



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



**Metacomunidades em riachos subtropicais  
preservados: singularidade ecológica e esforço de  
amostragem**

**Emanuela Cristina de Castro**

Orientadora: Fabiana Schneck

Rio Grande

2021



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



**Metacomunidades em riachos subtropicais preservados:  
singularidade ecológica e esforço de amostragem**

**Aluna:** Emanuela Cristina de Castro

**Orientadora:** Fabiana Schneck

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

Rio Grande

2021

### Ficha Catalográfica

C355m Castro, Emanuela Cristina de.  
Metacomunidades em riachos subtropicais preservados:  
singularidade ecológica e esforço de amostragem / Emanuela Cristina  
de Castro. – 2021.  
92 f.

Tese (doutorado) – Universidade Federal do Rio Grande –  
FURG, Programa de Pós-Graduação em Biologia de Ambientes  
Aquáticos Continentais, Rio Grande/RS, 2021.  
Orientador: Dr. Fabiana Schneck.

Emanuela Cristina de1. Diatomácea 2. Diversidade beta 3. Esforço  
amostral 4. Inseto aquático 5. Resolução taxonômica 6. Singularidade  
composicional I. Schneck, Fabiana II. Título.

CDU 556.53

Catálogo na Fonte: Bibliotecário José Paulo dos Santos CRB 10/2344



MINISTÉRIO DA EDUCAÇÃO  
UNIVERSIDADE FEDERAL DO RIO GRANDE - FURG  
INSTITUTO DE CIÊNCIAS BIOLÓGICAS - ICB  
PROGRAMA DE PÓS-GRADUAÇÃO EM BIOLOGIA DE AMBIENTES  
AQUÁTICOS CONTINENTAIS



**ATA DE DEFESA DE TESE DE DOUTORADO EM BIOLOGIA DE AMBIENTES  
AQUÁTICOS CONTINENTAIS – Nº 004/2021**

Às 08h30 (oito horas e trinta minutos) do dia 23 (vinte e três) do mês de setembro de 2021 (dois mil e vinte e um), via Webconferência no endereço eletrônico: (<https://conferenciaweb.rnp.br/events/defesa-emanuela-castro>) reuniram-se docentes, discentes e comunidade em geral, para a Defesa Pública da Tese de Doutorado da acadêmica Emanuela Cristina de Castro. A Tese intitulada “**Metacomunidades em riachos subtropicais preservados: singularidade ecológica e esforço de amostragem**” foi avaliada pela Banca Examinadora composta pela Prof<sup>ª</sup>. Dra. Fabiana Schneck (Orientadora e Presidente da Banca); Prof<sup>ª</sup> Dra Ana Silvia Rolon (FURG); Prof<sup>ª</sup> Dra. Danielle Katharine Petsch (UEM) e Prof. Dr. Kaoli Pereira Cavalcante (UVA). Após a defesa e arguição pública, a Banca Examinadora reuniu-se, para deliberação final, e considerou a acadêmica **APROVADA**. Desta forma, a acadêmica concluiu mais uma das etapas necessárias para a obtenção do grau de **DOUTOR EM BIOLOGIA DE AMBIENTES AQUÁTICOS CONTINENTAIS**. Nada mais havendo a tratar, às 11h30h (onze horas e trinta minutos) foi lavrada a presente ata, que lida e aprovada, foi assinada pelos membros da Banca Examinadora, pela Acadêmica e pelo Coordenador do Curso.

Prof<sup>ª</sup> Dra Fabiana Schneck

Prof<sup>ª</sup> Dra Ana Silvia Rolon

Prof<sup>ª</sup> Dra. Danielle Katharine Petsch

Prof. Dr. Kaoli Pereira Cavalcante

Emanuela Cristina de Castro

Prof. Dr. Rogério Tubino Vianna  
Coordenador do Curso

Dedico essa tese à minha mãe, Marisete, meu exemplo de força e ao meu avô Manoel, minha maior saudade e meu maior amor.

## AGRADECIMENTOS

Agradeço a Deus em primeiro lugar por estar comigo nos dias em que sorria e nos dias que pensei não conseguir continuar. Por ter me ensinado tanto em meio a muitas adversidades que já enfrentei, mas por ter me dado pessoas e momentos maravilhosos ao longo da minha vida.

À Fabiana Schneck pelo conhecimento compartilhado comigo, por ter me ensinado a ser uma profissional competente e uma pessoa mais forte. Agradeço por ter me incentivado a continuar nos muitos momentos em que pensei em desistir.

Ao PPGBAC e à Comissão do PPG por ter me dado a chance de terminar minha tese.

À Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) pela bolsa concedida durante todo meu doutorado.

Aos membros da banca: Dra. Ana Silvia Rolon, Dra. Danielle Katherine Petsch e Dr. Kaoli Pereira Cavalcante por aceitarem participar desse momento importante para mim.

Agradeço pelo aprendizado e oportunidade surreais em escrever um trabalho na parceria dos professores doutores Luis Mauricio Bini, Adriano Melo, Tadeu Siqueira e Victor Landeiro.

Aos professores Edélti Faria Albertoni e Cleber Palma Silva que conheci na FURG e guardo com carinho em meus pensamentos.

Ao Dr. Fernando Marques Quintela pela melhor disciplina que já cursei, sobre mamíferos aquáticos continentais, pelas boas risadas durante as aulas e por ter me proporcionado, na aula noturna no TAIM, visualizar um céu tão estrelado como nunca vi antes.

Aos técnicos do laboratório de Limnologia da FURG, Clara, Cláudio e Leonardo, por todo auxílio.

À Karoline Martins pelas parceiras nos trabalhos e almoços.

À Cíntia Negrine Fernandez pelas conversas de horas durante os cafés e pelas boas risadas que aliviavam o stress da pós.

À Daiana Kaster Garcez, Thaísa Bozzetti Gautério e Giselle Perazzo pelas risadas e almoços no RU.

À Yarin Puerta Quintana (minha “tica”) pelos dias convvidos na FURG e fora dela e por continuar tão próxima a mim mesmo estando a milhares de quilômetros de distância. Obrigada por compartilhar do seu espanhol comigo e por ter mentido dizendo que eu sabia falar como você (eu sempre acreditava). Te quiero mucho!

À Fabiana Gonçalves Barbosa por ter aberto as portas da sua casa, me acolhendo e me ouvindo quando mais precisei. Obrigada por seus conselhos, sua amizade e por ter me apresentado à *Keep cooler*.

À Maria Gabriela Junqueira e Tauana Morés por compartilharem comigo das suas dificuldades pessoais e por me permitirem desabafar também. Nessa reta final, as palavras de incentivo de vocês duas foram essenciais para que eu finalize a tese. Desejo de todo meu coração só o que há de melhor pra vocês duas. Muito obrigada!

Ao meu melhor amigo e amor, André Luiz Marques, por acreditar que eu poderia finalizar a tese e por fazer todo o possível para que isso acontecesse. Obrigada pelas risadas por qualquer motivo e pelos sorrisos por motivo nenhum, por me fazer ter bom humor de manhã, me apresentar ao mundo da Marvel e DC, por dividir comigo o vício no PS e pelas danças aleatórias na sala de casa. Amo você com todo meu coração.

Agradeço sempre pela minha mãe, por me incentivar a realizar cada sonho que tenho. A “mãe solteira”, que lutou sozinha durante minha infância, agora tem uma filha doutora.

Ao meu avô por ser meu grande exemplo de amor incondicional, sinto sua falta todos os dias. Amo você, seu Maneco.

Ao amor que recebi da minha tia Guaya e pelo seu bilhetinho me dizendo que eu deveria ser aquilo que quisesse. Espero que, de onde estiver, se orgulhe em ver a pessoa que me tornei.

Aos meus filhos de bigode: Maluzinha por ser rabugenta e amorosa, por sempre perceber quando não estava bem e se aconchegar ao meu lado; e ao Bruno, dengoso, carente, que ronronou desde a primeira vez que nos encontramos e segue assim todos os dias.

Às minhas amigas Francelize e Andressa que a vida me deu na 5ª série e que trago comigo até hoje. Por se fazerem presentes mesmo quando morei em outro Estado e por serem simplesmente que vocês são. À minha amiga Rosangela, pela sua loucura e amizade únicas.

Meus pensamentos durante a redação dos agradecimentos também está com mestrandxs e doutorandxs que adoecem mentalmente durante o período de estudos, especialmente por aqueles que desistem da vida por conta de doenças psicológicas relacionadas à pós. Meus maiores sentimentos a vocês.



## RESUMO

A diversidade beta é a variação na composição de espécies entre comunidades ao longo de um gradiente ambiental. A compreensão dos padrões de diversidade beta depende também de compreendermos o quanto cada comunidade local contribui para a diversidade beta regional, o que chamamos de singularidade composicional. Independente da escolha das várias métricas disponíveis para estimar a diversidade beta, o esforço amostral deve ser adequado para refletir os padrões de organização das comunidades. Nesta tese meu objetivo principal foi investigar aspectos relacionados à organização de metacomunidades de insetos aquáticos e diatomáceas perifíticas em riachos preservados. Ambas as comunidades representam importantes constituintes das comunidades biológicas de riachos, atuando desde a ciclagem de nutrientes até como recurso alimentar. Utilizei amostras de 90 corredeiras de nove riachos (10 corredeiras por riacho) localizados em área de proteção junto ao maior remanescente de Mata Atlântica do estado de São Paulo. No primeiro capítulo avaliei a singularidade na composição de comunidades locais (LCBD), tanto total como em termos de substituição de espécies e de aninhamento, e sua relação com a posição das corredeiras e com variáveis de singularidade ambiental (LCEH). Somente o LCBD de diatomáceas foi correlacionado com a singularidade na composição de substratos. Ainda, os valores de LCBD em termos de substituição de espécies e de aninhamento apresentaram relação negativa e positiva, respectivamente, com o LCEH de substratos. Estes resultados indicam que a singularidade composicional e a singularidade ambiental nem sempre apresentam relação significativa, contrariando nossa hipótese inicial. Dessa forma, nossos resultados demonstram que encontrar os fatores que regem a singularidade composicional é um desafio. No capítulo 2, analisei se resoluções taxonômicas mais grosseiras e esforço de contagem reduzido são suficientes para recuperar padrões de composição de espécies e de diversidade beta de comunidades de diatomáceas. Resoluções taxonômicas de gênero e família somente refletiram padrões de composição de

espécies com informações de abundância. Já a contagem de um número reduzido de valvas foi suficiente para recuperar os padrões de composição de espécies (400 valvas com dados de presença-ausência e 50 valvas com dados de abundância) e de diversidade beta (200 valvas com dados de presença-ausência ou com dados de abundância) gerados pelo conjunto total de valvas (500 valvas). Portanto, observamos que o número suficiente de valvas contadas pode variar dependendo do objetivo do estudo. Por fim, sugerimos estudos futuros que avaliem outros fatores que possam estar relacionados com a singularidade ecológica, uma vez que essa métrica tem se mostrado eficaz em avaliar como as comunidades biológicas são organizadas. Além disso, nossos resultados demonstraram que é possível reduzir o esforço de contagem em estudos de diatomáceas perifíticas, o que pode ser extremamente útil especialmente em programas de biomonitoramento.

**Palavras-chave:** diatomácea, diversidade beta, esforço amostral, inseto aquático, resolução taxonômica, singularidade composicional

## ABSTRACT

Beta diversity is the variation in species composition among communities across an environmental gradient. Understanding beta diversity patterns also depends on understanding how much each local community contributes to regional beta diversity, that is, the compositional uniqueness. Regardless of the choice of the various metrics available to estimate beta diversity, sampling effort must be adequate to reflect the assembling patterns of communities. In this thesis, my main objective was to investigate aspects related to the organization of aquatic insects and periphytic diatoms in pristine streams. Both communities represent important constituents of the biological community of streams, from nutrient cycling to food resource. I used samples from 90 riffles collected from nine streams (10 riffles per stream) at a protected area near the largest remnant of Atlantic Forest in the state of São Paulo. In the first chapter, I evaluated the uniqueness in the species composition of local communities (LCBD), both total and in terms of species replacement and nestedness, and its relationship with the position of the riffles and with variables of environmental uniqueness (LCEH). Only the LCBD of diatoms was correlated with uniqueness in substrate composition. Furthermore, the LCBD values in terms of species replacement and nestedness showed positive and negative relationships with the LCEH of substrates, respectively. These results indicate that compositional uniqueness and environmental uniqueness do not always have a significant relationship, contrary to our initial hypothesis. Thus, our results demonstrate that finding strong correlates of compositional uniqueness is a challenge. In chapter 2, I evaluated whether coarse taxonomic resolutions and reduced enumeration efforts are sufficient to recover patterns in species composition and in beta diversity of diatom metacommunities. Taxonomic resolutions of genus and family only reflected species composition patterns with abundance information. However, enumeration of a reduced number of valves was enough to recover the patterns in species composition (400 valves with presence-absence data and 50

valves with abundance data) and in beta diversity (200 valves with presence-absence data or with abundance data) generated by the total set of valves (500 valves). Therefore, we observed that the sufficient number of valves counted may vary depending on the purpose of the study. Finally, we conclude by suggesting that future studies should assess other factors that may be related to ecological uniqueness, since this metric has been shown to be effective in evaluating how biological communities are organized. Furthermore, our results showed that it is possible to reduce the counting effort in studies of periphytic diatoms, which can be extremely useful especially in biomonitoring programs.

**Key-words:** aquatic insect, beta diversity, compositional uniqueness, diatom, sampling effort, taxonomic resolution

## APRESENTAÇÃO

Meu projeto de pesquisa, que permitiu a realização da minha tese, faz parte de um projeto maior, intitulado *Scaling biodiversity in tropical and boreal streams: implications for diversity mapping and environmental assessment (ScaleBio)*. Esse projeto foi fomentado pela *Finnish Academy* e pela FAPESP (Fundação de Amparo à Pesquisa do Estado de São Paulo). Tem como pesquisadores principais: Dr. Tadeu Siqueira (Departamento de Ecologia, Universidade Estadual Paulista “Júlio de Mesquita Filho”, Brasil), Dr. Jani Heino (Departamento de Biologia, Universidade de Oulu, Finlândia) e Janne Soininen (Departamento de Geociências e Geografia, Universidade de Helsinki, Finlândia). O objetivo desse projeto foi avaliar a diversidade beta de insetos aquáticos e diatomáceas perifíticas em riachos boreais finlandeses e subtropicais brasileiros.

Eu realizei a análise biológica do material coletado dos riachos amostrados no Laboratório de Limnologia do Instituto de Ciências Biológicas da FURG (Universidade Federal do Rio Grande), logo no primeiro ano de doutorado. A identificação das espécies contou com a participação da Dra. Priscila Tremarin, do laboratório de Ficologia da UFPR (Universidade Federal do Paraná). Passei um ano e meio contando as valvas das diatomáceas das 90 amostras e dois meses identificando os indivíduos, que totalizaram 260 espécies. Todas as demais atividades como doutoranda (análises estatísticas, redação da Tese e outras) foram realizadas nas dependências da FURG, na cidade de Rio Grande (RS).

Minha tese está estruturada em dois capítulos: o primeiro, intitulado *Compositional uniqueness of diatoms and insects in subtropical streams is weakly correlated with riffle position and environmental uniqueness*, foi publicado na *Hydrobiologia* (<https://doi.org/10.1007/s10750-019-04037-8>). Nesse artigo, procuramos avaliar a singularidade composicional de metacomunidades de insetos aquáticos e diatomáceas perifíticas de nove riachos subtropicais preservados.

O segundo capítulo, denominado *Reduced enumeration effort, but not coarse taxonomic resolution, is sufficient to represent beta diversity patterns of stream benthic diatoms*, foi submetido ao periódico *Aquatic Sciences*. Nesse artigo avaliamos qual o esforço amostral suficiente que descreveria os padrões de diversidade beta de diatomáceas perifíticas de riachos subtropicais preservados. Os capítulos estão formatados de acordo com as normas dos periódicos *Hydrobiologia* (Capítulo 1) e *Aquatic Sciences* (Capítulo 2).

O restante da tese está formatado de acordo com as normas da ABNT (Associação Brasileira de Normas Técnicas), de acordo com o guia para elaboração de tese do Programa de Pós Graduação de Biologia de Ambientes Aquáticos Continentais - FURG. Após os capítulos, apresento uma seção de considerações finais, em que apresento meus principais resultados, assim como minhas perspectivas para estudos futuros.

## SUMÁRIO

<b>Resumo</b> .....	9
<i>Abstract</i> .....	11
<b>Apresentação</b> .....	13
<b>Lista de figuras</b> .....	16
<b>Lista de tabelas</b> .....	19
<b>Introdução geral</b> .....	20
Diversidade beta .....	22
Diversidade beta e a avaliação da singularidade na composição de espécies .....	24
Importância do esforço amostral e da resolução taxonômica na detecção de padrões em comunidades .....	25
Riachos .....	27
Insetos aquáticos .....	27
Diatomáceas .....	28
<b>Objetivo geral e objetivos específicos da tese</b> .....	29
<b>Capítulo 1</b> - <i>Compositional uniqueness of diatoms and insects in subtropical streams is weakly correlated with riffle position and environmental uniqueness</i> .....	36
<b>Capítulo 2</b> - <i>Reduced enumeration effort, but not coarse taxonomic resolution, is sufficient to represent beta diversity patterns of stream benthic diatoms</i> .....	68
<b>Considerações finais e perspectivas</b> .....	89
<b>Anexos</b> .....	93

## LISTA DE FIGURAS

### Capítulo 1 - Compositional uniqueness of diatoms and insects in subtropical streams is weakly correlated with riffle position and environmental uniqueness

**Figura 1.** Positions of the first and last riffles studied of each stream in Carmo River Basin (gray circles). Blue lines symbolize the hydrographic network of Carmo River and the numbers indicate the nine streams sampled in this study ..... 57

**Figura 2.** Simplified map of the Carmo River Basin showing compositional uniqueness (local contribution to beta diversity,  $LCBD_{Total}$  values) using presence-absence (Sørensen index) data for periphytic diatoms (a) and insects (b). Arrows inside boxes indicate flow direction. The size of the circles is proportional to the  $LCBD_{Total}$  value, blue and red circles represent significant ( $P < 0.05$ ) and non-significant values of  $LCBD_{Total}$ , respectively. A detailed map can be found in Fig. 1 ..... 58

**Figura 3.** Simplified map of the Carmo River Basin showing compositional uniqueness in terms of nestedness ( $LCBD_{NesS}$ ) (a) and species replacement ( $LCBD_{RepS}$ ) (b) using presence-absence (Sørensen index) data for periphytic diatoms. Arrows inside boxes indicate flow direction. The size of the circles is proportional to the  $LCBD$  values. Significance of the values was not evaluated. A detailed map can be found in Fig. 1 ..... 59

**Figura 4.** Simplified map of the Carmo River Basin showing compositional uniqueness in terms of nestedness ( $LCBD_{NesS}$ ) (a) and species replacement ( $LCBD_{RepS}$ ) (b) using presence-absence (Sørensen index) data for insects. Arrows inside boxes indicate flow direction. The



size of the circles is proportional to the LCBD values. Significance of the values was not evaluated. A detailed map can be found in Fig. 1 ..... 60

**Material suplementar 1**

**Figura 1.** Simplified map of the Carmo River Basin showing compositional uniqueness (local contribution to beta diversity,  $LCBD_{Total}$  values) using abundance (Bray-Curtis index) data for periphytic diatoms (a) and insects (b). Arrows inside boxes indicate flow direction. The size of the circles is proportional to the  $LCBD_{Total}$  value, blue and red circles represent significant ( $P < 0.05$ ) and non-significant values of  $LCBD_{Total}$ , respectively. A detailed map can be found in Fig. 1..... 61

**Figura 2.** Simplified map of the Carmo River Basin showing compositional uniqueness in terms of nestedness ( $LCBD_{NesBC}$ ) (a) and species replacement ( $LCBD_{RepBC}$ ) (b) using abundance (Bray-Curtis index) data for insects. Arrows inside boxes indicate flow direction. The size of the circles is proportional to the LCBD values. Significance of the values was not evaluated. A detailed map can be found in Fig. 1..... 62

**Capítulo 2 - Reduced enumeration effort, but not coarse taxonomic resolution, is sufficient to represent beta diversity patterns of stream benthic diatoms**

**Figura 1.** Histograms showing Procrustes correlations between PCoA scores using the complete enumeration effort (500 valves per riffle) and each of the 500 matrices generated using reduced enumeration efforts for both presence-absence (a) and abundance (b) data.... 84

**Figura 2.** Histograms showing Pearson correlations between beta diversity values obtained using the complete enumeration effort (500 valves per riffle) and each of the 500 subsets

generated using reduced enumeration efforts for both presence-absence (a) and abundance (b)  
data ..... 85

## LISTA DE TABELAS

### Capítulo 1 - Compositional uniqueness of diatoms and insects in subtropical streams is weakly correlated with riffle position and environmental uniqueness

