



Universidade Federal do Rio Grande do Sul
Instituto de Biociências
Programa de Pós-Graduação em Ecologia



Tese de Doutorado

*Efeitos da rugosidade de substratos, distúrbios físicos e organismos
pastejadores na estruturação de assembleias de algas bênticas e na
biomassa perifítica em um riacho subtropical:
uma abordagem experimental*

Fabiana Schneck

Porto Alegre, fevereiro de 2012

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Resumo

Os estudos apresentados nesta tese tiveram como objetivo compreender aspectos da organização de assembleias de algas bênticas em riachos. Foi avaliado especificamente o efeito da heterogeneidade de habitat na escala de rugosidade de substratos e sua interação com distúrbios hidrológicos e organismos pastejadores (*grazers*). Utilizou-se uma abordagem experimental *in situ* com substratos lisos e rugosos (com fendas) constituindo os tratamentos. Os experimentos foram conduzidos no Rio do Marco, um riacho de quarta ordem na região dos Campos de Cima da Serra, em São José dos Ausentes, Rio Grande do Sul. Após uma introdução geral acerca do assunto são apresentados os capítulos com os estudos. O Capítulo 1 avaliou os efeitos da rugosidade de substratos sobre a riqueza de espécies, densidade, composição e padrões de distribuição de assembleias de algas bênticas. Assembleias em substratos rugosos foram mais ricas que assembleias em substratos lisos, porém esse resultado diferiu entre grupos com diferentes formas de vida, assim como as diferenças na composição de espécies, indicando que somente alguns grupos foram beneficiados pela ocorrência de refúgios em substratos rugosos. Observou-se também que diferenças na composição da assembleia entre substratos lisos e rugosos resultaram de aninhamento e substituição de espécies. No Capítulo 2 foi testada a hipótese de que substratos rugosos abrigam assembleias mais persistentes que substratos lisos. Os resultados encontrados confirmaram esta hipótese e foram atribuídos ao efeito da rugosidade *per se* após a exclusão de alguns fatores de confundimento, como estabilidade física dos substratos e diferenças na riqueza de espécies. Concluiu-se que o mecanismo mais plausível para explicar a maior persistência em substratos rugosos é a maior disponibilidade de refúgios em relação a substratos lisos. O objetivo do Capítulo 3 foi avaliar o efeito da rugosidade de substratos sobre assembleias de algas frente a um distúrbio hidrológico experimental, testando especificamente se a resistência e a resiliência de algas é maior em substratos rugosos que em substratos lisos e se a resposta de

diferentes formas de vida é distinta. Os resultados indicaram que a rugosidade de substratos não apresenta efeitos pronunciados sobre a resistência e a resiliência de algas bênticas. Porém, grupos de algas com distintas formas de vida diferiram quanto à resistência e resiliência, de forma que o padrão observado está relacionado a atributos biológicos que conferem habilidades para suportar distúrbios. Os resultados deste estudo em conjunto com os resultados obtidos no Capítulo 2 e resultados obtidos por outros autores permitiram que fosse proposto que a importância da heterogeneidade de habitats e de refúgios é mediada pela intensidade de distúrbios. No Capítulo 4 foram avaliados os efeitos independentes e as interações de rugosidade de substratos, peixes pastejadores e tipos de mesohabitats (corredeiras e remansos) sobre a biomassa algal, matéria orgânica e peso seco total. O efeito de peixes pastejadores não foi mediado pelos mesohabitats, sendo que a exclusão de peixes causou aumento nas três variáveis resposta, independente do tipo de mesohabitat. Por outro lado, houve uma interação entre mesohabitats e substratos para determinar o acúmulo de peso seco total, sendo que substratos rugosos acumularam maior quantidade de material em remansos que em corredeiras, enquanto substratos lisos acumularam quantidades similares entre os dois tipos de mesohabitats. Já a biomassa algal e matéria orgânica apresentaram maior acúmulo nos substratos rugosos em relação aos lisos, independente do mesohabitat. Os resultados encontrados indicam que a perda de organismos pastejadores pode afetar o acúmulo de biomassa algal e material orgânico, importantes recursos alimentares em riachos. Finalmente, na última seção são apresentadas as considerações finais.

Palavras-chave: algas bênticas, aninhamento, distúrbios, diversidade, exclusão elétrica, heterogeneidade de habitats, organismos pastejadores, perifíton, persistência, rugosidade, refúgios, riachos, variabilidade temporal

Abstract

The studies presented in this thesis aimed at understanding some aspects of the organization of benthic algal assemblages in streams. Specifically, the studies evaluated the effect of habitat heterogeneity on the scale of substrate roughness and its interaction with hydrological disturbances and grazing organisms. An experimental *in situ* approach was used, in which the treatments were constituted by smooth and rough (with crevices) substrates. The experiments were conducted in the Marco River, a fourth-order stream at the region of Campos de Cima da Serra, São José dos Ausentes, Rio Grande do Sul, Brazil. After a general introduction on the subjects of this thesis, the chapters with the studies are presented. The Chapter 1 describes a study to evaluate the effects of substrate roughness on species richness, density, composition and distribution patterns of benthic algal assemblages. Algal assemblages on rough substrates were richer than on smooth substrates, but this result differed among algal life forms. Similarly, results on species composition also differed among life forms, indicating that only some groups were benefited by the occurrence of refuges on rough substrates. It was also observed that differences in the composition of assemblages between rough and smooth substrates resulted from nestedness and turnover. The Chapter 2 describes a study in which was tested the hypothesis that rough substrates harbor more persistent assemblages than smooth substrates. The results confirmed this hypothesis and were attributed to the effect of roughness *per se* after the exclusion of some confounding factors, such as the physical stability of substrates and differences in species richness. It was concluded that the most plausible mechanism that could explain the higher persistence in rough substrates is the greater availability of refuges in relation to smooth substrates. The main aim of the study described in Chapter 3 was to evaluate the effect of substrate roughness on algal assemblages in the face of an experimental hydrological disturbance. Specifically, it was tested if the resistance and resilience of algae is higher on rough than on smooth substrates and if algal life

forms differ in their response to disturbance. The results indicated that substrate roughness had no pronounced effects on benthic algal resistance and resilience. However, algal life forms differ in their resistance and resilience, and the observed pattern was mostly related to biological traits that confer abilities to support disturbances. The results of this study, in addition to the results obtained in Chapters 1 and 2, allowed us to suggest that the importance of habitat heterogeneity and refuges is mediated by the intensity of disturbances. The Chapter 4 describes a study that evaluated the independent and interacting effects of substrate roughness, grazing fish and mesohabitats (pools and riffles) on algal biomass, organic matter and total dry mass accrual. The effect of grazers was not mediated by mesohabitats, and their exclusion caused an increase in the three response variables, regardless of mesohabitat. On the other hand, mesohabitats and substrates interacted to determine the accrual of total dry mass, since rough substrates accumulated greater amounts of dry mass in pools than in riffles, while smooth substrates accumulated similar amounts in both mesohabitats. Algal biomass and organic matter showed greater accrual on rough than on smooth substrates, independently of mesohabitat. These results indicate that the loss of grazers may affect the accrual of algal biomass and organic matter, which are important food resources in streams. Finally, in the last section are presented the concluding remarks.

Keywords: benthic algae, disturbance, diversity, electrical exclusion, grazers, habitat heterogeneity, nestedness, periphyton, persistence, refuges, roughness, streams, temporal variability

Sumário

Lista de Figuras	x
Lista de Tabelas	xiii
Introdução geral	1
Capítulo 1. Substrate roughness affects stream benthic algal diversity, assemblage composition, and nestedness	11
Abstract	11
Introduction	13
Methods	14
Results	18
Discussion	20
Acknowledgements	22
Literature Cited	23
Capítulo 2. High assemblage persistence in heterogeneous habitats: an experimental test with stream benthic algae	32
Abstract	32
Introduction	33
Methods	35
Results	39
Discussion	40
Acknowledgements	42
References	42
Capítulo 3. Hydrological disturbance intensity overrides substrate roughness effects on the resistance and resilience of stream benthic algae	48
Summary	48

Introduction.....	50
Methods.....	52
Results.....	56
Discussion.....	57
Acknowledgments.....	61
References.....	61
Capítulo 4. Mesohabitat type, grazers and substrate roughness interact to determine algal biomass and sediment accrual in a high-altitude subtropical stream	72
Abstract.....	72
Introduction.....	74
Materials and methods	76
Results.....	80
Discussion.....	81
Acknowledgements.....	84
References.....	84
Considerações finais.....	91
Referências.....	96
Apêndice 1.....	104
Apêndice 2.....	105
Apêndice 3.....	109

Lista de Figuras

Capítulo 1

Figura 1. Box-and-whisker plot of difference in species richness of benthic algae between rough and smooth substrates ($S_{\text{rough}} - S_{\text{smooth}}$). The experiment was stratified within 66 blocks (i.e., pairs of treatments) tested using a paired *t*-test. Therefore, differences between treatments are presented rather than mean values. The heavy line shows the median, box ends show quartiles, and whiskers show the minimum and maximum. Total = total assemblage, Adn/pro = Adnate/prostrate life form, Ere/sta = Erect/stalked, Fil = Filamentous, Met = Metaphyton..... 28

Figura 2. Box-and-whisker plot of difference in $\log(\text{density} + 1)$ of benthic algae between rough and smooth substrates ($N_{\text{rough}} - N_{\text{smooth}}$). The experiment was stratified within 66 blocks (i.e., pairs of treatments) tested using a paired *t*-test. Therefore, differences between treatments are presented rather than mean values. The heavy line shows the median, box ends show quartiles, and whiskers show the minimum and maximum. Total = total assemblage, Adn/pro = Adnate/prostrate life form, Ere/sta = Erect/stalked, Fil = Filamentous, Met = Metaphyton..... 29

Figura 3. Principal Coordinates Analysis (PCoA) plots of benthic algal assemblages on smooth and rough substrates in stream reaches a–k. The analysis was run using the Bray–Curtis index on quantitative data ($\log[\text{density} + 1]$). Only 1 PCoA was run, but the axis scores are shown in 11 different plots for clarity (1 plot for each stream reach). Numbers (1–6) indicate the 6 sampling occasions. Lines connect rough and smooth substrates collected on the same sampling occasion. 30

Figura 4. Principal Coordinates Analysis (PCoA) plots of benthic algal assemblages on smooth and rough substrates in stream reaches a–k. Data were analyzed with the subsampling procedure and the Sørensen index. Symbols indicate averages of 100 ordinations of subsamples containing the same number of species as the experimental unit with the fewest species in the block. Only 1 PCoA was run, but the axis scores are shown in 11 different plots for clarity (1 plot for each stream reach). Numbers (1–6) indicate the 6 sampling occasions. Lines connect rough and smooth substrates collected on the same sampling occasion..... 31

Capítulo 2

Figura 1. Box-and-whisker plot of difference in the temporal variability of stream benthic algal assemblages on smooth and rough substrates (distances smooth – distances rough) for a) mean distances to the group centroid in the multi-dimensional space obtained in a PCoA and b) total Euclidean distance of the assemblage along the six sampling occasions in the space obtained by a multivariate correspondence analysis (EDCA). The experiment was carried out in 11 blocks, and the hypothesis of differences in temporal variability (or persistence) between assemblages was tested using paired t-tests. Therefore, differences between treatments are presented rather than mean values. The dashed horizontal lines indicate the case of no difference between smooth and rough substrates. The minimum and maximum (whiskers), the lower and upper quartiles (box ends), and the median (heavy line) for each type of data are shown. P/a = presence-absence data; Sub p/a = subsampled presence-absence; Log = log-transformed; Sub log = subsampled log-transformed; Max = standardized by the species maximum; Sub max = subsampled standardized by the species maximum..... 46

Capítulo 3

Figura 1. Relative densities (mean \pm standard error) of adnate/prostrate (a), erect/stalked (b), filamentous (c), motile (d) and metaphytic (e) algal life forms on smooth and rough substrates at four sampling times during an experimental hydrological disturbance (B = before, A = immediately after, 7A = 7 days after, 15A = 15 days after the disturbance). 69

Figura 2. Boxplots of species richness (a) and log (density + 1) (b) of stream benthic algae on smooth and rough substrates for four sampling times before and after an experimental hydrological disturbance. The heavy line represents the median, box ends are the first and third quartiles, and whiskers are the minimum and maximum values. 70

Figura 3. Boxplots of the resistance and resilience of each algal life form on smooth and rough substrates. The indices were calculated for qualitative (presence-absence; a, b, c) and quantitative data (log [density +1]; d, e, f). The heavy line represents the median, box ends are the first and third quartiles, and whiskers are the minimum and maximum values. And/pro = adnate/prostrate, Ere/sta = erect/stalked, Fila = filamentous, Meta = metaphytic life form. ... 71

Capítulo 4

Figura 1. Mean (\pm standard error) of total dry mass (a), ash free dry mass (b), and chlorophyll *a* (c) in pools and riffles (separated by a dashed line), in exclusion (hatched bars) and control (non-hatched bars) treatments for large grazers, and on smooth (S, white) and rough (R, grey) substrates. 90

Lista de Tabelas

Capítulo 3

Tabela 1. Summary of the results of repeated-measures ANOVA for the effects of substrate roughness on species richness (a) and log (density + 1) (b) of stream benthic algae after an experimental hydrological disturbance. *P* values were corrected using Huynh-Feldt method for repeated-measures ANOVA. Significant *post hoc* pairwise comparisons are shown. B = before, A = immediately after, 7A = 7 days after, 15A = 15 days after the disturbance. *P* values = < 0.05 are in bold. 67

Tabela 2. Summary of the results of ANOVAS comparing resistance and resilience among algal life forms and substrate types (smooth x rough) after an experimental hydrological disturbance. *Post hoc* pairwise comparisons are shown for results with *P* < 0.05. Ad = adnate/prostrate, E = erect/stalked, F = filamentous, Me = metaphytic, Mo = motile life form. *P* values = < 0.008 (α' for Bonferroni correction) are in bold. 68

Capítulo 4

Tabela 1. Summary of the results of split-split-plot ANOVAs for the effects of mesohabitat (pools and riffles), large grazers (exclusion and control), and substrate roughness (smooth and rough) on total dry mass (a), ash free dry mass (AFDM) (b), and chlorophyll *a* (c). *P* values < 0.05 are in bold. 89

Introdução geral

Assembleias bióticas são espacialmente e temporalmente dinâmicas, sendo que um dos principais objetivos dos estudos em ecologia de comunidades é reconhecer os padrões espaciais e temporais na organização de assembleias (e.g., Bengtsson et al. 1997; Cottenie 2005; Soininen et al. 2005; Heino et al. 2010). Porém, compreender os mecanismos geradores desses padrões não é tarefa simples, uma vez que é preciso conhecer a importância relativa dos diversos filtros ecológicos que atuam sobre as comunidades, como restrições ambientais e bióticas em escala local e limitações de dispersão em escala regional (Belyea & Lancaster 1999; Cottenie 2005; Vanormelingen et al. 2008).

Em ambientes aquáticos, além das interações bióticas, as condições físicas do habitat, especialmente velocidade da corrente, tipo de substratos, disponibilidade de luz, aporte de matéria orgânica e características químicas da água, são fatores chave na determinação da composição, abundância e distribuição de organismos em escala local (Allan & Castillo 2007). A combinação específica destes fatores determina a comunidade que está associada a cada habitat (Costa & Melo 2008). Porém, a simplificação de habitats é uma ameaça à diversidade destes ecossistemas (Cardinale et al. 2002). Perturbações antrópicas vêm simplificando a estrutura dos ecossistemas aquáticos através da homogeneização de habitats, de alterações na frequência e intensidade de distúrbios naturais e da homogeneização da biota (Rahel 2000; Cardinale et al. 2002).

Os estudos que desenvolvi durante meu doutorado tiveram como objetivo compreender aspectos da organização de assembleias de algas bênticas, um importante componente da diversidade de ecossistemas aquáticos (como exemplificado na próxima seção desta introdução). Especificamente, avaliei o efeito da heterogeneidade de habitats na escala

de rugosidade de substratos, incluindo sua interação com distúrbios hidrológicos e com organismos pastejadores (*grazers*). Para isso, utilizei experimentos *in situ* que permitiram a obtenção de resultados que pudessem ser atribuídos aos efeitos da heterogeneidade de habitats *per se* sobre a organização destas assembleias.

Algas bêmicas em riachos

As algas bêmicas são as principais produtoras primárias em muitos rios e riachos ao redor do mundo, especialmente em riachos não sombreados (Biggs 1996), como é o caso dos riachos na região dos Campos de Cima da Serra onde o presente estudo foi desenvolvido (Apêndice 1). As algas bêmicas compõem a base das cadeias alimentares de ambientes aquáticos, sendo que alterações na composição, estrutura ou biomassa podem acarretar em mudanças na dinâmica das cadeias alimentares destes ecossistemas (Lowe & Pan 1996). Além disso, as algas bêmicas são importantes moduladoras químicas, pois transformam uma série de compostos inorgânicos em formas orgânicas. Esse grupo de organismos pode ainda exercer papel importante na estabilização de substratos e macroalgas bêmicas podem servir de habitat para diversos organismos, como fases larvares e juvenis de invertebrados e peixes (Stevenson 1996). Não obstante, as algas bêmicas constituem uma parcela muito representativa da diversidade de ambientes lóticos. Estimativas conservadoras sugerem a existência de aproximadamente 26.000 espécies de algas (e.g., Stevenson 1996), enquanto Round et al. (1990) estimam que existam até 100.000 espécies somente de diatomáceas (Bacillariophyceae), o grupo de algas geralmente dominante em riachos.

