentheses) of biotic variables for the entire samples from the 18 study sites (six sites for each disturbance category)

Characteristics of the macroinvertebrate assemblages (whole samples)	Category of human disturbance of the streams		
	Most-disturbed	Intermediate-disturbed	Least-disturbed
Total assemblage family richness	19 (13–32)	29 (21–39)	30 (27–40)
Total assemblage number of individuals	415 (172–986)	780 (184–1,620)	676 (215–1,423)
Total assemblage equitability	0.53 (0.33–0.74)	0.61 (0.52–0.68)	0.62 (0.59–0.71)
Total assemblage Shannon–Wiener index	1.63 (0.89–2.25)	2.10 (1.77–2.25)	2.20 (2.03–2.36)
Total assemblage Simpson index	0.65 (0.38–0.72)	0.79 (0.70–0.82)	0.81 (0.73–0.85)

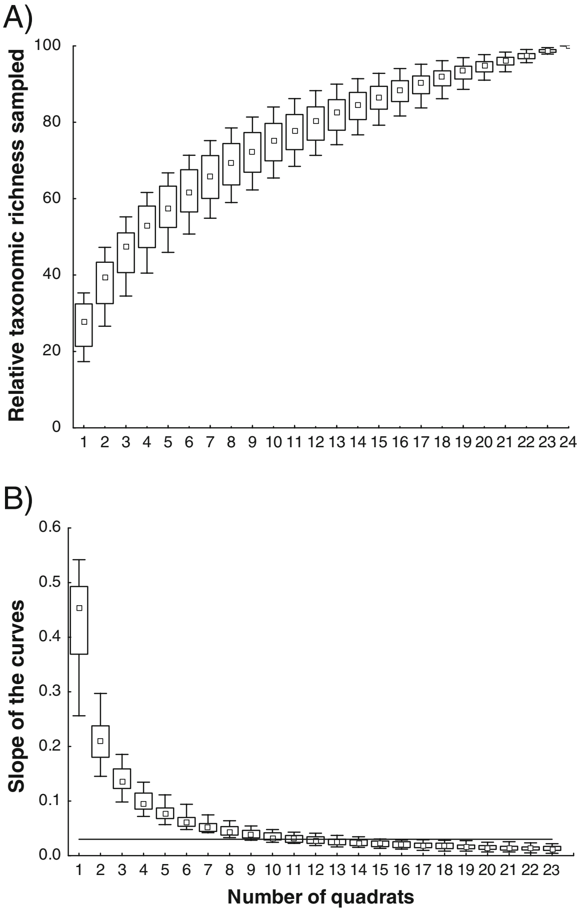

Fig. 1 Subsampling effort (1 to 24 quadrats) versus **A** relative taxonomic richness sampled and **B** curve slope. The *black line* at the bottom of the graph indicates the 3 % slope threshold. In both graphs, the *boxes* represent the median/interquartile of the 18 streams' mean values generated in the randomization procedure (1,000 times per subsampling effort per site). The *error bars* represent the range (maximum and minimum values)

and obtaining viable and timely answers (e.g., Vinson and Hawkins 1996; Hughes and Peck 2008). Currently, an increasing number of research groups are opting for subsampling methods (Carter and Resh 2001) and, besides knowing about the uncertainty associated with the subsampling methods, it is important to ensure that the methods are appropriate and that the data obtained are being properly interpreted.

Hurlbert (1971) distinguished between *numerical species richness* (or simply species richness), calculated as a function between the number of species observed and the number of individuals present in a sample, and *areal species richness* (or species density), calculated as a function of the number of species observed in a given field plot. Some researchers argue that fixed-area subsampling approaches are a solution to standardize areal richness among different samples, enabling comparisons among them (e.g., Courtemanch 1996; Petkovska and Urbanic 2010). However, we observed a strong relationship between the number of individuals in the whole sample and the difficulty in representing its macroinvertebrate areal richness (i.e., to reach some proportion of the total number of taxa in the sample). That is, the fewer the individuals in a sample, the greater the proportion of that sample that must be processed to represent any proportion of its total richness. In our study, for a most-disturbed site with low abundance of individuals, a mean of 16 quadrats were necessary to achieve 80 % of the total richness of the sample. On the other hand, in a least-disturbed site with high abundance of individuals, a mean of nine quadrats sufficed to achieve 80 % of the total richness of the sample; almost half of the number of quadrats needed in the first case. Other characteristics of the samples, like equitability and diversity indices, were not affected by subsampling effort. Initially, we assumed that when few individuals were present in the whole sample, it would be easier to determine sample taxonomic richness by processing few quadrats; however, we observed the opposite. When a sample contained few individuals distributed evenly among the quadrats (the standard subsampling procedure), the taxa were not evenly distributed in the tray. Consequently, we continued to find new taxa even after processing many quadrats. This pattern was strengthened by the existence of many rare families in the samples, as mentioned previously.

Our results indicate that for a given proportional subsampling effort (any fixed number of quadrats), samples with different numbers of individuals will be represented differently, in terms of the percentage of the total number of taxa of the samples. Samples with high numbers of individuals will be better represented than samples with low numbers of individuals. This pattern is likely to create a bias that is difficult to avoid using a fixed-area subsampling approach when one has samples with a wide range of abundances, thereby artificially enhancing statistical differentiation of taxonomic richness between low and high density sites. Therefore, although knowing about areal richness would be useful and ecologically meaningful in some cases (Courtemanch 1996; Gotelli and Cowell 2001), it is only possible to measure and analyze it reliably

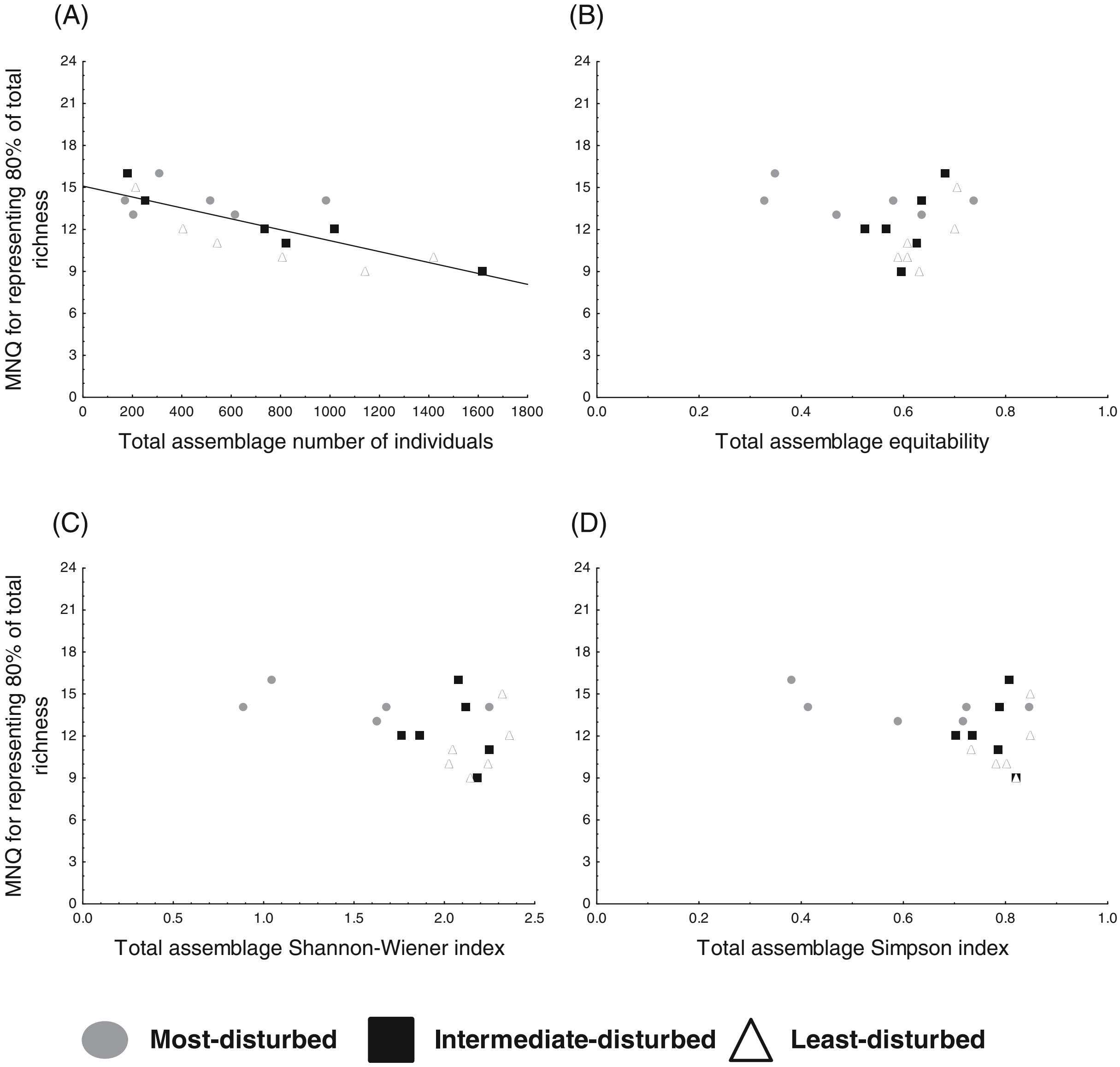

Fig. 2 Minimum number of quadrats (*MNQ*) necessary to represent 80 % of the total macroinvertebrate richness of the samples regressed against **A** total assemblage number of individuals, **B** total assemblage equitability, **C** total assemblage Shannon–Wiener index, and **D** total assemblage Simpson index. The stream disturbance levels are represented by *symbols*

when the entire sample is processed. This finding adds to the list made by Larsen and Herlihy (1998) regarding some practical disadvantages of the use and implementation of fixed-area subsampling approaches.

Sensu Gotelli and Cowell (2001), who revisited established concepts in community ecology (Arrhenius 1921; Preston 1948), stated that taxonomic richness found in a given sample depends on both the area sampled in the field and the number of individuals collected. Gotelli and Cowell (2001) clearly demonstrated that sites usually differ in the "densities" of their richness distribution, and if the number of individuals is not standardized, it is likely to produce erroneous richness comparisons and interpretations. Gotelli and Cowell (2001) compared tree species richness of old-growth and second-growth forests. Given

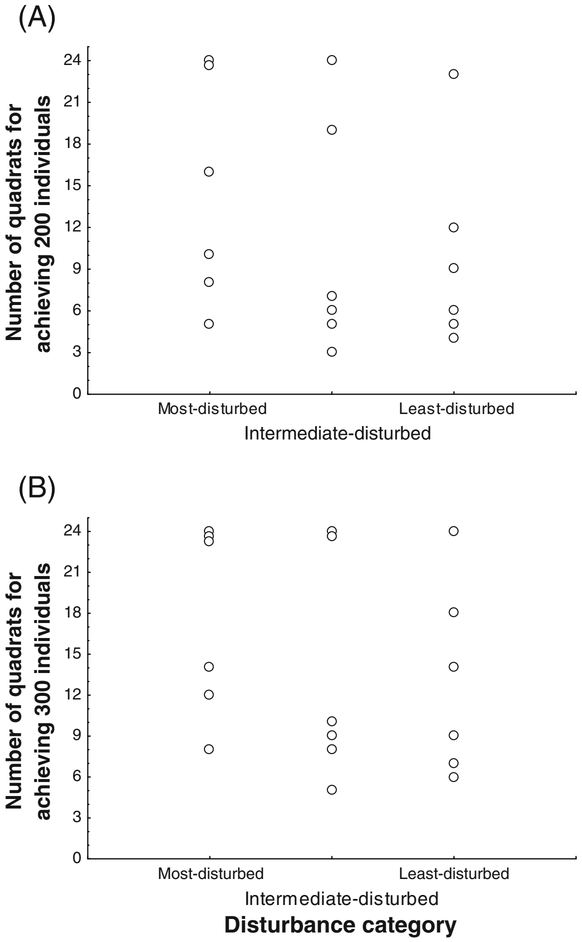

Fig. 3 Average number of quadrats needed to achieve a fixed-count of **A** 200 individuals and **B** 300 individuals from the samples

the same area sampled, they observed greater tree richness in second-growth forests, a pattern that is inverted when the tree density of the plots was also considered and the comparisons calibrated through individual rarefaction. The same analytical artifact was observed in a study with stream macroinvertebrates, regarding the effect of disturbance on the macroinvertebrate richness of artificial substrates (McCabe and Gotelli 2000). Not surprisingly, Barbour and Gerritsen (1996), Vinson and Hawkins (1996), and King and Richardson (2002) found individual rarefaction a more efficient way to compare sites. The standardization of the site area sampled has become a common procedure in field protocols (e.g., Barbour et al. 1999; Hering et al. 2004; Peck et al. 2006; Hughes and Peck 2008; Oliveira et al. 2011). The standardization of the number of individuals has not been used with the same frequency, but increasingly researchers are using rarefaction, statistical estimators, or sampling effort standardization to calibrate statistical comparisons (Cao et al. 2007).

If a given number of individuals is defined (fixed-count method), it is also pointless to set a minimum number of quadrats to process. Because samples usually vary greatly in their individual abundances, the numbers of quadrats necessary to achieve a given number of individuals will also vary greatly. In our study, this varied from as few as three quadrats to the whole sample if the goal was to reach 200 individuals. Setting a minimum number of quadrats can overestimate richness in highly abundant samples, which for instance need fewer quadrats to reach any given count of organisms.