**Tabela 1.** Mean and range of values of environmental variables (within riffles) in the nine streams sampled in southeast Brazil ..... 53

**Tabela 2.** Results of linear mixed-effects model using compositional uniqueness (local contribution to beta diversity,  $LCBD_{Total}$ ) of periphytic diatoms and insects as response variables (degrees of freedom = 78).  $LCEH_{phy}$  and  $LCEH_{sub}$ : local contribution to environmental heterogeneity resulting from stream physical characteristics and from substrate composition, respectively. Significant t-values ( $P \leq 0.05$ ) are in bold ..... 54

**Tabela 3.** Results of linear mixed-effects model using compositional uniqueness in terms of nestedness ( $LCBD_{Nes}$ ) and species replacement ( $LCBD_{Rep}$ ) of periphytic diatoms as response variables (degrees of freedom = 78).  $LCEH_{phy}$  and  $LCEH_{sub}$ : local contribution to environmental heterogeneity resulting from stream physical characteristics and from substrate composition, respectively. Significant t-values ( $P \leq 0.05$ ) are in bold ..... 55

**Tabela 4.** Results of linear mixed-effects model using compositional uniqueness in terms of nestedness ( $LCBD_{Nes}$ ) and species replacement ( $LCBD_{Rep}$ ) of insects as response variables (degrees of freedom = 78).  $LCEH_{phy}$  and  $LCEH_{sub}$ : local contribution to environmental heterogeneity resulting from stream physical characteristics and from substrate composition, respectively..... 56

## Material suplementar 2

**Tabela 1.** Results of linear mixed-effects model using compositional uniqueness in terms of richness difference (LCBD<sub>Nes</sub>) and species replacement (LCBD<sub>Rep</sub>) of periphytic diatoms as response variables (degrees of freedom = 78) using the approach of Podani & Schmera (2011). LCEH<sub>phy</sub> and LCEH<sub>sub</sub>: local contribution to environmental heterogeneity resulting from stream physical characteristics and from substrate composition, respectively. Significant t-values ( $P \leq 0.05$ ) are in bold .....63

**Tabela 2.** Results of linear mixed-effects model using compositional uniqueness in terms of richness difference or abundance difference (LCBD<sub>Nes</sub>) and species replacement (LCBD<sub>Rep</sub>) of insects as response variables (degrees of freedom = 78) using the approach of Podani and Schmera (2011). LCEH<sub>phy</sub> and LCEH<sub>sub</sub>: local contribution to environmental heterogeneity resulting from stream physical characteristics and from substrate composition, respectively. Significant t-values ( $P \leq 0.05$ ) are in bold..... 64

## Capítulo 2 - Reduced enumeration effort, but not coarse taxonomic resolution, is sufficient to represent beta diversity patterns of stream benthic diatoms

**Tabela 1.** Procrustes and Pearson correlations between data using species and coarse taxonomic resolutions for both presence-absence (Sørensen coefficient) and abundance (Bray-Curtis coefficient) data. Procrustes was used to compare ordination patterns ( $n = 90$ ), and Pearson correlation was used to quantify the relationship between beta diversity values estimated with species data and data with lower taxonomic resolutions ( $n = 9$ ) ..... 82

**Tabela 2.** Procrustes and Pearson mean correlations ( $\pm$  standard deviation) between data using the complete enumeration effort (500 valves per riffle) and reduced efforts for both presence-absence (Sørensen coefficient) and abundance (Bray-Curtis coefficient) data. Procrustes was used to compare ordination patterns ( $n = 90$ ), and Pearson correlation was used to quantify the relationship between beta diversity values estimated with complete enumeration (500 valves) and reduced enumeration efforts (from 50 to 400) ( $n = 9$ ). Correlations are the mean values ( $\pm$  standard deviation) from 500 matrices generated for each enumeration effort ..... 83

## INTRODUÇÃO GERAL

### Diversidade beta

A distribuição de espécies ocorre de maneira irregular na Terra e explicar essa configuração na organização dos indivíduos ao longo dos ambientes é um grande desafio (GASTON, 2000). O reconhecimento de que comunidades biológicas ocorrem em metacomunidades, ou seja, um conjunto de comunidades ligadas entre si pela dispersão de espécies (WILSON, 1992; LEIBOLD *et al.*, 2004), tem auxiliado na compreensão sobre como as comunidades interagem entre si (LEIBOLD *et al.*, 2004) e sobre o papel das características ambientais em sua organização, inclusive na capacidade de influenciar na variação das comunidades entre os locais. Em uma metacomunidade, a estruturação das comunidades locais é influenciada tanto pelas interações locais (interação entre espécies e entre espécies e ambiente), quanto pelos processos regionais (dispersão, heterogeneidade ambiental, etc.) (LOGUE *et al.*, 2011). Dessa forma, não só a composição local da comunidade, mas também a diversidade regional de espécies, norteiam a estrutura das metacomunidades (LOGUE *et al.*, 2011).

Whittaker (1960) definiu a diversidade beta ( $\beta$ ) como a mudança na composição de espécies entre comunidades ao longo de um gradiente ambiental. Além disso, ele descreveu a diversidade beta como um componente da diversidade gama ( $\gamma$ ), que abrange o total de espécies em uma região, e da diversidade alfa ( $\alpha$ ), o número de espécies em uma comunidade local. O conceito de diversidade beta vem sendo discutido desde então e foi ressignificado por Anderson *et al.* (2011), que separaram a diversidade beta em duas facetas: substituição e variação de espécies. A diversidade beta como a substituição de espécies descreve a variação direcional na composição de comunidades de uma unidade amostral à outra ao longo de um gradiente (espacial, temporal ou ambiental). Já a variação das espécies como faceta da diversidade beta desconsidera a direcionalidade entre as unidades amostrais, avaliando apenas

a mudança espacial ou temporal na composição de espécies entre os locais (ANDERSON *et al.*, 2011).

A diversidade beta pode ser observada em diferentes escalas, desde escalas globais e regionais até escalas locais (*e.g.*, entre microhabitats). Em escalas maiores, como entre regiões, a diversidade beta é comumente dirigida pela distância espacial causando restrições de dispersão. Já em escalas menores, em que a limitação de dispersão não é importante, a heterogeneidade ambiental parece ser o principal fator a gerar diversidade beta (HEINO *et al.*, 2013). A heterogeneidade ambiental pode ser definida como a variação nas condições ambientais entre locais em uma determinada região (HEINO *et al.*, 2015). Conhecer a relação entre diversidade beta e características ambientais é um passo importante para entender como as comunidades são estruturadas e sua relação com os fatores abióticos, especialmente em ambientes preservados. Por exemplo, Heino *et al.* (2013), ao estudarem riachos pouco impactados em uma mesma bacia hidrográfica da Finlândia, reduzindo assim a limitação de dispersão, observaram que a variação na composição da comunidade de macroinvertebrados foi maior entre riachos do que dentro de riachos, devido à maior variação ambiental entre os riachos. Ainda, avaliando a diversidade beta em riachos também preservados de uma mesma bacia hidrográfica da Finlândia, a comunidade de diatomáceas também mostrou ser mais heterogênea entre os riachos do que dentro deles, de maneira similar ao estudo anterior (JYRKÄNKALLIO-MIKKOLA *et al.*, 2016). Dentre as características que comumente têm um importante papel na organização de comunidades em riachos, podem ser citadas variáveis de qualidade da água, como pH e nutrientes (SOININEN *et al.*, 2016) e variáveis físicas, como tipos de substrato, velocidade de correnteza e profundidade (ASTORGA *et al.*, 2014). Ou seja, várias são as condições que dirigem a estrutura das comunidades em ambientes aquáticos continentais, especialmente em riachos, uma vez que esses ambientes variam amplamente em suas características físicas e químicas (ALLAN; CASTILLO, 2007).

## **Diversidade beta e a avaliação da singularidade na composição de espécies**

Como comentado anteriormente, a diversidade beta pode ser avaliada a partir da substituição direcional das espécies ao longo das comunidades em um gradiente ou da variação espacial ou temporal não direcional das espécies entre as comunidades locais (ANDERSON *et al.*, 2011). Além disso, várias são as métricas existentes para estimar a diversidade beta (*e.g.*, BASELGA, 2010; ANDERSON *et al.*, 2011; LEGENDRE; DE CÁCERES, 2013), sendo que a escolha dos índices de dissimilaridades a serem empregados em tais análises pode também ser crucial (BASELGA; ORME, 2012). Entretanto, independente de todas estas possibilidades, estudos indicavam uma crescente necessidade em quantificar e determinar a diversidade beta a partir de um único número (LEGENDRE; DE CÁCERES, 2013). Neste sentido, Legendre e De Cáceres (2013) propuseram uma maneira de quantificar a diversidade beta regional e a contribuição de cada comunidade local, o que os autores chamaram de LCBD (*local contribution to beta diversity*). Portanto, o LCBD permite avaliar o quanto cada comunidade local contribui para a diversidade beta regional, ou seja, o quanto cada comunidade local é singular em relação à comunidade ‘típica’ da região (LEGENDRE; DE CÁCERES, 2013).

Para quantificar a singularidade de cada comunidade local, a diversidade beta regional ( $BD_{Total}$ ) é estimada através da variância total da matriz de dados de sites e espécies (chamada de tabela  $Y$ ) de acordo com determinado índice de dissimilaridade, permitindo analisar a diferenciação na identidade de espécies entre as comunidades (LEGENDRE; DE CÁCERES, 2013). Uma vez que se tem a variância total, é possível particioná-la para obter os valores de singularidade de cada comunidade local, ou seja, os valores de LCBD. Estes valores variam de 0 a 1, sendo 0 nenhuma contribuição local para a diversidade beta total e 1 o valor máximo da contribuição local para a diversidade beta total (LEGENDRE; DE CÁCERES, 2013).

Unidades amostrais com valores altos de LCBD podem refletir, por exemplo, áreas degradadas que devem ser selecionadas para fins de recuperação (LEGENDRE; DE



CÁCERES, 2013). De fato, alterações antrópicas podem fazer com que poucas espécies sejam capazes de sobreviver em condições pouco favoráveis, refletindo a baixa riqueza de espécies nesses locais, fazendo com que tais comunidades locais sejam mais singulares quando comparadas à comunidade média regional (LEGENDRE; DE CÁCERES, 2013; HEINO *et al.*, 2017; SOR *et al.*, 2018; BENITO *et al.*, 2020). Tais resultados também podem revelar locais que abrigam espécies raras que contribuem para a variação na diversidade regional de metacomunidades (HEINO *et al.*, 2017). Além disso, estudos analisando a singularidade na composição de comunidades trazem novos conhecimentos à Ecologia, especialmente em metacomunidades. Por exemplo, Li *et al.* (2020) verificaram que comunidades ligadas entre si que abrigavam indivíduos com baixa capacidade de dispersão apresentavam valores alto de LCBD. Entretanto, ainda são incipientes os estudos que avaliam o LCBD e os fatores que determinam uma maior ou menor singularidade na composição de espécies, especialmente em ambientes subtropicais preservados. Dessa forma, estudos futuros analisando o LCBD desses ambientes em particular são necessários para o entendimento de quais fatores determinam a singularidade das comunidades biológicas e seu impacto para a diversidade beta regional.

### **Importância do esforço amostral e da resolução taxonômica na detecção de padrões em comunidades**

Como discutido até aqui, reconhecer os mecanismos que regem a organização das comunidades biológicas é um grande desafio. Muitas são as métricas de avaliação da diversidade, diversos os índices de dissimilaridade que podem ser aplicados e assim, infindáveis podem ser as respostas obtidas. Entretanto, outra questão vem à tona na tomada de decisão avaliando a organização das comunidades biológicas: definir o esforço amostral do estudo e a resolução taxonômica utilizada para a identificação dos indivíduos. De fato, definir o número de amostras a serem coletadas e quantos indivíduos devem ser quantificados são fatores centrais que podem determinar a qualidade de um estudo (BENNETT *et al.*, 2017) e

garantir um bom custo-benefício, evitando despender tempo e recursos financeiros desnecessários. Isso é especialmente importante para otimizar programas de biomonitoramento em regiões subtropicais e tropicais, devido aos desafios financeiros comumente enfrentados para a efetiva manutenção de tais programas.

Com o intuito de reconhecer a estrutura das comunidades biológicas, a identificação dos organismos em nível específico geralmente é requerida. No entanto, tais trabalhos são intensos, uma vez que podem exigir custo de tempo e pessoal, além de delineamento amostral amplo e outras demandas dependendo do objetivo do estudo (CARNEIRO *et al.*, 2013). Sendo assim, abordagens alternativas, como o uso de resoluções taxonômicas mais grosseiras (*e.g.*, gênero, família e ordem), vêm sendo avaliadas na tentativa de descrever a composição de comunidades sem perder as informações geradas pelas espécies. Alguns estudos vêm demonstrando que a identificação em resoluções taxonômicas menores é satisfatória para a descrição de padrões espaciais ou temporais, como já observado em nível de família para metacomunidades de macroinvertebrados (MELO, 2005) e em nível genérico para comunidades de diatomáceas bênticas (RIMET; BOUCHEZ, 2012), comunidades planctônicas (CARNEIRO *et al.*, 2010; 2013) e insetos aquáticos (HEINO; SOINENEN, 2007). Dessa maneira, é possível reduzir o tempo utilizado na identificação dos organismos, dispensar conhecimento específico de estruturas peculiares das espécies e daquelas que possuem ampla variabilidade morfológica (CARNEIRO *et al.*, 2010). Contudo, trabalhos específicos avaliando metacomunidades com o uso de menores resoluções taxonômicas de diatomáceas perifíticas em ambientes subtropicais pouco impactados ainda são incipientes. Tais estudos são fundamentais especialmente em ambientes aquáticos tropicais e subtropicais que apresentam alta diversidade de espécies.

Estudos também apontam outra possibilidade que reduziria substancialmente os custos e tempo despendido em programas de biomonitoramento que visam descrever os padrões de estruturação de comunidades: a redução no número de indivíduos contados por amostra

(BENNETT *et al.*, 2017; SGARBI *et al.*, 2020). Por exemplo, estudos com macroinvertebrados de riachos indicam que é possível reduzir substancialmente o número de indivíduos contados por amostra em relação a protocolos comumente utilizados (*e.g.*, SCHNECK; MELO, 2010; SGARBI *et al.*, 2020). Já para diatomáceas, protocolos indicam a necessidade de contagem de 400 a 600 valvas para avaliar as condições de qualidade da água em programas de biomonitoramento (CHARLES *et al.*, 2021). Entretanto, há uma escassez de estudos que avaliem a necessidade de um esforço de contagem tão elevado para riachos tropicais e subtropicais.

### **Riachos**

Riachos, especialmente aqueles inseridos em locais subtropicais, são ambientes aquáticos que apresentam variação sazonal bem definida, especialmente na profundidade, velocidade e química da água (JR, 2008, p. 2). Esses ambientes aquáticos apresentam grande variação ambiental, como quantidade de luz solar disponível, temperatura, nutrientes e tipos de substratos (CUSHING; ALLAN, 2001).

As comunidades biológicas presente em riachos se caracteriza por organismos que se adaptam às condições abióticas e bióticas desses ambientes, como a variação ambiental física e interações interespecíficas, respectivamente (ALLAN; CASTILLO, 2007). Produtores primários são os principais fornecedores de energia dos riachos, principalmente as diatomáceas, que constituem a principal fonte de alimento dos consumidores (ALLAN; CASTILLO, 2007).

### **Insetos aquáticos**

Atualmente existem cerca de 100.000 espécies de insetos aquáticos (DIJKSTRA *et al.*, 2014). Os insetos aquáticos dividem seu ciclo de vida na água, onde vivem dentro de ovos ou na forma larval; e na terra, como insetos adultos (DIJKSTRA *et al.*, 2014). Se reproduzem

sexuadamente e o comportamento e estratégias sexuais diferem para cada grupo (VILELA; SANMARTÍN-VILLAR, 2019, p. 263). Os insetos possuem como uma de suas principais características a capacidade de dispersão facilitada pela capacidade de voar pela presença de asas (HEINO; MYKRÄ, 2008). Como mecanismos de sobrevivência frente a predadores, os insetos aquáticos apresentam diversos mecanismos de defesa como estridulação, reações de escape, defesa química e mecânica (DETTNER, 2019, p. 191).

A importância ecológica e serviços dos insetos aquáticos é notável, uma vez que servem como fonte alimentar para outros animais aquáticos, controle de doenças, purificação dos corpos aquáticos e participam da ciclagem de nutrientes (SUNDAR *et al.*, 2019). São abundantes em riachos e são capazes de suportar uma ampla variação nas condições ambientais (HEINO; PECKARSKY, 2014). Os filtros ambientais, especialmente em riachos, promovem a diversidade de espécies nas comunidades aquáticas (HEINO; MYKRÄ, 2008).

### **Diatomáceas**

Diatomáceas são algas unicelulares eucarióticas com sua parede celular impregnada de sílica, composta por duas valvas ligadas pelo cingulo, denominada de frústula (ROUND; CRAWFORD; MANN, 1990). A frústula das diatomáceas constitui sua principal característica, exibe uma ampla variedade ornamental e é utilizada na taxonomia do grupo (MANN, 1999). Estima-se que haja 250 gêneros abrangendo cerca de 100.000 espécies de diatomáceas (VAN DEN HOEK; MANN; JAHNS, 1995).

As diatomáceas podem ser encontradas em ambientes continentais e marinhos (POTAPOVA; CHARLES, 2002), especialmente nas comunidades aquáticas bentônicas e planctônicas (MANN; DROOP, 1996), sendo abundantes principalmente em riachos (SOININEN, 2007). Constituem o grupo mais abundante de algas, participando ativamente dos ciclos de carbono e sílica (MANN, 1999). A grande maioria das diatomáceas é fotossintética, sendo poucas as espécies heterotróficas (ROUND *et al.*, 1990). No perifiton,

uma complexa comunidade de microorganismos aderidos a diversos tipos de substratos (WETZEL, 1993), as diatomáceas constituem o grupo algal mais representativo (BURLIGA; SCHWARZBOLD, 2013).

Várias são as aplicações ecológicas das diatomáceas: como bioindicadoras da qualidade da água, na reconstrução da história dos ambientes aquáticos em estudos paleontológicos, em estudos da ciclagem de nutrientes e até mesmo na ciência forense (SMOL; STOERMER, 2010).

## **OBJETIVO GERAL DA TESE E OBJETIVOS ESPECÍFICOS**

O objetivo geral desta tese é buscar compreender aspectos relacionados à organização de comunidades de insetos aquáticos e diatomáceas perifíticas em riachos preservados.

Nessa seção, vou dividir meus objetivos gerais e específicos correspondentes aos dois capítulos da minha tese.

### **Capítulo 1 - Compositional uniqueness of diatoms and insects in subtropical streams is weakly correlated with riffle position and environmental uniqueness**

- Objetivo geral:

- Analisar a contribuição local para a diversidade beta (LCBD) de diatomáceas perifíticas e insetos de riachos preservados.

- Objetivos específicos:

- Testar a ocorrência de uma relação positiva entre a singularidade na composição de espécies e a singularidade ambiental de corredeiras e a posição da corredeira no riacho.
- Testar se há uma relação positiva entre a singularidade na composição de espécies em termos de substituição de espécies e a singularidade ambiental.
- Testar se há uma relação positiva entre a singularidade composicional em termos de diferenças de riqueza e a posição da corredeira no riacho.

## **Capítulo 2 - Reduced enumeration effort, but not coarse taxonomic resolution, is sufficient to represent beta diversity patterns of stream benthic diatoms**

- Objetivo geral:
  - Analisar a suficiência de resoluções taxonômicas grosseiras ou esforços de contagem reduzidos em refletir os padrões de composição e de diversidade beta de diatomáceas em riachos.
  
- Objetivo específico
  - Testar o uso de gênero, família e ordem e de esforços de contagem reduzidos (subconjuntos de 50, 100, 200, 300 e 400 valvas de diatomáceas contadas em cada corredeira) refletem os padrões de composição de 90 comunidades e de diferenças na diversidade beta entre nove metacomunidades.

### **Referências bibliográficas**

ALLAN, J. D.; CASTILLO, M. M. **Stream ecology**. Structure and function of running waters. 2nd ed. Amsterdam: Springer, 2007.

ANDERSON, M. J.; CRIST, T. O.; CHASE, J. M.; VELLEND, M.; INOUE, B. D.; FREESTONE, A. L.; SANDERS, N. J.; CORNELL, H. V.; COMITA, L. S.; DAVIES, K. F.; HARRISON, S. P.; KRAFT, N. J. B.; STEGEN, J. C.; SWENSON, N. G. Navigating the multiple meanings of  $\beta$  diversity: a roadmap for the practicing ecologist. **Ecology Letters**, v. 14, p. 19-28, 2011.

ASTORGA, A.; DEATH, R.; DEATH, F.; PAAVOLA, R.; CHAKRABORTY, M.; MUOTKA, T. Habitat heterogeneity drives the geographical distribution of beta diversity: the case of New Zealand stream invertebrates. **Ecology and Evolution**, v. 4, p. 2693-2702, 2014.