As algas bêmicas desenvolveram uma variedade de formas de vida, revelando grande diversidade estrutural. Uma importante característica de muitas espécies é a capacidade de adesão ao substrato através de estruturas especializadas que secretam mucilagem, como a rafe nas diatomáceas, ou células basais diferenciadas para fixação em algumas algas filamentosas.

As formas de vida incluem formas prostradas e adnatas em que toda superfície da célula está em contato e aderida ao substrato; formas pedunculares em que a célula está colocada sobre longos pedúnculos mucilaginosos e perpendicularmente ao substrato; formas eretas, aderidas basalmente ao substrato por uma ‘almofada’ de mucilagem; formas filamentosas, que podem estar aderidas ao substrato ou emaranhadas em substratos. Algumas espécies podem ainda ser móveis e se locomover através do biofilme. Já outras espécies, que não são tipicamente bênticas e vivem na interface do biofilme com a coluna d’água, podem ocasionalmente ficar presas na matriz mucilaginosa do biofilme perifítico e são definidas como formas metafíticas (Lowe et al. 1986; Stevenson 1996; Biggs et al. 1998).

Heterogeneidade de habitats: definições e mecanismos

O termo ‘heterogeneidade de habitat’ pode ser definido de diversas formas, dependendo da escala espacial do estudo e do grupo taxonômico (Tews et al. 2004). Por exemplo, em ambientes aquáticos, heterogeneidade de habitat pode significar a rugosidade na superfície de substratos (Bergey 2005), a complexidade estrutural gerada por macrófitas aquáticas (e.g., Thomaz & Cunha 2010), a heterogeneidade vertical nas condições ambientais em lagos e reservatórios (Becker et al. 2009), ou ainda o gradiente ambiental ao longo de uma bacia hidrográfica (Vannote et al. 1980). Tews et al. (2004) citam mais de 10 termos que são utilizados como sinônimo de heterogeneidade de habitat, como por exemplo, ‘complexidade de habitat’, ‘heterogeneidade espacial’, ‘heterogeneidade estrutural’ e ‘diversidade de habitats’. Li & Reynolds (1995) definem, para descritores categóricos, heterogeneidade espacial (ou de habitat) como a complexidade na composição e configuração de elementos físicos (e.g., macrófitas aquáticas, fendas), ou seja, o número e a proporção de diferentes tipos de elementos, além do arranjo espacial, da forma e da conectividade destes elementos. Em uma definição mais estrita, Taniguchi et al. (2003) apresentam significados distintos para

heterogeneidade de habitat e complexidade de habitat. O primeiro termo levaria em conta somente “o número de diferentes tipos de estruturas”. Já complexidade de habitat consideraria tanto o número como as características morfológicas das estruturas (Taniguchi et al. 2003). Em frente às inúmeras definições existentes, nesta tese não faço distinção entre os diferentes termos, utilizando ‘heterogeneidade de habitat’ tanto em um contexto geral como para me referir à heterogeneidade espacial gerada por fendas na superfície de substratos (i.e., rugosidade).

O papel da heterogeneidade de habitats sobre os padrões e processos ecológicos tem atraído a atenção de ecólogos há muitas décadas, sendo reconhecidamente um componente-chave dos sistemas naturais. Um dos estudos pioneiros neste tema foi desenvolvido por MacArthur & MacArthur (1961), no qual os autores observaram que uma maior diversidade na distribuição vertical de plantas (*‘foliage height diversity’*) em florestas tinha um efeito positivo sobre a diversidade de aves. Durante os anos seguintes, diversos estudos em ambientes terrestres corroboraram os resultados de MacArthur & MacArthur (e.g. répteis: Pianka 1967; insetos: Murdoch et al. 1972; aves: Roth 1976), tornando a relação positiva entre heterogeneidade de habitats e diversidade um padrão amplamente reconhecido na ecologia.

Em ambientes aquáticos, vários autores têm demonstrado que a heterogeneidade de habitats pode afetar a estruturação de assembleias e o funcionamento de ecossistemas de diversas formas (ver Kovalenko et al. 2012 para uma revisão). Por exemplo, a heterogeneidade de habitats pode influenciar a densidade de organismos e a diversidade de espécies (Taniguchi & Tokeshi 2004), a composição de assembleias (Bergey 1999), modular interações bióticas (Padial et al. 2009), ou ainda afetar a retenção de matéria orgânica (Taniguchi & Tokeshi 2004) e o metabolismo de riachos (Cardinale et al. 2002).

Alguns mecanismos intrínsecos à heterogeneidade de habitat são citados na literatura como os responsáveis pelas relações apresentadas acima. Entre eles, o mecanismo mais simples é a maior área em habitats heterogêneos. Um aumento na área pode levar ao aumento na abundância de organismos e, conseqüentemente, ao aumento na riqueza de espécies. Neste caso, a riqueza de espécies aumentaria de forma ‘passiva’ em função da coleta de um maior número de organismos, ou seja, o padrão observado seria essencialmente de relação espécie-área e não da heterogeneidade *per se* (Johnson et al. 2003; Taniguchi et al. 2003). Para evitar esse viés, é necessário separar os efeitos simultâneos da abundância e da área sobre a riqueza de espécies. Uma maneira de excluir os efeitos da abundância e da área é através da padronização do número de indivíduos amostrados ou contados (Gotelli & Colwell 2001), método que utilizei nesta tese (ver Thomaz & Cunha 2010 para outras possibilidades). Um segundo mecanismo é a disponibilidade de uma maior quantidade e variedade de recursos e de microhabitats em habitats heterogêneos, possibilitando a partição dos recursos e a coexistência de um maior número de espécies (e.g., MacArthur & MacArthur 1961; Downes et al. 2000; Brown 2003). Por fim, sabe-se também que habitats heterogêneos possuem um maior número de refúgios contra distúrbios ou predação (Lancaster & Hildrew 1993). A disponibilidade de refúgios, por sua vez, pode afetar a variabilidade temporal de assembleias, além da resistência e resiliência de diferentes grupos de organismos frente a distúrbios.

Heterogeneidade de habitats e diversidade

Provavelmente a relação melhor documentada em estudos envolvendo heterogeneidade de habitats é o aumento da densidade e diversidade (seja riqueza de espécies ou outra medida de diversidade, como o índice de Shannon-Wiener) em função da heterogeneidade, nas mais diversas escalas. Por exemplo, em escala regional abrangendo nove reservatórios, a diversidade de peixes esteve positivamente relacionada com a riqueza de macrófitas, i.e., com

a heterogeneidade estrutural gerada por diferentes espécies de macrófitas (Agostinho et al. 2003). Na escala de ‘manchas’ dentro de riachos, áreas com maior heterogeneidade na composição de substratos apresentaram maior riqueza de espécies de insetos aquáticos (Brown 2003) e ‘manchas’ com maior heterogeneidade estrutural promovida por detritos lenhosos apresentaram faunas mais ricas de peixes e macroinvertebrados (Schneider & Winemiller 2008). Em escalas mais finas, maior riqueza de invertebrados bentônicos ocorre em macrófitas aquáticas com morfologia foliar mais complexa (Taniguchi et al. 2003; Thomaz et al. 2008). Também a rugosidade na superfície de substratos pode afetar positivamente a densidade e riqueza de invertebrados bentônicos (Downes et al. 2000; Taniguchi & Tokeshi 2004), rotíferos (Vieira et al. 2007) e algas bênticas (Bergey 1999)

Apesar da extensa literatura relacionando diversidade e heterogeneidade de habitats, poucos estudos têm explorado os possíveis mecanismos associados com essas relações. Se habitats heterogêneos e homogêneos diferem quanto à composição de espécies (e.g., Bergey 1999; Vieira et al. 2007), esta diferença pode resultar da substituição e/ou da perda de espécies. No primeiro caso, seria observada a substituição de espécies (*turnover*), enquanto que no segundo caso haveria uma distribuição aninhada em que biotas pobres seriam um subconjunto de biotas ricas (Wright et al. 1998; Baselga 2010). Além disso, em estudos sobre heterogeneidade de habitats realizados com peixes e invertebrados, uma hipótese sempre levantada para a maior densidade e riqueza de espécies, além de diferenças na composição, é a maior disponibilidade de alimento (principalmente algas) em habitats heterogêneos. De fato, o aumento na biomassa de algas bênticas em ambientes heterogêneos já foi demonstrado em diversos estudos (e.g., Clifford et al. 1992; Bergey 2005; Murdock & Dodds 2007). Porém, ainda é necessário compreender como esta assembleia responde à heterogeneidade de habitats quanto a uma série de outros atributos. Por exemplo, alterações na composição e fisionomia de assembleias de algas bênticas em decorrência da simplificação de habitats podem afetar

interações entre algas bênticas e macroinvertebrados (e.g., Tall et al. 2006). Assim, no **Capítulo 1** desta tese avaliei os efeitos da rugosidade de substratos sobre a riqueza, densidade, composição e padrões de distribuição de assembleias de algas bênticas em ambientes lóticos, considerando não somente a assembleia total, mas também as respostas separadas de grupos de algas com distintas formas de vida.

Heterogeneidade de habitats e variabilidade temporal de assembleias

Outro aspecto interessante é a hipótese de habitats heterogêneos abrigarem assembleias mais persistentes, ou seja, a heterogeneidade de habitats poderia não apenas aumentar a coexistência de espécies, mas também diminuir a variabilidade temporal de assembleias. Essa hipótese foi levantada ainda na década de 1980 por Pickett & White (1985), porém só veio a ser testada recentemente por Brown (2003) e depois por Mykrä et al. (2011). Brown (2003) observou uma diminuição média de 42% na variabilidade temporal da assembleia de invertebrados bentônicos ao longo do gradiente natural de heterogeneidade espacial gerada por substratos de diferentes tamanhos e tipos no leito de um riacho norte-americano. De forma semelhante, Mykrä et al. (2011) amostraram invertebrados em 32 riachos de uma bacia hidrográfica na Finlândia e observaram que as assembleias eram mais persistentes em riachos com maior cobertura de briófitas (i.e., um indicador de maior heterogeneidade estrutural). Porém, os dois estudos foram observacionais, o que não permitiu excluir um possível fator de confundimento: a estabilidade física do habitat gerada por substratos de tamanhos maiores no primeiro estudo e pela maior densidade de briófitas no segundo (e.g., Gurtz & Wallace 1984; Stream Bryophyte Group 1999). Além disso, esta hipótese ainda precisa ser testada com outros grupos taxonômicos e em outros ecossistemas.

No **Capítulo 2** busquei elucidar a importância da heterogeneidade de habitats na persistência de assembleias utilizando como modelo algas bênticas. Através de um

experimento manipulativo em campo pode excluir alguns fatores de confundimento que não haviam sido levados em conta em estudos anteriores e reavaliei a hipótese de que habitats heterogêneos (i.e., substratos rugosos em relação a substratos lisos) abrigam assembleias mais persistentes.

Heterogeneidade de habitats e distúrbios físicos

Distúrbios físicos naturais certamente estão entre os fatores chave na estruturação de assembleias em ambientes aquáticos. Os distúrbios hidrológicos, como enchentes em riachos, exercem fortes efeitos sobre a biota e sobre o ambiente, pois removem organismos, provocam a suspensão de sedimentos, afetam o fluxo d'água, a concentração de nutrientes e alteram o tamanho e a distribuição de substratos (Reice et al. 1990; Lake 2000; Death 2010). A remoção de organismos pode ocorrer através de três mecanismos: (i) remoção direta por raspagem do substrato pela ação da elevada velocidade da água; (ii) abrasão causada por sedimentos suspensos; (iii) deslocamento dos substratos (Melo et al. 2003; Francoeur & Biggs 2006).

Além de ser consenso que distúrbios hidrológicos e heterogeneidade de habitats regulam separadamente a organização de assembleias, a interação entre estes dois fatores também pode desempenhar papel fundamental (Poff & Ward 1990; Townsend & Hildrew 1994). Esta interação é mediada pela disponibilidade de refúgios em habitats heterogêneos, diminuindo os efeitos de distúrbios hidrológicos e aumentando a resistência e resiliência das populações protegidas nos refúgios (Poff & Ward 1990; Lancaster & Hildrew 1993; Townsend & Hildrew 1994; Lake 2000).

No artigo apresentado no **Capítulo 3** testei o efeito da heterogeneidade de habitats gerada pela rugosidade de substratos sobre assembleias de algas frente a um distúrbio hidrológico experimental. Especificamente, testei se a resistência e a resiliência de algas bênticas era maior em substratos rugosos. Avaliei também a resposta de grupos de algas com

diferentes formas de vida, uma vez que as distintas características biológicas dos grupos proporcionam maior ou menor capacidade de resistência e resiliência frente a distúrbios.

Heterogeneidade de habitats e organismos pastejadores

Evidências de que a modificação de habitats por organismos pode ser um processo central na organização de comunidades bióticas são consistentes em muitos ecossistemas (Steinman 1996). Algumas espécies são particularmente importantes, sendo que sua ausência (ou presença) pode modificar significativamente a organização de comunidades e o funcionamento de ecossistemas (e.g., Power 1990a, 1990b; Pringle & Blake 1994; Flecker 1996; Moulton et al. 2004; Bertrand & Gido 2007). Organismos pastejadores (*grazers*) em riachos podem afetar a composição, o acúmulo de biomassa e a produtividade de assembleias de algas bênticas tanto diretamente, através de consumo, como indiretamente, atuando como organismos engenheiros (Jones et al. 1994) ao modificar fisicamente a estrutura do habitat através da remoção de sedimentos.

Porém, as forças de interação entre organismos pastejadores e o perifíton podem ser mediadas pelas condições físicas do ambiente, como velocidade da corrente e profundidade, podendo impor restrições energéticas e ecológicas a ambos os grupos (Opsahl et al. 2003). Apesar da reconhecida importância das características do ambiente sobre o desempenho das espécies (Poff et al. 2003), poucos estudos sobre os efeitos de organismos pastejadores tem incluído mesohabitats (por exemplo, corredeiras e remansos) em estudos experimentais *in situ* em riachos (Feminella & Hawkins 1995, mas veja Flecker 1997).

Ainda, assim como no caso de distúrbios físicos, a interação entre organismos pastejadores e algas bênticas pode ser afetada pela disponibilidade de refúgios em substratos rugosos. Algas estabelecidas nas fendas dos substratos estariam protegidas, pelo menos em parte, da remoção por organismos pastejadores (Bergey & Weaver 2004).

No **Capítulo 4** realizei um experimento fatorial *split-split-plot* no qual os peixes pastejadores foram excluídos utilizando tratamentos de exclusão elétrica. Avaliei os efeitos independentes e as interações entre mesohabitats (corredeiras e remansos), peixes pastejadores (controle e exclusão) e rugosidade de substratos (lisos e rugosos) sobre a biomassa algal (clorofila *a*), matéria orgânica (peso seco livre de cinzas) e peso seco total (material orgânico e inorgânico) do perifíton.

Finalmente, a última seção desta tese, **Considerações finais**, integra os resultados obtidos pelos diferentes capítulos. Os Capítulos 1 a 4 estão formatados de acordo com as normas do periódico no qual foram publicados ou submetidos (ver notas de rodapé nos respectivos capítulos). Sendo assim, cada capítulo conta com uma formatação distinta.

Capítulo 1

Substrate roughness affects stream benthic algal diversity, assemblage composition, and nestedness¹

Abstract. Heterogeneity generated by irregularities on the surface of streambed substrates is an important determinant of local species diversity of algae. However, few investigators have examined the effects of substrate roughness on the composition of algal life forms² and on patterns of species distribution. We examined the influence of substrate roughness on stream benthic algal assemblages through a field experiment with 2 treatments (smooth and rough artificial substrates for algal colonization). We assessed whether species richness, density, and assemblage composition of benthic algae (all taxa and those in 5 life-form groups) differed between treatments and whether differences in species composition between substrates were the result of species turnover or nestedness. We also used a data subsampling procedure to investigate the effect of differences in species richness between treatments. Total species richness was higher on rough than on smooth substrates, but density did not differ between treatments. Species richness, density, and composition of the adnate/prostrate life form did not differ between treatments. The erect/stalked life form had higher species richness on rough substrates, but did not differ in density between treatments. All other life forms (filamentous, motile, and metaphyton) had higher species richness and density on rough substrates and differed in species composition between substrates. The results of the

¹ Schneck, F., Schwarzbald, A. & Melo, A. S. 2011. Substrate roughness affects stream benthic algal diversity, assemblage composition, and nestedness. *Journal of the North American Benthological Society*, 30: 1049-1056. DOI: 10.1899/11-044.1

² No artigo original foi utilizado o termo “*growth forms*”. Porém, para unificar a terminologia ao longo de toda a tese, optou-se por utilizar o termo “*life forms*” também no presente capítulo.

subsampling analysis indicated that assemblage composition was affected by differences in species richness and by changes in species composition (i.e., turnover). Species distribution had a nested pattern, in which the assemblages on smooth substrates were a subgroup of the species occurring on rough substrates. We suggest that the differences in assemblage composition between smooth and rough substrates resulted from variability in species' capabilities to colonize substrates with or without crevices. This variability resulted in both nestedness and turnover.