Conclusions

Although measurement of taxonomic density (the number of species found in a certain area) is the goal of some researchers, it is not reliably accomplished though fixed-area subsampling procedures. The consequence of fixing any fraction of the sample is an overestimation of the areal richness in some samples and an underestimation of it in others. Considering this, and also the importance of the use of individual-based rarefaction to compare taxonomic richness of different samples, we do not recommend the use of fixed-area subsampling alone if a key purpose of the research is to measure and analyze taxonomic richness. We encourage researchers, as is being implemented by many groups, to set a given number of individuals per sample as a goal (preferably enough to reach some stability in richness) and at the same time to record the number of quadrats processed, thereby also providing information about individual densities.

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Capítulo 3

Macroinvertebrate responsiveness to anthropogenic disturbances in streams varies with field and laboratory methodologies

Raphael Ligeiro [a]*, Robert M. Hughes* [b]*, Philip R. Kaufmann* [c]*, Adriano S. Melo* [d] *and Marcos Callisto* [a]

[a] Universidade Federal de Minas Gerais, Instituto de Ciências Biológicas, Departamento de Biologia Geral, Laboratório de Ecologia de Bentos, Av. Antônio Carlos, 6627, CP 486, CEP 30161-970, Belo Horizonte, Minas Gerais, Brazil.

[b] Oregon State University and Amnis Opes Institute, Department of Fisheries & Wildlife, Nash Hall, 97331-4501, Corvallis, Oregon, USA.

[c] U.S. Environmental Protection Agency, Office of Research & Development, National Health & Environmental Effects Lab., Western Ecology Division, 200 SW 35 Street, 97333, Corvallis, Oregon, USA.

[d] Universidade Federal de Goiás, Instituto de Ciências Biológicas, Departamento de Ecologia, Campus II, Samambaia, CP 131, CEP 74001-970, Goiânia, Goiás, Brazil.

Macroinvertebrate responsiveness to anthropogenic disturbances in streams varies with field and laboratory methodologies.

Raphael Ligeiro[1]*, Robert M. Hughes*[2]*, Philip R. Kaufmann*[3]*, Adriano S. Melo*[4] *and Marcos Callisto*[1]

[1] Universidade Federal de Minas Gerais, Instituto de Ciências Biológicas, Departamento de Biologia Geral, Laboratório de Ecologia de Bentos, Av. Antônio Carlos, 6627, CP 486, CEP 30161-970, Belo Horizonte, Minas Gerais, Brazil.

[2] Oregon State University and Amnis Opes Institute, Department of Fisheries & Wildlife, Nash Hall, 97331-4501, Corvallis, Oregon, USA.

[3] U.S. Environmental Protection Agency, Office of Research & Development, National Health & Environmental Effects Lab., Western Ecology Division, 200 SW 35 Street, 97333, Corvallis, Oregon, USA.

[4] Universidade Federal de Goiás, Instituto de Ciências Biológicas, Departamento de Ecologia, Campus II, Samambaia, CP 131, CEP 74001-970, Goiânia, Goiás, Brazil.

Abstract

Detection of human disturbance impacts using stream macroinvertebrates depends on proper field and laboratory methods. We compared the responsiveness to human disturbances for macroinvertebrate assemblage metrics and composition using commonly employed alternative combinations of field sampling and taxonomic enumeration. Six datasets for our comparisons were derived by: full processing of all individuals or simulations of fixed-count subsampling of 300 individuals per sample of samples obtained from 1) multihabitat sampling, 2) targeted sampling on leaf packs, or 3) the sum of the individuals collected in the two previous sampling methods. Our study was conducted on 39 headwater stream reaches in a relatively small basin of the Brazilian Cerrado. We used a previously published quantitative disturbance index to define least- and most-disturbed sites. When processing all individuals, differences in taxonomic richness and assemblage composition of macroinvertebrate assemblages between least- and most-disturbed sites were more pronounced in multihabitat sampling than sampling on leaf packs. However, multihabitat sampling produced weaker results than targeted sampling if 300 individuals were subsampled. No methodology was best in all cases, the choice depending on the particular objectives of the study and time and resource constraints. We caution researchers that biomonitoring studies conducted across small areas may be more sensitive to changes in field sampling methods than studies conducted over large areas.

Key-words: Biomonitoring programs, multihabitat sampling, leaf packs, subsampling procedures, methodology performance.

Introduction

Macroinvertebrate assemblages are good indicators of stream ecological condition (Karr and Chu 1999, Bonada 2006). Total taxa richness and the percentage of Ephemeroptera, Plecoptera and Trichoptera (EPT) individuals are among the most commonly used metrics in multimetric indices (MMIs; Barbour et al. 1999, Klemm et al. 2003, Stoddard et al. 2008). Most macroinvertebrate metrics are derived from the assemblage composition of the samples (e.g., presence, absence or relative abundance of some group) and predictive models are primarily based on taxonomic composition expected at the sites (Wright 1995, Reynoldson et al. 1997, Hawkins et al. 2000, Clarke et al. 2003). However, different field and laboratory procedures generate dissimilar datasets and alter how macroinvertebrate structure and composition appear to respond to disturbance gradients (Stoddard et al. 2008, Cao and Hawkins 2011). In this context, it is important to know which methods generate the best responses (Gerth and Herlihy 2006).

There are two basic protocols for sampling benthic macroinvertebrates in stream biomonitoring: multihabitat (MH), and targeted (TGT) sampling. In the first type, all common substrates and hydraulic habitats at the stream sites are sampled, usually yielding a composite sample to represent the entire site (Hughes and Peck 2008). The different habitats can be sampled systematically along the site (Li et al. 2001, Stoddard et al. 2005, Hughes and Peck 2008), or in proportion to a researcher's visual estimate of their occurrence (Barbour et al. 1999, Hering et al. 2004). These multihabitat samples will yield similar assemblage collections if actual and perceived habitat distributions at the site are similar. In TGT sampling, the sampled habitat type (e.g., riffles, snags, channel edge) usually is fixed for all sites and defined prior to the field sampling (e.g., Reynoldson et al. 1999). However, some national biomonitoring programs allow multiple targeted habitats (Davies 2000, Stark et al. 2001, Moulton et al. 2002).

Many authors have discussed the possible advantages and disadvantages of each approach (e.g., Kerans et al. 1992, Roy et al. 2003). Among the advantages of the TGT sampling include the intrinsic standardization obtained by not comparing sites where different kinds of habitats were sampled. The drawbacks of TGT sampling include insensitivity to changes in the proportion of suitable habitat, and the difficulty of finding the same pre-defined habitat type in all stream sites to be compared across an entire state or nation (Gerth and Herlihy 2006).

In the laboratory, researchers can opt to process the whole sample or a subsample of it (Carter and Resh 2001). Processing the whole sample is preferable if the main concern is to enumerate rare species (Courtemanch 1996). Whole sample processing is also preferred for obtaining unbiased estimations of *areal taxonomic richness* (Ligeiro et al. 2013a), defined as the number of taxa found in a given sampled area (Gotelli and Colwell 2001).

Subsampling procedures are implemented to reduce costs and make biomonitoring feasible (Vinson and Hawkins 2006, Hughes and Peck 2008). Arguably, the approach most commonly used is fixed-count subsampling (Carter and Resh 2001), which consists of identifying a fixed number of individuals from each sample to generate rarefied measurements of taxonomic richness (called *numerical taxonomic richness*, Hurlbert 1971) and other related variables. This is important because the number of taxa detected depends on the number of individuals processed (Gotelli and Colwell 2001). The number of subsampled individuals specified in biomonitoring protocols varies from as few as 100 individuals for rapid assessments (Plafkin et al.

1989), to 500 individuals national monitoring programs (Hughes and Peck 2008). However, 300 individuals is a common choice among many USA states and other research groups around the globe (Carter and Resh 2001).

An important task is to define the efficiency of the competing methods to detect known disturbance gradients (Ostermiller and Hawkins 2004). Therefore, we formed six different datasets through a combination of methodologies commonly used in the field (MH, TGT on leaf packs, and the sum of the two) and in the laboratory (processing the whole sample, and subsampling 300 individuals). We compared assemblage metrics and taxonomic composition calculated from the six different macroinvertebrate datasets to evaluate the effect of field and laboratory methodologies on the responsiveness of these variables to anthropogenic pressure. That is, we analyzed taxonomic richness, % EPT individuals, and taxonomic composition generated from these datasets against a quantitative disturbance gradient calculated across the sample sites within the study basin.

We hypothesized that targeted sampling datasets would achieve the best performances because standardizing microhabitat conditions among the sites would introduce less environmental variability. We also hypothesized that processing entire samples would provide the clearest distinction of the disturbance gradient because it would maximize differences in taxonomic richness resulting from rare species.

Methods

Study area and site selection

We sampled streams in the Upper Araguari River Basin, southeastern Brazil, located in the Cerrado biome of Minas Gerais State. The Cerrado is the second largest biome of Brazil, originally covering 2,045,064 km^2. It is marked by predominantly savannah-like vegetation and two well-defined seasons: a wet season from October to March and a dry season from April to September, with 1200-1800 mm of rainfall per year (Brasil 1992). The Cerrado is considered a terrestrial biodiversity hotspot (Myers et al. 2000) because of its high floral and faunal diversity and endemism (Oliveira and Marquis 2002) and high rates of habitat loss over the past 40 years (Wantzen et al. 2006). The Araguari Basin has an extensive and well-developed system of irrigated/mechanized agriculture, mainly of soy, coffee, corn, and sugar cane. Pasture and small patches of relatively undisturbed vegetation are also present. Most people dwell in small towns, although a few small cities up to 80,000 inhabitants are present.

Thirty-nine stream sites were selected from the pool of 1st to 3rd order (map scale 1:100,000) stream reaches available in a basin area of 7,376 km^2. They were randomly selected through a computerized probability-based design (Olsen and Peck 2008) that assures a spatially balanced distribution of sites (Stevens and Olsen 2003).

Field sampling and laboratory procedures

Field sampling was performed in September of 2009. In each selected site we applied habitat and macroinvertebrate sampling protocols as described in Peck et al. (2006), and used by the US-EPA in its national biomonitoring program (Paulsen et al. 2008). At each site, a length of stream reach equal to 40 x the mean wetted width was defined, with a minimum reach length set at 150 m. Then, 11 equidistant cross-sectional transects were marked from downstream to upstream, defining 10 longitudinal sections of the same length within each sampled reach. A wide variety of habitat measurements was performed at each transect and along the sections, including measurements of human disturbances.

For the MH sampling, one macroinvertebrate sample unit was taken per transect, following a systematic zig-zag pattern (right-middle-left) along the transects. Each of these 11 sample units was taken through use of a D-net (30 cm mouth width, 500 μm mesh), summing to 0.99 m^2 of stream bottom area sampled per site. This methodology assures that many types of habitats (substrates and water velocities) are sampled at each site. It is expected that the habitats will be sampled in proportion to their occurrence within each stream site.

For the TGT sampling, eight leaf packs were sampled per site, preferably located in different reach sections. The same D-net apparatus was used, summing to 0.72 m^2 of leaf pack area sampled per site. Other protocols commonly target riffles; however, we chose leaf packs for our study, given their ecological importance in tropical streams (Dudgeon 2008).

The individual sample units of each method were placed in separate plastic buckets, generating one composite sample for MH sampling and one composite sample for TGT sampling per stream site. Both composite samples were preserved with 10% formalin in the field.

In the laboratory, all samples were fully processed (all individuals counted). Insects and gastropods were identified to family through use of taxonomic keys (Pérez 1988, Fernández and Domínguez 2001, Costa et al. 2006, Mugnai et al. 2010). Only 7 taxa, representing < 4% of all individuals collected, were not identified to family

(Collembola, Hydracarina, Planariidae, Nematoda, Hirudinea, Oligochaeta and Bivalvia). Thus, for simplifying we will refer to all identified taxa as families.

Anthropogenic disturbance gradient

To quantitatively describe the exposure of the stream sites to human pressures, we used the disturbance index described in detail by Ligeiro et al. (2013b). This Integrated Disturbance Index (IDI) was calculated for each site based on the disturbances observed at the local scale (in-channel and riparian vegetation) and at the catchment scale (land use). We estimated local disturbance through use of the habitat metric *W1_hall* (Kaufmann et al. 1999), which is the mean number of specified types of human disturbances observed at each transect (buildings, channel revetment, pavement, roads, pipes, trash and landfill, parks and lawns, row crop agriculture, pasture, logging and mining), distance-weighted relative to its proximity to the stream channel. Catchment disturbance was calculated by summing the proportional areas of human land uses (pasture, agriculture and urban) in each catchment. The different land uses were weighted according to their potential to impair the environment (Rawer-Jost et al. 2004, Maloney et al. 2011). Higher the IDI of a site, higher the intensity of human alterations observed on that site, a zero value representing a site absent of the human disturbances measured. For the pool of sites analyzed in the present study, the IDI values ranged from 0.05 to 0.93.

Data analysis

Comparisons between field sampling methods

To compare the values obtained in MH and TGT sampling methods, we performed paired t-tests (39 stream sites) on the number and density (individuals/m^2) of organisms, family richness, and % EPT individuals (arcsine square root transformed).

For this purpose we processed all individuals in each composite sample. The t-tests were run on STATISTICA 7.0 software (StatSoft, Inc. 2004).