BASELGA, A. Partitioning the turnover and nestedness components of beta diversity. **Global Ecology and Biogeography**, v. 19, p. 134-143, 2010.

BASELGA, A.; ORME, C. D. L. Betapart: an R package for the study of beta diversity. **Methods in Ecology and Evolution**, v. 3, p. 808-812, 2012.

BENITO, X.; VILMI, A.; LUETHJE, M.; CARREVEDO, M. L.; LINDHOLM, FRITZ, M.; SHERILYN, C. Spatial and temporal ecological uniqueness of Andean diatom communities are correlated with climate, geodiversity and long-term limnological change. **Frontiers in Ecology and Evolution**, v. 8, p. 260, 2020.

BENNETT, J. R.; RÜHLAND, K. M.; SMOL, J. P. No magic number: determining cost-effective sample size and enumeration effort for diatom-based environmental assessment analyses. **Canadian Journal of Fisheries and Aquatic Sciences**, v. 74, p. 208-215, 2017.

BURLIGA, A. L.; SCHWARZBOLD, A. Perifiton: diversidade taxonômica e morfológica. In SCHWARZBOLD, A.; BURLIGA, A. L.; TORGAN, L. C. (Edits). **Ecologia do perifiton**. São Carlos: Rima, 2013, p.1-6.

CARNEIRO F. M.; BINI, L. M.; RODRIGUES, L. C. Influence of taxonomic and numerical resolution on the analysis of temporal changes in phytoplankton communities. **Ecological Indicators**, v. 10, p. 249-255, 2010.

CARNEIRO, F. M.; NABOUT, J. C.; VIEIRA, L. C. G.; LODI, S.; BINI, L. M. Higher taxa predict plankton beta-diversity patterns across an eutrophication gradient. **Natureza & Conservação**, v. 11, p. 43-47, 2013.

CHARLES, D. F.; KELLY, M. G., STEVENSON, R. J.; POIKANE, S.; THEROUX, S.; ZGRUNDO, A.; CANTONATI, M. Benthic algae assessment in the EU and the US: striving for consistency in the face of great ecological diversity. **Ecological Indicators**, v. 121, p. 107082, 2021.

CUSHING, C. E.; DAVID, ALLAN, J. **Streams: Their Ecology and Life**. 1 st ed. California: Academic Press, 2001.

DETTNER, K. Defenses of water insects. In: DEL-CLARO, K.; GUILLERMO, R. (Edits). **Aquatic Insects Behavior and Ecology**. Switzerland: Springer Nature Switzerland, 2019. p. 191-262.

DIJKSTRA, K. D. B.; MONAGHAN, M. T.; PAULS, S. U. Freshwater biodiversity and aquatic insect diversification. **Annual Review of Entomology**, v. 59, p. 143-163, 2014.

GASTON, K. J. Global patterns in biodiversity. **Nature**, v. 405, p. 200-227, 2000.

HEINO, J.; MYKRÄ, H. Control of stream insect assemblages: roles of spatial configuration and local environmental factors. **Ecological Entomology**, v. 33, p. 614-622, 2008.

HEINO, J.; PECKARSKY, B. L. Integrating behavioral, population and large-scale approaches for understanding stream insect communities. **Current Opinion in Insect Science**, v. 2, p.7-13, 2014.

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

HEINO, J.; BINI, L. M.; ANDERSSON, J.; BERGSTEN, J.; BJELKE, U.; JOHANSSON, F. Unravelling the correlates of species richness and ecological uniqueness in a metacommunity of urban pond insects. **Ecological Indicators**, v. 73, p. 422-431, 2017.

HEINO, J.; MELO, A.S.; BINI, L.M. Reconceptualising the beta diversity-environmental heterogeneity relationship in running water systems. **Freshwater Biology**, v. 60, p. 223-235, 2015.

HEINO, J.; GRÖNROOS, M.; ILMONEN, J., KARHU, T., NIVA, M.; PAASIVIRTA, L. Environmental heterogeneity and  $\beta$  diversity of stream macroinvertebrate communities at intermediate spatial scales. **Freshwater Science**, v. 32, p. 142-154, 2013.

JR, W. M. L. Physical and Chemical Features of Tropical Flowing Waters. In: DUDGEON, D. **Tropical Stream Ecology**. Oxford: Elsevier, 2008. p. 2-20.



JYRKÄNKALLIO-MIKKOLA, J.; HEINO, J.; SOININEN, J. Beta diversity of stream diatoms at two hierarchical spatial scales: implications for biomonitoring. **Freshwater Biology**, v. 61, p. 239-250, 2016.

LANCASTER, J.; DOWNES, B. J. **Aquatic Entomology**, 1st ed. Oxford: Oxford University Press, 2013.

LI, F.; TONKIN, J. D., HAASE, P. Local contribution to beta diversity is negatively linked with community-wide dispersal capacity in stream invertebrate communities. **Ecological Indicators**, v. 108, p. 105715, 2020.

LEGENDRE, P.; DE CÁCERES, M. Beta diversity as the variance of community data: dissimilarity coefficients and partitioning. **Ecology Letters**, v. 16, p. 951-963, 2013.

LEIBOLD, M. A.; HOLYOAK, M.; MOUQUET, N.; AMARASEKARE, P.; CHASE, J. M.; HOOPES, M. F.; HOLT, R. D.; SHURIN, J. B.; LAW, R.; TILMAN, D.; LOREAU, M.; GONZALEZ, A. The metacommunity concept: a framework for multi-scale community ecology. **Ecology Letters**, v. 7, p. 601-613, 2004.

LOGUE, J. B; MOUQUET, N.; HILLEBRAND, H. P. H. Empirical approaches to metacommunities: a review and comparison with theory. **Trends in Ecology and Evolution**, v. 26, p. 482-491, 2011.

MANN, D. G. The species concept in diatoms. **Phycologia**, v. 38, p. 437-495, 1999.

MANN, D. G. Size and sex. In: SECKBACH, J.; KOCIOLEK, J. P. (Edits). **The Diatom World**. Springer: Dordrecht, 2011, p. 147-166.

MANN, D. G.; DROOP, S. J. M. Biodiversity, biogeography and conservation of diatoms. **Hydrobiologia**, v. 336, p. 19-32, 1996.

MELO, A.S. Effects of taxonomic and numeric resolution on the ability to detect ecological patterns at a local scale using stream macroinvertebrates. **Archiv für Hydrobiologie**, v. 164, p. 309-323, 2005.

POTAPOVA, M.; CHARLES, D. F. Benthic diatoms in USA rivers: distributions along spatial and environmental gradients. **Journal of Biogeography**, v. 29, p. 67-187, 2002.

REBORA, M.; SALERNO, G; PIERSANT, S. Aquatic insect sensilla: morphology and function. In: DEL-CLARO, K.; GUILLERMO, R. (Edits). **Aquatic Insects Behavior and Ecology**. Switzerland: Springer Nature Switzerland, 2019. p. 139-166.

RIMET, F.; BOUCHEZ, A. Biomonitoring river diatoms: Implications of taxonomic resolution. **Ecological Indicators**, v. 15, p. 92-99, 2012.

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

SCHNECK, F.; MELO, A. S. Reliable sample sizes for estimating similarity among macroinvertebrate assemblages in tropical streams. **Annales de Limnologie - International Journal of Limnology**, v. 46, p. 93-100, 2010.

SGARBI, L. F.; BINI, L. M.; HEINO, J.; JYRKÄNKALLIO-MIKKOLA, J.; LANDEIRO, V. L.; SANTOS, E. P.; SCHNECK, F.; SIQUEIRA, T.; SOININEN, J.; TOLONEN, K. T.; MELO, A. S. Sampling effort and information quality provided by rare and common species in estimating assemblage structure. **Ecological Indicators**, v. 110, p. 105937, 2020.

SMOL, J. P.; STOERMER, E. F. **The Diatoms**. Applications for the Environmental and Earth Sciences. 2nd ed. Cambridge: Cambridge University Press, 2010.

SOININEN, J. Environmental and spatial control of freshwater diatoms – a review. **Diatom Research**, v. 22, p. 473-490, 2007.

SOININEN, J.; JAMONEAU, A.; ROSEBERY, J.; PASSY, S. I. Global patterns and drivers of species and trait composition in diatoms. **Global Ecology and Biogeography**, v. 25, p. 940-950, 2016.

SOR, R.; LEGENDRE, P.; LEK, S. Uniqueness of sampling site contributions to the total variance of macroinvertebrate communities in the Lower Mekong Basin. **Ecological Indicators**, v. 84, p. 425-432, 2018.

SUNDAR, S.; HEINO, J.; ROQUE, F. de O.; SIMAIKA, J. P.; MELO, A. S.; TONKIN, J. D.; NOGUEIRA, D. G.; SILVA, D. P. Conservation of freshwater macroinvertebrate biodiversity in tropical regions. **Aquatic Conservation: Marine and Freshwater Ecosystems**, v. 30, p. 1238-1250, 2020.

VAN DEN HOEK, C.; MANN, D. G.; JAHNS, H. M. **Algae - an introduction to phycology**. Cambridge: Cambridge University Press, 2010.

VILELA, D. S.; SANMARTÍN-VILLAR, I. Reproductive behavior and sexual selection. In: DEL-CLARO, K.; GUILLERMO, R. (Edits). **Aquatic Insects Behavior and Ecology**. Switzerland: Springer Nature Switzerland, 2019. p. 263-293.

WETZEL, R. G. **Limnologia**. 2nd ed. Lisboa: Saunders College, 1993.

WHITTAKER, R. H. Vegetation of the Siskiyou Mountains, Oregon and California. **Ecological Monographs**, v. 30, p. 279-338, 1960.

WILSON, D. S. Complex interactions in metacommunities, with implications for biodiversity and higher levels of selection. **Ecology**, v. 73, p. 1984-2000, 1992.

## CAPÍTULO 1

*Compositional uniqueness of diatoms and insects in subtropical streams is weakly correlated with riffle position and environmental uniqueness*

Manuscrito publicado pela revista *Hydrobiologia*.

Castro et al., 2019. <https://doi.org/10.1007/s10750-019-04037-8>

Instruções aos autores disponível em <https://www.springer.com/journal/10750/submission-guidelines>

De acordo com as normas do periódico, a fonte Times New Roman deve ter tamanho 10, entretanto, para melhor visualização e leitura, optei por aumentar a fonte para o tamanho 12 (exceto para o conteúdo das tabelas).

## **Abstract**

Some local communities contribute more to beta diversity than others, which has been known as compositional uniqueness or local contribution to beta diversity. Compositional uniqueness should correlate positively with environmental uniqueness and site isolation. We evaluated compositional uniqueness (total and in terms of species replacement and nestedness) of periphytic diatoms and insects in subtropical streams and tested for correlates of these metrics. We sampled 90 riffles in a near-pristine catchment in the southeast Atlantic Forest of Brazil. The total compositional uniqueness for diatoms and insects were not associated to riffle position. However, the total compositional uniqueness of diatoms (presence-absence data), was correlated with the uniqueness of streambed substrate composition, while the total compositional uniqueness of insects did not correlate with any explanatory variable. The compositional uniqueness in terms of nestedness (presence-absence data) for diatoms and for insects (abundance data) was correlated positively with the uniqueness of substrate composition. Compositional uniqueness in terms of species replacement (abundance data) for insects was correlated negatively with the uniqueness of substrate composition. Our results indicate that subtle differences in environmental uniqueness, play a role in determining beta diversity in near-pristine streams. However, finding strong correlates of compositional uniqueness proved to be a difficult task.

**Keywords:** compositional variation, LCBD, replacement, nestedness

## Introduction

Understanding the mechanisms underlying biodiversity patterns and community assembly is a major goal in community ecology (Mori et al., 2018), particularly in the current scenario of increasing threats to biodiversity (Dudgeon et al., 2006). The study of beta diversity (i.e. variation in species composition among sites; Anderson et al., 2011) may offer insights regarding the factors driving biodiversity patterns (Socolar et al., 2016), especially in highly heterogeneous environments (Petsch, 2016; Mori et al., 2018). For instance, environmental heterogeneity (Heino et al., 2015; Leibold & Chase, 2018), dispersal (Padial et al., 2014) and habitat productivity (Chase, 2010; Bini et al., 2014) are regarded as major drivers of beta diversity. Environmental heterogeneity promotes beta diversity because, when dispersal is sufficient, differences in abiotic conditions among sites allow the colonization of suitable sites by different sets of species (Leibold & Chase, 2018). Higher productivity allows for a stronger role of stochastic assembly processes, mainly through more intense priority effects, leading to higher compositional variation among communities in more productive environments (Chase, 2010). However, there is a paucity of studies on the extent to which each local community contributes to the total variation in community composition within a metacommunity, namely, the local contribution to beta diversity (LCBD; Legendre & De Cáceres, 2013; Vilmi et al., 2017; Landeiro et al., 2018).

Sites (e.g. streams) with higher LCBD values exhibit substantial dissimilarity in species composition when compared to the regional (e.g. catchment) typical community composition (Legendre & De Cáceres, 2013). It is also possible to investigate how unique sites are in terms of species replacement ( $LCBD_{Rep}$ ) or nestedness/richness difference ( $LCBD_{Nes}$ ) (Legendre, 2014; Szabó et al., 2019). Thus, sites with higher  $LCBD_{Rep}$  are those with higher species replacement in relation to the typical community, such as streams in transition zones between headwaters and mainstreams where environmental characteristics are unique (Legendre, 2014). On the other hand, higher  $LCBD_{Nes}$  values reflect sites with very

low species richness, as may be the case of headwater or polluted sites, or very high species richness, such as downstream sites (Legendre, 2014).

So far, research suggests that species compositional uniqueness in freshwater ecosystems can be driven by water characteristics (Pajunen et al., 2017; Szabó et al., 2019) and land use (Heino et al., 2017; Winegardner et al., 2017). To our knowledge, studies searching for correlates of species compositional uniqueness tend to focus on testing the importance of specific environmental variables (e.g. Vilmi et al., 2017; Sor et al., 2018; Szabó et al., 2019). An alternative and interesting approach consists in testing the relationship between species compositional uniqueness and environmental uniqueness. This issue is especially important considering the homogenization of abiotic conditions due to human-induced disturbances (Petsch, 2016). For instance, a positive relationship between compositional and environmental uniqueness would indicate that more environmentally different sites should be prioritized because they include species compositions deserving either conservation or restoration efforts (Legendre, 2014).

Streams are subject to different environmental gradients (size, hydrology, physical and chemical conditions) and contain groups of organisms with different traits and environmental requirements (e.g. periphytic microalgae and insects). Near-pristine streams may show high variation in physical conditions (e.g. water flow and substrate composition) even within the same reach (Heino et al., 2013), but are not expected to show much variation in chemical conditions, such as nutrients and dissolved oxygen (Allan & Castillo, 2007). Also, as streams have unidirectional flow, site position within streams may be an important factor structuring communities (Carrara et al., 2012; Durães et al., 2016). For example, environmental selection and dispersal limitation may be major forces driving variation in upstream reaches, while environmental selection and intensive dispersal shape communities in more connected reaches downstream (Brown & Swan, 2010; but see Henriques-Silva et al., 2019). Thus, because downstream sites receive organisms from upstream sites by drift (Swan & Brown, 2014;

Lancaster & Downes, 2017), it is expected that upstream sites would have more unique communities and that compositional uniqueness in terms of nestedness would be more strongly related to site position than uniqueness in terms of species replacement.

We investigated the compositional uniqueness (i.e. the local contribution to beta diversity, LCBD) of periphytic diatoms and insects of near-pristine streams and tested whether compositional uniqueness of riffles within streams was positively correlated with environmental uniqueness and riffle position (from downstream to upstream reaches). We also tested whether compositional uniqueness in terms of species replacement was positively related to environmental uniqueness and whether compositional uniqueness in terms of nestedness was positively correlated with riffle position.

## **Methods**

### Study area

The study was conducted in the Carmo River Basin (Fig. 1), located in the Intervales State Park (24°18'S, 48°25'W; São Paulo State, Brazil). This protected area has 48,000 ha and encompasses part of the largest portion of the preserved Atlantic Forest in the State of São Paulo (Morellato et al., 2000). The area of the Carmo River drainage is entirely located within the park. The region is characterized by an average annual rainfall of 2040 mm; the air temperature ranges from 15 °C to 30 °C during the austral summer (September-March) and from 0 °C to 25 °C during the winter (April-August) (Costa & Melo, 2008). The climate is classified as humid subtropical (Alvares et al., 2013).

### Biological and environmental data

We sampled 10 riffles in nine streams (totaling 90 riffles) in April 2015. Stream order ranged from 2nd to 4th. Within each stream, the riffles were ~ 10 to 50 m distant from each other and the distances between the most upstream and the most downstream riffles ranged from 190 m



to 430 m. At each riffle, we sampled periphytic diatoms from 10 stones and pooled the material to constitute a single sample for each riffle. For each stone, we brushed an area of 25 cm<sup>2</sup> using a soft bristle brush. In the laboratory, we oxidized the samples using potassium permanganate (KM<sub>N</sub>O<sub>4</sub>) and concentrated hydrochloric acid (HCl) and mounted permanent slides using Naphrax<sup>®</sup> mounting medium. We counted 500 valves from each sample using a Zeiss Primo Star microscope with 1000× magnification. Diatoms were identified to species or variety level using specialized literature (Metzeltin & Lange-Bertalot, 1998; Metzeltin et al., 2005; Metzeltin & Lange-Bertalot, 2007). We sampled insects by using a kick-net with a mesh size of 0.50 mm. At each riffle, we took a 2-minute kick-net sample by gently kicking the stony bottom of different microhabitats (e.g. which considered differences in current velocity, depth, and substrate particle size; Heino et al., 2018; Valente-Neto et al., 2018). The samples were preserved in 70% ethanol and taken to the laboratory for further processing and identification using specialized literature (Domínguez et al., 2006; Heckman, 2006; Domínguez & Fernández, 2009). Different groups (Coleoptera, Ephemeroptera, Megaloptera, Odonata, Plecoptera and Trichoptera) were identified to genus level. We chose these orders because they include many genera that are usually abundant in riffles and because their larvae exhibit varying responses to changes in the local habitat (Rosenberg & Resh, 1993).

We measured five physical variables per riffle: water depth, water velocity, stream width, substrate composition and percentage of canopy cover. These variables are thought to be important drivers of stream communities (Heino et al., 2013; Astorga et al., 2014). We measured water depth and water velocity in nine points on each riffle using a ruler and a water flow probe (Global Water FP111), respectively. Stream width was measured in three points of each riffle using a measuring tape. The characterization of streambed substrates was performed in three random quadrats of 50 × 50 cm at each riffle. In each quadrat, the percentages of the following substrate types were estimated visually: sand (0.25-2mm), gravel (2-16mm), pebble (16-64mm), cobble (64-256mm) and boulders (> 256mm). Percentage of

canopy cover (stream shading) was visually estimated in three points of each riffle. We also took water samples in three points of each stream (upstream riffle, middle riffle and downstream riffle) to determine total nitrogen ( $\text{mg.L}^{-1}$ ) and total phosphorus ( $\mu\text{g.L}^{-1}$ ) concentrations following standard methods (APHA, 2017) and we used a Horiba<sup>®</sup> multiparameter probe to obtain *in situ* data on pH, dissolved oxygen and turbidity (Table 1).

### Data analysis

We quantified the compositional uniqueness of riffles within streams separately for periphytic diatoms and insects using the Local Contribution to Beta Diversity (LCBD), an approach developed by Legendre & De Cáceres (2013). LCBD values vary from 0 to 1. The higher a LCBD value of a site, the higher its contribution to total beta diversity. We calculated LCBD for riffles separately for each of the nine streams and used it as response variable in our models (nine streams  $\times$  10 riffles = 90 LCBD values). LCBD was calculated for presence-absence (Sørensen index) and raw abundance data (Bray-Curtis index). We refer to total compositional uniqueness ( $\text{LCBD}_{\text{Total}}$ ) here to differentiate from LCBD in terms of species replacement and nestedness. We opted to use Sørensen and Bray-Curtis dissimilarity coefficients because both metrics are recommended by Legendre & De Cáceres (2013) and by Legendre (2014). Moreover, by using coefficients based on presence-absence and abundance data we can detect patterns not only derived from changes in species incidence, but also from changes in species abundances (e.g. Siqueira et al., 2015). This is especially important at the small spatial scale of our study (within-stream riffles) because it is more likely that species would differ in their abundances than in their incidences (Legendre, 2014). Also, we calculated LCBD in terms of species replacement ( $\text{LCBD}_{\text{RepS}}$  and  $\text{LCBD}_{\text{RepBC}}$ , for presence-absence and abundance data, respectively) and nestedness ( $\text{LCBD}_{\text{NesS}}$  and  $\text{LCBD}_{\text{NesBC}}$ , for presence-absence and abundance data, respectively) (Legendre, 2014) using the methods of Baselga (2010, 2013). As an alternative, we used the methods developed by Podani &

Schmera (2011) and Podani et al. (2013) to calculate the respective LCBD values for presence-absence (Sørensen index) and abundance data (Bray-Curtis index) (Legendre, 2014).  $LCBD_{NesBC}$  and  $LCBD_{RepBC}$  for diatoms were not calculated because we counted a fixed number of valves per riffle, which precludes the detection of abundance gradients between riffles.