Key words: algal life form, nested habitats, species turnover, distribution, periphyton.

Introduction

Habitat heterogeneity is a major driver of diversity in lotic ecosystems and acts at different spatial scales, such as reaches, pools and riffles, and streambed substrates (Murdock and Dodds 2007). The heterogeneity generated by irregularities on the surface of streambed substrates (e.g., crevices, projections) is an important determinant of local species diversity of algae (Bergey 1999, 2005), rotifers (Vieira et al. 2007), and macroinvertebrates (Downes et al. 2000, Taniguchi and Tokeshi 2004, Hutchens et al. 2004). In 7 of 11 studies of the effects of surface texture on stream invertebrate richness, species richness was significantly higher on more complex surfaces (Palmer et al. 2010). These differences in species diversity can be attributed to several mechanisms. For example, fine-scale substrate texture can affect retention of food resources and sediment and can provide refuges from high-discharge events, abrasion, desiccation, and predators (Dudley and D'Antonio 1991, Taniguchi and Takeshi 2004, Bergey 2005).

Rough substrates influence algal colonization (Bergey et al. 2010) and protect algae from grazers and disturbances better than smooth substrates do (Dudley and D'Antonio 1991). Algal biomass increases with substrate roughness and crevice availability (Murdock and Dodds 2007, Bergey 2005), and crevice size influences algal size distribution and species composition (Bergey 1999). However, the effects of substrate roughness on algal life-form composition or patterns of species distribution are not well understood.

The positive effect of substrate roughness on species diversity (Bergey 1999, Vieira et al. 2007) led us to hypothesize that rough substrates might allow coexistence of species able to colonize smooth substrates with species that need crevices for their establishment. Thus, a nested pattern of species distribution might be expected to occur between substrates with different degrees of roughness if species that are more susceptible to grazers and disturbance are selectively removed from smooth substrates. However, nestedness is not the only pattern

that could emerge. Algal assemblages on smooth and rough substrates also could vary because of species turnover between substrate types. Nestedness and patterns caused by turnover are not mutually exclusive (Lennon et al. 2001, Baselga 2010).

We conducted a field experiment to study the influence of substrate roughness on species richness, density, life-form composition, and species distribution of stream benthic algae. We tested whether species richness and density were higher on rough than on smooth substrates and whether assemblage composition (all taxa and 5 life-form groups) differed between smooth and rough substrates. We also assessed whether differences in species composition between substrates were the result of species turnover or nestedness.

Methods

Study area

We conducted our experiment in Marco Stream (lat 28°36'S, long 49°51'W) in the state of Rio Grande do Sul, southern Brazil. The study site is a 4th-order stream at ~1100 m asl. The stream drains a catchment with low human impact and has clear, oligotrophic (Buckup et al. 2007), well oxygenated water (10 mg/L), a pH of ~6.6, low conductivity (22 µS/cm), and a mean current velocity of 26 cm/s. The stream bed is composed of basaltic stones, boulders, and bedrock. Stream width varies from 2 to 5 m, and depth varies from 0.2 to 0.4 m in the study reaches.

Experimental design

We conducted the experiment from May through August 2009 (austral autumn and winter). We designed a 1-factor field experiment with 2 levels: smooth substrate and rough substrate. During the experiment, we took samples on 6 occasions (every 15 d) in 11 stream reaches (66 blocks). We sampled 2 artificial substrates of each type per block to yield 264 sampling units

(2 sampling units \times 2 substrate types \times 66 blocks = 264). Each pair of sampling units collected under the same conditions was pooled for analysis and constituted 1 experimental unit ($n = 132$).

We used acrylic substrates (5 \times 5 cm) for algal colonization. The surfaces of the acrylic substrates were either smooth or had longitudinal crevices (substrate treatments). All rough substrates had the same number of crevices (9), and all crevices had the same width (1 mm) and depth (1 mm). We glued substrates on 50 \times 50 \times 8-cm flat paving stones. We placed 1 stone in each of the 11 stream reaches and aligned the substrate crevices in a cross-stream direction. Each stone supported all substrates needed for the entire experiment to minimize variation of physical variables, such as current velocity and water depth, between treatments. We placed substrates on the stream bed for benthic algal colonization 45 d before the start of the experiment. Growth was evident on all substrates on day 45. No spates occurred during the period of the experiment.

Sampling and laboratory analyses

We brushed substrates with a toothbrush to remove periphyton, and the samples were preserved with 4% formaldehyde. We determined benthic algal composition and cell density by counting 500 cells from each experimental unit with an inverted microscope at 400 \times magnification. We cleaned additional subsamples with acid, mounted them on glass slides using Naphrax, and examined them at 1000 \times through a light microscope (Lowe and LaLiberte 2007) to identify diatom species.

We classified algae into 5 life-form groups: 1) adnate or prostrate (species of the genera *Achnanthes*, *Achnantheidium*, *Cocconeis*, *Epithemia*, *Lemnicola*, and *Psammothidium*), 2) erect or with mucilage stalks (species of *Cymbella*, *Encyonema*, *Eunotia*, *Fragilaria*, *Gomphonema*, *Meridion*, *Synedra*, and *Ulnaria*), 3) motile algae (species of *Frustulia*,

Hantzschia, *Luticola*, *Navicula*, *Neidium*, *Nitzschia*, *Pinnularia*, *Stauroneis*, *Surirella*, and *Tryblionella*), 4) filamentous (species of *Aphanochaete*, *Bulbochaete*, *Heteroleibleinia*, *Oedogonium*, *Spirulina*, *Stigeoclonium*, *Tolypothrix*, an unidentified filamentous green alga, an unidentified filamentous desmid, and an unidentified filamentous cyanobacterium), and 5) metaphyton, i.e., species without a fixation structure (species of *Closterium*, *Cosmarium*, *Desmodesmus*, *Euastrum*, *Merismopedia*, *Pleurotaenium*, *Scenedesmus*, *Staurastrum*, *Staurodesmus*, *Synechocystis*, and unidentified Chlorococcales). The classification was based on Lowe et al. (1986), Round et al. (1990), Biggs et al. (1998), Burliga et al. (2004), Passy (2007), Bixby et al. (2009), and Lange et al. (2011).

Data analyses

We assessed the effect of substrate roughness on species richness and density of the entire assemblage and of each life-form group with paired *t*-tests and adjusted α values by the number of tests performed (Bonferroni correction; $\alpha' = 0.004$). We used ordination diagrams resulting from 2 Principal Coordinates Analyses (PCoA) of qualitative (presence/absence; Sørensen dissimilarity index) and quantitative ($\log[\text{density} + 1]$; Bray–Curtis dissimilarity index) data (Legendre and Legendre 1998) to explore differences in assemblage composition and structure between smooth and rough substrates.

We conducted 2 distance-based multivariate analyses of variance (db-MANOVA; Anderson 2001) to test for differences in assemblage composition (measured with qualitative and quantitative data) between substrate types. We also used db-MANOVAs to test for differences in composition of each algal life-form group between substrate types. We ran 9999 permutations stratified within blocks for each db-MANOVA and corrected α values by the number of tests performed ($\alpha' = 0.004$). In addition, we identified species associated with each type of substrate with Indicator Species Analysis (Dufrêne and Legendre 1997).

Species richness differed between substrate types (see Results). Differences in richness can cause distortions when assessing resemblance (Dargie 1986), so we used a subsampling procedure to investigate the effect of differences in species richness among experimental units on the PCoA ordination and db-MANOVA (qualitative data only). We removed the effects of species richness by reducing the number of species in the richest treatment (per block) to the same number found in the poorest treatment. The procedure consisted of: 1) random subsampling of the richest experimental unit of each of the 66 pairs of treatments (i.e., blocks) to yield the same number of species as the poorest experimental unit of the pair (i.e., equalizing the number of species between treatments), 2) calculation of a dissimilarity matrix for the new subsampled data set (Sørensen index), and 3) ordination of subsamples using PCoA and calculation of a db-MANOVA (9999 permutations stratified within blocks). Steps 1 to 3 were repeated 100 times. We used Procrustean analysis to compare the ordination of the original data set with each of the 100 ordinations of subsampled data sets and plotted the mean values of the Procrustean scores of the subsamples in an ordination diagram to obtain a synthetic PCoA representation. We obtained the mean value of the statistics (F and p) generated by the db-MANOVA conducted for each of the 100 subsampled data sets. A similar subsampling procedure was used by Ferro and Melo (2011) to assess the robustness of PCoA results for localities differing in tiger-moth species richness in the Brazilian Atlantic Forest.

We tested our hypothesis that assemblages on smooth substrates are a subgroup of the species present on rough substrates with the Nestedness metric based on Overlap and Decreasing Fill (NODF; Almeida-Neto et al. 2008). The metric ranges from 0 to 100, with NODF = 100 representing a perfectly nested assemblage. Our goal was to test the occurrence of a nested pattern in species composition between our treatments, so we calculated the NODF only for rows. We generated 66 presence/absence matrices, 1 for each pair of

treatments per block, with the row representing the rough substrate placed above the row representing the smooth substrate. For each of the 66 matrices, we generated 2 null models. The 1st model maintained the number of presences but assigned these anywhere so that neither species nor site total were preserved (rows equiprobable, columns equiprobable). The 2nd model maintained species frequencies, but site frequencies were not preserved (rows equiprobable, columns fixed; Wright et al. 1998, Gotelli 2000, Jonsson 2001). Then, we calculated nestedness for each observed matrix and the equivalent average of 10 random matrices generated by each null model to obtain 2 sets of 66 pairs of nestedness values (the observed value and its respective expected value under each null model). We ran a 1-tailed paired *t*-test for each null model to test whether the observed nestedness was larger than the expected. We conducted all analyses in the R environment (R Development Core Team, Vienna, Austria).

Results

We recorded 92 benthic algal species. Diatoms were the most common and species-rich group, with 56 species and 85% of the total density in both treatments. The dominant species were the diatoms *Achnantheidium minutissimum* (Kützing) Czarnecki, *Cocconeis placentula* Ehrenberg, and *Ulnaria ulna* (Nitzsch) Compère.

Species richness of benthic algae was significantly higher on rough than on smooth substrates ($F_{1,64} = 199.05$, $p < 0.001$; Fig. 1), whereas density did not differ between treatments ($F_{1,64} = 0.72$, $p = 0.439$; Fig. 2). The adnate/prostrate life form did not differ in richness ($F_{1,64} = 15.50$, $p = 0.020$) or in density between treatments ($F_{1,64} = 2.92$, $p = 0.092$), and the erect/stalked life form showed higher species richness on rough than on smooth substrates ($F_{1,64} = 38.48$, $p < 0.001$) but did not differ in density ($F_{1,64} = 2.61$, $p = 0.112$). Motile, filamentous, and metaphytic life forms were more species-rich ($F_{1,64} = 52.83$, $F_{1,64} =$

33.77, $F_{1,64} = 127.06$, respectively; all $p < 0.001$) and abundant ($F_{1,64} = 26.60$, $F_{1,64} = 18.05$, $F_{1,64} = 48.77$, respectively; all $p < 0.001$) on rough than on smooth substrates (Figs 1, 2).

Substrate roughness affected assemblage composition. Assemblages were significantly different between treatments for both the qualitative Sørensen index ($F_{1,130} = 8.68$, $p < 0.001$) and the quantitative Bray–Curtis index ($F_{1,130} = 6.71$, $p < 0.001$; Fig. 3). Furthermore, differences between treatments were not caused by differences in species richness because distinct groups persisted when we used the subsampling procedure (mean $F_{1,130} = 4.63$, mean $p < 0.001$; Fig. 4). The pattern obtained with the PCoA ordination of the subsampled data set (Fig. 4) was similar to that obtained in the ordination of the original qualitative data set, a result indicating that the pattern was not dependent on the number of species. Indicator Species Analysis revealed that only *Gomphonema angustatum* (Kützing) Rabenhorst was significantly ($p < 0.05$) associated with smooth substrates. On the other hand, 14 species were associated with rough substrates: the stalked species *Encyonema minutum* (Hilse ex Rabenhorst) Mann in Round et al., *Eunotia incisa* Smith, *Eunotia praerupta* Ehrenberg, and *Eunotia faba* (Ehrenberg) Grunow; the motile species *Navicula cryptocephala* Kützing, *Surirella tenera* Gregory, and *Surirella* sp. 1; the filamentous species *Stigeoclonium* sp. and *Heteroleibleinia* sp.; and the metaphytic species *Cosmarium angulosum* Brébisson, *Cosmarium* sp. 2, *Cosmarium* sp. 3, *Closterium incurvum* Brébisson, and *Staurastrum punctulatum* (Brébisson) Ralfs.

Composition of the adnate/prostrate life-form assemblage did not differ between substrates for either qualitative ($F_{1,130} = 4.09$, $p = 0.012$) and quantitative ($F_{1,130} = 2.44$, $p = 0.006$) data sets. On the other hand, composition differed between substrate types for assemblages of erect/stalked (qualitative: $F_{1,130} = 6.61$, $p < 0.001$; quantitative: $F_{1,130} = 4.51$, $p < 0.001$), motile (qualitative: $F_{1,130} = 3.80$, $p = 0.003$; quantitative: $F_{1,130} = 3.08$, $p < 0.001$), filamentous (qualitative: $F_{1,130} = 5.40$, $p < 0.001$; quantitative: $F_{1,130} = 5.78$, $p < 0.001$), and

metaphytic (qualitative: $F_{1,130} = 5.28$, $p < 0.001$; quantitative: $F_{1,130} = 4.70$, $p < 0.001$) life forms.

Benthic algal assemblages had a nested pattern of species distribution (mean nestedness = 73.71). The species on smooth substrates were a subset of the species on rough substrates in analyses with the rows equiprobable/columns equiprobable ($F_{1,64} = 41.06$, $p < 0.001$) and with the rows equiprobable/columns fixed ($F_{1,64} = 75.20$, $p < 0.001$) null models.

Discussion

The occurrence of higher species richness on rough substrates and distinct assemblages between treatments can be attributed to differences in species' natural histories and abilities to deal with different environmental conditions. The adnate/prostrate life-form assemblage, which had similar species richness, density and assemblage composition between the 2 substrate types, was composed of species that occupy the low strata in the biofilm matrix and are strongly attached, such as *A. minutissimum*, *C. placentula*, and *Psammothidium subatomoides* (Hustedt) Bukhtiyarova and Round. The adnate/prostrate life form is resource-stressed because it occupies the low strata that may have nutrient and light limitations, but it is resistant to grazing and physical disturbance (Passy 2007), which might enable these algae to occur equally on smooth and rough substrates. Another possible explanation for this result is that adnate and prostrate algae can be dominant on smooth substrates (e.g., Bergey 2005). Our rough substrates had both crevices and smooth surfaces (>50% of the surface of rough substrates), so the similar composition between treatments may have occurred as a result of preferential colonization of smooth substrates.

Metaphytic species (mainly *Cosmarium* spp., *Staurastrum* spp., and *Closterium* spp.) and motile species (e.g., the diatoms *Navicula* spp. and *Surirella* spp.) were strongly associated with the rough substrates. These unattached organisms can occur in the mucilage

secreted by other algae, lie loose on the sediment, or be freely motile species living on the sediment (Round 1984, Gerrath 2003, Lowe 2003). Rough substrates may accrue a greater biofilm matrix and accumulate more sediment than smooth substrates (Clifford et al. 1992, Bergey 1999), thereby facilitating colonization by benthic algae in streams (Wehr and Sheath 2003) and providing additional nutrients to support benthic algal growth (Murdock and Dodds 2007). For example, motile algae can benefit from crevices by using the vertical dimension to exploit available resources and obtain protection from grazers or disturbances (Murdock and Dodds 2007). Authors of some studies have reported higher abundance of filamentous algae on rough substrates than on smooth substrates (Downes et al. 1998, Bergey 1999), and Dudley and D'Antonio (1991) found that zoospores of the filamentous green alga *Cladophora glomerata* (Linnaeus) Kützing selected crevices for settlement.

Species of the erect/stalked life form differed in their response to the treatments. For instance, *Encyonema minutum*, a short-statured species, occurred mainly on rough substrates, whereas *Gomphonema* species were associated with smooth substrates. *Gomphonema* is highly tolerant to flow conditions, and most species produce stalks that attach firmly to the substrate (Kociolek and Spaulding 2003). Therefore, they may be able to establish more efficiently than other algal species on smooth substrates.

Our results indicate that both nestedness and turnover affected assemblage composition. The subsampling procedure showed that differences in assemblage composition between treatments were independent of species richness and that assemblages showed species turnover. The nestedness analysis indicated that species in assemblages on smooth substrates were a subset of the species in richer assemblages on rough substrates. Thus, nestedness and turnover are not mutually exclusive, but can occur simultaneously to influence assemblage composition in a manner similar to the simultaneous occurrence of nestedness, checkerboardness and turnover reported by Heino (2005) for stream midges. Nestedness and

turnover can occur simultaneously because species autecological traits and dispersal processes both influence assemblage distribution patterns (Soininen 2008). Some species might depart from the nested pattern (idiosyncratic taxa) by not responding to the main factors that control nestedness (McAbedroth et al. 2005). For instance, Soininen (2008) found that benthic stream diatom assemblages were highly nested, but were characterized by a large number of idiosyncratic species that did not follow the nesting pattern. We suggest that the differences in composition between assemblages on smooth and rough substrates in our experiment resulted from species' different capabilities to establish on substrates with or without crevices, causing both nestedness and species turnover.