Datasets compared and subsampling procedures

We used six datasets for testing macroinvertebrate structure and composition against the disturbance gradient. The field methods included: 1) multihabitat sampling (MH), 2) targeted sampling (TGT), and 3) the sum of the individuals collected in the MH and TGT methods (MT). Concerning the laboratory methods, we employed two processing approaches for each field method mentioned above; A) processing of all individuals, and B) subsampling of 300 individuals per sample.

We subsampled via computer simulations in R software (R Development Core Team, 2012). Starting from the whole datasets of the three field sampling methods, we used the R function *rrarefy*, available in the *vegan* package (Oksanen et al. 2012), to simulate the random subsampling of 300 individuals for each site. Samples that originally yielded < 300 individuals were kept unaltered. There are protocols that use counts of 400 or more individuals (Carter and Resh 2001, Hughes and Peck 2008), but many sites did not yield these numbers, precluding simulations with more than 300 individuals. We performed 200 subsampling simulations for each field sampling methodology, totaling 600 simulations.

Assemblage metrics versus disturbance gradient

To test the performance of the six datasets in detecting the intensity of anthropogenic disturbances, family richness and % EPT individuals (arcsine square root transformed) were regressed through simple linear regressions (SLR), against the IDI values of the sites. We generated one regression model for each whole dataset; but we generated 200 regression models for each subsampled dataset (one model per subsampling simulation). Were conducted the regression models with STATISTICA 7.0 software (StatSoft, Inc. 2004).

The strength of each combined methodology was measured by the *F* values of their regression models, with higher *F* values indicating greater responsiveness to the disturbance gradient. To determine the degree which the two laboratory processing methods differed in strength we compared the single *F* values obtained from the whole datasets with the respective 200 *F* values obtained from the simulations through a standardized measurement of differentiation (*Z* values):

$$Z = (F_{\text{observed}} - \text{Mean } F_{\text{simulations}}) / \text{Standard deviation of } F_{\text{simulations}}$$

Higher the modular value of *Z*, higher the difference between the observed and the simulated *F* values. Although it is not properly an statistical test, *Z* values > 1.96 (or < -1.96) can be considered expressive (Zar 1996).

Assemblage composition dissimilarities

We included in the least-disturbed category all sites with IDI values < 0.3 and in the most-disturbed category all sites with IDI values > 0.6. These thresholds clearly distinguish two groups of stream sites in terms of the intensity of exposure to anthropogenic pressures, as suggested by Ligeiro et al. (2013b). The least- and the most-disturbed categories were represented by six and seven sites, respectively.

To test which methodology best discriminated between the assemblage compositions of least- and most-disturbed sites we performed PERMANOVAs (Permutational Multivariate Analysis of Variance, Anderson 2001) between these two groups of sites. We used the *adonis* function in the *vegan* package of R software and employed 10,000 randomizations in each model to test model significance. Again, we generated a single PERMANOVA model for each whole dataset, whereas we generated 200 PERMANOVA models for each subsampled dataset (one model per subsampling simulation). We used as dissimilarity measures the Jaccard index (presence/absence data) and the modified Gower distance for proportional abundance, data transformed by $(\log_2 X) + 1$, but with zeros left as zeros (Anderson et al. 2006). According to Anderson et al. (2006), the modified Gower distance gives a clearer and more effective representation of differences on relative abundances than other more popular dissimilarity measures (e.g., Bray-Curtis index).

Once more, the *F* values of PERMANOVA models were used to measure the discrimination strength of each combined methodology, and *Z* values were calculated to determine the degree which the two laboratory processing methods differed in strength.

Results

Comparisons between field sampling methods

We collected a total of 22,345 and 21,508 individuals in the MH and TGT field samplings, respectively. The number of families found was also similar; 69 in MH and 66 in TGT, totaling 77 families in the combined sampling methodologies. These methods did not differ significantly in the number of individuals (Table 1). However, MH sampling produced significantly more families whereas TGT sampling produced a higher density of macroinvertebrates per site (Table 1). The difference between the two methods was marginally significant for % EPT individuals, MH sampling presenting higher values (Table 1).

In general, the composition and relative abundance of the major macroinvertebrate groups differed little between the two sampling methods (Figure 1). In both cases, insects comprised around 96% of the individuals collected, with Diptera the dominant insect order and Chironomidae the most abundant family. MH sampling produced more EPT individuals (28%, versus 20.8% on TGT sampling), particularly Ephemeroptera (17.6%, versus 10.6% on TGT sampling), whereas TGT sampling produced more Chironomidae (46.2%, versus 40.4% on MH sampling) (Figure 1).

Assemblage metrics versus disturbance gradient

The response of family richness to the disturbance gradient differed between the whole and the subsampled datasets, for all field sampling methods (Table 2). There was a decrease in MH model strength when subsampling simulations were applied (Z value = 0.67), whereas there was a marked increase in TGT and MT model strength with subsampling (Z values = -2.41 and -1.54, respectively).

The response of % EPT individuals to the disturbance gradient differed little between the whole and subsampled datasets (Table 2), although whole samples performed slightly better than subsampling for the MH sampling (Z value = 0.25). Therefore, % EPT individuals can be considered more stable than family richness with respect to laboratory methodologies (processing of whole samples versus subsampling of individuals).

For both whole and subsampled datasets, the strength of MH sampling for discriminating the intensity of human disturbances was higher for family richness than for % EPT individuals (Table 2, Figure 2). Conversely, the responsiveness of TGT sampling to human disturbances was lower for family richness than for % EPT individuals. Considering family richness, the F value of the TGT regression based on whole samples was very low and insignificant ($F_{(1,37)} = 1.87$, $p = 0.18$). However, it increased, and most of the regression models were significant, when subsampling of individuals was employed (Median $F_{(1,37)} = 6.33$, % significant tests > 92.5; Table 2).

For both family richness and % EPT individuals the *F* results of the combined (MT) sampling were mostly intermediate between the MH and TGT sampling (Table 2). Also, MT sampling produced more variable *F* values with the simulated subsampling models of family richness (Figure 2).

Assemblage composition dissimilarities

The altered Gower distance was more discriminating (higher PERMANOVA *F* values) than the Jaccard index for all six methodologies (Table 3, Figure 3). The dissimilarities between the macroinvertebrate assemblages of least- and most-disturbed sites usually decreased after employing subsampling of individuals for five out of the six combinations (Table 3). This decrease in performance was most pronounced in the MH field sampling method, for which *F* values were almost halved in the subsampled datasets for both dissimilarity measures. In the other hand, dissimilarity of TGT sampling increased following subsampling when considering the altered Gower distance (Table 3), and the Jaccard index results for TGT sampling were also only slightly different between the whole and subsampled datasets (*Z* value = 0.62).

When considering the processing of whole samples, MH sampling produced greater dissimilarity between most- and least-disturbed sites than the TGT and MT sampling for both dissimilarity measures (Table 3). This situation was reversed when subsampling of individuals was employed; TGT and MT sampling produced greater dissimilarities than MH sampling, especially for the altered Gower distance (Figure 3,

Table 3). Again, in all these comparisons the MT sampling results were intermediate between the MH and the TGT field sampling methods.

Discussion

Choice of targeted habitat

Leaf packs are microhabitats formed by a mixture of leaves from many plant species and in different stages of decomposition (Moretti et al. 2007), usually harboring a high density and taxonomic richness of aquatic macroinvertebrates (Wallace et al. 1997, Mathuriau and Chauvet 2002, Kobayashi and Kagaya 2005). In contrast with the pulsed input of leaf litter to temperate streams, leaf detritus inputs continue throughout the year in Cerrado streams, allowing the persistence of leaf packs in stream channels in all seasons (Gonçalves et al. 2006, França et al. 2009). Thus, given its ecological importance for tropical streams (Dudgeon 2008) we targeted our sampling on leaf packs, although other options were possible (e.g., riffles, pools, boulders, snags, macrophytes).

We found significantly higher densities of macroinvertebrate individuals in leaf pack samples than in multihabitat samples, suggesting that they concentrate benthos. However, the number of families was higher in MH samples, confirming the importance of habitat/microhabitat heterogeneity for macroinvertebrate diversity (Vinson and Hawkins 1998, Robson and Chester 1999, Costa and Melo 2008, Ligeiro et al. 2010).

Influence of field methods on the responsiveness to human disturbances

Macroinvertebrate metrics and assemblage composition responded to the intensity of anthropogenic disturbances to different degrees, depending on the field methodology employed. Frequently, the multihabitat and the targeted field sampling methods yielded opposite results. Consequently, the combined methods (MT sampling) produced intermediate results for most assemblage variables analyzed, these average results reflecting the sum of the weaknesses and strengths of each individual sampling methodology.

Regarding the univariate assemblage metrics, family richness was more associated to the disturbance gradient using the MH sampling, whereas % EPT individuals best correlated with the disturbance gradient using the TGT sampling. Initially we had hypothesized that sampling leaf packs would standardize to some degree the physical environment that sustains macroinvertebrates (Gerth and Herlihy 2006). By reducing natural habitat variability among streams by analyzing leaf pack samples, we expected that the human disturbance signal would be stronger. However, the low *F* value of the regression model (whole dataset) indicates that the number of families was only slightly higher in least-disturbed sites than in most-disturbed sites. This means that leaf packs served as a refuge for a considerable diversity of organisms even when the stream was disturbed by human activities, in agreement with Buss et al. (2004).

Accordingly, when whole samples were considered, TGT samples weakly discriminated assemblage composition between least- and most-disturbed sites. Thus, leaf packs seem to impair the detection of disturbances when the whole sample is processed and taxonomic richness and assemblage composition are considered as indicators. Chessman et al. (2006) also found that multihabitat sampling performed better discriminating the assemblages of least- and most-disturbed sites than targeted sampling. Still, the % EPT individuals responded more strongly to the disturbance gradient (higher *F* values of the regression models) when TGT sampling was considered. This demonstrates that, although the number of families and general assemblage composition of leaf packs varies little along the disturbance gradient, the number of sensitive individuals decreases greatly.

The dissimilarity measures used in this study aimed to describe assemblage differences between least- and most-disturbed sites considering pure compositional variation (Jaccard Index) and compositional plus proportionate abundance variations (altered Gower distance). In this study, all datasets presented higher dissimilarities between the groups when both proportionate abundances and taxonomic composition were considered together. Human disturbances on streams can change macroinvertebrate proportionate abundances (decreasing the number of individuals of some taxa and increasing the number of others) and taxonomic composition (via extirpation of some taxa and immigration of others) (Karr 1999, Norris and Thoms 1999). This explains the better performance obtained by the altered Gower distance.

Effects of subsampling procedures on disturbance assessment

Some studies have found that subsampling impairs taxonomic richness comparisons, defending the processing of whole samples (Courtemanch 1996, Doberstein et al. 2000), while others defend subsampling and the standardization of the number of organisms (Barbour and Gerritsen 1996, Vinson and Hawkins 1996, Walsh 1997). Neither areal or numerical taxonomic richness is necessarily the "correct" way to measure diversity, each method enlightening different aspects of the diversity patterns (Gotelli and Cowell 2001). Thus, the key aspect of this discussion is to define what aspect of taxonomic richness better describes the effects of anthropogenic disturbances. Vinson and Hawkins (1996) recommended the use of subsampling of individuals when comparing sites of different ecological conditions, a practice widely performed nowadays (Cao and Hawkins 2011). In the other hand, Courtemanch (1996) argued that counting all individuals (i.e., considering areal richness) enhance the importance of the rare taxa, which encompasses the majority of macroinvertebrate diversity. Doberstein et al. (2000) achieved weaker models when employing subsampling to analyze taxonomic richness, and they also advocated counting all individuals in samples for a more comprehensive understanding of human alterations.

Given these competing views, the question "what is the right way to process samples?" is not resolved. Indeed, the primary reasons for researchers to subsample is to reduce costs in order to make regional and national biomonitoring programs feasible (Vinson and Hawkins 1996, Hughes and Peck 2008), and provide a standard method that would facilitate national data syntheses (Hughes and Peck 2008, Cao and Hawkins 2011). So, the real questions become more pragmatic: "how much information is being lost?" and "are site conditions being reliably interpreted?" (Barbour and Gerritsen 1996, Vinson and Hawkins 1996, but see Doberstein et al. 2000 for a criticism of this general pragmatism).

We showed that subsampling of individuals had differing effects on our ability to detect assemblage response to the disturbance gradient, depending primarily on the field sampling method employed. In this way, our second hypothesis was just partially corroborated. It is expected that subsampling procedures impair to some degree the characterization of assemblage composition, mainly relative abundances (Shneck and Melo 2010). For MH field sampling, subsampling impaired the response to disturbances of all variables analyzed. However, with TGT sampling both the metrics and the assemblage composition responses to the disturbance gradient were mostly strengthened by subsampling individuals. If this stabilizing effect can be attributed to sampling leaf packs specifically or for sampling any standardized habitat/microhabitat it is a matter for future studies.

In contrast with other variables assessed, the % EPT individuals gave fairly uniform results for all whole and subsampled datasets. This result is in agreement with Courtemanch (1996) which argued that, once individuals are collected randomly during subsampling procedures, metrics that deal with proportions of individuals would be more stable than richness metrics.

Methodological procedures: a closed question?

Two general ideas have become gradually more established through the past 15 years regarding field and laboratory methodologies employed in biomonitoring programs; 1) subsampling of individuals is the proper manner to handle macroinvertebrate samples for biomonitoring purposes, and 2) detections of environmental/disturbance gradients are fairly robust to the type of habitat sampled. Our results contradict these tenets in many aspects. We highlight the possible reasons for the differences between our results and those from previous studies.