We calculated the local contribution to environmental heterogeneity (LCEH), that is, the uniqueness of each riffle in terms of environmental characteristics. A high value of LCEH indicates that a riffle exhibits particularized environmental conditions. LCEH was calculated for each riffle using a standardized Euclidean distance (Legendre & Legendre, 1998). In addition to calculating LCEH using the total environmental matrix ( $LCEH_{tot}$ ), we also deconstructed this matrix and calculated two other metrics of environmental uniqueness: LCEH generated by stream physical characteristics not related to streambed substrates ( $LCEH_{phy}$ ; width, canopy cover, velocity and depth) and by substrate heterogeneity ( $LCEH_{sub}$ ; sand, gravel, pebble, cobble and boulder). We also included the position of each riffle within streams in the models (see below) as a rank variable (1, for the most downstream riffle, up to 10, for the most upstream riffle), since riffle position within streams may be an important factor structuring communities (Brown & Swan, 2010).

We did not include  $LCEH_{tot}$  in our models because it was strongly correlated with  $LCEH_{sub}$  ( $r = 0.84$ ). We used linear mixed-effects models (LMMs, Lindstrom & Bates, 1988) with restricted maximum likelihood to test the relationships between compositional uniqueness (LCBD, separately for insects and diatoms), as a response variable, and  $LCEH_{phy}$ ,  $LCEH_{sub}$  and riffle position, as explanatory variables. The variance inflation factor (VIF) was lower than 3 and thus, we included all three variables in the models (Zuur et al., 2009). We used stream identity as a random factor in these analyses. We reported the marginal  $R^2$  for each model, following Nakagawa & Schielzeth (2013). Marginal  $R^2$  in LMMs describes the proportion of variance explained by the fixed factors alone and is less susceptible to

mathematical problems (e.g. negative or lower  $R^2$  in larger models) than other  $R^2$  metrics proposed for LMMs (Nakagawa & Schielzeth, 2013).

All analyses were done using R (R Core Team, 2018). Package *vegan* (Oksanen et al., 2018) was used to standardize the environmental variables and to obtain the environmental distance matrices used to calculate LCEH, *adespatial* (Dray et al., 2018) to obtain replacement and nestedness components and to calculate LCBD and LCEH metrics, *nlme* (Pinheiro et al., 2018) to calculate LMMs, *car* (Fox & Weisberg, 2018) to calculate VIF and MuMIn (Barton, 2018) to obtain the variance explained by fixed factors in LMMs (marginal  $R^2$  values).

## Results

We recorded 260 periphytic diatom taxa and 81 insect taxa in the Carmo River Basin. The most abundant diatoms were *Achnantheidium minutissimum* (Kützing) Czarnecki, *Nupela praecipua* (Reichardt) Reichardt and *Eolimna minima* (Grunow) Lange-Bertalot. For insects, an unidentified genus of the family Hydropsychidae Curtis, the genera *Traverhyphes* Molineri, *Farrodes* Peters and *Baetodes* Needham & Murphy were the most abundant. Species richness in streams ranged from 69 to 146 for diatoms, while genus richness varied from 44 to 70 for insects.

There was no clear spatial pattern of total compositional uniqueness ( $LCBD_{Total}$ ) for diatoms or insects among riffles within streams (Fig. 2; Online Resource 1, Fig. S1). Within-stream  $LCBD_{Total}$  values for diatoms ranged from 0.06 to 0.15 for presence-absence data and from 0.06 to 0.25 for abundance data. Similarly, for insects,  $LCBD_{Total}$  values ranged from 0.05 to 0.15 and from 0.06 to 0.23 for presence-absence and abundance data, respectively.

Results for compositional uniqueness using Baselga's and Podani's approaches were similar and, thus, for brevity, we show here only the results based on Baselga's indices (see Online Resource 2 for results based on Podani's indices). The compositional uniqueness in terms of species replacement and nestedness of diatoms (Fig. 3) and insects (Fig. 4; Online

Resource 1, Fig. S2) did not show spatial patterns. In general, for both diatoms and insects, compositional uniqueness generated by replacement showed similar values among riffles within each stream, while compositional uniqueness generated by nestedness showed high variability among riffles, with usually one riffle per stream showing a much higher value than the others (Figs. 3, 4; Online Resource 1, Fig.S2).

Only uniqueness in substrate composition of riffles ( $LCEH_{sub}$ ) showed a positive relationship with total compositional uniqueness of diatoms (presence-absence data) (Table 2). For insects, we did not find any relationship between  $LCBD_{Total}$  and the explanatory variables (Table 2).  $LCBD_{Nes}$  (obtained using both Baselga and Podani methods) showed a significant, but weak, positive relationship with uniqueness in substrate composition ( $LCEH_{sub}$ ) for both diatoms (presence-absence data) and insects (abundance data) (Tables 3 and 4, Online resource 2, Tables S1 and S2).  $LCBD_{Rep}$  was not correlated with the explanatory variables independently of the type of data (presence-absence or abundance) and the biological group (Tables 3 and 4). However, using the Podani's approach,  $LCBD_{Rep}$  of insects (abundance data) was negatively correlated with  $LCEH_{sub}$  (Online resource 2, Table S2).

## **Discussion**

Contrary to our expectations, total compositional uniqueness of both periphytic diatoms and insects were poorly explained by environmental uniqueness and riffle position. Also, our expectation that compositional uniqueness in terms of species replacement and nestedness would be explained by different factors was only partly corroborated. These results indicate that understanding the mechanisms responsible for beta diversity patterns is far from trivial, as more unique habitats are not necessarily the ones harboring more unique communities. This is also in line with other studies indicating the difficulty in finding consistent correlates of beta diversity (Heino & Grönroos, 2017; Lopes et al., 2017; Ceschin et al., 2018).

We expected that compositional uniqueness would be positively explained by riffle isolation within the stream network (i.e. more upstream reaches would contain more unique communities). This relationship should be even stronger when analyzing compositional uniqueness in terms of nestedness because upstream sites are isolated and tend to be dispersal-limited and thus to present lower species richness than downstream sites (Carrara et al., 2012; Jyrkänkallio-Mikkola et al., 2018). Although several studies have shown that site position within the stream network may be a proxy for isolation and thus a key factor structuring communities (Brown & Swan, 2010; Jyrkänkallio-Mikkola et al., 2018), our prediction was not supported. Riffles were relatively close to each other in the streams we sampled (the distance between the first and last reaches ranged from 190 m to 430 m) and, due to these short distances, dispersal among riffles was likely high. Aquatic insects may have high dispersal rates along the stream channel (Lancaster & Downes, 2017), especially in regions where many species have multiple reproduction events per year (Vásquez et al., 2009). Thus, the predicted relationship is more likely to be found among streams of different orders (e.g. Finn et al., 2011; Jyrkänkallio-Mikkola et al., 2018) than among riffles within the same stream.

While previous studies tested for an association between compositional uniqueness and mean environmental characteristics, we evaluated whether more unique communities would be found in more unique environments (here called LCEH, local contribution to environmental heterogeneity). In general, compositional uniqueness at the riffle scale has been found to be weakly correlated with local (average) environmental variables and some studies argue that this result may be due to the lack of key explanatory variables (Vilmi et al., 2017), to the reduced species pool in disturbed streams (Tonkin et al., 2016) or to the fact that compositional uniqueness is more affected by large-scale than by local variables (Heino et al., 2017). Our analyses suggest that compositional uniqueness was only weakly related to LCEH in different tests. We, thus, suggest that finding correlates of compositional uniqueness is a

challenging and pressing task, as LCBD is a potentially useful metric to select priority areas for restoration (Legendre & De Cáceres, 2013) and conservation (Landeiro et al., 2018).

Our results show that total compositional uniqueness of diatoms and insects was not related to  $LCEH_{phy}$  (i.e. to uniqueness generated by width, canopy cover, velocity and depth), but that total compositional uniqueness of diatoms was positively related to substrate uniqueness (i.e.  $LCEH_{sub}$ ). This means that riffles that mostly deviate from the average composition of substrates within a stream are those that support more unique communities. Because  $LCBD_{Total}$  of diatoms was positively correlated with species richness ( $r = 0.23$ ,  $P = 0.030$  for presence-absence;  $r = 0.26$ ,  $P = 0.011$  for abundance data), it could be suggested that those unique communities in more environmentally unique riffles are more species rich than communities in less unique riffles. This result contrasts with the commonly observed negative correlation between compositional uniqueness and species richness (e.g. Legendre & De Cáceres, 2013; Heino et al., 2017; Vilmi et al., 2017; Jyrkänkallio-Mikkola et al., 2018), which could be because we sampled near-pristine streams not affected by human-induced changes in streambed composition. Moreover, despite  $LCBD_{Rep}$  did not correlate with any variable of environmental uniqueness (using Baselga's method), the positive relationship between  $LCBD_{Nes}$  for both diatoms and insects and  $LCEH_{sub}$  reinforces the importance of the uniqueness of substrate composition in streams. On the other hand, the unexpected negative relationship between  $LCBD_{Rep}$  for insects and  $LCEH_{sub}$  deserves further attention despite the poor explanatory power, for it indicates higher species replacement in sites with more common substrate composition.

In general, the composition of streambed substrates has been found to be an important factor structuring communities (e.g. Bergey, 2005; Brown & Lawson, 2010; Petsch et al., 2017). For example, in an experimental study, Petsch et al. (2017) found higher beta diversity of periphytic diatoms among rough than among smooth substrates. Further, Brown & Lawson (2010) found a positive relationship between temporal variation in species composition of

macroinvertebrates and substrate heterogeneity. Our results provide evidence that not only within-site substrate heterogeneity but also substrate uniqueness is a key factor in the organization of biological communities in streams.

Our results highlight that subtle differences in environmental uniqueness play a role in determining beta diversity of diatoms and insects in near-pristine streams. However, most of our findings suggest that the mechanisms generating biodiversity patterns at small spatial scales are even more complex to understand in undisturbed ecosystems, indicating that beta diversity of different groups of organisms may be explained by different factors. We argue that despite the difficulty in finding consistent correlates of beta diversity, uniqueness in streambed substrate composition is a driver of compositional uniqueness in near-pristine streams and, consequently, a determinant of regional gamma and beta diversity patterns. Thus, these results have implications in the understanding of the effects of human-induced habitat homogenization, especially in regions strongly affected by deforestation and land-use intensification, as is the case of most tropical streams. Finally, we suggest that studies aiming to understand the extent to which local communities contribute to the total variation in a metacommunity should encompass not only mean environmental characteristics but also different metrics of environmental uniqueness.

### **Acknowledgments**

We thank Priscila Tremarin for helping with diatom species identification. This study was partially funded by grant 2013/50424-1 from the São Paulo Research Foundation (FAPESP). EC was supported by a student fellowship by Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) - Finance Code 001. ASM, LMB and VLL are supported by research grants from the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq no. 307587/2017-7, 304314/2014-5 and 307961/2017-6, respectively).



## References

- Allan, J. D. & M. M. Castillo, 2007. *Stream Ecology. Structure and function of running waters*. Springer, Amsterdam.
- Alvares, C. A., J. L. Stape, P. C. Sentelhas, J. L. de Moraes Gonçalves & G. Sparovek, 2013. Köppen's climate classification map for Brazil. *Meteorologische Zeitschrift* 22: 711–728.
- American Public Health Association, 2017. *Standard Methods for the Examination of Water and Wastewater*, 23rd ed. American Public Health Association, Washington, DC.
- Anderson, M. J., T. O. Crist, J. M. Chase, M. Vellend, B. D. Inouye, A. L. Freestone, N. J. Sanders, H. V. Cornell, L. S. Comita, K. F. Davies, S. P. Harrison, N. J. B. Kraft, J. C. Stegen & N. G. Swenson, 2011. Navigating the multiple meanings of  $\beta$  diversity: A roadmap for the practicing ecologist. *Ecology Letters* 14: 19–28.
- Astorga, A., R. Death, F. Death, R. Paavola, M. Chakraborty & T. Muotka, 2014. Habitat heterogeneity drives the geographical distribution of beta diversity: the case of New Zealand stream invertebrates. *Ecology and Evolution* 4: 2693–2702.
- Barton, K., 2018. MuMIn: Multi-Model Inference. R package version 1.42.1.
- Baselga, A., 2010. Partitioning the turnover and nestedness components of beta diversity. *Global Ecology and Biogeography* 19: 134–143.
- Baselga, A., 2013. Separating the two components of abundance-based dissimilarity: balanced changes in abundance vs. abundance gradients. *Methods in Ecology and Evolution* 4: 552–557.
- Bergey, E. A., 2005. How protective are refuges? Quantifying algal protection in rock crevices. *Freshwater Biology* 50: 1163–1177.
- Bini, L. M., V. L. Landeiro, A. A. Padiál, T. Siqueira & J. Heino, 2014. Nutrient enrichment is related to two facets of beta diversity of stream invertebrates across the continental United States. *Ecology* 95: 1569–1578.

- Brown, B. L. & C. M. Swan, 2010. Dendritic network structure constrains metacommunity properties in riverine ecosystems. *Journal of Animal Ecology* 79: 571–580.
- Brown, B. L. & R. L. Lawson, 2010. Habitat heterogeneity and activity of an omnivorous ecosystem engineer control stream community dynamics. *Ecology* 91: 1799–1810.
- Carrara, F., F. Altermatt, I. Rodriguez-Iturbe & A. Rinaldo, 2012. Dendritic connectivity controls biodiversity patterns in experimental metacommunities. *Proceedings of the National Academy of Sciences* 109: 5761–5766.
- Ceschin, F., L. M. Bini & A. A. Padial, 2018. Correlates of fish and aquatic macrophyte beta diversity in the Upper Paraná River floodplain. *Hydrobiologia* 805: 377–389.
- Chase, J. M., 2010. Stochastic community assembly causes higher biodiversity in more productive environments. *Science* 328: 1388–1391.
- Costa, S. S. & A. S. Melo, 2008. Beta diversity in stream macroinvertebrate assemblages: among-site and among-microhabitat components. *Hydrobiologia* 598: 131–138.
- Domínguez, E., C. Molineri, M. Pescador, M. Hubbard & C. Nieto, 2006. Ephemeroptera of South America. Pensoft Publishers, Bulgaria.
- Domínguez, E. & H. R. Fernández, 2009. Macroinvertebrados bentónicos sudamericanos: Sistemática y biología. Fundación Miguel Lillo, Tucuman.
- Dray, S., D. Bauman, G. Blanchet, D. Borcard, S. Clappe, G. Guenard, T. Jombart, G. Larocque, P. Legendre, N. Madi & H. H. Wagner, 2018. *adespatial: Multivariate Multiscale Spatial Analysis*. R package version 0.3-0.
- Dudgeon, D., A. H. Arthington, M. O. Gessner, Z. Kawabata, D. J. Knowler, C. Lévêque, R. J. Naiman, A.-H. Prieur-Richard, D. Soto, M. L. J. Stiassny & C. A. Sullivan, 2006. Freshwater biodiversity: importance, threats, status and conservation challenges. *Biological Reviews* 81: 163–182.

- Durães, L., F. O. Roque, T. Siqueira, A. M. Santos, M. A. Borges & R. S. Rezende, 2016. Simulating the role of connectivity in shaping stream insect metacommunities under colonization cycle dynamics. *Ecological Modelling* 334: 19–26.
- Finn, D. S., N. Bonada, C. Murria & J. M. Hughes, 2011. Small but mighty: headwaters are vital to stream network biodiversity at two levels of organization. *Journal of the North American Benthological Society* 30: 963–980.
- Fox, J. & S. Weisberg, 2018. *An R Companion to Applied Regression*. SAGE Publications, California.
- Heckman, C. W., 2006. *Encyclopedia of South American Aquatic Insects: Odonata - Anisoptera*. Springer, The Netherlands.
- Heino, J., A. S. Melo, J. Jyrkänkallio-Mikkola, D. K. Petsch, V. S. Saito, K. T. Tolonen, L. M. Bini, V. L. Landeiro, T. S. F. Silva., V. Pajunen, J. Soininen & T. Siqueira, 2018. Subtropical streams harbour higher genus richness and lower abundance of insects compared to boreal streams, but scale matters. *Journal of Biogeography* 45: 1983–1993.
- Heino, J., A. S. Melo & L. M. Bini, 2015. Reconceptualising the beta diversity-environmental heterogeneity relationship in running water systems. *Freshwater Biology* 60: 223–235.
- Heino, J., L. M. Bini, J. Andersson, J. Bergsten, U. Bjelke & F. Johansson, 2017. Unravelling the correlates of species richness and ecological uniqueness in a metacommunity of urban pond insects. *Ecological Indicators* 73: 422–431.
- Heino, J. & M. Grönroos, 2017. Exploring species and site contributions to beta diversity in stream insect assemblages. *Oecologia* 183:151–160.
- Heino, J., M. Grönroos, J. Ilmonen, T. Karhu, M. Niva & L. Paasivirta, 2013. Environmental heterogeneity and  $\beta$  diversity of stream macroinvertebrate communities at intermediate spatial scales. *Freshwater Science* 32: 142–154.

- Henriques-Silva, R., M. Logez, N. Reynaud, P. A. Tedesco, S. Brosse, S. R. Januchowski-Hartley, T. Oberdorff & C. Argillier, 2019. A comprehensive examination of the network position hypothesis across multiple river metacommunities. *Ecography* 42: 284–294.
- Jyrkänkallio-Mikkola, J., M. Siljander, V. Heikinheimo, P. Pellikka & J. Soininen, 2018. Tropical stream diatom communities – The importance of headwater streams for regional diversity. *Ecological Indicators* 95: 183–193.
- Lancaster, J. & B. J. Downes, 2017. A landscape-scale field experiment reveals the importance of dispersal in a resource-limited metacommunity. *Ecology* 98: 565–575.
- Landeiro, V. L., B. Franz, J. Heino, T. Siqueira & L. M. Bini, 2018. Species-poor and low-lying sites are more ecologically unique in a hyperdiverse Amazon region: Evidence from multiple taxonomic groups. *Diversity and Distributions* 24: 966–977.
- Leibold, M. A. & J. M. Chase, 2018. *Metacommunity Ecology*. Princeton University Press, New Jersey.
- Legendre, P., 2014. Interpreting the replacement and richness difference components of beta diversity. *Global Ecology and Biogeography* 23: 1324–1334.
- Legendre, P. & L. Legendre, 1998. *Numerical Ecology*. Elsevier, Amsterdam.
- Legendre, P. & M. De Cáceres, 2013. Beta diversity as the variance of community data: Dissimilarity coefficients and partitioning. *Ecology Letters* 16: 951–963.
- Lindstrom, M. J. & D. M. Bates, 1988. Newton-Raphson and EM algorithms for linear mixed-effects models for repeated-measures data. *Journal of the American Statistical Association* 83: 1014–1022.
- Lopes, V. G., C. W. C. Branco, B. Kozłowsky-Suzuki, I. F. Sousa-Filho, L.C. e Souza & L. M. Bini, 2017. Predicting temporal variation in zooplankton beta diversity is challenging. *PLoS ONE* 12: e0187499.
- Metzeltin, D. & H. Lange-Bertalot, 1998. *Tropical Diatoms of South America*. *Iconographia Diatomologica* 5. Koeltz Botanical Books, Germany.

- Metzeltin, D. & H. Lange-Bertalot, 2007. Tropical Diatoms of South America II. *Iconographia Diatomologica* 18. Koeltz Botanical Books, Germany.
- Metzeltin, D., H. Lange-Bertalot & F. Garcia-Rodríguez, 2005. Diatoms of Uruguay. *Iconographia Diatomologica* 15. Koeltz Botanical Books, Germany.
- Morellato, P. L., D. C. Talora, A. Takahasi, C. C. Bencke, E. C. Romera & V. B. Zipparro, 2000. Phenology of Atlantic Rain Forest trees: A comparative study. *Biotropica* 32: 811–823.
- Mori, A. S., F. Isbell & R. Seidl, 2018.  $\beta$ -diversity, community assembly and ecosystem functioning. *Trends in Ecology & Evolution* 33: 549–564.
- Nakagawa, S. & H. Schielzeth, 2013. A general and simple method for obtaining  $R^2$  from Generalized Linear Mixed-effects Models. *Methods in Ecology and Evolution* 4: 133–142.
- Oksanen J., F. G. Blanchet, M. Friendly, R. Kindt, P. Legendre, D. McGlenn, P. R. Minchin, R. B. O'Hara, G. L. Simpson, P. Solymos, M. H. H. Stevens, E. Szoecs & H. H. Wagner, 2018. *vegan: Community Ecology Package*. R package version 2.5-2. <https://CRAN.R-project.org/package=vegan>
- Padiál, A. A., F. Ceschin, S. A. J. Declerck, L. De Meester, C. C. Bonecker, F. A. Lansac-Tôha, L. Rodrigues, L. C. Rodrigues, S. Train, L. F. M. Velho & L. M. Bini, 2014. Dispersal ability determines the role of environmental, spatial and temporal drivers of metacommunity structure. *PLoS ONE* 9: e111227.
- Pajunen, V., M. Luoto & J. Soininen, 2017. Unravelling direct and indirect effects of hierarchical factors driving microbial stream communities. *Journal of Biogeography* 44: 2376–2385.
- Petsch, D. K., 2016. Causes and consequences of biotic homogenization in freshwater ecosystems. *International Review of Hydrobiology* 101: 113–122.
- Petsch, D. K., F. Schneck & A. S. Melo, 2017. Substratum simplification reduces beta diversity of stream algal communities. *Freshwater Biology* 62: 205–213.