Our study provides evidence of the role of substrate roughness in structuring benthic algal assemblages. Substrate roughness strongly affected species richness, assemblage composition, and nestedness. Thus, substrate roughness should be considered in studies of benthic algal assemblages. Further investigations will be necessary to disentangle the possible mechanisms (e.g., occurrence of refuges, sediment deposition patterns) related to fine-scale substrate texture that affect algal assemblage composition.

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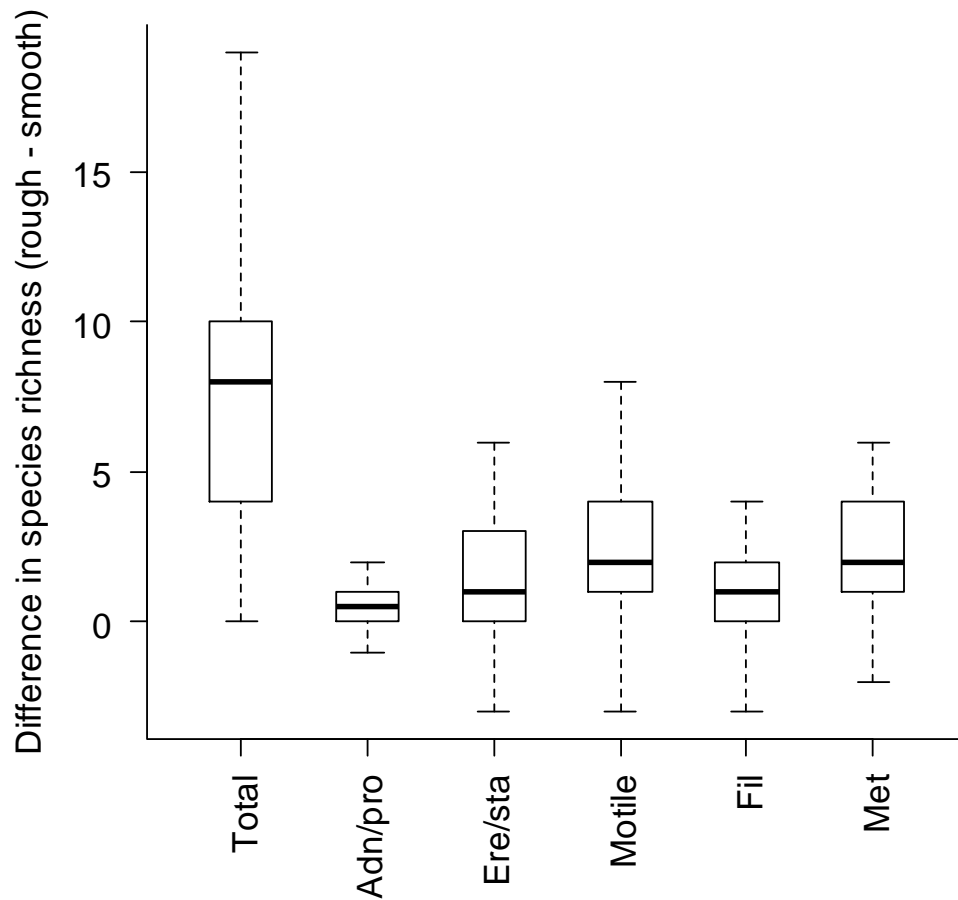


Fig. 1. Box-and-whisker plot of difference in species richness of benthic algae between rough and smooth substrates ($S_{\text{rough}} - S_{\text{smooth}}$). The experiment was stratified within 66 blocks (i.e., pairs of treatments) tested using a paired t-test. Therefore, differences between treatments are presented rather than mean values. The heavy line shows the median, box ends show quartiles, and whiskers show the minimum and maximum. Total = total assemblage, Adn/pro = Adnate/prostrate life form, Ere/sta = Erect/stalked, Fil = Filamentous, Met = Metaphyton.

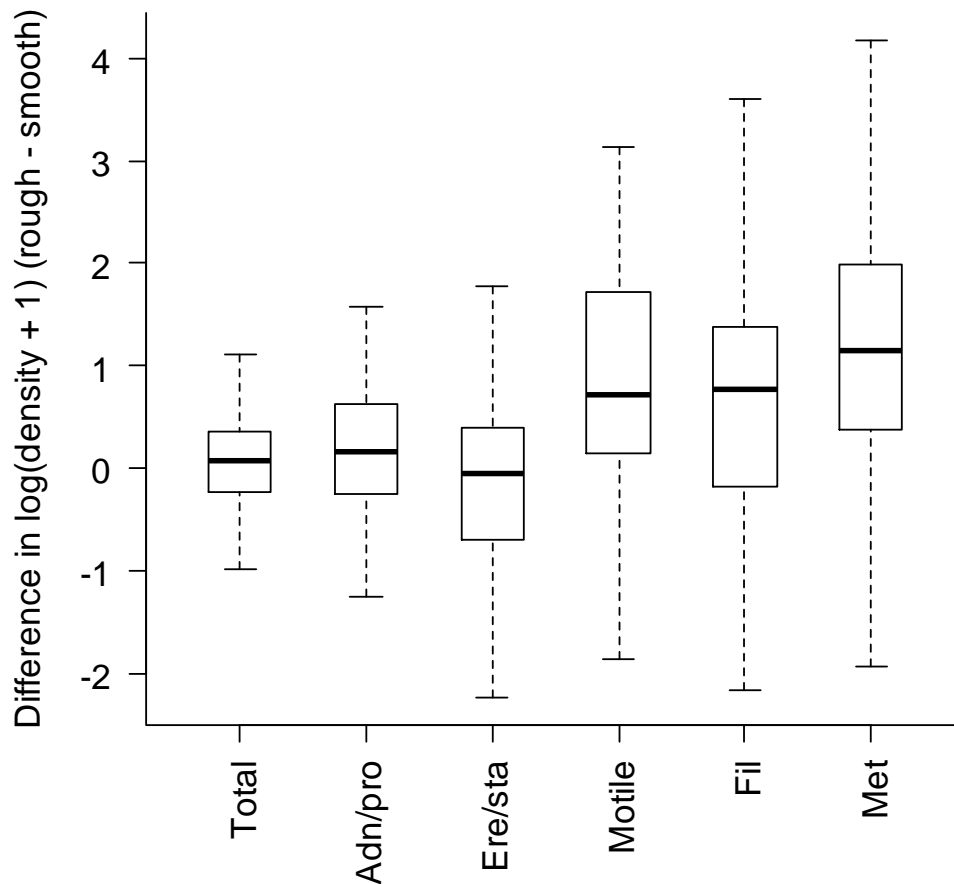


Fig. 2. Box-and-whisker plot of difference in $\log(\text{density} + 1)$ of benthic algae between rough and smooth substrates ($N_{\text{rough}} - N_{\text{smooth}}$). The experiment was stratified within 66 blocks (i.e., pairs of treatments) tested using a paired t -test. Therefore, differences between treatments are presented rather than mean values. The heavy line shows the median, box ends show quartiles, and whiskers show the minimum and maximum. Total = total assemblage, Adn/pro = Adnate/prostrate life form, Ere/sta = Erect/stalked, Fil = Filamentous, Met = Metaphyton.

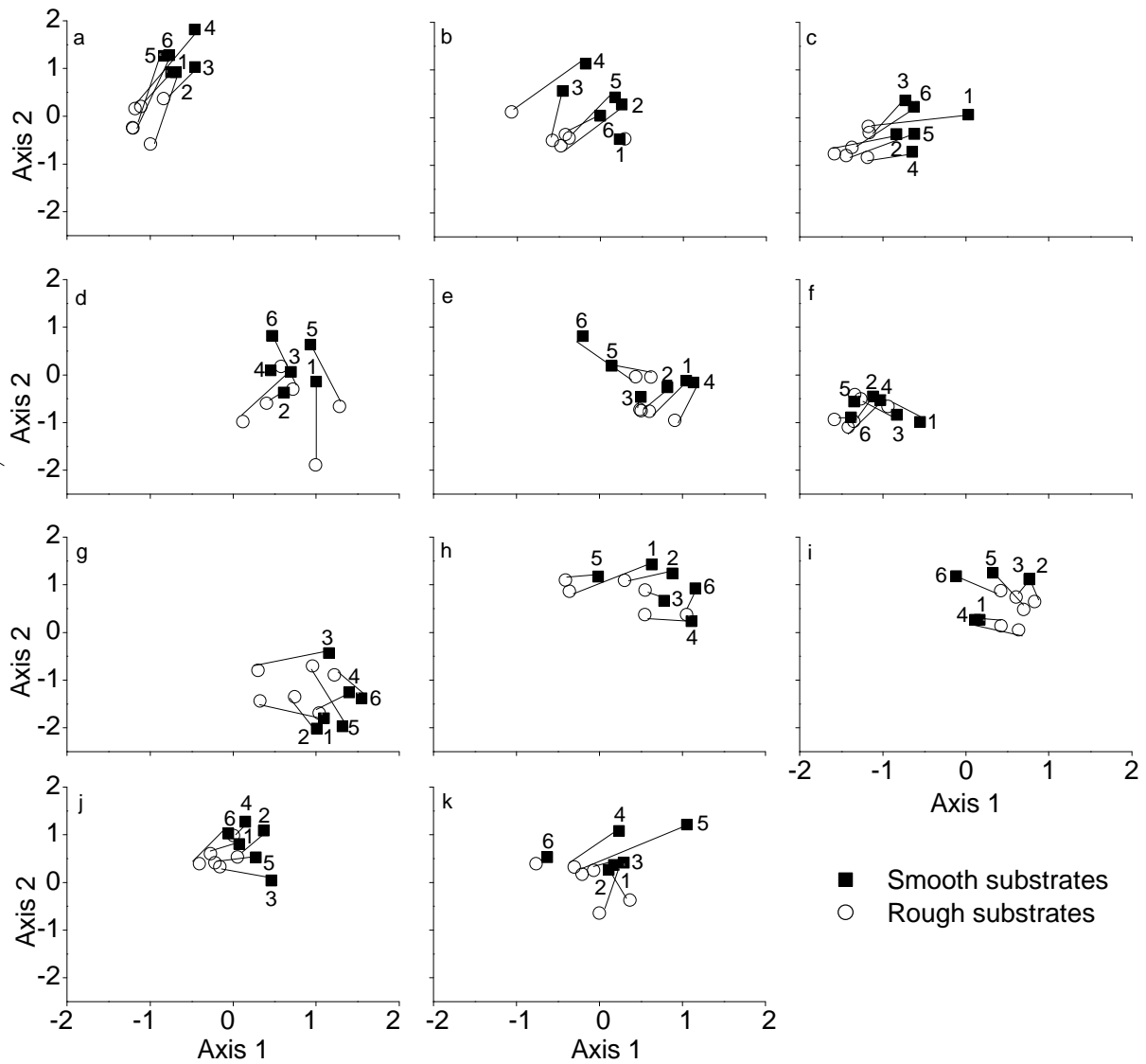


Fig. 3. Principal Coordinates Analysis (PCoA) plots of benthic algal assemblages on smooth and rough substrates in stream reaches a–k. The analysis was run using the Bray–Curtis index on quantitative data ($\log[\text{density} + 1]$). Only 1 PCoA was run, but the axis scores are shown in 11 different plots for clarity (1 plot for each stream reach). Numbers (1–6) indicate the 6 sampling occasions. Lines connect rough and smooth substrates collected on the same sampling occasion.

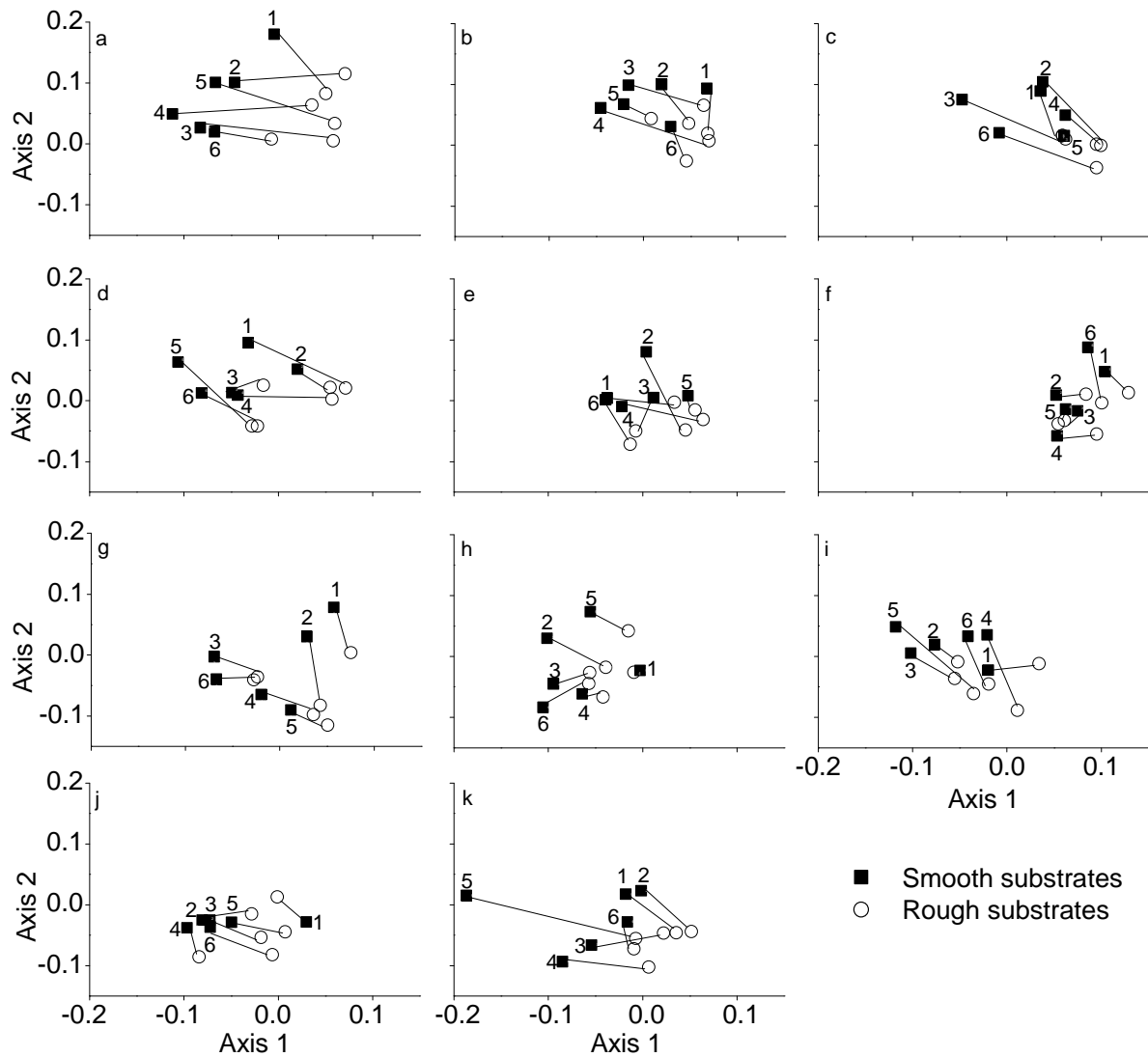


Fig. 4. Principal Coordinates Analysis (PCoA) plots of benthic algal assemblages on smooth and rough substrates in stream reaches a–k. Data were analyzed with the subsampling procedure and the Sørensen index. Symbols indicate averages of 100 ordinations of subsamples containing the same number of species as the experimental unit with the fewest species in the block. Only 1 PCoA was run, but the axis scores are shown in 11 different plots for clarity (1 plot for each stream reach). Numbers (1–6) indicate the 6 sampling occasions. Lines connect rough and smooth substrates collected on the same sampling occasion.

Capítulo 2

High assemblage persistence in heterogeneous habitats: an experimental test with stream benthic algae³

Abstract. It has been suggested that the persistence of biological assemblages is positively affected by spatial heterogeneity. This influence may be indirect, through increased species richness. Another possibility is the increased availability of refuges from disturbances, which would prevent local extinctions. We tested the hypothesis that greater roughness (a type of spatial heterogeneity) on the surface of substrates allows higher persistence of assemblages of stream benthic algae, and that this relation does not depend on species richness. We conducted a field experiment in which samples were taken on six occasions from smooth and rough artificial substrates used for algal colonization. We calculated the persistence of assemblages using two analytical approaches: the mean distance to group centroid in a multivariate space and the sum of the Euclidean distances between consecutive sampling occasions. We also used data subsampling procedures to evaluate the effect of species richness on persistence. Assemblages on rough substrates were more persistent than assemblages on smooth substrates. The effects detected were not due to increased species richness on the rough substrates, since a higher persistence of the assemblages on rough substrates remained after the subsampling procedures. Our results indicate a strong positive relationship between substrate roughness and the persistence of stream benthic algal assemblages. We suggest that this is due to the presence of physical refuges in heterogeneous habitats.

³ Schneck, F. & Melo, A. S. Manuscrito submetido ao periódico *Oikos* em outubro de 2011.