Subsampling of individuals in the laboratory was already a widespread practice more than 10 years ago (Carter and Resh 2001), and it is probably even more ubiquitous now (Hughes and Peck 2008, Cao and Hawkins 2011). However, it is clear that it is a decision taken considering mostly the logistics point of view (Vinson and Hawkins 1996). As discussed before, areal and numerical taxonomic richness account for different aspects of local diversity, and can be complementary for interpreting effects of stream impairment (Courtemanch 1996, Gotelli and Cowell 2001). The results comparing the performance of whole and subsampled datasets for detecting disturbances are often conflicting (Barbour and Gerritsen 1996, Courtemanch 1996, Vinson and Hawkins 1996, Doberstein et al. 2000, King and Richardson 2002). Our results add to this discussion because they indicate that the sensitivity of subsampled data can vary with the field method employed. In any case, if choosing not to process whole samples, fixed-counts of individuals are the preferred approach because fixed-proportion subsampling methods are likely to lead to biased comparisons between sites (Ligeiro et al. 2013a).

Many authors have agreed that multihabitat or targeted sampling rarely lead to differences in assessment of the responses of macroinvertebrate assemblages to human disturbances (Plafkin et al. 1989, Hewlett 2000, Ostermiller and Hawkins 2004, Gerth and Herlihy 2006, Rehn et al 2007). Parsons and Norris (1996) and Hewlett (2000) explained that those similarities resulted from redundancy in the information obtained by the two sampling methods. However this emerging conclusion was generated mostly from studies dealing with much larger spatial extents (> 200,000 km^2). As the spatial scale increases, the main determinants of changes in the structure and composition of the assemblages migrates from local-scale factors (e.g. substrate, water velocity) to large-scale factors (e.g. precipitation, geomorphology, land use) (Wu and Loucks 1995, Wiens 2002, Bonada et al. 2008), reducing the variability contributed by the sampled habitat (Gerth and Herlihy 2006). Thus, our study complements those studies, demonstrating that the choice of the field sampling methodology can affect the responsiveness of assemblage metrics and composition to disturbances in a smaller scale assessment. Chessman et al. (2007), studying a relatively small spatial extent in western Australia, also found differences in the performances of metrics derived from different habitats in detecting human alterations in streams, corroborating our findings.

Summary and conclusions

Initially we had predicted that standardizing the microhabitat conditions in macroinvertebrate samples by targeting leaf packs would increase the responsiveness of macroinvertebrate assemblages to anthropogenic disturbances. This seemed to be true only when % EPT individuals was considered. The differences in taxonomic richness and assemblage composition between least- and most-disturbed sites were diminished when these variables were based on leaf pack sampling, especially when whole samples were processed.

There is probably no methodology that is best in all cases, the choice depending on the particular objectives of the study and time and resource constraints. If processing the whole sample is an option, multihabitat sampling revealed the strongest responses for most macroinvertebrate variables assessed. However, this field sampling methodology was less sensitive at detecting anthropogenic disturbance if 300 individuals were subsampled; targeted leaf pack sampling was more sensitive in those cases. Equilibrating the strengths and weaknesses of the two sampling methods, MT sampling usually generated average results in all cases, both in the whole and subsampled datasets. The increased effort of sampling both targeted leaf packs and multiple habitat composites may be an option depending on the research question and funding availability.

Compared with results of previous studies, our results suggests that biomonitoring conducted on smaller spatial extents tends to be more sensitive to changes in field sampling methods than that conducted on larger spatial extents. Therefore, researchers should be cautious about the properties and efficiencies of the methodologies employed, and additional comparative research on sampling methods is recommended.

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Table 1. Comparisons between macroinvertebrate variables obtained with multihabitat (MH) and targeted (TGT) field sampling methods, showing means (± standard errors) and paired t-test results (38 degrees of freedom). Significant *p* values are followed by an asterisk.

Variable	Sampling method		Statistics (paired t-tests)	
	MH	TGT	*t* value	*p* value
Number of individuals	573 (± 68)	551 (± 58)	0.35	0.727
Density (ind./m^2)	579 (± 69)	766 (± 80)	- 2.59	0.013 *
Number of families	24 (± 1)	21 (± 1)	3.29	0.002 *
% EPT individuals	26 (± 3)	21 (± 2)	1.96	0.058

Table 2. Comparisons of simple linear regression models for macroinvertebrate family richness and % EPT individuals against the disturbance index. The six datasets were generated from three different field sampling methods; 1) multihabitat (MH), 2) targeted (TGT), and 3) multihabitat+targeted (MT), and two laboratory procedures; A) processing of whole samples, and B) subsampling of 300 individuals. For the subsampled datasets (200 simulations per field sampling method) we show the median *F* values and the proportion of significant regression models (with $p < 0.05$). Finally, we compare the single *F* values obtained from the whole datasets with the respective 200 *F* values obtained from the simulations through a standardized measurement of differentiation (Z values). Significant *p* values are followed by an asterisk.

		Whole datasets		Subsampled datasets (simulations)		
Metric	Sampling method	*F* value	*p* value	Median *F* values	% significant models	*Z* value
Family richness	MH	14.76	< 0.001 *	12.75	100	0.67
	TGT	1.87	0.18	6.33	92.5	-2.41
	MT	8.99	0.005 *	16.95	100	-1.54
% EPT individuals	MH	4.79	0.035 *	4.66	89.5	0.25
	TGT	17.43	< 0.001 *	17.37	100	0.07
	MT	12.3	0.001 *	12.26	100	0.01

Table 3. Comparisons of PERMANOVA models performed between least- and most-disturbed stream sites using the altered Gower distance and the Jaccard index. The six datasets were generated from three different field sampling methods; 1) multihabitat (MH), 2) targeted (TGT), and 3) multihabitat+targeted (MT), and two laboratory procedures; A) processing of whole samples, and B) subsampling of 300 individuals. For the subsampled datasets (200 simulations per field sampling method) we show median *F* values and the proportion of significant PERMANOVA models ($p < 0.05$). Finally, we compare the single *F* values obtained from the whole datasets with the respective 200 *F* values obtained from the simulations through a standardized measurement of differentiation (Z values). Significant *p* values are followed by an asterisk.

		Whole datasets		Subsampled datasets (simulations)		
Dissimilarity measure	Sampling method	*F* value	*p* value	Median *F* values	% significant models	*Z* value
Altered Gower distance	MH	3.6	0.003 *	1.75	87	12.52
	TGT	2.05	0.005 *	2.63	100	-2.95
	MT	2.96	0.001 *	2.47	100	2.08
Jaccard index	MH	2.73	0.002 *	1.58	48	4.39
	TGT	1.95	0.01 *	1.74	69.5	0.62
	MT	2.29	0.005 *	1.68	68	1.97

Figure Legends

Figure 1. Macroinvertebrate assemblage composition (sum of all stream sites) observed in multihabitat (MH) and targeted (TGT) field sampling methods from processing all individuals. COH = Coleoptera+Odonata+Heteroptera.

Figure 2. Distribution (median, interquartiles and extremes) of the *F* values obtained from simple linear regression models (taxonomic richness and % EPT individuals versus the disturbance index). We used simulated subsampled datasets of each field sampling method (multihabitat, targeted, and multihabitat+targeted; 200 simulations on each). S = family richness; EPT = % EPT individuals; MH = multihabitat sampling; TGT = targeted sampling (leaf packs); MT = multihabitat+targeted sampling.

Figure 3. Distribution (median, interquartiles and extremes) of the *F* values obtained on PERMANOVA models made between least- and most-disturbed sites with the altered Gower Distance and the Jaccard index. We used simulated subsampled datasets of each field sampling method (multihabitat, targeted, and multihabitat+targeted; 200 simulations on each). Gower = altered Gower distance; Jaccard = Jaccard index; MH = multihabitat sampling; TGT = targeted sampling (leaf packs); MT = multihabitat+targeted sampling.

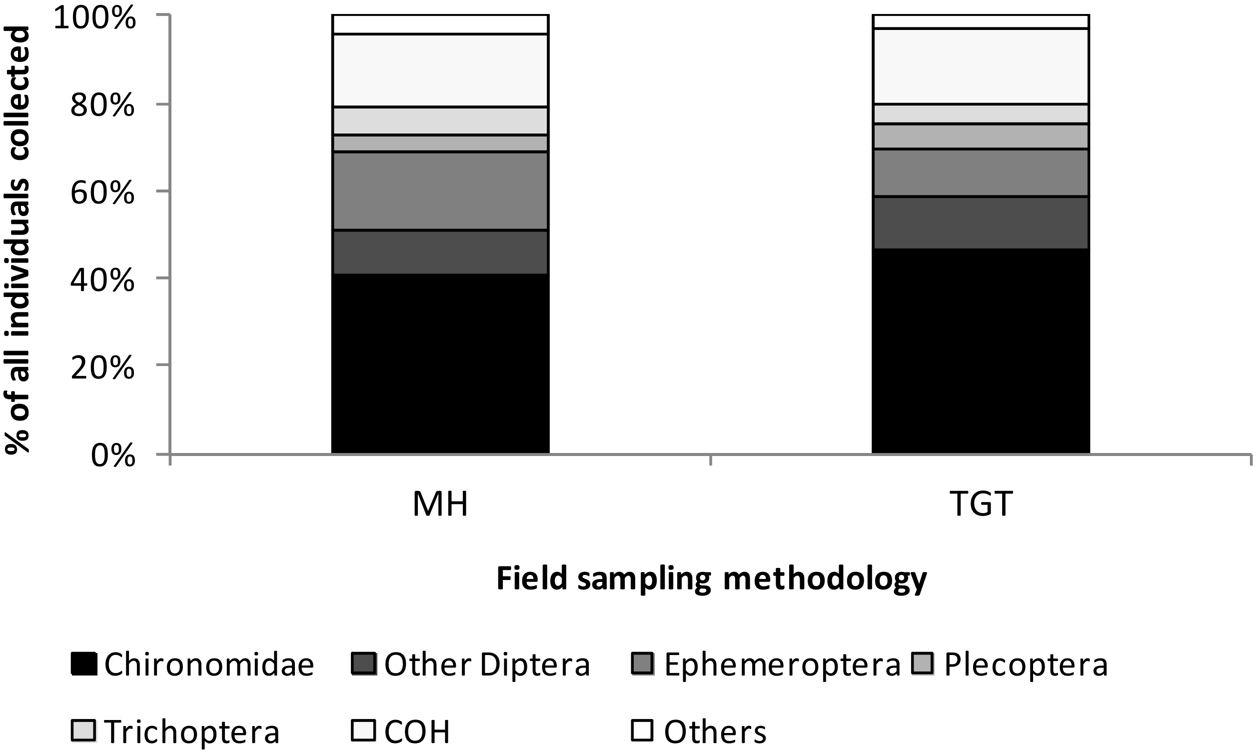
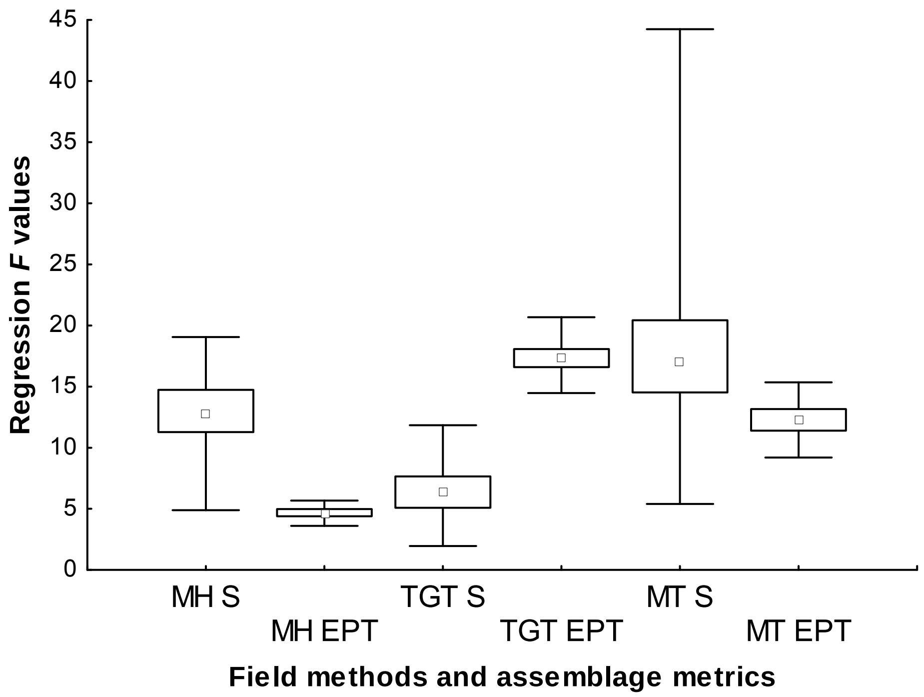

100%
80%
60%
40%
20%
0%
% of all individuals collected
MH
TGT
Field sampling methodology
Chironomidae
Other Diptera
Ephemeroptera
Plecoptera
Trichoptera
COH
Others

Figure 1.



45
40
35
30
25
20
15
10
5
0
Regression *F* values
MH S
MH EPT
TGT S
TGT EPT
MT S
MT EPT
Field methods and assemblage metrics

Figure 2.