- Pinheiro J., D. Bates, S. DebRoy & D. Sarkar, 2018. nlme: Linear and Nonlinear Mixed Effects Models. R package version 3.1-137.
- Podani, J. & D. Schmera, 2011. A new conceptual and methodological framework for exploring and explaining pattern in presence–absence data. *Oikos* 120: 1625–1638.
- Podani, J., C. Ricotta & D. Schmera, 2013. A general framework for analyzing beta diversity, nestedness and related community-level phenomena based on abundance data. *Ecological Complexity* 15: 52–61.
- R Core Team, 2018. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL <https://www.R-project.org>
- Rosenberg, D. M. & V. H. Resh, 1993. Freshwater biomonitoring and benthic macroinvertebrates. Springer, Switzerland.
- Siqueira, T., C. G. L. T. Lacerda & V. S. Saito, 2015. How does landscape modification induce biological homogenization in tropical stream metacommunities? *Biotropica* 47: 509–516.
- Socolar, J. B., J. J. Gilroy, W. E. Kunin & D. P. Edwards, 2016. How should beta-diversity inform biodiversity conservation? *Trends in Ecology & Evolution* 31: 67–80.
- Sor, R., P. Legendre & S. Lek, 2018. Uniqueness of sampling site contributions to the total variance of macroinvertebrate communities in the Lower Mekong Basin. *Ecological Indicators* 84: 425–432.
- Swan, C. M. & B. L. Brown, 2014. Using rarity to infer how dendritic network structure shapes biodiversity in riverine communities. *Ecography* 37: 993–1001.
- Szabó, B., E. Lengyel, J. Padisák & C. Stenger-Kovács, 2019. Benthic diatom metacommunity across small freshwater lakes: driving mechanisms,  $\beta$ -diversity and ecological uniqueness. *Hydrobiologia* 828: 183–198.

- Tonkin, J. D., J. Heino, A. Sundermann, P. Haase & S. C. Jähnig, 2016. Context dependency in biodiversity patterns of central German stream metacommunities. *Freshwater Biology* 61: 607–620.
- Valente-Neto, F., L. Durães, T. Siqueira & F. O. Roque, 2018. Metacommunity detectives: Confronting models based on niche and stochastic assembly scenarios with empirical data from a tropical stream network. *Freshwater Biology* 63: 86–99.
- Vásquez, D., R. W. Flowers & M. Springer, 2009. Life history of five small minnow mayflies (Ephemeroptera: Baetidae) in a small tropical stream on the Caribbean slope of Costa Rica. *Aquatic Insects* 31: 319–332.
- Vilmi, A., S. M. Karjalainen & J. Heino, 2017. Ecological uniqueness of stream and lake diatom communities shows different macroecological patterns. *Diversity and Distributions* 23: 1042–1053.
- Winegardner, A. K., P. Legendre, B. E. Beisner & I. Gregory-Eaves, 2017. Diatom diversity patterns over the past c. 150 years across the conterminous United States of America: Identifying mechanisms behind beta diversity. *Global Ecology and Biogeography* 26: 1303–1315.
- Zuur, A. F., E. N. Ieno, N. J. Walker, A. A. Saveliev & G. M. Smith, 2009. Mixed effects models and extensions in Ecology with R. Springer, New York.

Table 1. Mean and range of values of environmental variables (within riffles) in the nine streams sampled in southeast Brazil.

Variable	Stream 1	Stream 2	Stream 3	Stream 4	Stream 5	Stream 6	Stream 7	Stream 8	Stream 9
Width (m)	2.8 (1.3-4.4)	2.8 (1.8-4.6)	4.5 (2.0-8.5)	10.1 (6.0-14.0)	4.0 (2.0-7.0)	4.8 (2.2-8.0)	3.0 (1.4-5.0)	9.8 (7.0-13.0)	5.4 (2.6-11.5)
Canopy cover (%)	74 (50-95)	65 (40-80)	64 (10-95)	54 (10-95)	77 (40-100)	66 (20-90)	89 (70-95)	77 (50-95)	90 (60-95)
Sand (%)	4 (0-20)	7 (0-50)	4 (0-30)	13 (0-90)	16 (0-80)	12 (0-40)	10 (0-40)	7 (0-40)	9 (0-40)
Gravel (%)	17 (0-90)	16 (0-60)	5 (0-25)	9 (0-40)	16 (0-50)	11 (0-25)	17 (0-70)	9 (0-40)	11 (0-40)
Pebble (%)	28 (0-90)	20 (0-80)	9 (0-40)	8 (0-40)	16 (0-50)	11 (0-60)	11 (0-50)	13 (0-60)	16 (0-60)
Cobble (%)	41 (0-85)	24 (0-80)	34 (0-90)	36 (0-80)	31 (0-100)	31 (0-70)	34 (0-80)	43 (0-80)	38 (0-90)
Boulder (%)	11 (0-70)	33 (0-100)	47 (0-100)	34 (0-100)	22 (0-90)	35 (0-100)	29 (0-90)	29 (0-80)	27 (0-90)
Velocity (m.s <sup>-1</sup> )	0.3 (0-0.7)	0.4 (0.1-1.0)	0.5 (0.1-0.9)	0.5 (0.1-1.1)	0.3 (0.1-0.7)	0.4 (0.1-0.9)	0.3 (0.1-0.9)	0.4 (0.1-1.0)	0.4 (0.1-0.9)
Depth (cm)	14 (3-48)	11 (2-25)	20 (2-45)	21 (8-40)	11 (3-23)	14 (0-28)	13 (2-30)	21 (6-40)	12 (4-30)
pH	7.3 (6.6-7.5)	7.5 (7.4-7.5)	7.3 (7.2-7.5)	7.3 (7.2-7.4)	7.4 (7.3-7.5)	7.2 (7.0-7.5)	7.6 (7.6-7.7)	7.4 (7.4-7.5)	8.2 (8.1-8.2)
Turbidity (NTU)	1.3 (0.2-4.1)	2.5 (1.2-6.7)	0.7 (0-2.1)	0.0	0.6 (0-4.2)	0.0	0.2 (0-0.7)	0.0	0.0
Dissolved oxygen (mg.L <sup>-1</sup> )	8.4 (8.0-8.7)	8.2 (7.6-8.8)	8.8 (8.2-9.7)	8.5 (8.0-9.0)	8.4 (7.6-9.2)	8.8 (8.0-9.5)	8.6 (8.1-9.3)	8.6 (8.0-9.3)	8.2 (7.8-8.7)
Total phosphorus (µg.L <sup>-1</sup> )	5.96 (4.94-7.34)	5.14 (4.89-5.43)	5.61 (5.11-6.48)	3.96 (3.10-4.82)	3.41 (2.39-3.93)	3.27 (2.67-3.98)	2.28 (1.09-3.28)	3.24 (2.58-4.46)	3.50 (3.12-3.74)
Total nitrogen (mg.L <sup>-1</sup> )	0.12 (0.11-0.13)	0.15 (0.14-0.17)	0.25 (0.20-0.29)	0.18 (0.15-0.20)	0.13 (0.13-0.14)	0.21 (0.17-0.27)	0.15 (0.14-0.15)	0.22 (0.21-0.24)	0.23 (0.22-0.25)



Table 2. Results of linear mixed-effects model using compositional uniqueness (local contribution to beta diversity,  $LCBD_{Total}$ ) of periphytic diatoms and insects as response variables (degrees of freedom = 78).  $LCEH_{phy}$  and  $LCEH_{sub}$ : local contribution to environmental heterogeneity resulting from stream physical characteristics and from substrate composition, respectively. Significant t-values ( $P \leq 0.05$ ) are in bold.

Explanatory variables	Estimate	Std. Error	t-value	P	$R^2$
<b>Periphytic diatoms</b>					
<b>Sørensen</b>					0.10
Intercept	0.0925	0.0063	14.6566		
$LCEH_{sub}$	0.0816	0.0308	<b>2.6474</b>	0.010	
$LCEH_{phy}$	0.0146	0.0341	0.4293	0.669	
Riffle position	-0.0004	0.0007	-0.5619	0.576	
<b>Bray-Curtis</b>					0.07
Intercept	0.0913	0.0164	5.5575		
$LCEH_{sub}$	0.0791	0.0612	1.2926	0.200	
$LCEH_{phy}$	0.0811	0.0898	0.9031	0.369	
Riffle position	-0.0013	0.0013	-1.0597	0.293	
<b>Invertebrates</b>					
<b>Sørensen</b>					0.03
Intercept	0.1071	0.0074	14.4434		
$LCEH_{sub}$	-0.0519	0.0435	-1.1937	0.236	
$LCEH_{phy}$	0.0154	0.0452	0.3402	0.735	
Riffle position	-0.0006	0.0010	-0.6145	0.541	
<b>Bray-Curtis</b>					0.03
Intercept	0.0960	0.0147	6.5209		
$LCEH_{sub}$	0.0538	0.0507	1.0609	0.292	
$LCEH_{phy}$	0.0251	0.0706	0.3552	0.723	
Riffle position	-0.0007	0.0012	-0.5771	0.566	

Table 3. Results of linear mixed-effects model using compositional uniqueness in terms of nestedness (LCBD<sub>Nes</sub>) and species replacement (LCBD<sub>Rep</sub>) of periphytic diatoms as response variables (degrees of freedom = 78). LCEH<sub>phy</sub> and LCEH<sub>sub</sub>: local contribution to environmental heterogeneity resulting from stream physical characteristics and from substrate composition, respectively. Significant t-values ( $P \leq 0.05$ ) are in bold.

Explanatory variables	Estimate	Std. Error	t-value	P	R <sup>2</sup>
<b>Sørensen</b>					
<b>Nestedness</b>					0.07
Intercept	0.0552	0.0275	2.0045		
LCEH <sub>sub</sub>	0.2473	0.1218	<b>2.0305</b>	0.046	
LCEH <sub>phy</sub>	0.0852	0.1515	0.5623	0.575	
Riffle position	0.0021	0.0026	0.7934	0.430	
<b>Replacement</b>					0.04
Intercept	0.0983	0.0090	10.9570		
LCEH <sub>sub</sub>	0.0556	0.0498	1.1156	0.268	
LCEH <sub>phy</sub>	0.0143	0.0514	0.2776	0.782	
Riffle position	-0.0010	0.0010	-0.9958	0.322	

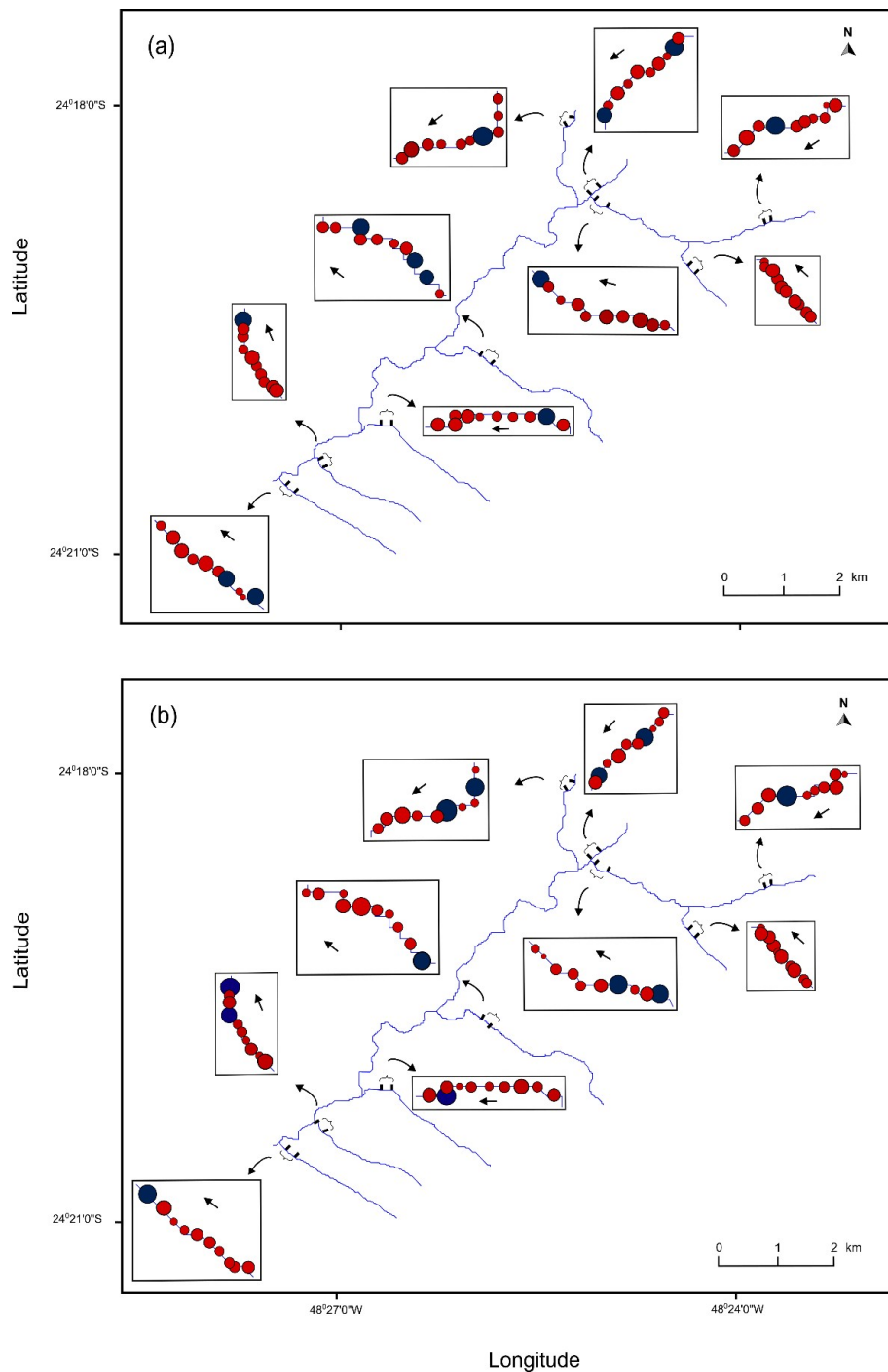
Table 4. Results of linear mixed-effects model using compositional uniqueness in terms of nestedness (LCBD<sub>Nes</sub>) and species replacement (LCBD<sub>Rep</sub>) of insects as response variables (degrees of freedom = 78). LCEH<sub>phy</sub> and LCEH<sub>sub</sub>: local contribution to environmental heterogeneity resulting from stream physical characteristics and from substrate composition, respectively.

Explanatory variables	Estimate	Std. Error	t-value	P	R <sup>2</sup>
<b>Sørensen</b>					
<b>Nestedness</b>					0.05
Intercept	0.1193	0.0300	3.9749		
LCEH <sub>sub</sub>	-0.2057	0.1207	-1.7045	0.092	
LCEH <sub>phy</sub>	-0.0645	0.1767	-0.3653	0.716	
Riffle position	0.0014	0.0033	0.4308	0.668	
<b>Replacement</b>					0.01
Intercept	0.1065	0.0125	8.5481		
LCEH <sub>sub</sub>	-0.0160	0.0747	-0.2140	0.831	
LCEH <sub>phy</sub>	0.0268	0.0870	0.3081	0.759	
Riffle position	-0.0014	0.0018	-0.7574	0.451	
<b>Bray-Curtis</b>					
<b>Nestedness</b>					0.12
Intercept	0.0485	0.0493	0.9825		
LCEH <sub>sub</sub>	0.3744	0.1804	<b>2.0751</b>	0.041	
LCEH <sub>phy</sub>	0.1521	0.2006	0.7584	0.450	
Riffle position	-0.0002	0.0038	-0.0536	0.957	
<b>Replacement</b>					0.05
Intercept	0.1170	0.0108	10.8542		
LCEH <sub>sub</sub>	-0.0779	0.0434	-1.7932	0.077	
LCEH <sub>phy</sub>	-0.0091	0.0839	-0.1079	0.914	
Riffle position	-0.0015	0.0012	-1.2992	0.198	

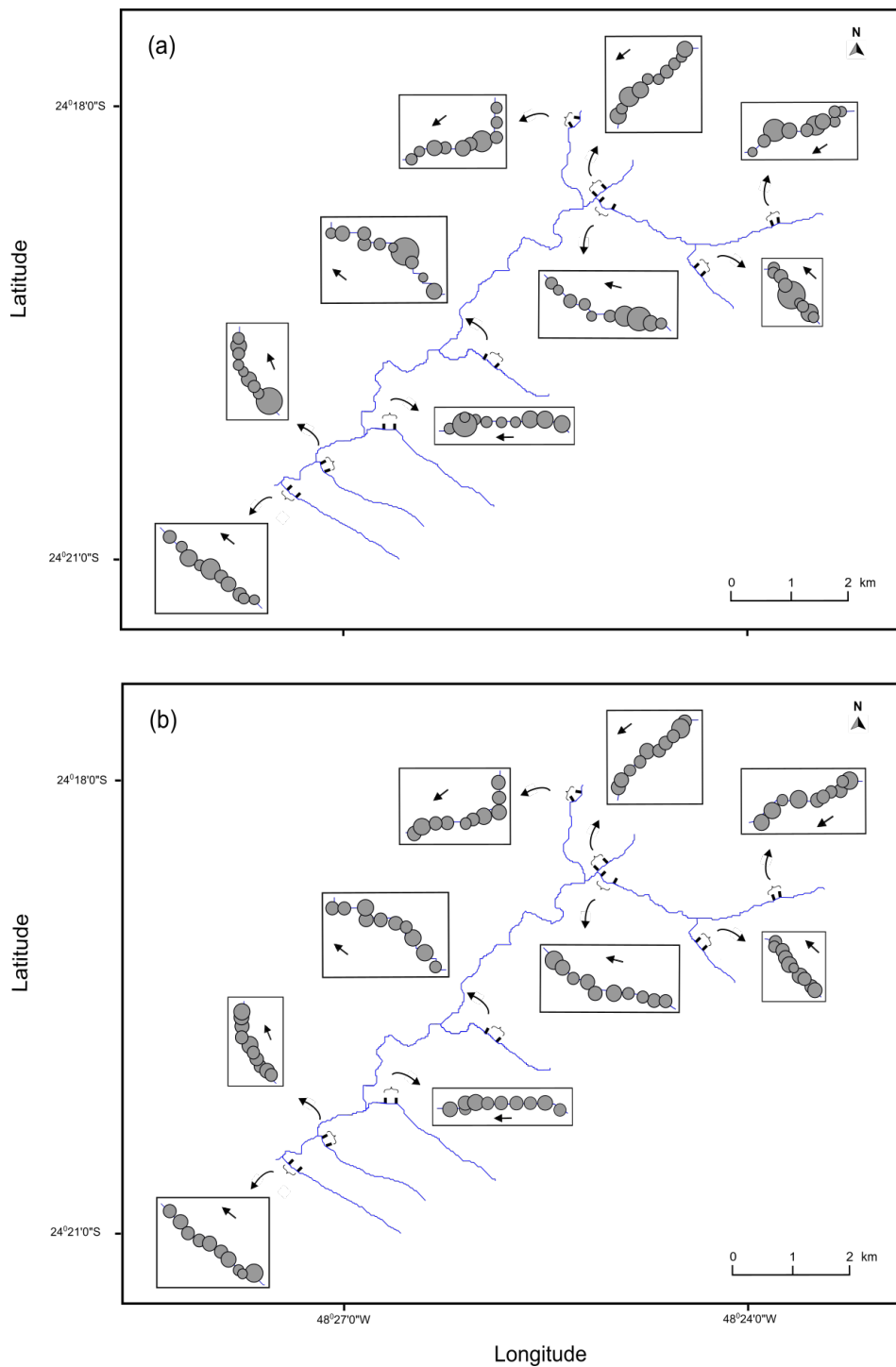
**Fig. 1** Positions of the first and last riffles studied of each stream in Carmo River Basin (gray circles). Blue lines symbolize the hydrographic network of Carmo River and the numbers indicate the nine streams sampled in this study.