Introduction

Assemblages vary in space and time and it is of major importance to identify the mechanisms responsible for this variability, as this information may provide insights into the processes governing the assembly of communities and the functioning of ecosystems. For instance, understanding the temporal variability of assemblages is important for both basic and applied ecology, since it may enhance our ability to detect and predict impacts and thus improve management and conservation efforts (Cottingham et al. 2001).

The temporal variability of assemblages is influenced by the physical environment, particularly its temporal variability, and by biotic interactions (Bengtsson et al. 1997). Some studies suggest that the persistence of assemblages (i.e., “... constancy in absolute abundance, abundance ranking, or the presence or absence of species over time”, accordingly to Rahel 1990) is shaped by interspecific interactions, while others show that the persistence of assemblages is most likely affected by environmental factors. For example, Hildrew and Townsend (1982) suggested that the dynamics of a stream insect community is dominated by predation, in which predatory insects determine the temporal variability of prey. On the other hand, Bengtsson et al. (1997) found that bird assemblages were more persistent in a mature forest than in a forest that was experiencing vegetation succession, and suggested that this was related to higher habitat stability in the former forest. Similarly, studies with phytoplankton (Schneck et al. 2011a) and fish (Oberdorff et al. 2001) showed patterns of increased persistence with environmental stability.

Recently, physical spatial heterogeneity has been suggested to play a prominent role in the temporal variability of assemblages (Brown 2003, Mykrä et al. 2011). Although the increase in species richness as a result of spatial or habitat heterogeneity is widely recognized in ecology (Begon et al. 2006) and has been documented for many taxa in a variety of environments (e.g., MacArthur and MacArthur 1961, Murdoch et al. 1972, Downes et al.

2000, Thomaz et al. 2008), the effect of spatial heterogeneity on the persistence of assemblages remains unclear. According to Brown (2003), two mechanisms inherent to spatial heterogeneity may affect the persistence of assemblages, either indirectly, through an increase in species richness, or directly, through an increase in the number of refuges. The first mechanism is related to the fact that heterogeneous habitats provide large quantities and wide varieties of resources, and may allow resource partitioning and promote diversity (MacArthur and MacArthur 1961). An increased diversity may increase the persistence of assemblages by reducing extinction and/or colonization rates, and thus preventing large population variability in assemblages with mostly weak interspecific interactions (McCann et al. 1998, Shurin et al. 2007).

The second mechanism through which spatial heterogeneity may affect persistence is by generating a high availability of physical refuges (Brown 2003). Refuges may protect organisms against predation, disturbance and other adverse abiotic factors (Milchunas and Noy-Meir 2002, Bergey 2005). This may prevent local extinctions that could occur as a result of increased population variability of one or more species in the face of disturbance (Pimm 1991). Consequently, refuges tend to reduce the variability of assemblages. Additionally, the organisms protected within refuges can rapidly recolonize the habitat after an adverse condition ceases (Bergey 2004).

The study conducted by Brown (2003) was the first to disentangle the effects of high diversity and habitat heterogeneity on the persistence of assemblages. He took into account the effect of statistical averaging caused by increased species richness, and found an increased persistence in stream invertebrate assemblages along a gradient of spatial heterogeneity. Accordingly, he concluded that the increased persistence was likely due to the larger number of refuges. However, Brown's study could have been partially confounded by the correlation between habitat heterogeneity and physical stability of the substrate. For instance, his three

measures of habitat heterogeneity, the Simpson index, evenness and contagion, were correlated with the mean size of the substrates in each plot along the gradient of heterogeneity (respectively 0.75, -0.70 and 0.58; data from his Table 1), and large substrates lead to high assemblage persistence (Gurtz and Wallace 1984). Accordingly, his observational study did not allow unequivocal conclusions as to whether the effect of habitat heterogeneity leads to high persistence of assemblages. We avoided this pitfall, and reevaluated the hypothesis raised by Brown, by conducting a field experiment in which we set up two treatments composed by smooth and rough artificial substrates for algal colonization. The roughness of substrates (e.g., crevices, small projections) plays an important role in regulating the composition and structure of benthic algal assemblages in streams (e.g., Dudley and D'Antonio 1991, Bergey 2005), including increased species richness (Schneck et al. 2011b). We tested the hypothesis that small-scale spatial heterogeneity increases the persistence of stream benthic algae, and that this relation does not depend on species richness.

Methods

Study area

We conducted the experiment in the Marco stream (28°36'S; 49°51'W), a fourth-order stream at an elevation of approximately 1100 m asl, in the state of Rio Grande do Sul, southern Brazil. The regional climate is high-altitude subtropical, with uniform precipitation throughout the year. Annual mean rainfall ranges from 1400 to 2200 mm, and annual mean temperature ranges from 12°C to 18°C (Behling 2002). The vegetation is *Campos* grassland with patches of Araucaria Forest. The stream drains a catchment with low local human impact. The water is oligotrophic (Buckup et al. 2007) and well oxygenated (10 mg/L), with a mean pH of approximately 6.6, low conductivity (22 µS/cm), and a mean current velocity of 26 cm/s during the period of the study. The streambed is composed mainly of basaltic stones,

boulders, and bedrock. The stream width varies from 2 to 5 m, and the depth varies from 0.2 to 0.4 m in the reaches studied.

Experimental design

We designed a field experiment in which we used smooth and rough substrates as the two levels of substrate heterogeneity. Samples were obtained every 15 d on six sampling occasions from May to August 2009 (austral autumn and winter) in 11 stream reaches. On each sampling occasion we sampled two substrates of each treatment per stream reach. Each pair of substrates collected under the same conditions was pooled for analysis, and constituted one experimental unit ($n = 132$).

We used acrylic substrates (5×5 cm) with either a smooth surface or a surface with longitudinal crevices for algal colonization. All rough substrates had nine crevices, and all crevices were 1 mm in width and 1 mm in depth. The size of the crevices was defined after Bergey and Weaver (2004) which found that crevices with openings smaller than 2 mm protected algal assemblages from grazers. The substrates were glued on $50 \times 50 \times 8$ cm flat paving stones, and one stone was placed in each of the 11 stream reaches. Each stone had all substrates necessary for the entire experiment, minimizing the variation of physical variables, such as current velocity and water depth, between treatments. The substrates were left in place to be colonized during a period of 45 d. Reaches were considered blocks in statistical analyses.

Sampling and laboratory analyses

The upper surfaces of the substrates were scraped with a toothbrush to remove the biofilm. The samples were adjusted to a defined volume (50 ml) and preserved with 4% formaldehyde (Lowe and LaLiberte 2007). For each experimental unit, 500 cells or units (each unit

corresponded to 10 μm -long units of fine-celled filaments) were counted and identified, using an inverted microscope. For the identification of diatoms, additional subsamples were acid-cleaned using standard techniques (Lowe and LaLiberte 2007) and examined at 1000 \times through a light microscope. We identified 92 species of benthic algae. Additional details on the experiment and on the composition of the assemblage can be found in Schneck et al. (2011b).

Data analyses

We used two approaches to test for differences in the persistence of assemblages between smooth and rough substrates. In the first, the distances from individual experimental units to their group centroid in multi-dimensional space obtained in a Principal Coordinates Analysis (PCoA) were used as a measure of the variability of assemblages (Anderson 2006). PCoAs were generated for both qualitative (presence-absence; Sørensen dissimilarity index) and two forms of quantitative data (density; Bray-Curtis index). The first form consisted of log-transformed data, and the second of standardization by the species maximum. In the qualitative analysis, rarity is not taken into account and all species have the same importance in the analysis. Using the data standardized by the maximum, species still retain the same overall importance but relative densities are maintained within species. Log-transformed data maintain part of the original relative densities and importance of each species. Since the use of non-metric dissimilarity coefficients may produce principal coordinates axes with negative eigenvalues, we computed the square root of distances before the analyses (Legendre and Legendre 1998). We conducted separate PCoAs for each stream reach (block) and used the mean multivariate distances to the group centroid of each treatment per block to perform a one-tailed paired t-test for each type of data.

In the second approach, we conducted a procedure similar to that employed by Brown (2003) and Brown and Lawson (2010), which calculates the distance in the multivariate taxa space that an assemblage moved between sampling occasions (EDCA; Brown 2003). For each type of data (presence-absence, log-transformed, and standardized by species maximum), we first conducted separate Correspondence Analyses (CA) of the experimental units for each of the 11 blocks. Next, using the scores of experimental units in the first 11 CA axes, we calculated the Euclidean distance in the ordination space between experimental units of a given treatment on two consecutive sampling occasions. The distance between the experimental units for each interval is a direct measure of temporal variability, and the greater the Euclidean distance, the greater the variability of the assemblage (Brown 2003). Since there were six sampling occasions, five intervals were calculated and summed to generate a total Euclidean distance for each treatment per block. We used these total Euclidean distances for the two treatments to perform a one-tailed paired t-test for each type of data.

We conducted subsampling procedures to investigate the effect of increased species richness on the persistence of assemblages, since rough substrates were more species-rich than smooth substrates (Schneck et al. 2011b). The procedure was conducted for each of the two approaches and for all three types of data (presence-absence, log-transformed, and standardized by species maximum). First, we randomly subsampled the richest treatment of each of the 11 blocks to contain the same number of species of the poorest treatment in the block. For the first approach we conducted a separate PCoA for each of the 11 subsampled blocks, followed by calculation of the mean distance to the group centroid in each block. For the EDCA approach, we conducted a CA for each subsampled block, followed by calculation of the total Euclidean distance for each treatment. The procedure was repeated 100 times. Next, using all 100 subsamples we calculated the mean distance to the centroid for each treatment per block (first approach) and the mean total Euclidean distances for each treatment

per block (EDCA approach). Finally, we used the two sets of 11 pairs of values to perform one-tailed paired t-tests. We adjusted the α -value by the number of tests performed (Bonferroni correction; $\alpha' = 0.004$). We conducted all analyses in the R environment (The R Development Core Team 2009) using functions in the *vegan* package (Oksanen et al. 2010), and routines that we wrote for the subsampling procedures.

Results

Assemblages on rough substrates were less dispersed in the multivariate space of the PCoA (i.e., were more persistent) than assemblages on smooth substrates, for presence-absence ($F_{1,9} = 44.27$; $P < 0.001$), log-transformed ($F_{1,9} = 37.00$; $P < 0.001$) and standardized by species maximum data ($F_{1,9} = 28.67$; $P < 0.001$) (Fig. 1a). The subsampling procedures indicated that the persistence of assemblages on rough substrates remained higher than the persistence on smooth substrates for all types of data, even after taking into account differences in species richness between treatments ($F_{1,9} = 43.91$; $F_{1,9} = 36.22$; $F_{1,9} = 24.74$, respectively for presence-absence, log-transformed and standardized data; in all cases $P < 0.001$; Fig. 1a).

Similarly, the EDCA approach indicated that the assemblages on rough substrates were more persistent than the assemblages on smooth substrates, for presence-absence, log-transformed and standardized by species maximum data ($F_{1,9} = 24.67$; $F_{1,9} = 20.71$; $F_{1,9} = 51.46$, respectively; in all cases $P < 0.001$; Fig. 1b). These results persisted after the subsampling procedure ($F_{1,9} = 20.35$; $F_{1,9} = 18.31$; $F_{1,9} = 32.12$, respectively for presence-absence, log-transformed and standardized data; in all cases $P < 0.001$; Fig. 1b).

Notably, the persistence was higher for assemblages on rough substrates in 31 of the 33 (11 blocks and 3 data types) pairs in the distance-to-centroid approach, and in all 33 pairs in the EDCA approach for the observed data set. In the cases of subsample data, persistence on rough substrates was higher than on smooth substrates in 31 of the 33 pairs for the

distance-to-centroid approach, and in 30 of 33 pairs for the EDCA approach, indicating a strong effect of substrate roughness.

Discussion

The results found agree with the hypothesis raised by Brown (2003), that habitat heterogeneity increases the persistence of assemblages. Our results also show that the increase in persistence with increasing habitat heterogeneity was not influenced by the potential underlying mechanisms intrinsic to spatial heterogeneity that may affect the temporal variability of assemblages (e.g., substrate physical stability, species richness). The experiment was carried out within blocks in which the substrates for both treatments were subjected to the same physical conditions, enabling an effective control of possible confounding factors and thus a response that is unequivocally attributed to spatial heterogeneity (i.e., substrate roughness).

The positive effect of substrate roughness on the persistence of stream benthic algal assemblages was irrespective of the role that rare and dominant species might play in affecting the temporal variability, as the data emphasizing different aspects of the assemblage structure showed similar results. Accordingly, high persistence in spatially heterogeneous habitats should be valid for both rare and common species.

We suggested that the effects of spatial heterogeneity on the persistence of assemblages in the study of Brown (2003) may have been confounded by its correlation with the stability of the physical habitat. In his study, the size of the substrates and the bryophyte cover may have increased not only the spatial heterogeneity, but also the stability of the habitat for invertebrates (Gurtz and Wallace 1984, Stream Bryophyte Group 1999). Similarly, Mykrä et al. (2011) found a high persistence of stream invertebrates in heterogeneous habitats, defined as those with high bryophyte cover. They inferred that the bryophyte cover

provides refuges and thus favors high persistence of assemblages. However, the authors recognized that bryophytes also confer high substrate stability (Stream Bryophyte Group 1999) which in turn could have been the cause of the observed high persistence of invertebrate assemblages. Our experiment was set up to avoid this correlation, as both types of substrates were equally stable and subject to the same physical environment.

The subsampling procedures indicated that the higher persistence of the assemblages on rough substrates was related to the effect of substrate roughness *per se*, rather than to the increased species richness on these substrates. Since the substrates were very simple in design, and the only difference between the two treatments was the presence or absence of crevices, the most plausible mechanism that could mediate the higher persistence on rough substrates is the occurrence of refuges. The efficacy of refuges in protecting algae from disturbances has been shown by some studies. For instance, Bergey (2005) quantified the protection of algae within crevices after an experimental disturbance caused by scrubbing, and found that the amount of total biomass that remained on stones increased with surface roughness, concluding that physical refuges (i.e., crevices) effectively protected the algae. Refuges have also been shown to be effective in protecting algae from grazers (Dudley and D'Antonio 1991). Further, a study combined with the present experiment showed that the two types of substrates differed in the composition of assemblages (Schneck et al. 2011b). Motile, filamentous and metaphytic algal life forms were more species-rich and dense on the rough substrates, whereas adnate and prostrate algae did not differ in species richness and density between the substrates. Notably, the algal life forms that showed a positive response to substrate roughness are those most likely to benefit from the occurrence of refuges, by both enhancing their protection and the availability of resources within crevices (Murdock and Dodds 2007).

Our experiment provided clear-cut evidence of the major role that small-scale spatial heterogeneity plays in the persistence of stream benthic algal assemblages. The results also reinforce the hypothesis that the occurrence of physical refuges in heterogeneous habitats is an important ecological factor, especially in frequently disturbed ecosystems.

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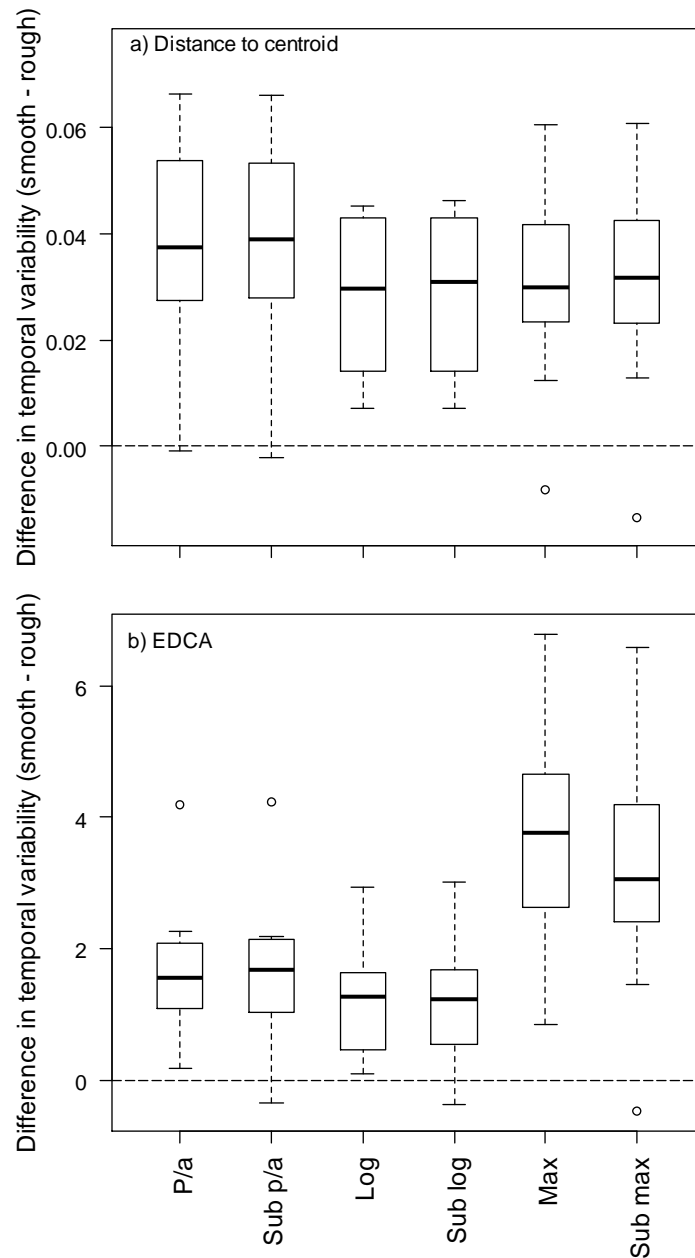


Figure 1. Box-and-whisker plot of difference in the temporal variability of stream benthic algal assemblages on smooth and rough substrates (distances smooth – distances rough) for a) mean distances to the group centroid in the multi-dimensional space obtained in a PCoA and b) total Euclidean distance of the assemblage along the six sampling occasions in the space obtained by a multivariate correspondence analysis (EDCA). The experiment was carried out in 11 blocks, and the hypothesis of differences in temporal variability (or persistence) between assemblages was tested using paired t-tests. Therefore, differences between

treatments are presented rather than mean values. The dashed horizontal lines indicate the case of no difference between smooth and rough substrates. The minimum and maximum (whiskers), the lower and upper quartiles (box ends), and the median (heavy line) for each type of data are shown. P/a = presence-absence data; Sub p/a = subsampled presence-absence; Log = log-transformed; Sub log = subsampled log-transformed; Max = standardized by the species maximum; Sub max = subsampled standardized by the species maximum.