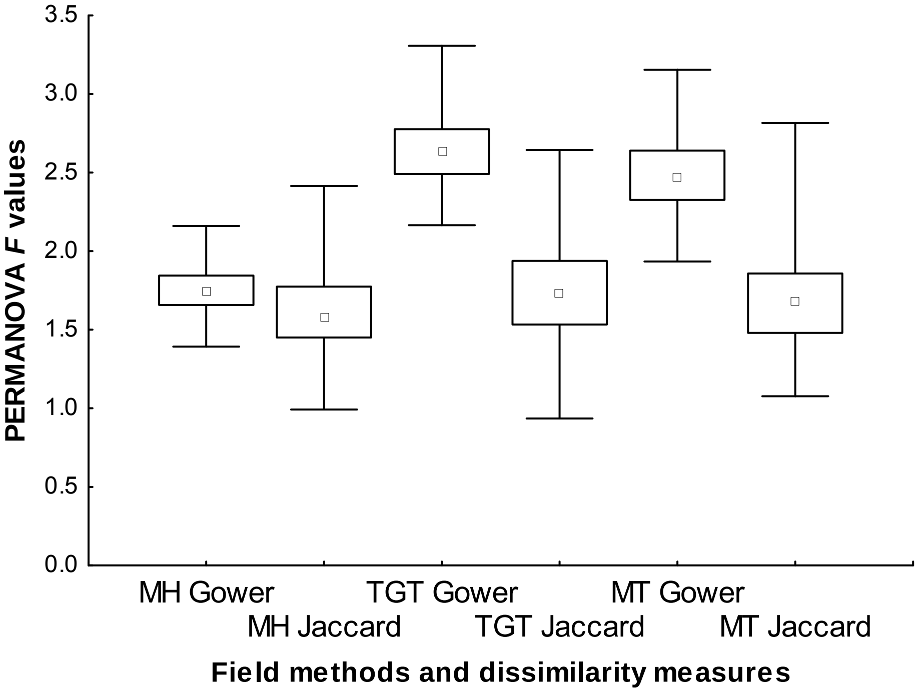

3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
PERMANOVA *F* values
MH Gower
MH Jaccard
TGT Gower
TGT Jaccard
MT Gower
MT Jaccard
Field methods and dissimilarity measures

Figure 3.

Capítulo 4

Anthropogenic disturbances alter alpha and beta diversity patterns of macroinvertebrate assemblages in tropical headwater streams

Raphael Ligeiro [a], Robert M. Hughes [b], Adriano S. Melo [c] and Marcos Callisto [a]

[a] Universidade Federal de Minas Gerais, Instituto de Ciências Biológicas, Departamento de Biologia Geral, Laboratório de Ecologia de Bentos, Av. Antônio Carlos, 6627, CP 486, CEP 30161-970, Belo Horizonte, Minas Gerais, Brazil.

[b] Oregon State University and Amnis Opes Institute, Department of Fisheries & Wildlife, Nash Hall, 97331-4501, Corvallis, Oregon, USA.

[c] Universidade Federal de Goiás, Instituto de Ciências Biológicas, Departamento de Ecologia, Campus II, Samambaia, CP 131, CEP 74001-970, Goiânia, Goiás, Brazil.

Anthropogenic disturbances alter alpha and beta diversity patterns of macroinvertebrate assemblages in tropical headwater streams

Raphael Ligeiro[1], Robert M. Hughes[2], Adriano S. Melo[3] and Marcos Callisto[1]

[1] Laboratório de Ecologia de Bentos, Departamento de Biologia Geral, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, Av. Antônio Carlos, 6627, CP 486, CEP 30161-970, Belo Horizonte, Minas Gerais, Brazil.

[2] Department of Fisheries & Wildlife, Oregon State University and Amnis Opes Institute, Nash Hall, 97331-4501, Corvallis, Oregon, USA.

[3] Departamento de Ecologia, Instituto de Ciências Biológicas, Universidade Federal de Goiás, Campus II, Samambaia, CP 131, CEP 74001-970, Goiânia, Goiás, Brazil.

Abstract

Knowing how stream macroinvertebrates are distributed along the gradient of anthropogenic disturbances is essential for understanding impairment processes and for rational ecosystem management. Disturbance effects on either alpha (site) or beta (variation between sites) diversities can ultimately decrease gamma (regional) diversity. Much is known about anthropogenic impacts on the alpha diversity of stream macroinvertebrates, but little attention has been given to the beta component. Therefore, the objective of this study was to measure the effects of human alterations on alpha and beta diversities of stream Ephemeroptera, Plecoptera and Trichoptera (EPT) assemblages. Specifically, we hypothesized that anthropogenic pressures will 1) reduce alpha diversity, 2) generate a nested pattern in which the more disturbed sites will constitute a subset of species present in the less disturbed sites and 3) reduce beta diversity among the most-disturbed sites. Forty stream sites ranging from least- to most-disturbed conditions were sampled in a Neotropical basin. The intensity of human alterations was measured using an index that incorporates both in-stream/riparian vegetation and catchment alterations. Site richness of EPT genera decreased with the intensity of human disturbances and a significant nestedness pattern was observed in the assemblages along the disturbance gradient, with more-disturbed sites being subsets of the less-disturbed ones. Beta diversity analyses revealed greater variation among most-disturbed sites than among least-disturbed sites, not corroborating our initial hypothesis or the traditional paradigm of homogenization of disturbed sites. We conclude that human disturbances decreased regional taxonomic richness mainly by reducing site (alpha) diversity. We recommend further study of diversity distribution in disturbed sites in markedly different ecoregions, thereby clarifying how alterations in alpha and beta components affect species richness across stream networks.

Key-words: Multivariate dispersion, additive partitioning, nestedness patterns, NODF, impairment process, macroinvertebrate distribution.

Introduction

Understanding the spatial distribution of assemblages in riverine landscapes (*sensu* Wiens 2002) is one of the main goals of stream community ecologists (Poff 1997, Fausch et al. 2002, Heino 2009). Whittaker (1960) was the first to define regional diversity, which he called gamma diversity, in terms of alpha (site) and beta (variability between sites) diversity components, in this way providing a general framework to study spatial distribution of species.

Alpha and gamma diversities, although varying at the scale analyzed, are usually measured by the same descriptors (species richness or diversity indices), being grouped as "inventory diversities" (Jurasinski et al. 2009). On the other hand, the initial generalist concept of beta diversity took many different meanings through the years, being described by different aspects of the variations among assemblages (e.g., Legendre et al. 2005, Jurasinski et al. 2009, Melo et al. 2011), from multiplicative and additive approaches through multivariate and gradient analyses (Anderson et al. 2011).

Most studies of stream ecosystems, especially those of macroinvertebrate assemblages, are focused on describing and explaining the distribution of species at the local scale (stream reaches or smaller scales), i.e., on understanding alpha diversity (Clarke et al. 2008). Studies dealing with beta diversity are rarer in the literature and often focused on preserved or least-disturbed areas (Mykrä et al. 2007, Lecraw and Mackereth 2010, Ligeiro et al. 2010, Hepp et al. 2012, Hepp and Melo 2012). Nevertheless, anthropogenic disturbances are increasingly prevalent on streams and it is important to know how human actions are altering the beta diversity patterns of the assemblages (Maloney et al. 2011).

Mirroring studies of preserved environments, effects of human activities on stream macroinvertebrates typically focus on the alpha diversity component. In such cases, reduced taxonomic richness is often observed at most-disturbed sites, with the loss of more sensitive species (Karr 1991, Rosenberg and Resh 1993, Malmqvist and Rundle 2002). Consequently, it might be expected that loss of sensitive species produces a nestedness pattern (*sensu* Patterson and Atmar 1986). That is, macroinvertebrate assemblages in the more-disturbed sites will be composed of subsets of those of the less-disturbed sites. This prediction was rarely tested directly with nestedness analyses (but see Merovich Jr. and Petty 2010). Loss of sensitive species may cause fauna homogenization among impaired sites as the same set of resistant species will be present everywhere, leading to diminished beta diversity (Rahel 2002, Lougheed et al. 2008).

Maintaining the regional pool of species is one of the main purposes of most conservation programs (Chandy et al. 2006, Clarke et al. 2010). Once regional diversity is determined by the interaction of alpha and beta diversities, alterations of either component by anthropogenic disturbances can lead to overall reduction of gamma diversity. Therefore, the objective of this study was to describe the effects of human disturbances on alpha and beta diversity components in stream ecosystems. For this purpose we sampled 40 stream sites in a Neotropical basin that ranged from very good to very poor ecological condition. We focused on the Ephemeroptera, Plecoptera and Trichoptera (EPT) assemblage given the high sensitivity of most of these organisms to human disturbances (Rosenberg and Resh 1993, Stoddard et al. 2008). We hypothesized that; 1) alpha (site) EPT genera richness decreases with the intensity of human disturbance, 2) EPT assemblages are nested along a gradient of anthropogenic disturbances, and 3) most-disturbed sites are biologically more homogeneous among themselves (have lower beta diversity of EPT genera) than least-disturbed sites.

Methods

Study area and site selection

Streams were sampled in the Upper Araguari River Basin, southeastern Brazil. This area is located in the Cerrado biome of Minas Gerais state. The Cerrado is the second largest biome of Brazil and it is characterized predominantly by a savanna-like vegetation and two well-defined seasons: a wet season from October to March and a dry season from April to September, with 1200-1800 mm of rainfall per year (Brasil 1992). This biome is considered a terrestrial biodiversity hotspot (Myers et al. 2000) because of its high floral and faunal diversity and endemism (Oliveira and Marquis 2002) and high rates of habitat loss, mainly over the past 40 years (Wantzen et al. 2006, Diniz-Filho et al., 2009). The studied area has an extensive and well-developed system of irrigated/mechanized agriculture. Pasture and small patches of relatively undisturbed vegetation are also present.

Forty stream sites were selected from the pool of 1st to 3rd order stream reaches available (map scale 1:100,000) inside a basin area of 7,376 km^2. They were randomly selected through a computerized probability-based sampling procedure (Olsen and Peck 2008) that assures a spatially balanced distribution of sites (Stevens and Olsen 2003).

Field sampling and laboratory procedures

We sampled the sites in September 2009. In each site we applied a habitat/macroinvertebrate sampling protocol as described in Peck et al. (2006), which is used by the US-EPA in its national biomonitoring program (Paulsen et al. 2008). In each site, a longitudinal reach equal to 40 x the mean wetted width was defined, with a minimum reach length of 150 m. Then, eleven equidistant cross-sectional transects were marked from downstream to upstream, defining 10 longitudinal sections of the same length in each stream site. A wide variety of habitat measurements was performed at each transect and along the sections, including measurements of human disturbances.

For macroinvertebrate sampling, one sample unit was taken per transect, following a systematic zig-zag pattern along the reach. Each of the 11 sample units was taken through use of a D-net (30 cm mouth width, 500 μm mesh), summing to 0.99 m^2 of stream bottom area sampled per site. The individual sample units were placed together in plastic buckets (generating one composite sample per stream site) and preserved with 10% formalin in the field.

In the laboratory, all samples were fully processed (all individuals counted). The EPT individuals were identified to genus through use of taxonomic keys (Wiggins 1996, Pés et al. 2005, Dominguez et al. 2006, Salles 2006, Mugnai et al. 2010).

Anthropogenic disturbance gradient

To quantitatively describe the level of exposure of the stream sites to human pressures, we used the disturbance index described in Ligeiro et al. (2013). An Integrated Disturbance Index (IDI) was calculated for each stream site based on the disturbances observed at the local scale (in-channel and riparian vegetation) and at the catchment scale (land use). An IDI value of zero indicates the absence of observed disturbances at both spatial scales. The higher the IDI, the more disturbed is the site. We used the habitat metric *W1_hall* (Kauffman et al. 1999) for estimating the local disturbance. This metric is the sum of observations made at each transect of 11 types of disturbance (buildings, channel revetment, pavement, roads, pipes, trash and landfill, parks and lawns, row crop agriculture, pasture, logging and mining), distance-weighted relative to its proximity to the stream channel (Kauffman et al. 1999). Catchment disturbance was calculated by summing the proportional areas of human land uses (pasture, agriculture and urban) in each catchment. The different land uses were weighted according to their potential to impair the environment (Rawer-Jost et al. 2004, Maloney et al. 2011). The IDI values of the sampled sites ranged from 0.05 to 1.09, for the least- and the most-disturbed sites, respectively (Ligeiro et al. 2013).

Data analysis

Effects of disturbances on alpha diversity

In a previous work (Ligeiro et al. 2013), we assessed the effects of human alterations on local richness by regressing the number of EPT genera found in the sites against the IDI values (simple linear regression). The results are presented in Fig. 8A of Ligeiro et al. (2013). We used these previous results on alpha diversity, presenting them also in this study, to best understand human impacts on beta and gamma diversities.

Nestedness patterns in the taxonomic composition of the assemblages

We assessed whether the taxonomic compositions of EPT assemblages (presence/absence data) followed a nested pattern according to the disturbance gradient. For this purpose the sites were ordered according to the IDI values (from least- to most-disturbed) and we used the nestedness metric NODF (Nestedness metric based on Overlap and Decreasing Fill, Almeida-Neto et al. 2008). This metric analyzes separately nested patterns on rows (sites) and columns (taxa), giving for each one statistical values that range from 0 (complete absence of nestedness) to 100 (perfect nestedness). The hypothesis of this study only concerns the nestedness of sites. The observed NODF value was tested against a null model that kept the genera frequencies but randomized the site frequencies (Jonsson 2001). The rationale for the choice of this method is that it keeps the regional relative rarity or commonness of all genera but randomizes their presences in the sites, in this way disrupting the disturbance effect and simulating random site gradients. We performed 10,000 matrix simulations and computed NODF for each one. The proportion of simulated matrices that achieved NODF metric values higher than the observed one was deemed the *p* value of the test.