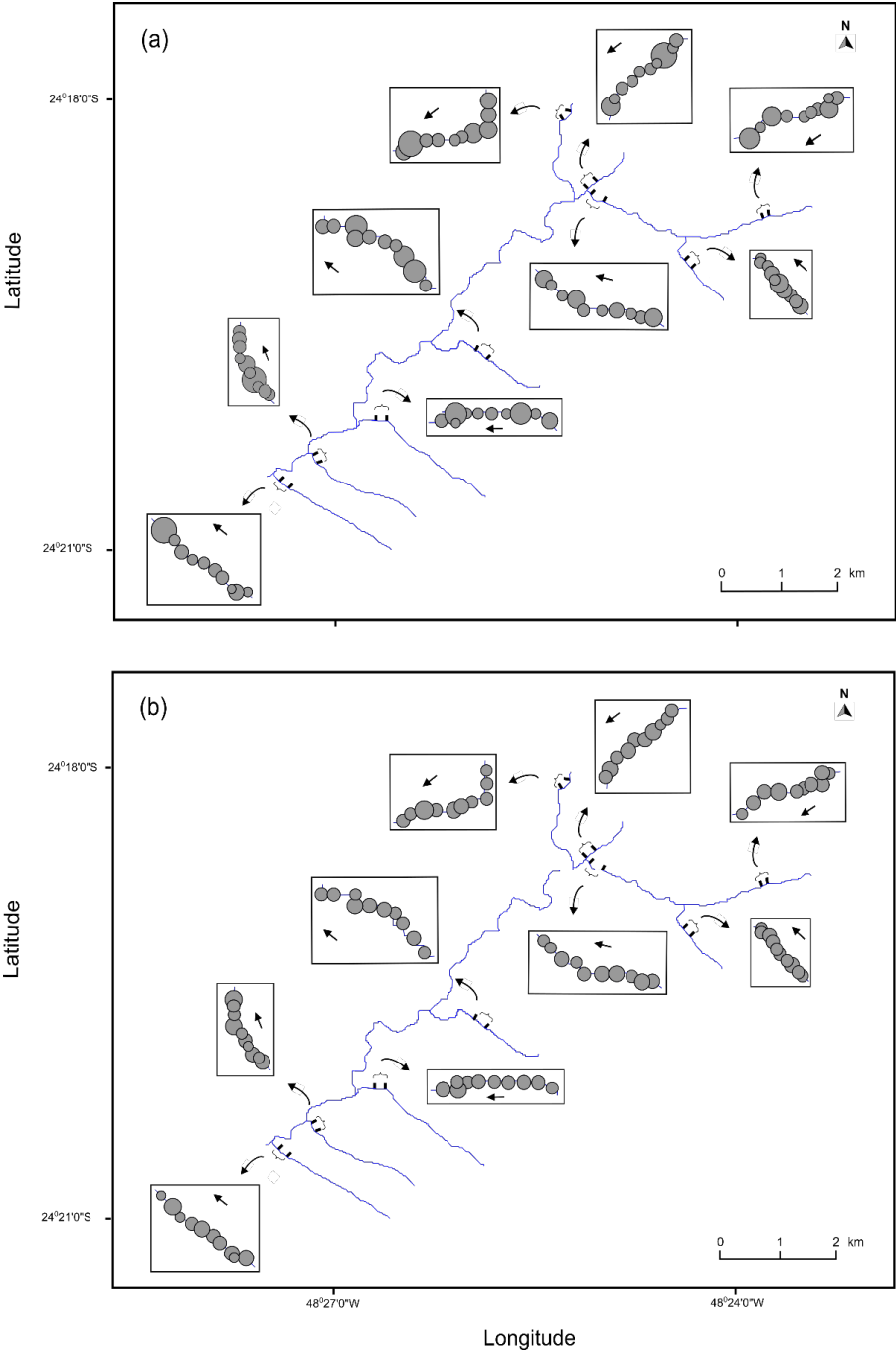
**Fig. 2** Simplified map of the Carmo River Basin showing compositional uniqueness (local contribution to beta diversity,  $LCBD_{Total}$  values) using presence-absence (Sørensen index) data for periphytic diatoms (a) and insects (b). Arrows inside boxes indicate flow direction. The size of the circles is proportional to the  $LCBD_{Total}$  value, blue and red circles represent significant ( $P < 0.05$ ) and non-significant values of  $LCBD_{Total}$ , respectively. A detailed map can be found in Fig. 1.



**Fig. 3** Simplified map of the Carmo River Basin showing compositional uniqueness in terms of nestedness ( $LCBD_{NesS}$ ) (a) and species replacement ( $LCBD_{RepS}$ ) (b) using presence-absence (Sørensen index) data for periphytic diatoms. Arrows inside boxes indicate flow direction. The size of the circles is proportional to the LCBD values. Significance of the values was not evaluated. A detailed map can be found in Fig. 1.

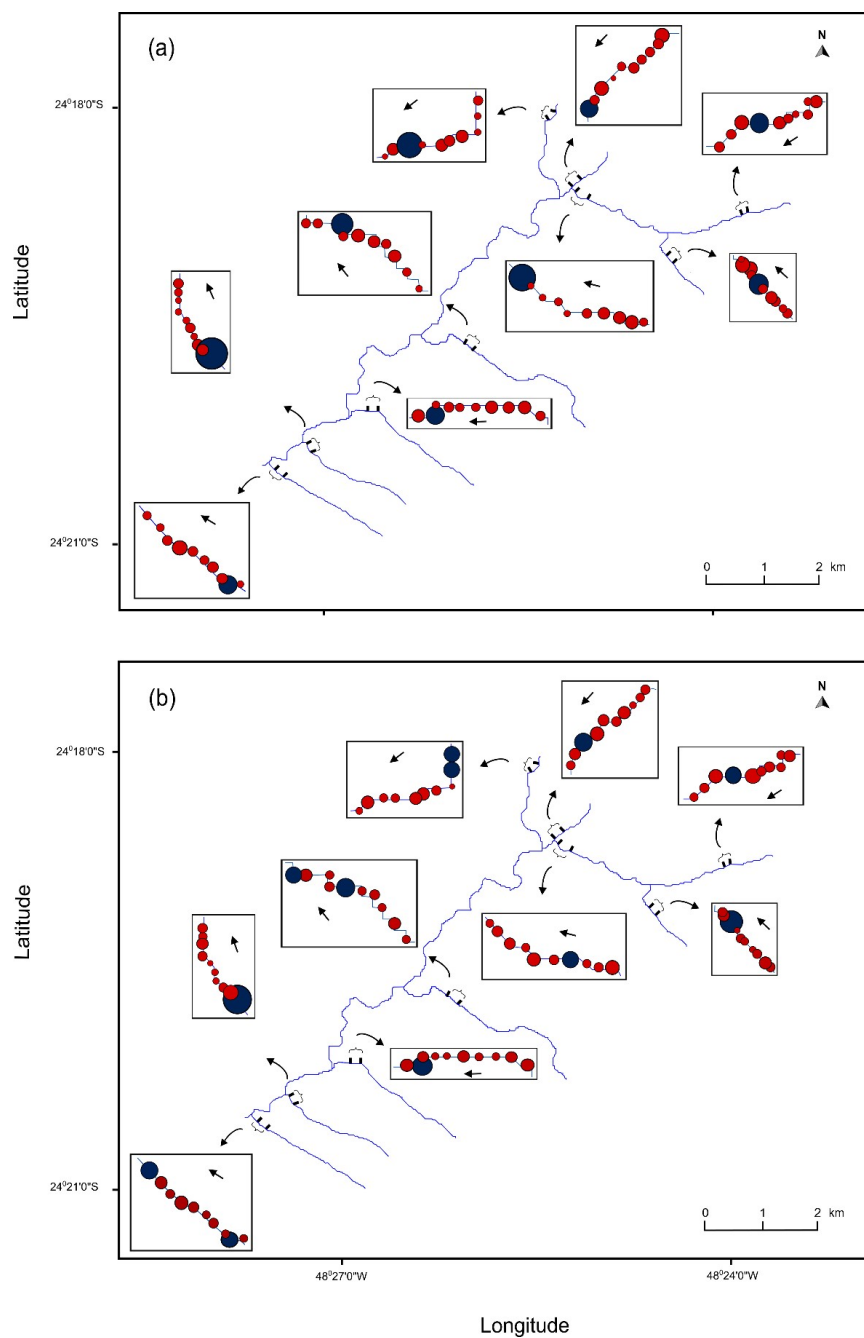


**Fig. 4** Simplified map of the Carmo River Basin showing compositional uniqueness in terms of nestedness ( $LCBD_{NesS}$ ) (a) and species replacement ( $LCBD_{RepS}$ ) (b) using presence-absence (Sørensen index) data for insects. Arrows inside boxes indicate flow direction. The size of the circles is proportional to the LCBD values. Significance of the values was not evaluated. A detailed map can be found in Fig. 1



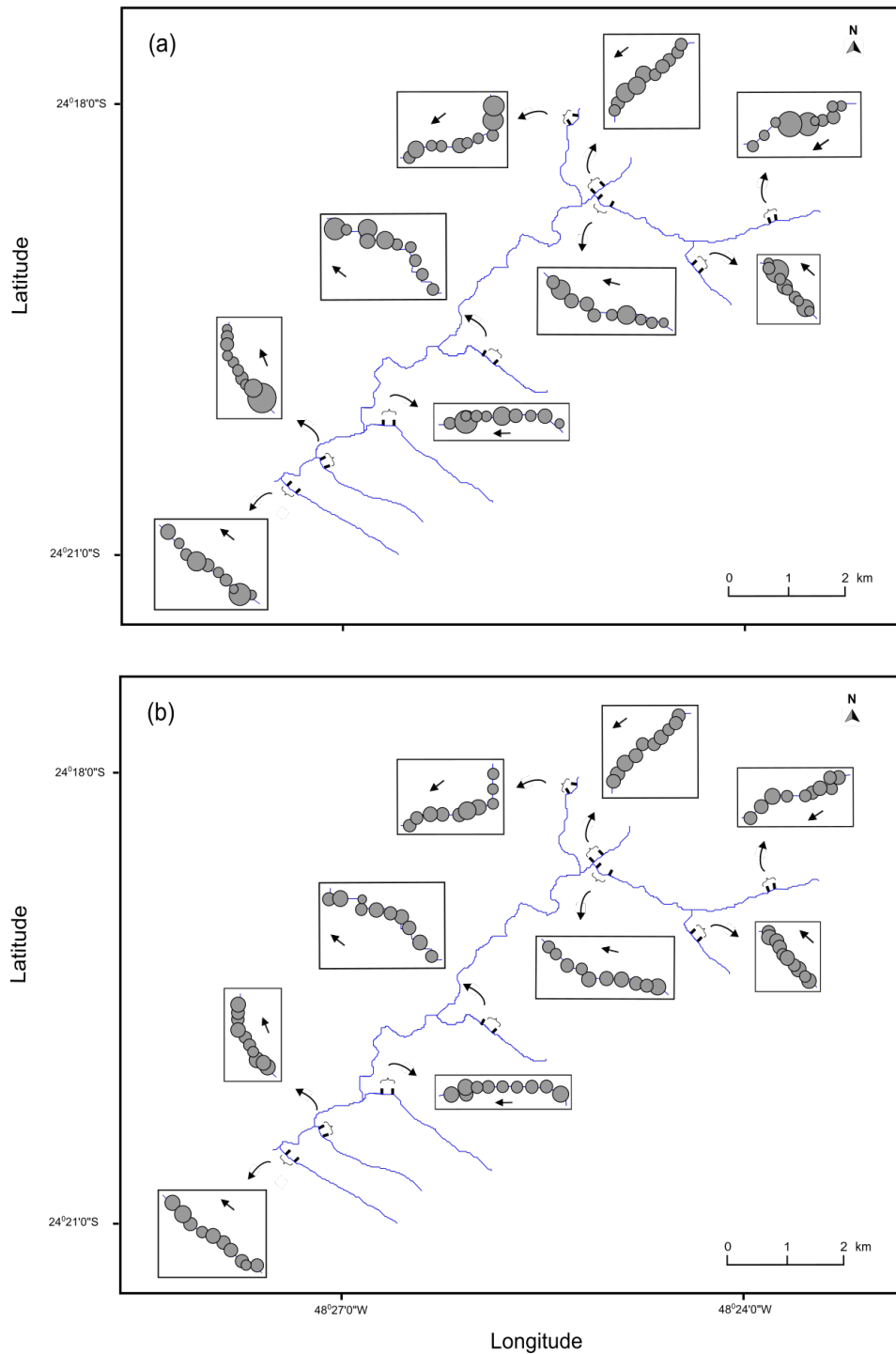
## Online resource 1

**Fig. S1** Simplified map of the Carmo River Basin showing compositional uniqueness (local contribution to beta diversity,  $LCBD_{Total}$  values) using abundance (Bray-Curtis index) data for periphytic diatoms (a) and insects (b). Arrows inside boxes indicate flow direction. The size of the circles is proportional to the  $LCBD_{Total}$  value, blue and red circles represent significant ( $P < 0.05$ ) and non-significant values of  $LCBD_{Total}$ , respectively. A detailed map can be found in Fig. 1.





**Fig. S2** Simplified map of the Carmo River Basin showing compositional uniqueness in terms of nestedness ( $LCBD_{NesBC}$ ) (a) and species replacement ( $LCBD_{RepBC}$ ) (b) using abundance (Bray-Curtis index) data for insects. Arrows inside boxes indicate flow direction. The size of the circles is proportional to the LCBD values. Significance of the values was not evaluated. A detailed map can be found in Fig. 1.



## Online resource 2

**Table S1.** Results of linear mixed-effects model using compositional uniqueness in terms of richness difference (LCBD<sub>Nes</sub>) and species replacement (LCBD<sub>Rep</sub>) of periphytic diatoms as response variables (degrees of freedom = 78) using the approach of Podani & Schmera (2011). LCEH<sub>phy</sub> and LCEH<sub>sub</sub>: local contribution to environmental heterogeneity resulting from stream physical characteristics and from substrate composition, respectively. Significant t-values ( $P \leq 0.05$ ) are in bold.

Explanatory variables	Estimate	Std. Error	t-value	P	R <sup>2</sup>
<b>Sørensen</b>					
<b>Richness difference</b>					0.10
Intercept	0.0540	0.0249	2.1704		
LCEH <sub>sub</sub>	0.2772	0.1120	<b>2.4751</b>	0.015	
LCEH <sub>phy</sub>	0.0762	0.1332	0.5724	0.569	
Riffle position	0.0019	0.0024	0.8102	0.420	
<b>Replacement</b>					0.02
Intercept	0.1034	0.0100	10.3397		
LCEH <sub>sub</sub>	0.0261	0.0513	0.5086	0.612	
LCEH <sub>phy</sub>	0.0093	0.0573	0.1626	0.871	
Riffle position	-0.0013	0.0011	-1.1724	0.245	

**Table S2.** Results of linear mixed-effects model using compositional uniqueness in terms of richness difference or abundance difference (LCBD<sub>Nes</sub>) and species replacement (LCBD<sub>Rep</sub>) of insects as response variables (degrees of freedom = 78) using the approach of Podani and Schmera (2011). LCEH<sub>phy</sub> and LCEH<sub>sub</sub>: local contribution to environmental heterogeneity resulting from stream physical characteristics and from substrate composition, respectively. Significant t-values ( $P \leq 0.05$ ) are in bold.

Explanatory variables	Estimate	Std. Error	t-value	P	R <sup>2</sup>
<b>Sørensen</b>					
<b>Richness difference</b>					0.05
Intercept	0.1201	0.0277	4.3379		
LCEH <sub>sub</sub>	-0.2016	0.1118	-1.8029	0.075	
LCEH <sub>phy</sub>	-0.0538	0.1676	-0.3211	0.749	
Riffle position	0.0010	0.0030	0.3336	0.740	
<b>Replacement</b>					0.01
Intercept	0.1060	0.0134	7.8463		
LCEH <sub>sub</sub>	-0.0070	0.0794	-0.0872	0.931	
LCEH <sub>phy</sub>	0.0252	0.0920	0.2750	0.784	
Riffle position	-0.0013	0.0020	-0.7040	0.484	
<b>Bray-Curtis</b>					
<b>Abundance difference</b>					0.11
Intercept	0.0544	0.0477	1.1399		
LCEH <sub>sub</sub>	0.3482	0.1739	<b>2.0021</b>	0.049	
LCEH <sub>phy</sub>	0.1451	0.1958	0.7412	0.461	
Riffle position	-0.0007	0.0036	-0.1854	0.853	
<b>Replacement</b>					0.06
Intercept	0.1186	0.0126	9.3992		
LCEH <sub>sub</sub>	-0.1110	0.0511	<b>-2.1698</b>	0.033	
LCEH <sub>phy</sub>	-0.0242	0.0871	-0.2775	0.782	
Riffle position	-0.0009	0.0014	-0.6583	0.512	

## CAPÍTULO 2

*Reduced enumeration effort, but not coarse taxonomic resolution, is sufficient to represent beta diversity patterns of stream benthic diatoms*

Manuscrito submetido para o periódico *Aquatic Sciences*.

Instruções aos autores disponível em: <https://www.springer.com/journal/27/submission-guidelines>

## **Abstract**

The use of coarse taxonomic resolutions and the reduced enumeration effort per sample are potential strategies for cost-effective biomonitoring programs and biological assessments. Here, we evaluated whether coarse taxonomic resolutions or reduced enumeration efforts (i.e. subsets of counted valves) are sufficient to recover patterns in the species composition and in beta diversity of benthic diatom metacommunities. Diatoms were sampled in 90 riffles (the local communities) within nine near-pristine subtropical streams (each stream represented a metacommunity). We found that genus and family levels only reflected species-level composition patterns when abundance data are used. Reduced enumeration efforts were suitable to depict patterns in the composition of the metacommunities and in differences in beta diversity among metacommunities produced by full samples (500 valves per community). A total of 400 and 50 valves were necessary to obtain similar patterns observed using species composition and full samples for presence-absence and abundance data, respectively. Furthermore, beta diversity patterns were recovered with 200 valves per community for both presence-absence and abundance data. These results indicate that it is possible to reduce counting efforts to detect regional biodiversity patterns of benthic diatoms in subtropical streams. This is especially important due to financial challenges faced by monitoring programs in tropical regions.

**Key words:** metacommunity, periphyton, procrustes, sampling effort, subtropical stream, taxonomic sufficiency

## **Introduction**

The description of freshwater biodiversity patterns can be challenging because communities include diverse taxonomic groups with little information at the species level (Heino and Soininen 2007) and are highly time-consuming for sample processing (Carneiro et al. 2010; Bennett et al. 2017). Also, many samples are needed to detect regional biodiversity patterns (Saito et al. 2015). In this sense, ecological (e.g., long-term studies) and biomonitoring programs would greatly benefit from reduced effort employed for sample processing (Bennett et al. 2017; Oliveira Jr. et al. 2020).

A potential strategy to optimize studies aiming to describe compositional dissimilarities is the use of coarse taxonomic resolutions (or taxonomic sufficiency; Ellis 1985; see Oliveira et al. 2020 for a review). The aim of this approach is to use coarse taxonomic resolutions (e.g., genera, family, or order) to detect a given ecological pattern observed using species-level identifications (Oliveira et al. 2020). Indeed, coarse taxonomic resolutions have been effective in describing compositional patterns for several freshwater groups, as lake phytoplankton (Carneiro et al. 2010), zooplankton (Carneiro et al. 2013), diatoms (Vilmi et al. 2016) and invertebrates (Pires et al. 2021), as well as stream diatoms (Rimet and Bouchez 2012) and insects (Melo 2005). Moreover, the choice of coarse taxonomic resolutions, such as the use of family instead of species identification, can reduce by more than 60% the time spent to identify organisms (Tataranni et al. 2009). The use of this approach may, thus, be relevant to increase time and financial efficiency while processing samples. This is especially true when evaluating biological groups characterized by wide morphological diversity, such as diatoms, which makes precise identification more difficult (Rimet and Bouchez 2012). However, this approach has been mainly evaluated in the context of human-induced environmental change affecting species composition, where differences among samples are usually large (e.g., Rimet and Bouchez 2012; Carneiro et al. 2013). Its effectiveness needs to be further tested to detect biodiversity patterns in near-pristine

freshwater ecosystems where differences among samples are subtle. If suitable, it would allow increased spatial representation and/or longer time-series.