Capítulo 3

Hydrological disturbance intensity overrides substrate roughness effects on the resistance and resilience of stream benthic algae⁴

Summary

1. High habitat heterogeneity in lotic systems is usually associated with high availability of refuges. Heterogeneous habitats (here, rough substrates) should mediate the effect of high-flow disturbances by protecting benthic algae and thus increasing the resistance and resilience of algal assemblages. Additionally, the ability of algae to resist a disturbance and recover after it should be dependent on the algae life forms, because these differ in biological traits that confer resistance and resilience.

2. We designed a field experiment to test the effect of substrate roughness on the resistance (similarity between samples collected before and samples collected immediately after the disturbance) and resilience (similarity between samples collected before and samples collected 7 d and 15 d after the disturbance) of five algal life forms following an experimental hydrological disturbance. We evaluated whether algal resistance and resilience were higher on rough than on smooth substrates, and whether the life forms differed in their ability to resist and recover from a disturbance.

3. Rough substrates had higher species richness than smooth substrates, and this difference persisted even immediately after the disturbance. There was no significant effect of substrate

⁴ Schneck, F. & Melo, A. S. Manuscrito submetido ao periódico *Freshwater Biology* em novembro de 2011.

roughness on algal resistance and resilience, for both species richness and density of the total assemblage. Roughness also did not affect the resistance and resilience of the total algal assemblage or the algal life forms separately, when evaluated using multivariate datasets (presence-absence and quantitative). However, there was a non-significant tendency toward higher resilience on rough than on smooth substrates, independently of algal life forms.

4. On the other hand, algal life forms showed a clear pattern of differences in resistance and resilience, in which adnate/prostrate and erect/stalked species were more resistant and resilient than the other life forms (filamentous, motile and metaphytic). Additionally, motile species resisted and recovered better than did metaphytic species.

5. The results suggest that substrate roughness had no pronounced effect on benthic algal resistance and resilience. The results of this and some other studies allow us to suggest that the intensity of disturbance determines the importance of habitat heterogeneity and flow refuges for benthic algae in streams.

Key words: algal life form, floods, habitat heterogeneity, periphyton, refuges

Introduction

Natural physical disturbances are major components of many ecosystems, and generate much of the spatial and temporal heterogeneity in the functioning of communities (Sousa, 1984; Townsend, 1989). In streams, disturbances caused by high-flow events play a key role, as they rearrange the physical environment and reorganize the structure and dynamics of assemblages (Lake, 2000; Death, 2010). For example, hydrological disturbances can reduce algal biomass (Grimm & Fisher, 1989) and abundance of invertebrates (Melo et al., 2003) and macrophytes (Riis & Biggs, 2003), affect species richness (Biggs & Smith, 2002; Melo et al., 2003) and patchiness (Matthaei, Guggelberger & Huber, 2003; Melo et al., 2003), and alter ecosystem metabolism (Bertrand et al., 2009). The removal of organisms during high-flow events can result from shear stress caused by elevated water velocity, abrasion caused by suspended sediments, and displacement of substrates (Melo et al., 2003; Francoeur & Biggs, 2006).

One factor that likely mediates the effects of hydrological disturbances is habitat heterogeneity (Lancaster & Hildrew, 1993; Brown, 2007), which can be recognized at different spatial scales, from catchments (Vannote et al., 1980) to microhabitats (Bergey, 2005; Costa & Melo, 2008). Habitat heterogeneity generates distinct habitats that are affected to different degrees by high flows, so that more stable and sheltered patches may act as refuges, protecting organisms against hydraulic stress (Townsend, 1989; Palmer et al., 1996). Similarly to habitat heterogeneity, flow refuges occur at many spatial scales. In large basins, side channels in floodplains can act as refuges (Sedell et al., 1990), while at the scale of stream reaches, refuges may be provided by areas of reduced flow (Lancaster & Hildrew, 1993) and stable streambed substrates (Matthaei, Arbuckle & Townsend, 2000). At fine scales, rough substrates (Bergey & Resh, 2006) were found to protect organisms during high-flow events. Organisms protected within refuges are able to resist disturbances and to recolonize the habitat shortly after the disturbance (Lancaster & Hildrew, 1993). The availability of

refuges may increase the resistance of the biota to flow disturbance and provide migrants to the affected patches, increasing the resilience of the system (Lake, 2000).

The surface roughness of substrates, formed of crevices, pits and small projections, plays an important role in regulating the structure of benthic algal assemblages in streams (Bergey, 2005; Schneck, Schwarzbald & Melo, 2011). Rough substrates influence algal colonization (Bergey, Cooper & Phillips, 2010), support higher algal biomass (Bergey, 2005), and are more species-rich than smooth substrates (Schneck et al., 2011). There is also strong evidence of the efficacy of crevices as refuges for algae (Dudley & D'Antonio, 1991; Bergey, 2005; Bergey & Resh, 2006). Therefore, we could expect assemblages on rough substrates to be more resistant to disturbances and to recover more quickly (i.e., show higher resilience) than assemblages on smooth substrates.

Additionally, the ability of benthic algae to resist and to recover after a disturbance depends on their biological characteristics (Peterson, 1996). For instance, algal assemblages usually shift from the dominance of upright species to the dominance of tightly attached species after disturbances (Peterson & Stevenson, 1992; Francoeur, Biggs & Lowe, 1998). Species with prostrate growth forms and that are adnatly attached have tight adhesion structures and live on the understory layer of the biofilm, features that allow these species to resist disturbance and to colonize patches affected by disturbance (Steinman & McIntire, 1990; Peterson & Stevenson, 1992). Other life forms, such as motile and metaphytic species, are less resistant and may benefit mostly from the occurrence of refuges (Murdock & Dodds, 2007; Schneck et al., 2011).

We experimentally evaluated the effect of substrate roughness on the resistance and resilience of five algal life forms following a hydrological disturbance. Specifically, we tested the hypotheses that (i) resistance and resilience of algal assemblages would be higher on rough than on smooth substrates and (ii) the five algal life forms would differ in their ability

to resist and recover after a hydrological disturbance. For this last hypothesis, we predicted that the adnate/prostrate life form would be more resistant and resilient than the other life forms, and that the metaphytic life form would be the least resistant and resilient.

Methods

Study site

We conducted the experiment in the Marco stream (28°36'S; 49°51'W), São José dos Ausentes, state of Rio Grande do Sul, southern Brazil. The vegetation is *Campos* grassland with patches of Araucaria Forest, and the climate is high-altitude subtropical (Cfb), with uniform precipitation throughout the year (Behling, 2002). The stream is fourth-order at an elevation of approximately 1100 m asl, and the water is oligotrophic (Buckup et al., 2007), well oxygenated (10 mg L⁻¹), slightly acidic (pH 6.6), and with low electrical conductivity (22 μS cm⁻¹). The mean current velocity is 26 cm s⁻¹. Stream width varies from 2 to 5 m, and the depth varies from 0.2 to 0.4 m in the reaches studied. The streambed is composed mostly of basaltic stones, boulders, and bedrock.

Experimental design and biological analyses

We evaluated the effects of substrate roughness on the resistance and resilience of algal life forms through a field repeated-measures experiment designed in blocks (11 reaches in the stream). We used smooth and rough substrates as the two levels of substrate roughness, and took samples on four occasions: immediately before, immediately after, seven days after, and 15 days after the experimental disturbance. The experiment consisted of 88 experimental units (2 substrate types × 4 sampling occasions × 11 blocks) and each experimental unit consisted of two sampling units. Samples collected immediately before the experimental disturbance were considered the control. Samples collected immediately after the disturbance were used to

measure resistance, while samples collected 7 and 15 days after the disturbance measured the resilience of assemblages.

We used acrylic substrates (5×5 cm) as sampling units for algal colonization. The smooth substrates had an even surface and the rough substrates had a surface with nine longitudinal crevices. All crevices had the same width (1 mm) and depth (1 mm). We glued the substrates on $50 \times 50 \times 8$ cm flat paving stones and placed one stone in each of the 11 stream reaches. The substrate crevices were aligned in a cross-stream direction. Each stone had all the substrates needed for the entire experiment. We placed the stones with the artificial substrates in the stream in April 2009 for algal colonization, and conducted the study during July and August 2009.

We used a water compressor and a hose to simulate a high-flow event (mean current velocity $208 \text{ cm}\cdot\text{s}^{-1}$). Concomitantly, we added 2000 cm^3 of sediment to simulate the mobilization of suspended sediment caused by floods. The sediment was removed from the stream bank and sieved through a 1 mm mesh. The experimental disturbance was applied for 10 min in each of the 11 blocks. A similar experimental disturbance was employed by Melo et al. (2003) and significantly reduced species richness and abundance of stream invertebrates.

We scraped the upper surfaces of the substrates with a toothbrush to remove the biofilm and preserved the samples with 4% formaldehyde (Lowe & LaLiberte, 2007). We counted 500 cells or units (each unit corresponded to $10 \mu\text{m}$ -long fine-celled filaments) from each experimental unit within a known number of fields, using an inverted microscope at $400\times$ magnification (Utermöhl, 1958). We calculated the number of cells per unit area (cm^2) to estimate cell density of each species and total cell density of each experimental unit. We used additional subsamples to mount glass slides, using standard techniques to identify diatoms (Lowe & LaLiberte, 2007).

We classified algae into five life-form groups: 1) adnate or prostrate (species of *Achnanthes*, *Achnanthidium*, *Cocconeis*, *Coleochaete*, *Epithemia*, *Psammothidium* and *Rhopalodia*), 2) erect or with mucilage stalks or tubes (species of *Cymbella*, *Encyonema*, *Eunotia*, *Fragilaria*, *Gomphonema*, *Meridion*, *Synedra*, and *Ulnaria*), 3) motile algae (species of *Frustulia*, *Hantzschia*, *Luticola*, *Navicula*, *Neidium*, *Nitzschia*, *Pinnularia*, *Sellaphora*, *Stauroneis* and *Surirella*), 4) filamentous (species of *Aulacoseira*, *Bulbochaete*, *Heteroleibleinia*, *Melosira*, *Oedogonium*, *Stigeoclonium*, an unidentified filamentous green alga and an unidentified filamentous desmid), and 5) metaphyton (species of *Cosmarium*, *Euastrum*, *Pleurotaenium*, *Scenedesmus*, *Staurastrum*, and unidentified Chlorococcales). Some genera could fit in more than one life-form group, such as *Epithemia* and *Rhopalodia*, which although they may be motile, are commonly described as adnate or prostrate (Biggs, Stevenson & Lowe, 1998; Lowe, 2003) and therefore we opted to assign them to the latter life form. Other genera, such as the diatoms *Aulacoseira* and *Melosira* or some green algae, are filamentous algae that are not attached to substrates, but live entangled with other algae or substrates (Stevenson, 1996; Biggs et al., 1998). We based our classification on algal physiognomies, personal observations, and information provided by Lowe, Golladay & Webster (1986), Round, Crawford & Mann (1990), Biggs et al. (1998), Wehr & Sheath (2003), Bixby et al. (2009), Spaulding, Lubinski & Potapova (2010) and Lange et al. (2011).

Data analysis

We used a two-factor Analysis of Variance with repeated-measures in both factors (i.e., substrate roughness and time; RM-ANOVA) to test for the effect of substrate roughness on species richness and log (density +1) of algae after the experimental disturbance. In this analysis, stones containing the artificial substrates represented the unit in which the trials were obtained, also termed the subject. We corrected *P*-values using the Huynh-Feldt method for

RM-ANOVA (Looney & Stanley, 1989). In case of significant time effects, we made *post-hoc* pairwise comparisons based on estimated marginal means with *P*-values adjusted for multiple comparisons (Bonferroni correction).

We used the Bray-Curtis similarity index on $\log(\text{density} + 1)$ and its qualitative version, the Sørensen index (presence-absence data; Legendre & Legendre, 1998), to calculate the resistance and resilience of each algal life form on smooth and rough substrates. We defined a resistance index as the similarity between the control samples and the samples collected immediately after the disturbance. A resilience index was obtained separately for 7 d and 15 d after the disturbance, using the similarity values between the control samples and the samples from each date. We excluded from analysis, one experimental unit of the metaphytic life form on the smooth substrate, because no cells of this group were found in the control sample and only two cells were found in each of the post-disturbance samples, preventing the calculation of reliable values for the indices. We carried out an Analysis of Variance (ANOVA) for each index, using the values obtained for each algal life form in each treatment per block (for both qualitative and quantitative data). The models in the ANOVAs included life form and substrate type as factors, plus their interaction and the block factor. In all cases we used the logarithm of the indices to meet the assumption of normality and homoscedasticity. If the results of life forms were significant, we performed Tukey HSD *post hoc* tests for pairwise comparisons. We present the results of ANOVAs using the original values of *P* and discuss them based on the adjusted α value after the conservative Bonferroni correction ($\alpha' = 0.008$). We conducted the analyses in the R environment (The R Development Core Team, 2009).

Results

We found 81 species of algae. The adnate/prostrate life form was the dominant group, comprising on average more than 45% of the total density on smooth and rough substrates at each of the four sampling times (Fig. 1a). This group also showed an increase in its relative density immediately after the disturbance, while all other life-form groups decreased in their relative densities (Fig. 1). The erect/stalked life form was the second most common group, with an average relative density ranging from 28 to 42% (Fig. 1b).

There was no significant interaction between substrate type and time, either for species richness ($P = 0.201$) or density ($P = 0.096$) (Table 1), indicating that assemblages on smooth and rough substrates did not differ in their response to the disturbance with respect to resistance and resilience. Species richness and density differed significantly between sampling times (richness: $P < 0.001$, density: $P = 0.003$; Table 1), and this was mostly due to differences between samples obtained before vs. immediately after the disturbance (richness: $P = 0.004$, density: $P = 0.002$; Table 1; Fig. 2). Both species richness and density recovered after 7 days, showing similar values to those observed for samples collected before the disturbance (comparison of before vs. 7 days after, richness: $P = 0.999$, density: $P = 0.137$; comparison of before vs. 15 days after, richness: $P = 0.999$, density: $P = 0.999$; Table 1; Fig. 2). Species richness was significantly higher on rough than on smooth substrates ($P < 0.001$), whereas density did not differ ($P = 0.293$) (Table 1; Fig. 2).

There were no significant interactions between algal life forms and substrates in terms of similarity, for resistance or resilience (Table 2). The effect of substrate roughness on the resistance and resilience was statistically non-significant (Bonferroni correction, all $P > 0.008$; Table 2). However, there was a tendency toward higher resilience on rough substrates, for both the qualitative and quantitative indices (all P values between 0.013 and 0.050; Table 2; Fig. 3). Algal life forms differed in their resistance and resilience. The adnate/prostrate and

the erect/stalked life forms were more resistant and resilient than the other groups in most of the comparisons (Table 2, Fig. 3). Further, the motile life form was more resistant and resilient than the metaphyton (Table 2, Fig. 3).

Discussion

Effects of substrate roughness

Our hypothesis of higher resistance and resilience of benthic algal assemblages on rough substrates than on smooth substrates was not supported, as shown by the non-significant interaction terms for species richness and density in the RM-ANOVAs and by the non-significant differences of substrate roughness when using the indices based on similarity. However, we cannot completely exclude the protective effect of rough substrates, and the evidence is threefold. First, there was a tendency toward higher resilience on rough substrates. While this result cannot be explained by the occurrence of relict algae protected in crevices (because in this case we also should have found higher resistance on rough substrates), it could be explained by the possibility that new colonizers may settle easier on rough than on smooth substrates during base-flow (e.g., Dudley & D'Antonio, 1991). Second, rough substrates harbored more species-rich assemblages than smooth substrates at all sampling times, a pattern previously observed for algae and attributed to the high availability of resources and refuges on rough substrates (Schneck et al., 2011). Third, the metaphytic life form, the least resistant and resilient group, had higher relative density on rough than on smooth substrates (as shown in Fig. 1e). This life form has been shown to benefit from the occurrence of crevices, increasing species richness and density on rough substrates (Schneck et al., 2011). This evidence suggests that habitat heterogeneity affected the benthic algae to some extent, probably by protecting at least part of the assemblage in crevices and by facilitating recolonization after disturbance.