Assemblages are structured by many natural gradients other than disturbances. Therefore, it is expected that sites with similar IDI values have their assemblage compositions structured by other factors than the disturbance gradient tested (i.e., it is not expected that these sites will have nested patterns). A consequence of this assumption is that if only sites with large differences in IDI values were analyzed, the disturbance signal on assemblages would be enhanced, thereby creating a clearer nestedness pattern (higher NODF value). We tested this prediction through a separate nestedness analysis, hereafter called "maximized distance dataset" (MDD) analysis, keeping the least-disturbed site sampled and including in the analysis only sites with IDI values distant at least 0.05 from each other. For instance, starting with our least-disturbed site sampled (IDI=0.05) the next site of the sequence had to have an IDI score ≥ 0.1 (0.05+0.05) to be included in the MDD. Our second least-disturbed site sampled had an IDI score = 0.06, so it did not enter. Our third least-disturbed site sampled had an IDI score = 0.1, so it entered in the MDD. Following this rationale, the next site of the MDD sequence (the third one) had to have an IDI score at least 0.05 greater than the second site participating in the MDD, and so on until considering all 40 sites. The minimum distance employed of 0.05 represent about 5% of the total difference of IDI values observed between the least- and the most-disturbed site sampled. Naturally, the choice of this value was arbitrary. We are not stating that this distance is the only one possible, or even that it is the best one to use (this is a matter for future studies). We just used it as a practical example to examine the effects of the inclusion of a minimum gradient distance on nestedness analyses, in this way strengthening the gradient assessed.

Both nestedness analyses (all sites and MDD) and their null models were performed through use of R software (R Development Core Team, 2012) using the functions *nestednodf* and *oecosimu*, available in the *vegan* package (Oksanen et al. 2012).

Beta diversity of least- and most-disturbed sites

To define the groups to be compared, we included in the least-disturbed category all sites with IDI values < 0.3 and in the most-disturbed category all sites with IDI values > 0.6. These thresholds promote a clear distinction between the stream sites of the two groups in terms of the intensity of exposure to anthropogenic pressures, as suggested in Ligeiro et al. (2013). Thus, the least- and the most-disturbed categories were represented by six and eight sites, respectively.

First we compared the assemblage variation of the two groups using the analysis of homogeneity of multivariate dispersions (hereafter betadisper), described by Anderson (2006). This analysis is the multivariate equivalent of Levene's test of homogeneity of variances. In this comparison, a resemblance matrix is created using some appropriate measure of similarity/dissimilarity. Then the sites are embedded in a multivariate space using Principal Coordinates Analysis (PCoA) and the distances of each site to its group centroid (spatial median) is calculated. Betadisper then tests, through permutation procedures on least-absolute-deviation residuals, the null hypothesis that the average distances (dispersions) of the compared groups are similar. We were specifically interested in comparisons between the within-dispersions of the two groups, rather than the taxonomic compositions between the groups. This highlights an important distinction between "dispersion" and "location" effects (see Anderson et al. 2008, Warton et al. 2012). We used the Jaccard index (presence/absence data matrix) as the measure of dissimilarity. Average distances to group centroids were compared with 10,000 permutations on residuals. We analyzed just the taxonomic composition of the assemblages once we were interested in directly relate taxonomic richness, nestedness and beta diversity. Betadisper was performed through use of R software (R Development Core Team, 2012) using the function *betadisper*, available in the *vegan* package (Oksanen et al. 2012).

Second, we used an additive partitioning analysis to decompose the total (gamma) diversity observed in each group of sites on their alpha and beta components. Additive partitioning, using the general formula gamma = alpha + beta, was first conceived many decades ago (MacArthur et al. 1966, Levins 1968) and gained renewed importance after Lande (1996). It can be used to assess the distribution of diversity at many spatial scales simultaneously (Veech et al. 2002, Stendera and Jonhson 2005, Ligeiro et al. 2010) but here we were concerned with just one spatial level (stream reaches). This analysis calculates beta diversity subtracting the average site richness from the total richness found in the group of sites (beta = gamma – mean alpha). Additive partitioning was calculated separately for each group of sites and the patterns were then compared. In each analysis, a Type I null model (according to Crist et al.

2003) was employed using 10,000 permutations to verify if the alpha and beta components observed could be achieved randomly, i.e., could be unrelated to ecological processes. Each permutation shuffles the individuals between all sites and then calculates the alpha and beta diversities generated. A very high (≥ 0.95) or very low (≤ 0.05) proportion of simulations that achieve a higher diversity value than the observed one indicates that the observed diversity is significantly lower or higher than that expected, respectively (Crist et al. 2003). This analysis was performed through use of PARTITION 2.0 software (Veech and Crist 2007).

Results

Effects of disturbances on alpha diversity

A total of 5,463 EPT individuals (61 genera) were collected from the 40 studied sites. From these, 3,291 individuals (30 genera) belonged to the order Ephemeroptera, 871 individuals (5 genera) to the order Plecoptera and 1,301 individuals (26 genera) to the order Trichoptera.

The intensity of human disturbances measured in the sites (IDI values) significantly decreased the EPT genera richness of the sites ($F_{(1,38)} = 24.6$, $R^2 = 0.39$, $p < 0.001$) (Figure 1).

Nestedness patterns in the composition of the assemblages

Nestedness of EPT assemblages along the disturbance gradient was significant under the null model when all 40 stream sites were considered ($NODF_{(rows)} = 41.79$, matrix fill = 0.23, $p < 0.001$) (Figure 2A). When a minimum IDI difference of 0.05 was established (MDD analysis) the number of sites dropped to 13 and included 56 genera.

The maximized distance dataset had a stronger nestedness pattern than the dataset with all sites ($NODF_{(rows)} = 56.54$, matrix fill = 0.3, $p < 0.001$) (Figure 2B).

Beta diversity on least- and most-disturbed sites

The average distance to group centroid (dispersion) was significantly higher among the most-disturbed sites (PCoA multivariate distance = 0.54) than among least-disturbed sites (PCoA multivariate distance = 0.39) for the dissimilarity index used (Betadisper, $F_{(1,12)} = 6.68$, $p = 0.03$). This means that beta diversity was higher among most-disturbed sites than among least-disturbed sites (Figure 3).

The additive partitioning analysis also revealed relatively greater beta diversity among the most-disturbed sites than among the least-disturbed sites (Figure 4). Although the total EPT richness observed in the least-disturbed sites was almost twice that of the most-disturbed sites (53 and 28, respectively), the proportional beta diversity was higher in most-disturbed sites (71.1%, versus 48.5% in the least-disturbed sites). Alpha diversity (average EPT richness of sites) markedly decreased in the most-disturbed group (8 genera, versus 27 genera in least-disturbed sites). In both groups of sites, alpha diversity was significantly lower than that expected under the null model applied ($p < 0.001$) and the beta diversity was significantly higher than that expected ($p < 0.001$) (Figure 4).

Discussion

Effects of human disturbances on alpha diversity

Local and regional anthropogenic disturbances can disrupt the ecological processes that maintain stream assemblages, mainly changing sediment dynamics and habitat heterogeneity (Bryce et al. 1999, Malmqvist and Rundle 2002, Walters et al. 2003, Allan 2004). In the present study we found a strong negative relationship between the intensity of human alterations and the number of EPT genera of the sites, corroborating our first hypothesis. This disturbance effect on alpha diversity is frequently reported for macroinvertebrate assemblages of streams, and especially for EPT assemblages (Strayer et al. 2003, Buss et al. 2004, Moreno and Callisto 2006, Urban et al. 2006, Allan and Castillo 2007). For this reason, taxonomic richness (total invertebrate or EPT richness) is commonly used in biotic multimetric indices as a metric to assess stream conditions (Barbour et al. 1999, Klemm et al. 2003, Stoddard et al. 2008, Moya et al. 2011).

Nestedness patterns along the disturbance gradient

Decreasing richness along the gradient analyzed is a pre-requisite for nestedness (Atmar and Patterson 1993, Ulrich et al. 2009). However decreasing richness will not necessarily generate a nested pattern, because species-poor assemblages may include species absent from species-rich assemblages (i.e. replacement or turnover; Almeida-Neto et al 2008, Ulrich and Almeida-Neto 2012). In our study, following the decrease in taxonomic richness, an evident nestedness pattern was observed along the disturbance gradient, with assemblages from more disturbed sites being subsets of assemblages from less disturbed ones, corroborating our second hypothesis. To illustrate the highly nested structure of the assemblages, from the 28 EPT genera found in the most-disturbed sites, only one genus (*Itaura*, Glossosomatidae, Trichoptera) was not found in the least-disturbed sites. This regional pattern is in agreement with the traditional idea that more sensitive species are progressively lost as the intensity of human disturbances increases (Rosenberg and Resh 1993, Allan 2004, Pond 2010). Nevertheless, as far as we know this assumption has rarely been tested through use of nestedness analysis.

Merovich Jr. and Petty (2010), studying stream macroinvertebrate assemblages in the United States, used the temperature metric of nestedness (Atmar and Patterson 1993) and also found a significant nestedness pattern according to a site classification based on physical and chemical water-quality characteristics. In most cases their more-disturbed sites were subsets of their less-disturbed sites. Yet, they worked with discrete water-quality categories and not with a continuous disturbance gradient like we used. Even more important, temperature metric only calculates the nestedness score after the matrix is ordered from the species-richest to the species-poorest site. Thus, they tested only indirectly the hypothesis of nestedness generated by human disturbances, identifying the disturbance categories along the inflexible ordination of the sites. On the other hand, NODF permit the entry of any sequence of sites, permitting the direct assessment of other gradients besides the richness gradient. This flexibility is an often overlooked advantage of this metric (Almeida-Neto et al. 2008).

The nestedness pattern was strengthened when a minimum disturbance distance between the sites was applied. The NODF metric value increased from 41.8 (considering all 40 sites) to 56.6 (considering just 13 MDD sites). Natural effects cause variation in invertebrate assemblages that can conceal disturbance effects if their disturbance signal is not strong enough (Parsons and Norris 1996, Moya et al. 2011, Ligeiro et al. 2013). By removing sites with similar IDI values we could emphasize the disturbance effects.

Effects of disturbances on beta diversity

The beta diversity concept has multiple interpretations and analytical approaches. Jurasinski et al. (2009) proposed a division of beta diversity analyses on 1) *differentiation diversity*, which deals with the differentiation of the assemblage composition among the sites, usually through the use of resemblance matrices (abundance, frequency or presence/absence data), and 2) *proportional diversity*, which expresses the relative distribution of inventory diversity across one or more scales. Moreno and Rodriguez (2008) and Anderson et al. (2011) further subdivided differentiation diversity on 1) *variation analyses*, when comparing discrete groups of sites, and 2) *turnover analyses*, when the differentiation is made along continuous gradients (environmental, spatial, etc).

In this study we aimed to assess the beta diversity of EPT assemblages among the least- and among the most-disturbed sites through both the differentiation (variation) and proportional approaches. The two approaches indicated greater beta diversity among most-disturbed sites, the opposite of the third hypothesis we had proposed initially. To date, few studies have assessed beta diversity patterns among disturbed streams. Many authors proposed that human impacts cause a regional homogenization of the biota (Rahel 2002, Lougheed et al. 2008), i.e., led to decreased beta diversity among disturbed sites. Indeed, this has been observed in the freshwater realm for lake fish (Rahel 2002), stream fish (Walters et al. 2003) and lake zooplankton (Beisner et al. 2003) assemblages.

Giving the previous expectations regarding beta diversity in disturbed sites, the results we obtained are somewhat contentious. Nevertheless, similar patterns have been observed elsewhere. Maloney et al. (2011) studied beta diversity of stream macroinvertebrates in the United States and found very weak negative correlations between beta diversity (measured via multivariate dispersion analysis) and anthropogenic land uses. The assemblage dispersion of their most-disturbed sites was not significantly different from the dispersion of their least-disturbed sites. Therefore, although regional biotic homogenization was their initial hypothesis, they could not observe it directly. Recently, Muotka et al. (2012), studying stream macroinvertebrates in Finland, and Dimitriadis and Koutsoubas (2011), working with marine benthic invertebrates in the Eastern Mediterranean Sea, also reported greater beta diversity among their most-disturbed sites than among their least-disturbed sites. As mentioned before, the scarcity of studies of beta diversity in altered environments precludes the formulation of a general theory. One plausible explanation for the patterns we found is that because different disturbance types can select differing sets of resistant taxa in each site (Urban et al. 2006, Marzin et al. 2012) alpha diversity can be reduced and the dissimilarity of the remaining biota can be increased in response to human alterations. Nevertheless, more specific studies are necessary to test this hypothesis.

Importance of studying beta diversity for regional aquatic conservation

Small headwater streams (1st to 3rd order), like the ones we worked with in this paper, encompass the majority of the total channel length in river networks (Benda et al. 2005). Thereby, conservation of small streams is essential for maintaining the ecological health of the whole basin (Finn et al. 2011). Because they are numerous, the variation between stream sites (beta diversity) is more important than the number of species found in each individual site (alfa diversity) for constituting regional (gamma) diversity (Clarke et al. 2008).