Another potential way to reduce sample processing time and costs without substantial loss of information consists in counting the minimal number of individuals necessary to detect an ecological pattern of interest (e.g., Bennett et al. 2017; Cozzoni et al. 2017). Whereas reduced enumeration effort may underestimate species richness (Cao and Hawkins 2005; Cozzoli et al. 2017), the estimation of compositional dissimilarity among communities is less affected (Cao and Hawkins 2005; Schneck and Melo 2010; Sgarbi et al. 2020). This is because estimates of dissimilarity are less dependent on rare species, especially when abundance data are used (Sgarbi et al. 2020), so that the main patterns may still be detected with the enumeration of few individuals (Cao and Hawkins 2005; Blanchet et al. 2016; Sgarbi et al. 2020). For instance, King and Richardson (2002) found that the use of more than 200 individuals of macroinvertebrates did not improve the differentiation between impaired and reference wetland sites. It has also been suggested that a number as low as 150 individuals can recover resemblance patterns of macroinvertebrate communities in pristine subtropical streams (Schneck and Melo 2010). Moreover, metrics based on the distribution of abundances among phytoplankton species have shown high accuracy with the enumeration of only 200 cells per sample (Cozzoli et al. 2017). For stream benthic diatoms, the European protocol for sampling diatoms recommends the enumeration of 300 to 500 valves (Kahlert et al. 2012), whereas the USA Rapid Bioassessment Protocol indicates 600 valves for the estimation of species richness and composition (Barbour et al. 1999). Thus, the fixed-count of 400-600 valves is routinely used worldwide to assess water quality conditions in stream monitoring programs (Charles et al. 2021). However, rigorous analyses on the need to count such an elevated number of valves per sample are still lacking for tropical and subtropical streams, particularly those not subjected to strong anthropogenic pressures.

Studies investigating the effect of taxonomic sufficiency and enumeration effort on the

detection of community composition patterns usually compare the location of samples in ordination analyses or directly through dissimilarity matrices (e.g., King and Richardson 2002; Rimet and Bouchez 2012; Saito et al. 2015; Vilmi et al. 2016; Sgarbi et al. 2020). However, the detection of differences in beta diversity (i.e., multivariate dispersion as proposed by Anderson 2006 and Anderson et al. 2006) could also benefit from reduced sample processing efforts (Terlizzi et al. 2009). For example, one could evaluate whether a coarse taxonomic resolution reflects not only differences in community composition across an environmental gradient, but also differences in beta diversity among distinct parts of the gradient. Hypothetically, a lower beta diversity between polluted sites, as compared to pristine ones, may indicate a process of biotic homogenization (e.g., see Zorzal-Almeida et al. 2017 for a study with diatoms in reservoirs).

We evaluated whether coarse taxonomic resolutions and reduced enumeration efforts are sufficient to depict patterns in the composition of metacommunities and in beta diversity. For this, we used a dataset with 260 species of benthic diatoms sampled in 90 riffle sites within nine near-pristine subtropical streams. We considered that each stream with 10 riffles sampled represents a metacommunity. We thus compared the patterns of community composition and beta diversity among riffles using datasets with different taxonomic resolutions and counting efforts. The use of coarse taxonomic resolutions and reduced enumeration efforts would be feasible if they produce negligible differences in multivariate location (composition of the nine metacommunities) and multivariate dispersion (differences in beta diversity among metacommunities) compared to those using species level identification and full samples. We anticipated our test to be very stringent as the data used do not include a strong environmental gradient and were obtained over a short spatial extent (riffles in a stream).

## **Materials and Methods**



### *Study area*

We conducted field work in the Carmo River Basin (Fig. 1), located within the Intervales State Park (24°18'S, 48°25'W; São Paulo State, Brazil). The park (48000 ha) is part of the largest preserved Atlantic Forest area in the State of São Paulo (Morellato et al. 2000). The climate is humid subtropical, with average annual rainfall of 2040 mm and air temperature varying between 15 °C and 30 °C during the austral summer, and from 0 °C to 25 °C during winter (Alvares et al. 2013).

### *Biological and environmental data*

We collected samples in April 2015 in 10 riffles of nine streams (totaling 90 riffles). Stream order ranged from 2nd to 4th. Riffles within each stream were approximately 10 to 50 m apart from each other. At each riffle, we sampled periphytic diatoms from 10 stones by brushing an area of 25 cm<sup>2</sup> from each stone. We pooled the material of the 10 stones and this constituted a sample. In the laboratory, we oxidized the samples with potassium permanganate (KMnO<sub>4</sub>) and concentrated hydrochloric acid (HCl) for mounting permanent slides using Naphrax<sup>®</sup>. We counted 500 valves from each sample using a Zeiss Primo Star microscope with 1000× magnification and identified diatoms to species level using specialized literature (Metzeltin and Lange-Bertalot 1998; Metzeltin et al. 2005; Metzeltin and Lange-Bertalot 2007). We recorded 260 species, 44 genera, 22 families and 12 orders of benthic diatoms (Castro et al. 2019).

### *Data analysis*

#### *Higher taxon approach*

To evaluate whether coarse taxonomic resolutions were sufficient to describe the patterns in biological composition among the nine metacommunities (i.e., streams), we constructed four data matrices, one for each taxonomic resolution (species, genus, family, and order). For each

matrix, we calculated biological dissimilarities using Sørensen (presence-absence) and Bray-Curtis (logarithmized abundance + 1) dissimilarity coefficients. We then used Principal Coordinate Analysis (PCoA) to ordinate samples. The ordination sample scores obtained with the species dataset were compared with the ordination sample scores obtained with data at coarser taxonomic resolutions using Procrustes Analysis ( $n = 90$ ; Jackson 1995; Peres-Neto and Jackson 2001). We used the first two axes in these analyses. The higher the Procrustes correlation coefficient  $r$ , the higher the similarity between the ordination plots under comparison.

Further, we investigated whether differences in beta diversity among metacommunities obtained with species-level identification were recovered by coarser taxonomic resolutions. We used the analysis of multivariate homogeneity of group dispersions (PERMDISP; Anderson 2006; Anderson et al. 2006) to obtain the distance of each sample (i.e., riffle) to its group centroid (i.e., stream) in the PCoA multidimensional space. The greater the mean distance of the samples from the centroid, the greater the beta diversity (Anderson et al. 2006). Next, we used the Pearson correlation coefficient to evaluate the relationship between beta diversity values obtained using the species dataset and each of the other taxonomic resolutions ( $n = 9$ ).

#### *Enumeration effort*

We tested the effect of reduced enumeration effort by using subsets of 50, 100, 200, 300, and 400 diatom valves out of a total of 500 valves counted for each riffle. To do that, we generated reduced datasets separately for each of the 90 riffles by randomly subsampling valves. The probability of species to be sampled was weighted by its abundance within each riffle. We constructed matrices with the reduced datasets from the 90 riffles and repeated this procedure 500 times for each of the five predetermined enumeration efforts, generating 2500 matrices. Next, we proceeded with the same analyses used for the higher taxon approach.

Thus, each matrix, for both presence-absence and abundance data, was subjected to a PCoA and to a Procrustes analysis to be compared with the complete dataset (i.e., 500 valves per riffle). This means that we had 500 Procrustes correlation coefficients  $r$  for each enumeration effort being tested. Finally, to evaluate whether beta diversity patterns among streams are affected by the reduced enumeration effort, we calculated the mean distance from riffles to their group centroid (i.e., stream) and estimated the Pearson correlations between the mean distances of each reduced dataset and the complete dataset. In this analysis, the higher the Pearson correlation coefficient, the lower the effect of reducing the enumeration effort on beta diversity.

All analyses were performed in the R environment (R Core Team 2021). We used package ‘vegan’ (Oksanen et al. 2020) to perform PCoA, PERMDISP, and Procrustes analysis, and package ‘ggplot2’ (Wickham 2016) to construct figures.

## Results

### *Higher taxon approach*

Patterns of community composition were preserved with the reduction of taxonomic resolution to genus and family, since Procrustes correlations were high ( $\geq 0.90$ ), but only for abundance data (Table 1). For presence-absence data, correlations were low ( $\leq 0.60$ ) for all comparisons (Table 1), indicating the loss of information with the reduction of taxonomic resolution. Beta diversity values estimated with coarse taxonomic resolutions were poorly correlated ( $\leq 0.73$ ) with those obtained with species data, independently of the use of presence-absence or abundance data (Table 1).

### *Enumeration effort*

In general, abundance data presented higher mean  $r$  values and lower variability in  $r$  values than presence-absence data to represent patterns of community composition (Table 2; Fig. 1)

and beta diversity (Table 2; Fig. 2). At least 400 valves were necessary to represent patterns of community composition for presence-absence data, reaching a mean correlation as high as  $r = 0.97$  and low variability ( $r$  always  $\geq 0.8$ ) (Table 2; Fig. 1a). In contrast, for abundance data, enumeration efforts as low as 50 valves were enough to describe patterns in community composition among streams ( $r \geq 0.95$ ) (Table 2; Fig. 1b). The strengths of the relationships between the beta diversity pattern generated with the highest enumeration effort (500 valves) and those with lower enumeration efforts were high ( $r \geq 0.92$ ) and less variable when 200 valves per riffle were counted, for both presence-absence and abundance data (Table 2; Fig. 2).

## **Discussion**

We showed that coarser taxonomic resolutions were only sufficient to reflect species-level patterns in community composition when relative abundance data were used. Reduced enumeration efforts performed better than the higher taxon approach in showing ordination and beta diversity patterns obtained with high resolution data. However, a larger number of valves was necessary to produce high correlation values for incidence than for abundance data, at least for depicting community composition patterns. In general, the number of valves counted per sample could be reduced by 20 to 90%, depending on the type of data (incidence or abundance) and on the aim of the study.

The higher taxon approach, and more specifically genus and family resolutions, was effective as a surrogate to species-level identifications of diatoms only to represent community resemblance patterns and only for abundance data. Numerous studies on taxonomic sufficiency with freshwater organisms also found that low resolutions, mainly genus and family, can be effective surrogates to species data (e.g. Melo 2005; Carneiro et al. 2010; Rimet and Bouchez 2012; Ribas and Padial 2015; Oliveira Jr. et al. 2020). The use of genus or family to describe compositional patterns of benthic diatoms has many advantages,

since the identification of diatom species is complex and time-consuming due to the high morphological variability of the group (Rimet and Bouchez 2012). Furthermore, it involves the examination of small structures, which requires trained taxonomists and up-to-date specialized literature (Irfanullah 2006; Rimet and Bouchez 2012). The use of higher taxa may not only reduce time and financial costs, but reduce inconsistencies in species-level taxonomic identification among analysts (Rimet and Bouchez 2012; Lee et al. 2019). However, our results indicated that the use of coarse taxonomic resolutions should be only employed with abundance data as presence-absence represented poorly the patterns obtained using species-level identifications.

Regarding beta diversity patterns among streams (i.e. multivariate dispersion), the higher taxon approach showed poor to moderate performances for either incidence or abundance data. This may be related to the small spatial extent of our study (all sampled streams are tributaries of the same mainstem) and to the near-pristine and environmentally homogeneous characteristic of the streams. At larger spatial extents or at stronger environmental gradients, it could be expected a change not only on species identities among habitats, but also changes on genus and family-level identities. In this case, genus and family-level beta diversity would follow species-level beta diversity (Terlizzi et al. 2009). However, as pointed out by Terlizzi et al. (2009), the applicability of the higher taxon approach to assess differences in beta diversity among environments still needs to be further explored at different environments and with distinct biological groups.

The most striking results we found were the strong correlations between the complete dataset and the various reduced enumeration efforts for both ordination and dispersion analyses based on abundance data. This suggests that little ecological information was lost when effort decreased from 500 to 50 valves (for ordination patterns) or 200 valves (for dispersion patterns) per sample. The reduction of at least 60% in enumeration effort compared to standard protocols of 500-600 valves (e.g. Tyree et al. 2020; Charles et al. 2021) may

optimize environmental monitoring programs by reducing the time spent in counting organisms in the laboratory and increasing the number of sampled sites. Our results are similar to the results of a previous study showing that increasing enumeration effort above 200 valves did not increase accuracy in the estimation of relationships between community composition and environmental variables for lake diatoms (Bennett et al. 2017). However, such a reduced number of valves should be used only for abundance datasets as we found that for recovering presence-absence patterns it is necessary a higher number of valves to be counted. Furthermore, reducing enumeration effort may impair the detection of species losses due to anthropogenic impacts, as suggested by Cao and Hawkins (2005) for stream invertebrates. Accordingly, despite providing reliable estimates of dissimilarity in community composition and of differences in beta diversity, the reduction in the number of counted valves may affect the estimation of other metrics not evaluated in this study.

We showed that reduced efforts were able to discriminate between subtle compositional and beta diversity differences among diatom communities from environmentally homogeneous streams. This indicates that even lower valve counts as the ones tested in our study may be enough to detect differences among ecosystems subjected to human-induced impacts. This is because strong environmental gradients should result in distinct species compositions and in beta diversity differences among sites due to replacement and extirpation of species (Olden and Poff 2003).

We recommend here the use of reduced enumeration efforts that showed both a strong relationship (i.e., high mean Procrustes or Pearson correlation) with the highest enumeration effort (500 valves) and a low variability (i.e., high similarity in  $r$  values among measurements). We highlight that high mean correlation is not enough to justify a reduction in effort, because values could be too variable. For example, had we taken into account only correlations, we would have concluded that 200 valves are enough to represent ordination patterns for presence-absence data ( $r = 0.80$ , but high variability; see Fig. 1a). Instead, we

showed that the use of presence-absence data requires at least 400 valves to achieve high correlation values and low variability because Procrustes  $r$  values were too variable among subsamples. Accordingly, both the strength of the relationships of interest and the variability among measurements need to be estimated to determine the representativeness of reduced sampling efforts (Cao et al. 2003) as practical users will only have a single sample and, thus, no way to estimate variability.

We suggest that counting 400 valves or 50 valves per sample when using presence-absence or abundance data, respectively, is sufficient to evaluate the composition of communities. When the aim is to analyze beta diversity patterns, we suggest that 200 valves is enough for both presence-absence and abundance data. These results indicate that it is possible to reduce identification and counting efforts necessary to detect regional biodiversity patterns of benthic diatoms in subtropical streams. However, more studies evaluating these issues at a variety of environmental gradients could help to determine minimum efforts needed for bioassessment and monitoring of tropical and subtropical streams. This is especially important due to financial challenges faced by monitoring programs in tropical regions.

## **Acknowledgments**

We thank Priscila Tremarin for helping with identification of diatom species.

## **References**

- Alvares CA, Stape JL, Sentelhas PC, de Moraes Gonçalves JL, Sparovek G (2013) Köppen's climate classification map for Brazil. *Meteorol Z* 22:711-728. <https://10.1127/0941-2948/2013/0507>
- Anderson MJ (2006) Distance-based tests for homogeneity of multivariate dispersions. *Biometrics* 62:245-253. <https://10.1111/j.1541-0420.2005.00440.x>

- Anderson MJ, Ellingsen KE, McArdle BH (2006) Multivariate dispersion as a measure of beta diversity. *Ecol Lett* 9:683-693. <https://doi.org/10.1111/j.1461-0248.2006.00926.x>
- Barbour MT, Gerritsen J, Snyder BD, Stribling JB (1999) Rapid bioassessment protocols for use in streams and wadeable rivers: periphyton, benthic macroinvertebrates and fish, 2nd edn. EPA 841-B-99-002. U.S. Environmental Protection Agency, Washington, D.C.
- Bennett JR, Rühland KM, Smol JP (2017) No magic number: determining cost-effective sample size and enumeration effort for diatom-based environmental assessment analyses. *Can J Fish Aquat Sci* 74:208-215. <https://doi.org/10.1139/cjfas-2016-0066>
- Blanchet FG, Legendre P, He F (2016) A new cost-effective approach to survey ecological communities. *Oikos* 125:975-987. <https://doi.org/10.1111/oik.02838>
- Cao Y, Hawkins CP (2005) Simulating biological impairment to evaluate the accuracy of ecological indicators. *J Appl Ecol* 42:954-965. <https://doi.org/10.1111/j.1365-2664.2005.01075.x>
- Cao Y., Hawkins CP, Vinson MR (2003) Measuring and controlling data quality in biological assemblage surveys with special reference to stream benthic macroinvertebrates. *Freshw Biol* 48: 1898-1911. <https://doi.org/10.1046/j.1365-2427.2003.01123.x>
- Carneiro FM, Bini LM, Rodrigues LC (2010) Influence of taxonomic and numerical resolution on the analysis of temporal changes in phytoplankton communities. *Ecol Indic* 10:249-255. <https://10.1016/j.ecolind.2009.05.004>
- Carneiro FM, Nabout JC, Vieira LCG, Lodi S, Bini LM (2013) Higher taxon predict plankton beta-diversity patterns across an eutrophication gradient. *Nat Conserv* 11:43-47. <http://dx.doi.org/10.4322/natcon.2013.006>
- Castro E, Siqueira T, Melo AS, Bini LM, Landeiro VL, Schneck F (2019) Compositional uniqueness of diatoms and insects in subtropical streams is weakly correlated with riffle position and environmental uniqueness. *Hydrobiologia* 842:219-232. <https://doi.org/10.1007/s10750-019-04037-8>



- Charles DF, Kelly MG, Stevenson RJ, Poikane S, Theroux S, Zgrundo A, Cantonati M (2021) Benthic algae assessment in the EU and the US: striving for consistency in the face of great ecological diversity. *Ecol Indic* 121:107082. <https://doi.org/10.1016/j.ecolind.2020.107082>
- Cozzoli F, Stanca E, Selmecky GB, Francé J, Varkitzi I, Basset A (2017) Sensitivity of phytoplankton metrics to sample-size: a case study on a large transitional water dataset (WISER). *Ecol Indic* 82:558-573. <https://doi.org/10.1016/j.ecolind.2017.07.022>
- Ellis D (1985) Taxonomic sufficiency in pollution assessment. *Mar Pollut Bull* 16:459. [https://10.1016/0025-326X\(85\)90362-5](https://10.1016/0025-326X(85)90362-5)
- Heino J, Soininen J (2007) Are higher taxa adequate surrogates for species-level assemblage patterns and species richness in stream organisms? *Biol Conserv* 137:78-89. <https://10.1016/j.biocon.2007.01.017>
- Irfanullah HMD (2006) Algal taxonomy in limnology: an example of the declining trend of taxonomic studies? *Hydrobiologia* 559:1-9. <https://doi.org/10.1007/s10750-005-9202-z>
- Jackson DA (1995) Protest: a PROcrustean Randomization TEST of community environment concordance. *Écoscience* 2:297-303
- Kahlert M, Kelly M, Albert RL, Almeida SFP, Besta T, Blanco S, Coste M, Denys L, Ector L, Fránková M, Hlúbiková D, Ivanov P, Kennedy B, Marvan P, Mertens A, Miettinen J, Picinska-Faltynowicz J, Rosebery J, Tornés E, Vilbaste S, Vogel A (2012) Identification versus counting protocols as sources of uncertainty in diatom-based ecological status assessments. *Hydrobiologia* 695:109-124. <https://doi.org/10.1007/s10750-012-1115-z>
- King RS, Richardson CJ (2002) Evaluating subsampling approaches and macroinvertebrate taxonomic resolution for wetland bioassessment. *J N Am Benthol Soc* 21:150-171. <https://doi.org/10.2307/1468306>
- Lee SS, Bishop IW, Spaulding SA, Mitchell RM, Yuan LL (2019) Taxonomic harmonization may reveal a stronger association between diatom assemblages and total phosphorus in large datasets. *Ecol Indic* 102:166-174. <https://doi.org/10.1016/j.ecolind.2019.01.061>

- Melo AS (2005) Effects of taxonomic and numeric resolution on the ability to detect ecological patterns at a local scale using stream macroinvertebrates. *Arch Hydrobiol* 164:309-323. <https://doi.org/10.1127/0003-9136/2005/0164-0309>
- Metzeltin D, Lange-Bertalot H (1998) Tropical diatoms of South America. *Iconographia Diatomologica* 5. Koeltz Botanical Books, Germany
- Metzeltin D, Lange-Bertalot H (2007) Tropical diatoms of South America II. *Iconographia Diatomologica* 18. Koeltz Botanical Books, Germany
- Metzeltin D, Lange-Bertalot H, Garcia-Rodríguez F (2005) Diatoms of Uruguay. *Iconographia Diatomologica* 15. Koeltz Botanical Books, Germany
- Morellato PL, Talora DC, Takahasi A, Bencke CC, Romera EC, Zipparro VB (2000) Phenology of Atlantic Rain Forest trees: a comparative study. *Biotropica* 32:811-823. <https://doi.org/10.1111/j.1744-7429.2000.tb00620.x>
- Olden JD, Poff NL (2003) Toward a mechanistic understanding and prediction of biotic homogenization. *Am Nat* 162:442-460. <https://doi.org/10.1086/378212>
- Oksanen J, Blanchet FG, Friendly M, Kindt R, Legendre P, McGlinn D, Minchin PR, O'Hara RB, Simpson GL, Solymos P, Stevens MHH, Szoecs E, Wagner H (2020) vegan: Community Ecology Package. R package version 2.5-7. <https://CRAN.R-project.org/package=vegan>
- Oliveira Jr. SS, Ortega JCG, Ribas LGS, Lopes VG, Bini LM (2020) Higher taxa are sufficient to represent biodiversity patterns. *Ecol Indic* 111:105994. <https://doi.org/10.1016/j.ecolind.2019.105994>
- Peres-Neto PR, Jackson DA (2001) How well do multivariate data sets match? The advantages of a Procrustean superimposition approach over the Mantel test. *Oecologia* 129:169-178. <https://doi.org/10.1007/s004420100720>
- R Core Team (2021) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. <https://www.R-project.org/>

- Ribas LGS, Padial AA (2015) The use of coarser data is an effective strategy for biological assessment. *Hydrobiologia* 747:83-95. <https://doi.org/10.1007/s10750-014-2128-6>
- Rimet F, Bouchez A (2012) Biomonitoring river diatoms: implications of taxonomic resolution. *Ecol Indic* 15:92-99. <https://doi.org/10.1016/j.ecolind.2011.09.014>
- Saito VS, Fonseca-Gessner AA, Siqueira T (2015) How should ecologists define sampling effort? The potential of procrustes analysis for studying variation in community composition. *Biotropica* 47:399-402. <https://doi.org/10.1111/btp.12222>
- Schneck F, Melo AS (2010) Reliable sample sizes for estimating similarity among macroinvertebrate assemblages in tropical streams. *Ann Limnol Int J Limnol* 46:93-100. <https://doi.org/10.1051/limn/2010013>
- Sgarbi LF, Bini LM, Heino J, Jyrkänkallio-Mikkola J, Landeiro VL, Santos EP, Schneck F, Siqueira T, Soininen J, Tolonen KT, Melo AS (2020) Sampling effort and information quality provided by rare and common species in estimating assemblage structure. *Ecol Indic* 110:105937. <https://doi.org/10.1016/j.ecolind.2019.105937>
- Tataranni M, Maltagliati F, Floris A, Castelli A, Lardicci C (2009) Variance estimate and taxonomic resolution: an analysis of macrobenthic spatial patterns at different scales in a Western Mediterranean coastal lagoon. *Mar Environ Res* 67:219-229. <https://doi.org/10.1016/j.marenvres.2009.02.003>
- Terlizzi A, Anderson MJ, Bevilacqua S, Frascchetti S, Włodarska-Kowalczyk M, Ellingsen KE (2009) Beta diversity and taxonomic sufficiency: do higher-level taxa reflect heterogeneity in species composition? *Divers Distrib* 15:450-458. <https://doi.org/10.1111/j.1472-4642.2008.00551.x>
- Tyree MA, Bishop IW, Hawkins CP, Mitchell R, Spaulding SA (2020) Reduction of taxonomic bias in diatom species data. *Limnol Oceanogr Methods*, 18:271-279. <https://doi.org/10.1002/lom3.10350>
- Vilmi A, Karjalainen SM, Nokela T, Tolonen K, Heino J (2016) Unravelling the drivers of

aquatic communities using disparate organismal groups and different taxonomic levels.