The absence of a relationship between habitat heterogeneity and high-flow disturbance was also observed in other studies, for both natural and experimental disturbances. For example, Grimm & Fisher (1989) observed low and similar algal resistance (measured as chlorophyll *a*) to natural floods between cobble-boulder riffles and sand-gravel runs, two habitats with different degrees of heterogeneity and substrate stability. An experimental study found that invertebrate assemblages inhabiting streams with greater habitat heterogeneity, and thus with more flow refuges (natural tussock streams), did not differ in resistance and resilience from invertebrate assemblages in less heterogeneous streams (pasture streams) (Melo et al., 2003).

In a previous experiment using the same type of artificial substrates and number and size of crevices, we found low temporal variability of algal assemblages during a period without high-flow events in rough substrates, and suggested that a plausible mechanism was the high availability of refuges compared to smooth substrates (F. Schneck & A. S. Melo, unpublished data). In the present study, the effect of substrate roughness was not as pronounced as in the above-mentioned study. This allows us to raise the hypothesis that crevice refuges were effective in buffering against less severe hydraulic disturbances and grazing, but were inefficient in protecting against a disturbance of high magnitude, indicating that the efficacy of refuges depends on the type and severity of disturbance (Sedell et al., 1990). Indeed, there is evidence of this dependency for invertebrate assemblages. While habitat heterogeneity decreased the temporal variability of stream invertebrates during base-flow, it had no influence on assemblage variability during high-flow and drought events (Brown, 2007). As suggested by Brown (2007) for stream invertebrates, it is likely that intensity of disturbance may also regulate the effectiveness of habitat heterogeneity and flow refuges to benthic algae.

Resistance and resilience of algal life forms

We found a clear pattern of differences in resistance and resilience among the five algal life forms. The ability of benthic algal species to resist and/or recover from a high-flow disturbance varies markedly (Peterson & Stevenson, 1992) and is mostly related to differences in biological traits, such as adhesion structures, growth rates, body size, and adaptation to live in resource-stressed environments (Grimm & Fisher, 1989; Steinman & McIntire, 1990; Townsend & Hildrew, 1994). In our study, the two most resistant and resilient life forms, adnate/prostrate and erect/stalked, together with the motile group, which was more resistant and resilient than metaphytic species, were composed mainly by diatoms. Diatoms usually resist disturbances better than other groups, and are almost always the first colonizers of denuded substrates (e.g., Grimm & Fisher, 1989; Peterson & Stevenson, 1992). These attributes are probably related to their ability to secrete mucilage, their rapid growth rates, and their large species pool (Steinman & McIntire, 1990). For instance, diatoms were more resistant and recovered faster than other groups of algae after several floods, and were followed by filamentous green algae and mats of cyanobacteria (Grimm & Fisher, 1989). Grimm & Fisher (1989) also observed that diatoms rapidly recolonized substrates after the flood ceased.

The adnate/prostrate life form primarily includes small and tightly attached resource-stressed species that live in the understory layer, with limited light and nutrients (Passy, 2007). Living in the understory layer enables them to avoid the shear stress of high-flow events and resist scour (Stevenson, 1996). Species of this group, such as *Achnanthydium minutissimum* (Kützing) Czarnecki and *Cocconeis placentula* Ehrenberg, are fast-growing and early colonizers of open spaces (Steinman & McIntire, 1990). In addition to their high resistance and resilience, the dominance of the adnate/prostrate life form at the four sampling times is an expected pattern for nutrient-poor and fast-current streams, such as the stream

studied here, in which pioneer fast-growing species are likely not replaced by competitively dominant species such as filamentous algae (Townsend & Hildrew, 1994).

Erect/stalked species are usually the second group in the succession, growing faster than filamentous species (which have higher light and nutrient requirements and are late colonists) (Steinman & McIntire, 1990; Peterson & Stevenson, 1992). The high resistance of the erect/stalked group is probably related to the ability of all these species to live attached to substrates, while the filamentous group includes attached and unattached species that may differ in resistance (Power & Stewart, 1987). Filamentous species may also have long filaments growing out of the boundaries of the biofilm, increasing their susceptibility to shear stress of floods (Grimm & Fisher, 1989; Steinman & McIntire, 1990). Finally, the difference in resistance and resilience between motile and metaphytic species can be explained by the ability of motile species to move through the biofilm and select habitats relatively free from disturbance but with sufficient resources (Passy, 2007). In contrast, metaphytic algae in our study occurred as detached single cells or small colonies that are easily carried away from the biofilm matrix by high flow.

Conclusion

We observed that the interplay between habitat heterogeneity and hydrological disturbance is not as simple and clear as expected. While there is much evidence that flow refuges protect organisms and that the occurrence of refuges is related to habitat heterogeneity, we showed that more heterogeneous habitats are not always effective in protecting organisms. Our study, in addition to some others, showed that severe disturbances may negatively affect benthic assemblages independently of habitat heterogeneity. Although in the present study we did not evaluate the effects of different intensities of disturbance, such as grazing, subtle hydraulic disturbances, and high-flow events, evidence from this study with benthic algae and other

studies with invertebrates allows us to infer that habitat heterogeneity may have a positive effect on the resistance and resilience of benthic assemblages during less-severe disturbances. We also observed a clear pattern of differences in the resistance and resilience of algal life forms, mostly related to biological traits that confer an ability to overcome disturbance. Further studies on habitat heterogeneity and disturbance should take into account the interaction among different intensities of disturbance and algal life forms, to disentangle the effects of habitat heterogeneity on benthic assemblages.

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Table 1 Summary of the results of repeated-measures ANOVA for the effects of substrate roughness on species richness (a) and log (density + 1) (b) of stream benthic algae after an experimental hydrological disturbance. *P* values were corrected using Huynh-Feldt method for repeated-measures ANOVA. Significant *post hoc* pairwise comparisons are shown. B = before, A = immediately after, 7A = 7 days after, 15A = 15 days after the disturbance. *P* values = < 0.05 are in bold.

	df	MS	<i>F</i>	<i>P</i>	Pairwise comparisons
(a) Richness					
Subject (Sj)	10	31.420			
Within-subjects					
Substrates (S)	1	585.557	144.339	< 0.001	Rough > Smooth
Sj x S	10	4.057			
Time (T)	3	140.830	15.426	< 0.001	(B, 7A, 15A) > A
Sj x T	30	9.130			
S x T	3	12.890	1.641	0.201	
Sj x S x T	30	7.857			
(b) Log (density + 1)					
Subject (Sj)	10	15.961			
Within-subjects					
Substrates (S)	1	0.444	1.233	0.293	
Sj x S	10	0.360			
Time (T)	3	3.873	7.518	0.003	B > A
Sj x T	30	0.515			
S x T	3	0.214	2.313	0.096	
Sj x S x T	30	0.093			

Table 2 Summary of the results of ANOVAS comparing resistance and resilience among algal life forms and substrate types (smooth x rough) after an experimental hydrological disturbance. *Post hoc* pairwise comparisons are shown for results with $P < 0.05$. Ad = adnate/prostrate, E = erect/stalked, F = filamentous, Me = metaphytic, Mo = motile life form. P values = < 0.008 (α' for Bonferroni correction) are in bold.

	df	MS	F	P	Pairwise comparisons
Qualitative data					
Resistance					
Algal life forms (Al)	4	0.448	17.546	< 0.001	(Ad, E) > (Mo, Me, F); Mo > Me
Substrates (S)	1	0.022	0.877	0.352	
Block	10	0.022			
Al x S	4	0.024	0.948	0.440	
Residuals	89	0.026			
Resilience after 7 days					
Algal life forms (Al)	4	0.295	13.503	< 0.001	(Ad, E) > (Mo, Me, F)
Substrates (S)	1	0.086	3.938	0.050	Rough > Smooth
Block	10	0.029			
Al x S	4	0.044	2.031	0.097	
Residuals	89	0.022			
Resilience after 15 days					
Algal life forms (Al)	4	0.401	17.052	< 0.001	(Ad, E) > (F, Me); Ad > Mo > Me
Substrates (S)	1	0.099	4.205	0.043	Rough > Smooth
Block	10	0.024			
Al x S	4	0.028	1.189	0.321	
Residuals	89	0.024			
Quantitative data					
Resistance					
Algal life forms (Al)	4	0.533	26.075	< 0.001	(Ad, E) > (Mo, Me, F); Mo > Me
Substrates (S)	1	0.048	2.330	0.131	
Block	10	0.019			
Al x S	4	0.012	0.599	0.664	
Residuals	89	0.020			
Resilience after 7 days					
Algal life forms (Al)	4	0.353	21.370	< 0.001	(Ad, E) > (Mo, Me, F); Mo > Me
Substrates (S)	1	0.105	6.372	0.013	Rough > Smooth
Block	10	0.016			
Al x S	4	0.040	2.407	0.055	
Residuals	89	0.017			
Resilience after 15 days					
Algal life forms (Al)	4	0.392	17.911	< 0.001	(Ad, E) > (F, Me); Ad > Mo > Me
Substrates (S)	1	0.097	4.447	0.038	Rough > Smooth
Block	10	0.019			
Al x S	4	0.028	1.271	0.287	
Residuals	89	0.022			

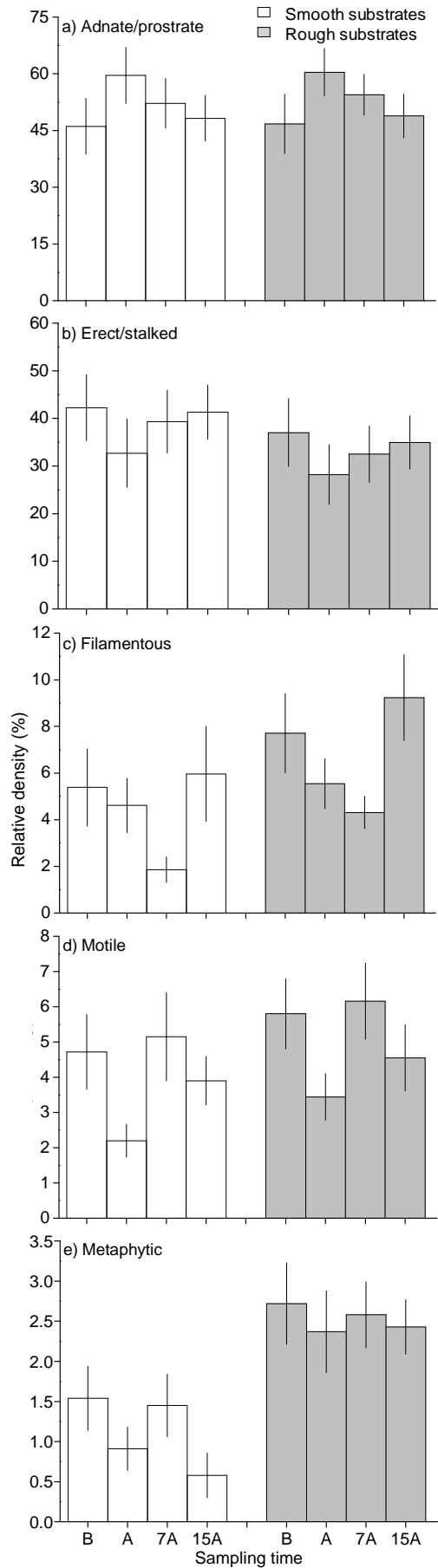


Fig. 1 Relative densities (mean \pm standard error) of adnate/prostrate (a), erect/stalked (b), filamentous (c), motile (d) and metaphytic (e) algal life forms on smooth and rough substrates at four sampling times during an experimental hydrological disturbance (B = before, A = immediately after, 7A = 7 days after, 15A = 15 days after the disturbance).

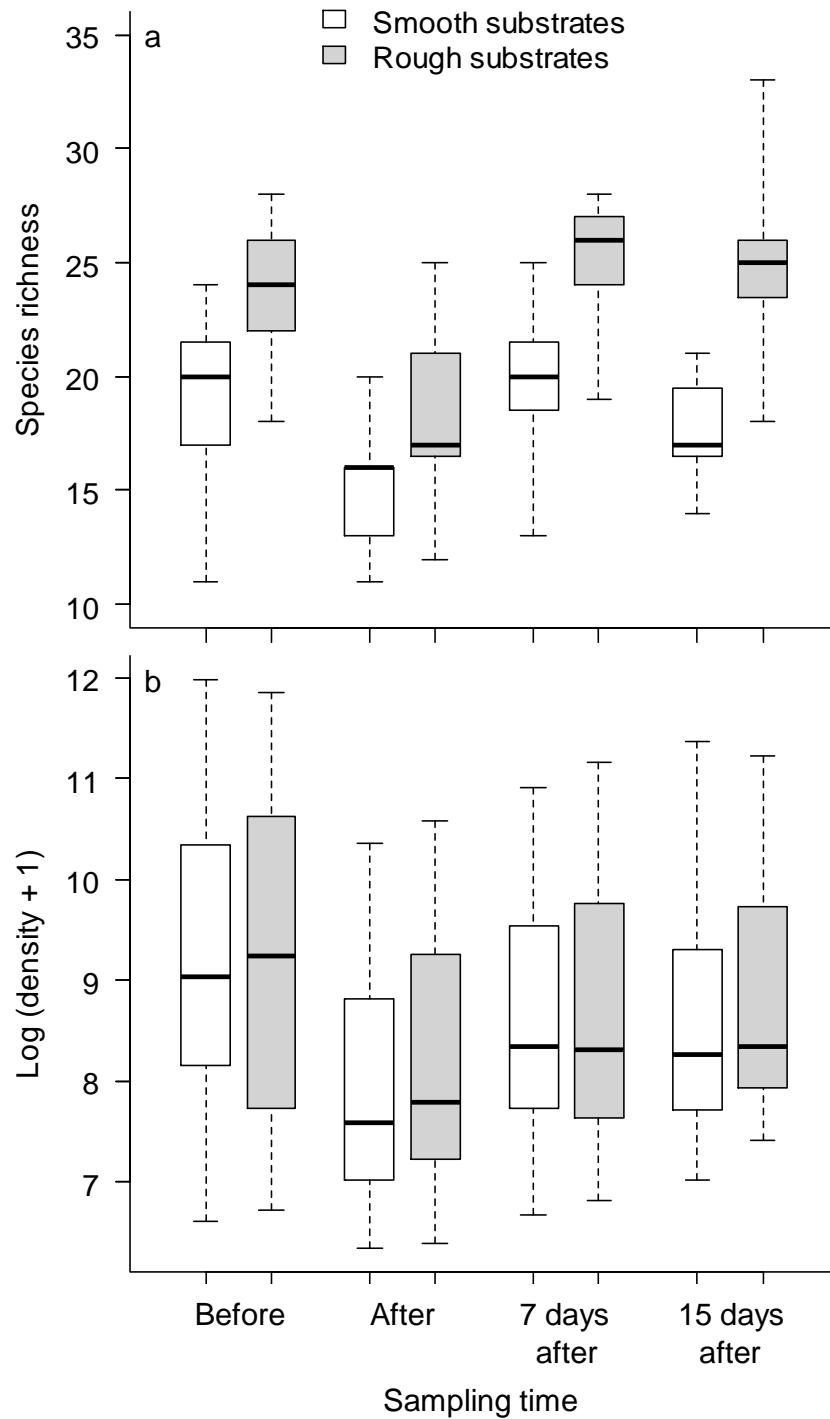


Fig. 2 Boxplots of species richness (a) and log (density + 1) (b) of stream benthic algae on smooth and rough substrates for four sampling times before and after an experimental hydrological disturbance. The heavy line represents the median, box ends are the first and third quartiles, and whiskers are the minimum and maximum values.