Our results from additive partitioning analysis revealed that beta diversity was higher than expected by chance in both groups of sites, meaning that in any case ecological processes (e.g., niche occupancy, dispersion of organisms) were causing dissimilarities among sites (Crist et al. 2003, Leibold et al. 2004, Thompson and Townsend 2006). The alpha and beta components were similar in our least-disturbed sites (Figure 4), i.e., the number of EPT genera found in a single stream site averaged half of what can be found in all least-disturbed sites. Similarly, high among-site beta diversity of macroinvertebrate assemblages has been reported in preserved catchments (Clarke et al. 2010, Ligeiro et al. 2010, Hepp and Melo 2012), confirming the importance of headwater streams for conservancy purposes.

Proportional beta diversity was higher among the most-disturbed sites than among the least-disturbed sites. Thus, the reduced regional EPT richness observed in the most-disturbed group likely results from reductions of alpha diversity in these sites, suggesting that anthropogenic alterations led to reduced regional richness primarily through local extinction of species, and not by homogenizing the macroinvertebrate biota among streams in the basin.

Conclusions

Knowing the regional distribution of the biota is essential for rational ecosystem management and conservation planning (Whittaker et al. 2005, Richardson and Whittaker 2010). As human activities are increasingly impairing headwater streams, it is important to study their effects on alpha, beta, and gamma diversities. Beta diversity is naturally high among least-disturbed sites and we provided evidence that it can be high among most-disturbed sites also, contrary to the prevalent paradigm of homogenization of disturbed sites. In our study basin, the regional reduction of EPT richness observed at the most-disturbed group of sites was caused mainly by a marked decrease of local richness. We recommend more studies describing and explaining patterns of macroinvertebrate distribution in disturbed streams to understand better the impairment processes and their consequences for regional species richness.

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Figure Legends

Figure 1. Linear regression between the number of EPT genera found in each stream site (alpha diversity) and the IDI (Integrated Disturbance Index) values (adapted from Ligeiro et al. 2013).

Figure 2. Incidence matrices showing the nestedness patterns of (A) all stream sites and (B) only sites with an IDI difference of at least 0.05 among each other (MDD, see text). Presences are represented by the black squares, and absences are represented by the grey spaces. Rows represent stream sites aligned by IDI values, from least-disturbed (top) to most-disturbed (bottom). Columns represent EPT genera from most common (left) to rarest (right).

Figure 3. Multivariate space of the two first axes of Principal Coordinates Analysis (PCoA) representing the relative positions of stream sites based on Jaccard index of dissimilarity scores. For each group of sites, the lines represent the distances between the sites and the group centroid (spatial median). The multivariate spatial ranges of the groups are delimitated by dashed lines.

Figure 4. Additive partitioning of total EPT richness (gamma diversity) of each group of sites (least- and most-disturbed sites) into alpha and beta components. For each group of sites; alpha diversity = average site richness, and beta diversity = gamma diversity minus alpha diversity. Mean expected results under the null model employed are to the right of each observed partition.

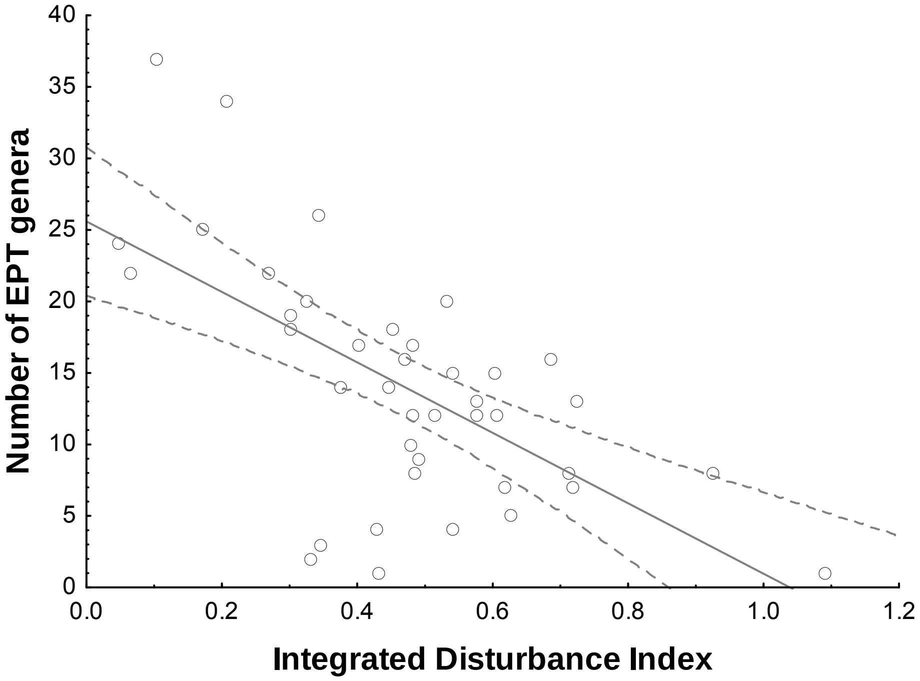
Number of EPT genera
0
5
10
15
20
25
30
35
40
0.0
0.2
0.4
0.6
0.8
1.0
1.2
Integrated Disturbance Index

Figure 1.

A)

B)

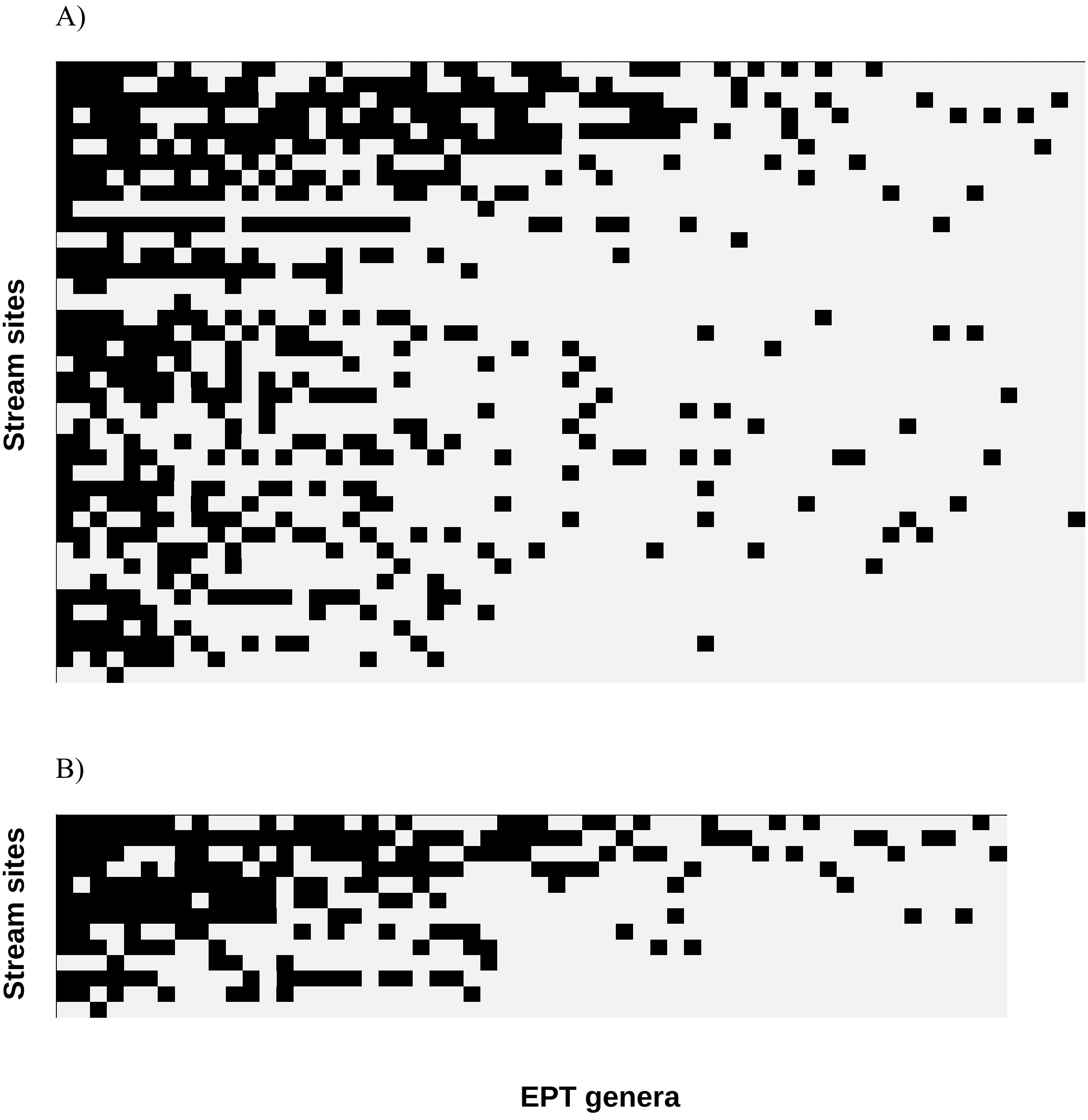

Figure 2.

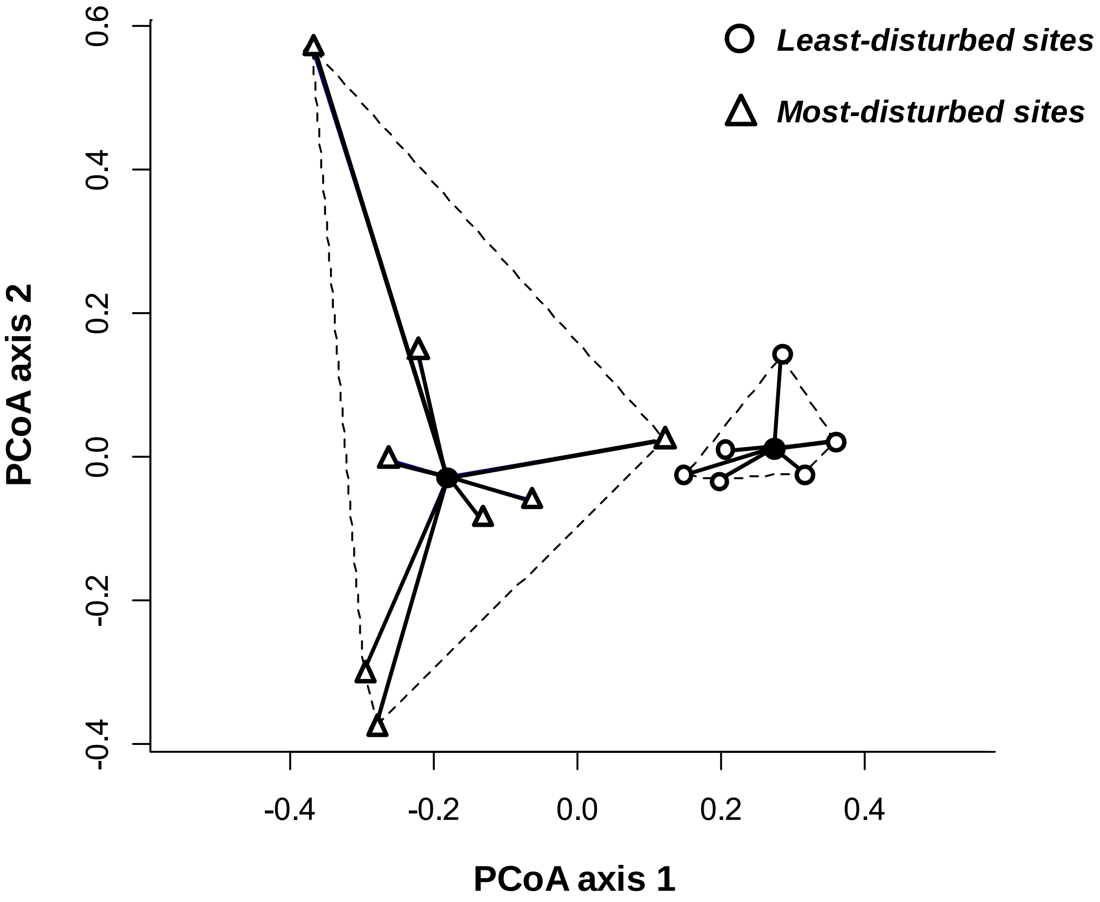
○ Least-disturbed sites
△ Most-disturbed sites
PCoA axis 2
0.6
0.4
0.2
0.0
-0.2
-0.4
-0.4
-0.2
0.0
0.2
0.4
PCoA axis 1

Figure 3.