Ecol Indic 60:108-118. <https://doi.org/10.1016/j.ecolind.2015.06.023>

Wickham H (2016) ggplot2: Elegant Graphics for Data Analysis. Springer-Verlag, New York.

Zorzal-Almeida S, Bini LM, Bicudo DC (2017) Beta diversity of diatoms is driven by environmental heterogeneity, spatial extent and productivity. *Hydrobiologia* 800:7-16.

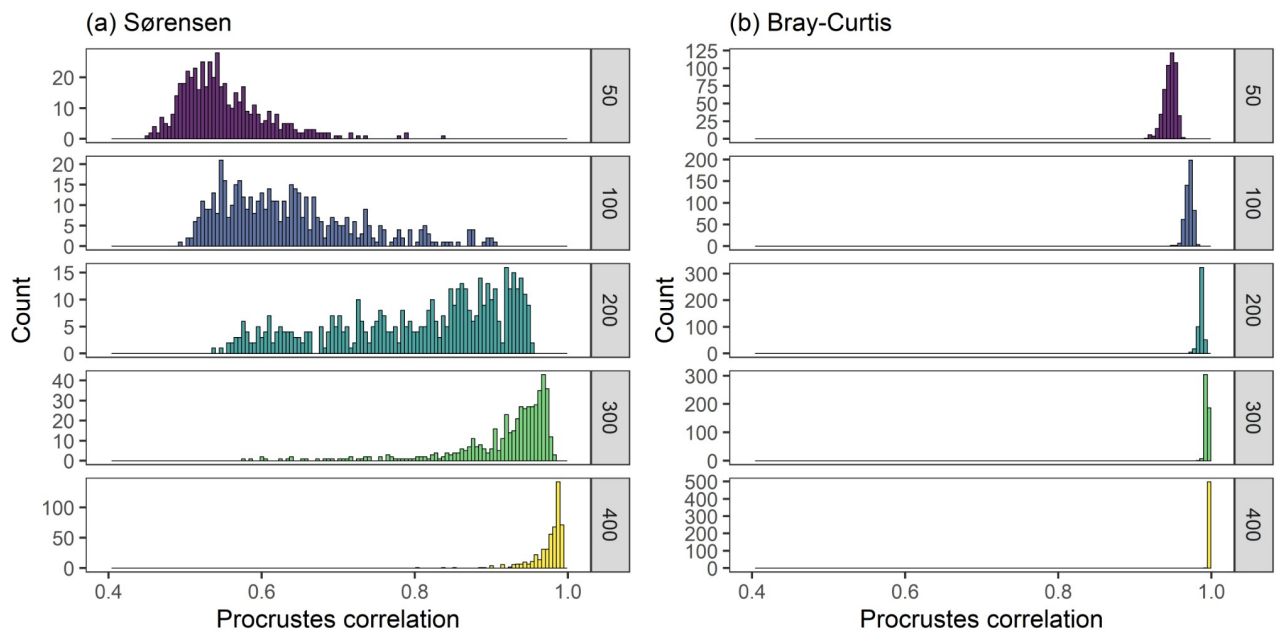
<https://doi.org/10.1007/s10750-017-3117-3>

**Table 1** Procrustes and Pearson correlations between data using species and coarser taxonomic resolutions for both presence-absence (Sørensen coefficient) and abundance (Bray-Curtis coefficient) data. Procrustes was used to compare ordination patterns ( $n = 90$ ), and Pearson correlation was used to quantify the relationship between beta diversity values estimated with species data and data with lower taxonomic resolutions ( $n = 9$ )

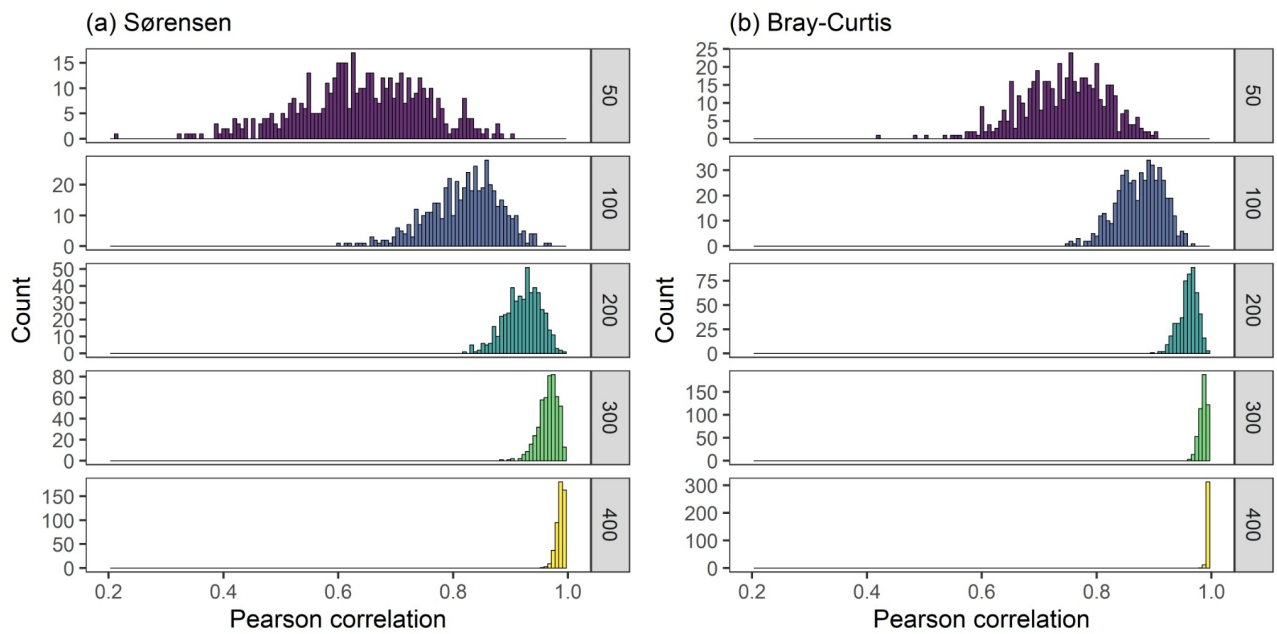
	Community composition (Procrustes correlation)		Beta diversity (Pearson correlation)	
	Sørensen	Bray-Curtis	Sørensen	Bray-Curtis
	<i>r</i>	<i>r</i>	<i>r</i>	<i>r</i>
Genus	0.60	0.95	0.73	0.63
Family	0.51	0.90	0.49	0.49
Order	0.39	0.76	0.66	0.60

**Table 2** Procrustes and Pearson mean correlations ( $\pm$  standard deviation) between data using the complete enumeration effort (500 valves per riffle) and reduced efforts for both presence-absence (Sørensen coefficient) and abundance (Bray-Curtis coefficient) data. Procrustes was used to compare ordination patterns ( $n = 90$ ), and Pearson correlation was used to quantify the relationship between beta diversity values estimated with complete enumeration (500 valves) and reduced enumeration efforts (from 50 to 400) ( $n = 9$ ). Correlations are the mean values ( $\pm$  standard deviation) from 500 matrices generated for each enumeration effort

	Community composition (Procrustes correlation)		Beta diversity (Pearson correlation)	
	Sørensen	Bray-Curtis	Sørensen	Bray-Curtis
	<i>r</i>	<i>r</i>	<i>r</i>	<i>r</i>
50	0.55 ( $\pm$ 0.06)	0.95 ( $\pm$ 0.01)	0.64 ( $\pm$ 0.11)	0.74 ( $\pm$ 0.07)
100	0.63 ( $\pm$ 0.09)	0.97 ( $\pm$ 0.005)	0.82 ( $\pm$ 0.06)	0.87 ( $\pm$ 0.04)
200	0.80 ( $\pm$ 0.11)	0.99 ( $\pm$ 0.003)	0.92 ( $\pm$ 0.03)	0.96 ( $\pm$ 0.02)
300	0.91 ( $\pm$ 0.07)	0.99 ( $\pm$ 0.002)	0.97 ( $\pm$ 0.02)	0.99 ( $\pm$ 0.01)
400	0.97 ( $\pm$ 0.02)	1.00 ( $\pm$ 0.001)	0.99 ( $\pm$ 0.01)	1.00 ( $\pm$ 0.002)



**Fig. 1** Histograms showing Procrustes correlations between PCoA scores using the complete enumeration effort (500 valves per riffle) and each of the 500 matrices generated using reduced enumeration efforts for both presence-absence (a) and abundance (b) data



**Fig. 2** Histograms showing Pearson correlations between beta diversity values obtained using the complete enumeration effort (500 values per riffle) and each of the 500 subsets generated using reduced enumeration efforts for both presence-absence (a) and abundance (b) data



## CONSIDERAÇÕES FINAIS E PERSPECTIVAS

Na minha tese, eu procurei compreender os determinantes que atuam na estruturação de metacomunidades de diatomáceas perifíticas e de insetos aquáticos em riachos subtropicais preservados. No primeiro capítulo, avaliei a singularidade composicional de cada site amostrado (i.e. corredeira) e o quanto cada comunidade local contribuía para a diversidade beta total da região (i.e. riacho). Minha hipótese era de que a heterogeneidade ambiental, medida como a singularidade ambiental (LCEH), teria relação positiva com o LCBD, uma vez que locais ambientalmente heterogêneos tendem a ter maior variação na composição de suas comunidades biológicas (HEINO *et al.*, 2013). Porém, meus resultados exibiram uma relação positiva apenas entre a singularidade composicional de diatomáceas (dados qualitativos) e a singularidade composicional de substratos no leito do riacho; para os insetos a singularidade composicional não foi relacionada com nenhuma variável explicativa.

Eu também analisei o LCBD em termos de aninhamento e substituição de espécies, avaliando se existia alguma correlação positiva com a posição da corredeira e a singularidade ambiental (LCEH), respectivamente. Apenas o LCBD em termos de aninhamento para diatomáceas (dados de presença-ausência) e para insetos (dados de abundância) foi correlacionado positivamente com o LCEH de substrato. Já a singularidade composicional em termos de substituição de insetos (dados de abundância) foi negativamente correlacionada com LCEH de substrato. Nossos resultados indicam que a estruturação das comunidades biológicas, especialmente quando avaliando a singularidade composicional, se dá por motivos ainda pouco conhecidos e que vão além das hipóteses comuns que estabelecemos.

Além disso, eu esperava que a singularidade composicional dentro de riachos (considerando cada uma das 90 corredeiras) tivesse uma relação positiva com a posição de cada corredeira (montante à jusante), uma vez que as corredeiras à montante abrangeriam espécies mais únicas, em relação às corredeiras à jusante que recebem as espécies das

corredeiras à montante por conta da direção do fluxo (SWAN; BROWN, 2014). Porém, os resultados para ambas as comunidades (diatomáceas e insetos) não mostraram resultados significativos para essa relação. Isso pode ser reflexo da baixa distância entre as corredeiras amostradas dentro dos riachos (distâncias entre a primeira corredeira e a última variaram de 190 a 430 metros), o que, provavelmente, facilitou a dispersão dos organismos.

Finalizo minhas conclusões a respeito do primeiro capítulo sugerindo o emprego de métricas alternativas e recentes para avaliação da diversidade beta, como a que usei em minha tese quando avaliei a singularidade composicional de cada site amostrado (e.g. LEGENDRE; DE CÁCERES, 2013). Tais análises exibem resultados bastante interessantes e que podem indicar caminhos futuros para estudos ecológicos. Minhas sugestões para estudos futuros avaliando o LCBD, são: (i) incorporar às variáveis explicativas outros fatores ambientais que possam estar relacionados à singularidade da composição das comunidades e (ii) avançar com estudos que enfoquem no uso da singularidade ambiental (LCEH), medida que propus nesta tese para avaliar a contribuição de cada site à heterogeneidade ambiental regional. O uso de medidas de LCEH é promissor, já que meus resultados confirmaram que mesmo diferenças sutis na heterogeneidade ambiental (especialmente de substrato) de riachos prístinos mostraram ter relação positiva com o LCBD das comunidades analisadas.

Em meu segundo capítulo, avaliei o esforço amostral que seria suficiente para refletir os padrões de composição de espécies de comunidades locais (i.e. posição multivariada) e de diferenças de diversidade beta entre metacomunidades (i.e. dispersão multivariada) de diatomáceas perifíticas de riachos preservados. Em relação ao emprego de resoluções taxonômicas grosseiras (resoluções acima de espécie), eu comparei o quanto cada conjunto de dados de gênero, família e ordem refletiria os padrões de composição de espécies e de diferenças de diversidade beta obtidos através do conjunto de dados de espécie. Os resultados foram satisfatórios somente para o uso de resoluções a nível de gênero e família na análise de composição de espécies utilizando dados de abundância. Ou seja, resoluções taxonômicas

maiores que espécie não podem ser utilizadas em estudos que objetivam analisar dados de presença-e-ausência ou em estudos cujo foco é a análise de diferenças na diversidade beta entre metacomunidades. Em relação à redução na contagem de indivíduos por amostra, nossos resultados mostraram que é possível reduzir em pelo menos 20% o número de valvas contadas por amostra, ou seja, a contagem de 400 valvas é tão suficiente quanto a contagem de 500 valvas para dados qualitativos. Esse número é ainda menor quando os dados são de abundância, uma vez que a contagem de apenas 50 indivíduos se mostrou hábil em refletir os padrões de ordenação de comunidades de diatomáceas. Ainda, a contagem de 200 valvas se mostrou suficiente quando o objetivo é analisar diferenças de diversidade beta, seja com dados de presença-e-ausência, seja com dados de abundância.

Tais resultados são bastante promissores, uma vez que o uso de resoluções taxonômicas grosseiras abreviaria o tempo despendido em identificações a nível específico, o que em muitos grupos biológicos que apresentam ampla variação morfológica requer taxonomistas especializados (CARNEIRO *et al.*, 2010; 2013), como é o caso de diatomáceas. Além disso, a redução no número de indivíduos contados por amostras possibilita aumentar o número de sítios amostrados, alternativa que pode ser extremamente proveitosa em estudos que abrangem áreas de monitoramento ambiental. Por fim, sugiro através dos resultados que obtive, que a contagem de 300 valvas por amostra é suficiente para análises de ordenação e de diversidade beta de diatomáceas perifíticas em riachos subtropicais. A minha expectativa é que estes resultados possam contribuir para que as pesquisas científicas se tornem mais produtivas e contínuas, especialmente quando grupos biológicos tão diversos como as diatomáceas forem utilizados.

## Referências bibliográficas

CARNEIRO F. M.; BINI, L. M.; RODRIGUES, L. C. Influence of taxonomic and numerical resolution on the analysis of temporal changes in phytoplankton communities. **Ecological Indicators**, v. 10, p. 249-255, 2010.

CARNEIRO, F. M.; NABOUT, J. C.; VIEIRA, L. C. G.; LODI, S.; BINI, L. M. Higher taxa predict plankton beta-diversity patterns across an eutrophication gradient. **Natureza & Conservação**, v. 11, p. 43-47, 2013.

HEINO, J.; GRÖNROOS, M.; ILMONEN, J., KARHU, T., NIVA, M.; PAASIVIRTA, L. Environmental heterogeneity and  $\beta$  diversity of stream macroinvertebrate communities at intermediate spatial scales. **Freshwater Science**, v. 32, p. 142-154, 2013.

LEGENDRE, P.; DE CÁCERES, M. Beta diversity as the variance of community data: dissimilarity coefficients and partitioning. **Ecology Letters**, v. 16, p. 951-963, 2013.

SWAN, C. M.; BROWN, B. L. Using rarity to infer how dendritic network structure shapes biodiversity in riverine communities. **Ecography**, v. 37, p. 993-1001, 2014.

**Anexo 1.** Primeira página do artigo da tese publicado na revista *Hydrobiologia* durante o doutorado.

*Hydrobiologia* (2019) 842:219–232  
<https://doi.org/10.1007/s10750-019-04037-8>.



PRIMARY RESEARCH PAPER

## Compositional uniqueness of diatoms and insects in subtropical streams is weakly correlated with riffle position and environmental uniqueness

Emanuela Castro · Tadeu Siqueira · Adriano Sanches Melo · Luis Mauricio Bini · Victor Lemes Landeiro · Fabiana Schneck

Received: 22 January 2019 / Revised: 23 July 2019 / Accepted: 27 July 2019 / Published online: 3 August 2019  
 © Springer Nature Switzerland AG 2019

**Abstract** Some local communities contribute more to beta diversity than others, which has been known as compositional uniqueness or local contribution to beta diversity. Compositional uniqueness should correlate positively with environmental uniqueness and site isolation. We evaluated compositional uniqueness (total and in terms of species replacement and nestedness) of periphytic diatoms and insects in subtropical streams and tested for correlates of these metrics. We sampled 90 riffles in a near-pristine

catchment in the southeast Atlantic Forest of Brazil. The total compositional uniqueness for diatoms and insects were not associated to riffle position. However, the total compositional uniqueness of diatoms (presence–absence data), was correlated with the uniqueness of streambed substrate composition, while the total compositional uniqueness of insects did not correlate with any explanatory variable. The compositional uniqueness in terms of nestedness (presence–absence data) for diatoms and for insects (abundance data) was correlated positively with the uniqueness of substrate composition. Compositional uniqueness in terms of species replacement (abundance data) for insects was correlated negatively with the uniqueness of substrate composition. Our results indicate that subtle differences in environmental uniqueness, play a role in determining beta diversity in near-pristine streams. However, finding strong correlates of compositional uniqueness proved to be a difficult task.

Handling editor: André Padial

**Electronic supplementary material** The online version of this article (<https://doi.org/10.1007/s10750-019-04037-8>) contains supplementary material, which is available to authorized users.

E. Castro · F. Schneck  
 Instituto de Ciências Biológicas, Universidade Federal do Rio Grande - FURG, Rio Grande, RS, Brazil  
 e-mail: decastro.emanuela@gmail.com

T. Siqueira  
 Instituto de Biociências, Universidade Estadual Paulista (UNESP), Rio Claro, SP, Brazil

A. S. Melo · L. M. Bini  
 Departamento de Ecologia, Universidade Federal de Goiás, Goiânia, GO, Brazil

V. L. Landeiro  
 Departamento de Botânica e Ecologia, Universidade Federal de Mato Grosso, Cuiabá, MT, Brazil

**Keywords** Compositional variation · LCBD · Replacement · Nestedness

### Introduction

Understanding the mechanisms underlying biodiversity patterns and community assembly is a major goal in community ecology (Mori et al., 2018), particularly in the current scenario of increasing threats to