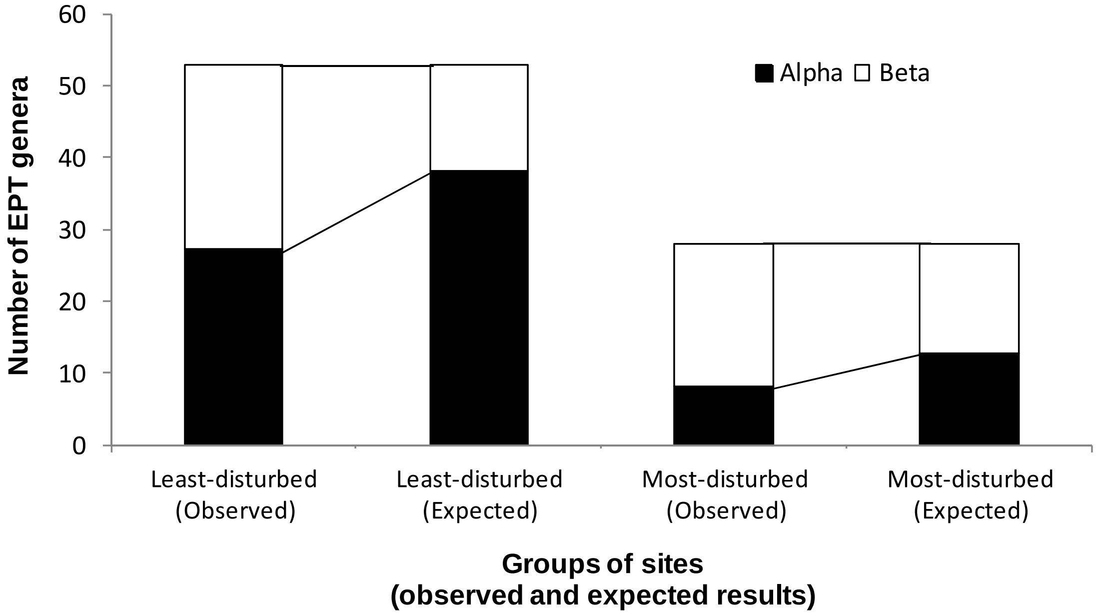
60
50
40
30
20
10
0
Number of EPT genera
■ Alpha ☐ Beta
Least-disturbed (Observed)
Least-disturbed (Expected)
Most-disturbed (Observed)
Most-disturbed (Expected)
Groups of sites
(observed and expected results)

Figure 4.

CONCLUSÕES

- A influência relativa de habitats físicos sobre a diversidade alfa de macroinvertebrados em riachos varia inversamente com a força do gradiente de distúrbios antrópicos nos riachos avaliados. Dessa forma, quanto menor a influência do distúrbio, maior a influência dos habitats para explicar a riqueza taxonômica nos riachos de uma bacia hidrográfica.

- A dificuldade de se representar a riqueza taxonômica das amostras (diversidade alfa) por meio de procedimentos de subamostragem por fração fixa ("*fixed-fraction subsampling*") depende mais da abundância de indivíduos de cada amostra do que da condição ecológica dos riachos. Quanto maior o número de indivíduos em uma amostra, menor a proporção da amostra necessária para representar sua riqueza taxonômica. Dessa forma, estes procedimentos de sub-amostragem geram estimativas de riqueza tendenciosas quando são comparadas amostras com número de indivíduos muito distintos. Se o uso de procedimentos de sub-amostragem faz-se necessário, a utilização de contagens fixas de indivíduos ("*fixed-count subsampling*") é mais aconselhada.

- A resposta das assembleias de macroinvertebrados ao gradiente de distúrbio depende da forma de amostragem em campo e da forma de processamento das amostras em laboratório. Amostragens em variados tipos de microhabitats são mais eficientes em diferenciar a composição e a riqueza taxonômica entre riachos de diferentes níveis de condição ecológica. Por outro lado, amostragens em bancos de folhas são mais eficientes para diferenciar o percentual de indivíduos EPT, sendo suas performances menos comprometidas por procedimentos de subamostragem.

- A composição taxonômica das assembleias de macroinvertebrados apresenta estrutura aninhada em função de gradientes de distúrbios, as assembleias dos riachos mais alterados no geral sendo subconjuntos das assembleias dos riachos mais preservados.
- Riachos mais alterados apresentaram maior heterogeneidade biológica entre si (diversidade beta) do que riachos mais preservados. Conclui-se que a diminuição da riqueza taxonômica regional (diversidade gama) observada nos riachos mais perturbados foi causada principalmente por extinções locais de espécies (diminuição da diversidade alfa), e não por meio de homogeneização biológica da paisagem aquática.

PERSPECTIVAS FUTURAS

Os resultados e produtos desta tese ressaltam a importância de se ter um modelo sólido e robusto como base para a definição de gradientes de distúrbio e para a subsequente definição de trechos de riachos mais preservados (em condições de "referência") e trechos mais alterados. Somente assim é possível a separação dos efeitos naturais e antrópicos sobre a estrutura e composição das assembleias aquáticas e o real entendimento dos padrões e causas da distribuição espacial dos organismos. Os seguintes tópicos devem ser futuramente aprofundados e explorados:

- Avaliar a performance da presente abordagem de definição de condição de distúrbio em outras áreas e em conjuntos de dados maiores (outras bacias, outros biomas, escala nacional), dessa forma permitindo a sua validação, adaptação e calibração.
- Investigar a relação dos diferentes tipos de distúrbios com a composição taxonômica de assembleias em riachos mais perturbados, dessa forma compreendendo melhor a razão da maior diversidade beta observada nesses locais.
- Analisar os padrões de diversidade alfa e beta em escala espacial mais ampla (regional, nacional), permitindo o entendimento da influência de fatores regionais (bacias, ecorregiões) sobre a estrutura e distribuição espacial de assembleias de macroinvertebrados aquáticos.

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ANEXOS

ANEXO I. Lista dos macroinvertebrados coletados nos riachos das bacias do Alto Rio Araguari e do Alto Rio São Francisco (Minas Gerais) durante as campanhas amostrais de setembro de 2009 e setembro de 2010, respectivamente. Estão representadas as abundâncias relativas de cada *taxa* (percentual em relação ao total de indivíduos coletados em cada bacia).

Taxa	Bacia do Alto Rio Araguari	Bacia do Alto Rio São Francisco
Arthropoda		
Insecta		
Megaloptera		
Corydalidae	0,2	< 0,1
Sialidae	0,1	< 0,1
Ephemeroptera		
Baetidae	3,6	8,5
Caenidae	0,6	1,7
Ephemeridae	-	< 0,1
Euthyplociidae	< 0,1	< 0,1
Leptohyphidae	6,2	4,3
Leptophlebiidae	6,4	3,7
Polymitarcyidae	-	0,2
Odonata		
Aeshnidae	< 0,1	< 0,1
Calopterygidae	0,3	0,1
Coenagrionidae	0,5	0,4
Corduliidae	< 0,1	< 0,1
Gomphidae	0,4	0,3
Libellulidae	0,3	0,4
Megapodagrionidae	0,1	< 0,1
Perilestidae	-	< 0,1
Plecoptera		
Gripopterygidae	2,4	< 0,1
Perlidae	1,6	0,3

Taxa	Bacia do Alto Rio Araguari	Bacia do Alto Rio São Francisco
Heteroptera		
Belostomatidae	< 0,1	< 0,1
Corixidae	< 0,1	0,1
Gelastocoridae	-	< 0,1
Gerridae	< 0,1	< 0,1
Hebridae	-	< 0,1
Mesoveliidae	< 0,1	< 0,1
Naucoridae	0,8	0,2
Notonectidae	< 0,1	< 0,1
Pleidae	0,3	0,1
Veliidae	0,2	< 0,1
Coleoptera		
Chrysomelidae	-	< 0,1
Curculionidae	< 0,1	< 0,1
Dryopidae	< 0,1	< 0,1
Dytiscidae	0,2	0,2
Elmidae	11,3	7,7
Gyrinidae	0,1	< 0,1
Hydrophilidae	0,7	< 0,1
Lutrochidae	< 0,1	0,1
Noteridae	< 0,1	< 0,1
Psephenidae	0,5	0,1
Ptilodactylidae	< 0,1	< 0,1
Salpingidae	-	< 0,1
Scirtidae	< 0,1	< 0,1
Staphylinidae	< 0,1	< 0,1
Lepidoptera		
Pyralidae	0,1	< 0,1

Taxa	Bacia do Alto Rio Araguari	Bacia do Alto Rio São Francisco
Trichoptera		
Calamoceratidae	0,2	0,1
Ecnomidae	< 0,1	< 0,1
Glossosomatidae	0,9	< 0,1
Helicopsychidae	< 0,1	0,1
Hydrobiosidae	< 0,1	0,1
Hydropsychidae	2,6	1,0
Hydroptilidae	0,1	0,4
Leptoceridae	1,0	0,2
Odontoceridae	0,6	0,1
Philopotamidae	0,1	1,1
Polycentropodidae	0,2	0,3
Sericostomatidae	< 0,1	-
Diptera		
Ceratopogonidae	0,7	1,9
Chaoboridae	-	0,1
Chironomidae	39,3	42,9
Culicidae	-	0,4
Dixidae	< 0,1	-
Dolichopodidae	0,1	< 0,1
Empididae	0,8	0,1
Muscidae	< 0,1	< 0,1
Phoridae	< 0,1	-
Psychodidae	< 0,1	< 0,1
Simuliidae	10,3	15,0
Stratiomyidae	< 0,1	< 0,1
Syrphidae	< 0,1	-
Tabanidae	0,1	0,1
Tipulidae	1,4	0,2

Taxa	Bacia do Alto Rio Araguari	Bacia do Alto Rio São Francisco
Aracnida		
Acari		
Hidracarina	0,1	0,1
Crustacea		
Ostracoda	< 0,1	-
Collembola	< 0,1	< 0,1
Anellida		
Hirudinea	0,1	0,1
Oligochaeta	3,5	4,5
Platyhelminthes		
Turbellaria		
Planariidae	0,1	0,1
Nematoda	< 0,1	< 0,1
Mollusca		
Gastropoda		
Ancylidae	-	0,1
Hydrobiidae	-	0,1
Limnaeidae	-	< 0,1
Physidae	-	< 0,1
Planorbiidae	-	0,2
Thiaridae	-	< 0,1
Bivalvia	0,5	2,1

ANEXO I!. Lista dos gêneros de EPT (Ephemeroptera, Plecoptera e Trichoptera) coletados nos riachos das bacias do Alto Rio Araguari e do Alto Rio São Francisco (Minas Gerais) durante as campanhas amostrais de setembro de 2009 e setembro de 2010, respectivamente. Estão representadas as abundâncias relativas de cada gênero (percentual em relação ao total de indivíduos EPT coletados em cada bacia).

Taxa	Bacia do Alto Rio Araguari	Bacia do Alto Rio São Francisco
Ephemeroptera		
Baetidae		
Americabaetis	3,9	11,6
Apobaetis	0,2	0,6
Aturbina	0,5	2,4
Baetodes	1,6	1,3
Callibaetis	0,1	9,5
Camelobaetidius	0,3	0,6
Cleodes	3,1	7,4
Cryptonympha	1,2	0,2
Paracleodes	1,2	1,2
Rivudiva	< 0,1	-
Spiritiops	-	0,1
Varipes	0,3	-
Waltzoyphius	0,4	2,2
Zelusia	1,2	0,4
Caenidae		
Caenis	2,1	7,6
Latineosus	-	0,1
Ephemeridae		
Hexagenia	-	< 0,1
Euthyplociidae		
Campylocia	0,2	0,2

Taxa	Bacia do Alto Rio Araguari	Bacia do Alto Rio São Francisco
Leptohyphidae		
Leptohyphes	1,3	0,2
Traverhyphes	10,8	18,0
Tricorithodes	1,1	1,3
Tricorythopsis	8,6	0,3
Leptophlebiidae		
Askola	0,9	0,1
Farrodes	2,8	5,0
Hagenulopsis	0,6	0,3
Hermanella	-	0,2
Hydrosmilodon	< 0,1	2,3
Hylister	< 0,1	-
Massartella	0,7	0,3
Miroculis	1,0	2,1
Needhamella	-	< 0,1
Paramaka	-	< 0,1
Simothraulopsis	-	0,3
Terpides	0,1	< 0,1
Thraulodes	14,4	4,2
Traverella	< 0,1	-
Ulmeritoides	1,9	1,8
Polymitarcyidae		
Asthenopus	-	0,4
Campsurus	-	0,6
Plecoptera		
Gripopterygidae		
Gripopteryx	0,6	< 0,1
Paragrypopteryx	5,1	-
Tupiperla	3,9	-

Taxa	Bacia do Alto Rio Araguari	Bacia do Alto Rio São Francisco
Perlidae		
Anacroneuria	5,3	1,4
Kempnyia	1,0	-
Macrogynoplax	-	< 0,1
Trichoptera		
Calamoceratidae		
Phylloicus	1,0	0,3
Ecnomidae		
Austrotinodes	0,2	< 0,1
Glossosomatidae		
Itaura	0,2	-
Mortoniella	3,4	< 0,1
Protoptila	0,1	< 0,1
Helicopsychidae		
Helichopsyche	0,1	0,2
Hydrobiosidae		
Atopsyche	0,1	0,3
Hydropsychidae		
Leptonema	0,8	0,3
Macronema	0,2	0,6
Macrostemum	0,2	< 0,1
Smicridea	9,6	3,9
Hydroptilidae		
Alisiotrichia	< 0,1	< 0,1
Anchitrichia	-	< 0,1
Hydroptila	0,1	1,0
Metrichia	0,1	0,5
Neotrichia	< 0,1	< 0,1
Ochrotrichia	0,1	< 0,1

Taxa	Bacia do Alto Rio Araguari	Bacia do Alto Rio São Francisco
Hydroptilidae		
Oxyethira	0,1	0,4
Taraxitrichia	-	< 0,1
Leptoceridae		
Nectopsyche	1,5	0,5
Oecetis	0,4	0,3
Triplectides	2,1	0,1
Odontoceridae		
Barypenthus	0,6	-
Marilia	1,8	0,7
Philopotamidae		
Chimarra	0,5	4,9
Wormaldia	-	< 0,1
Polycentropodidae		
Cyrnellus	-	0,2
Polycentropus	0,1	0,8
Polyplectropus	0,6	0,6
Sericostomatidae		
Grumicha	< 0,1	-