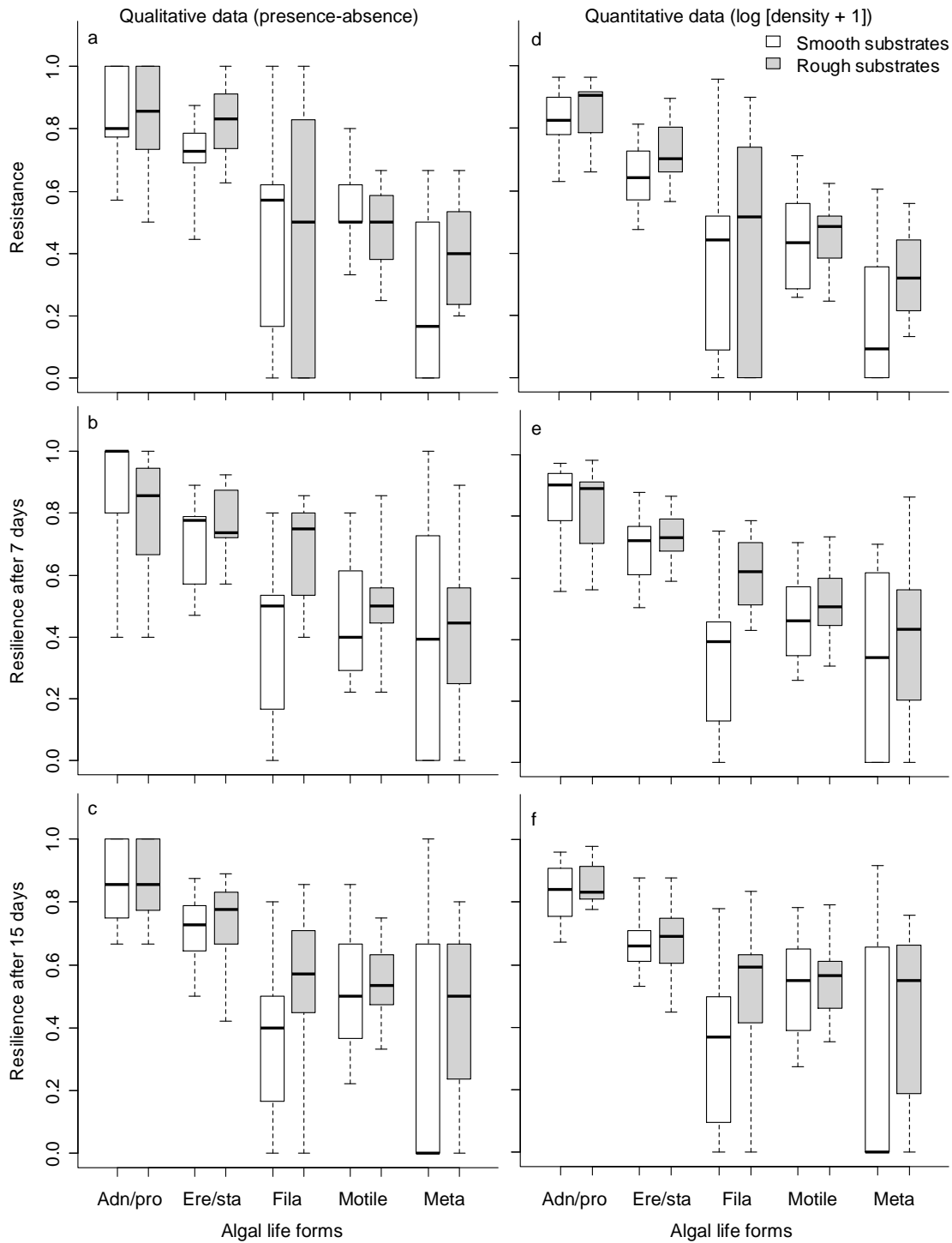


Fig. 3 Boxplots of the resistance and resilience of each algal life form on smooth and rough substrates. The indices were calculated for qualitative (presence-absence; a, b, c) and quantitative data (log [density +1]; d, e, f). The heavy line represents the median, box ends are the first and third quartiles, and whiskers are the minimum and maximum values. And/pro = adnate/prostrate, Ere/sta = erect/stalked, Fila = filamentous, Meta = metaphytic life form.

Capítulo 4

Mesohabitat type, grazers and substrate roughness interact to determine algal biomass and sediment accrual in a high-altitude subtropical stream⁵

Abstract

Benthic algae are the major constituents of the periphytic biofilm and are an important source of food in lotic ecosystems. In face of the increasing human perturbations of lotic ecosystems, it is of increasing importance to understand how key ecological factors, such as biotic interactions and habitat heterogeneity, affect the accrual and loss of algal biomass and sediment. We designed a field experiment to evaluate the independent and interacting effects of mesohabitat type (riffles and pools), grazing fish, and substrate roughness on chlorophyll *a*, ash free dry mass (AFDM) and total dry mass in a subtropical stream. We used electrical fences to exclude large grazers from smooth and rough substrates in pools and riffles during 8 d. Mesohabitat types did not affect the interaction strength between grazers and periphyton for the three response variables. However, mesohabitat types interacted with substrate roughness in the accrual of total dry mass. Rough substrates accumulated more total dry mass in pools than in riffles, while smooth substrates accumulated similar amounts of total dry mass in both mesohabitats. The accrual of AFDM and chlorophyll *a* was 3.1 and 1.4 times greater on rough than on smooth substrates, respectively, regardless of mesohabitat type. Exclusion treatments (without grazing fish) accrued 1.8 times more total dry mass, 2.2 times more ash free dry

⁵ Schneck, F., Schwarzbald, A. & Melo, A. S. Manuscrito redigido de acordo com as normas do periódico *Fundamental and Applied Limnology*.

mass, and 1.6 times more chlorophyll *a* than control treatments, showing that grazing fish play a major role in this stream by removing algal biomass and sediment. Additionally, there was a tendency toward an interaction between grazing fish and substrate roughness in the accrual of chlorophyll *a*, in which the difference between rough and smooth substrates was greater in control than in exclusion treatments, indicating the possible occurrence of a refuge effect on rough substrates. These results suggest that habitat homogenization and loss of large grazers may have strong and contrasting impacts on the accrual and loss of algal biomass and sediment in lotic ecosystems.

Key words: benthic algae, electrical exclusion, fish, interaction strength, periphyton

Introduction

Benthic algae are the major constituents of the periphytic biofilm and, in addition to heterotrophic microorganisms and organic sediments, are an important source of food in lotic ecosystems (Biggs 1996), and changes on its quantity and quality may affect the whole food web (Lowe & Pan 1996). Therefore, understanding how key ecological factors, such as biotic interactions and habitat heterogeneity, affect the accrual and loss of algal biomass, organic matter and sediment is of great importance in face of increasing human perturbations, which are simplifying habitats, altering the intensity of disturbances and causing the loss and homogenization of assemblages in lotic ecosystems (Rahel 2000, Cardinale et al. 2002).

Experimental studies have shown that the modification of habitats by grazers is a major mechanism structuring benthic communities in lotic ecosystems. For instance, the effects of grazers on the structure and productivity of streams and rivers have been documented for insects (Moulton et al. 2004, Álvarez & Peckarsky 2005), immature amphibians (Flecker et al. 1999), shrimps (Pringle & Blake 1994, Souza & Moulton 2005), and fish (Power 1990, Flecker 1996, Bertrand & Gido 2007). Grazers can affect benthic algae directly, by ingestion, and indirectly, by removing organic and inorganic sediments (Moulton et al. 2004). Benthic algal responses to grazing vary from changes in biomass to changes in composition, diversity, physiognomy, nutrient content and succession of communities (Feminella & Hawkins 1995, Steinman 1996).

Substrate roughness (a type of small-scale habitat heterogeneity, Bergey 2005) is an important ecological factor that strongly influences benthic algal biomass and sediment accrual in streams and likely mediates the effect of grazers. Rough substrates support higher algal biomass than smooth substrates (Bergey 2005, Murdock & Dodds 2007) and affect sediment retention (Bergey 1999, Taniguchi & Tokeshi 2004). Moreover, there is evidence of the efficacy of crevices on rough substrates as refuges for algae (Dudley & D'Antonio 1991,

Bergey & Weaver 2004). If crevices effectively protect algae from grazers, rough substrates should accumulate larger amounts of algal biomass than smooth substrates in the presence of grazers, while similar amounts of algal biomass should be accumulated on rough and smooth substrates in the absence of grazers.

In addition to grazers and substrate roughness, mesohabitat type should also affect directly and indirectly the accrual of algal biomass and organic and inorganic sediment. Distinct characteristics of pools and riffles, such as current velocity and retention of organic matter and inorganic sediment (Allan & Castillo 2007), may affect algal growth on rough and smooth substrates. Accordingly, we could expect hard substrates to accumulate more sediment in pools than in riffles and thus negatively affect algal growth. However, the distinct environmental conditions of pools and riffles may affect the interaction strength between grazers and the periphytic biofilm. For instance, Poff & Ward (1995) found that the interaction strength of a grazing caddisfly varied with current velocity. The caddisfly reduced algal biomass and altered algal assemblage composition at low velocities, but had only weak effects under high velocity regimes. Similarly, the detritivorous fish *Prochilodus mariae* Eigenmann strongly influenced sediment accrual in pools, but it had no influence in riffles in a tropical Andean stream (Flecker 1997).

We experimentally excluded large grazers (fish) from smooth and rough substrates used for algal colonization in pools and riffles. We evaluated the independent and interacting effects of mesohabitat type, large grazers, and substrate roughness on periphyton (algal biomass, organic matter, and total organic and inorganic mass). We hypothesized that the interaction strength between grazers and periphyton would be mediated by mesohabitat types and by substrate roughness. Additionally, we hypothesized that the effect of substrate roughness would be mediated by mesohabitat types. We predicted that grazers would have higher grazing impact in pools than in riffles, and that in the absence of large grazers, pools

would retain more biomass and total dry mass than riffles. We also predicted that in the presence of large grazers, crevices on rough substrates would act as refuges and protect algae, and thus rough substrates would accumulate more algal biomass than smooth substrates, while smooth and rough substrates would accumulate similar amount of algal biomass in the absence of grazers in the same mesohabitat type. For the last hypothesis we predicted that rough substrates would accumulate more organic matter and sediment in pools than in riffles, but the accrual on smooth substrates would not differ between mesohabitat types.

Materials and methods

Study area

We conducted the experiment in Marco stream (28°36'S; 49°54'W), São José dos Ausentes, state of Rio Grande do Sul, southern Brazil. The vegetation in the catchment is *Campos* grassland with patches of Araucaria Forest, and the climate is high-altitude subtropical (Cfb), with uniform precipitation throughout the year (Behling 2002). The study site is a fourth-order stream at approximately 1100 m asl that drains a catchment with low human impact and oligotrophic waters (Buckup et al. 2007). Stream width varies from 6 to 10 m, and the depth varies from 0.1 to 0.4 m in the reaches studied. The streambed is composed mostly of basaltic stones, boulders, and bedrock. During the period of the study the water was characterized by high dissolved oxygen concentrations (mean of 8.6 mg L⁻¹), low electrical conductivity (21 µS cm⁻¹) and slightly acidic pH (6.6). In pools, the current velocity varied from 0 to 7 cm s⁻¹, with a mean of 3.7 cm s⁻¹, while in riffles the velocity varied from 18 to 35 cm s⁻¹, with a mean of 25.6 cm s⁻¹.

The fish fauna of Marco stream is composed by 11 species, mostly of small Characiforms and Siluriforms (Winckler-Sosinski et al. 2009). Common grazers on algae and detritus in Marco stream include the armored catfishes (Loricariidae) *Rineloricaria* sp. and

Pareiorhaphis hystrix Pereira & Reis, and the Curimatidae *Steindachnerina biornata* (Bowen 1983, Winckler-Sosinski et al. 2009, Dias & Fialho 2011). The Poeciliidae *Cnesterodon brevirostratus* Rosa & Costa probably feeds mostly on detritus, as described for other species of the genera (e.g., Quintans et al. 2009). *Eurycheilichthys pantherinus* Reis & Schaefer is a common armored catfish in the stream, but differently of most Loricariidae, it was described as insectivore in Marco stream (Dias & Fialho 2011). The benthic algal assemblage is composed mostly of diatoms and some filamentous green algae (Schneck et al. 2011).

Experimental design

We designed a 3-factor split-split-plot field experiment with the following hierarchical treatments: i) mesohabitat, with two levels, pools and riffles (5 replications); ii) grazer occurrence, with presence or absence of fishes (10 replications); and iii) substrate roughness, with smooth and rough substrates (20 replications). We randomly selected 5 pools and 5 riffles along approximately 200 m in the stream reach. In each of these 10 locations we placed two flat paving stones (50 x 50 x 8 cm) that constituted the grazer factor, i.e., one stone was randomly assigned to receive the electrical exclusion treatment and the other to be the control. Each stone had smooth and rough acrylic substrates (5 × 5 cm) glued on it. The rough substrates had nine longitudinal crevices, each crevice with 1 mm width and 1 mm depth. The experiment consisted of 40 experimental units (2 substrate types × 2 grazer treatments × 10 locations) and each experimental unit consisted of two sampling units (acrylic substrates).

We excluded grazers using electrified fences that were constructed in a similar way as described by Landeiro et al. (2008). We used commercial fence electrifiers (Shock 8 Lite; JFL Equipamentos Eletrônicos Ind. Com. Ltda, Santa Rita do Sapucaí, MG, Brazil) labeled as having an electric pulse of 8000 V per second, 1.8 J, and maximum current of 1.6 A. Each electrifier was connected to a 12 V car battery. We fixed two copper wires on each 50 × 50 ×

8 cm flat paving stone. We fixed one of the wires on three sides of the stone (40 cm of wire on each side) and connected its ends to the exit and return connectors of the electrifier. The two parallel sides were stripped and acted as the negative electrodes. A second wire was also connected to the electrifier and its final 40 cm long stripped part was fixed between and parallel to the two stripped sides of the first wire. This second central wire acted as the positive electrode.

Since the electrifiers emit low-intensity pulses, they are able to exclude only large organisms. Landeiro et al. (2008) used an electrifier very similar as the one we used and were able to exclude organisms larger than 1 cm. In fact, during preliminary experiments, we observed that organisms larger than 1 cm were excluded. The electric shocks caused reactions in small fishes, which immediately left the electrified area. Macroinvertebrates were not excluded and species of Trichoptera, Ephemeroptera and Chironomidae were present on both electrified and control substrates (F. Schneck, personal observation). We considered that our exclusion treatment was effective against fish species, including grazers such as the small armored catfishes and poeciliids present in the studied stream.

We constructed the wire fences on all stones used for the experiment (i.e., in exclusion and control treatments), and placed the pair of stones approximately 50 cm apart. We placed the stones with the fences and artificial substrates in the stream in the end of September 2010 for algal colonization. The exclusion experiment started in 01 November 2010 when we randomly assigned one stone of the pair to be connected to the electrifier at each of the 10 locations. Sampling was carried out in 08 November. Biomass of periphyton and inorganic sediment accrual were visually higher on exclusion treatments than on control treatments after 7 d.

Sampling and laboratory analyses

We removed two sampling units of each substrate type per stone. We brushed the upper surfaces of the substrates using a toothbrush to remove the periphytic material, adjusted the samples to a defined volume (100 ml), and preserved them on ice in the dark until their processing in the laboratory (24-30 h after collection). In the laboratory each sample was divided into two subsamples for analyses of chlorophyll *a* (50 ml) and periphyton dry mass (50 ml). We determined chlorophyll *a* by extracting pigments with ethanol after filtration through a Whatman GF/C filter, followed by spectrophotometrically measuring chlorophyll *a* according to standard procedures (Biggs & Kilroy 2000).

For dry mass determination, we transferred the subsamples to pre-combusted and pre-weighted porcelain crucibles. We dried the samples at 70 °C until constant weight (total dry mass) and then burned the organic content in a muffle furnace at 500 °C for 3 h to estimate ash free dry mass (AFDM) (Schwarzbald 1990). We calculated the Autotrophic Index (AFDM/chlorophyll *a*) to determine the trophic status of the periphyton. The index usually ranges from 50 to 200 for an autotrophic periphyton, while larger ratios indicate a heterotrophic periphyton (Steinman et al. 2007). We also calculated the indices proposed by Lakatos (1989) based on total dry mass (low mass: < 2 mg cm⁻²; medium: 2-4; or high: > 4), on the proportions of organic and inorganic matter (inorganic periphyton: > 75% of ashes in relation to total dry mass; inorganic-organic: 50-75%; organic-inorganic: 25-50%; or organic: < 25%), and on the proportions of autotrophic and heterotrophic components (autotrophic periphyton: > 0.60% of chlorophyll *a* in relation to total dry mass; auto-heterotrophic: 0.25-0.60%; hetero-autotrophic: 0.10-0.25%; or heterotrophic: < 0.10%).

Data analysis

We used a 3-factor split-split-plot Analyses of Variance (ANOVAs) to test for the effects of treatments on total dry mass, AFDM, and chlorophyll *a*. Data were log-transformed to meet the assumptions of homogeneity of variance and normality of residuals. The 10 locations (5 riffles, 5 pools) were considered the main-plot (blocks), the grazer factor was the sub-plot (flat stones), and the substrate factor was the sub-sub-plot (acrylic substrates). Mesohabitat type was included in the model as a between-plot factor. The model used in the analyses was as follows: $\log(\text{response variable}) \sim (\text{mesohabitat} + \text{grazer} + \text{substrates})^3 + \text{error}(\text{blocks/grazer})$. Analyses were carried out in the R statistical environment using the function *aov* (R Development Core Team, 2010).

Results

The periphyton was mainly heterotrophic, since the Autotrophic Index ranged from 305 to 13,000, with an average of 3,812. According to the classification of Lakatos (1989), the periphytic biofilm had mostly low to medium amounts of biomass, was inorganic-organic to inorganic, and was heterotrophic.

Total dry mass accrual was dependent on the interaction between mesohabitat and substrates ($P = 0.037$; Table 1). Total dry mass on smooth substrates was similar between pools and riffles, while on rough substrates the accrual of total dry mass was 2.6 times greater in pools than in riffles (Figure 1a). On the other hand, the interactions between mesohabitat and grazers and between grazers and substrates were not significant (Table 1). The amount of total dry mass increased 1.8 times in the exclusion treatment in relation to the control, independently of mesohabitat and substrate type ($P = 0.001$; Table 1; Figure 1a).

No interaction term was important for ash free dry mass (Table 1). Exclusion treatments accrued 2.2 times more AFDM than control treatments ($P = 0.023$; Table 1; Figure

1b), and rough substrates accumulated 3.1 times more AFDM than smooth substrates ($P < 0.001$; Table 1; Figure 1b).

There were no significant interactions in the determination of the amounts of chlorophyll *a* between treatments (Table 1). Chlorophyll *a* was 1.6 times greater in the exclusion treatment than in the control ($P = 0.005$; Table 1), and 1.4 times greater on rough than on smooth substrates ($P = 0.006$; Table 1) (Figure 1c). However, it is worth noting that, despite not significant, there was a tendency toward an interaction between presence/absence of fishes and substrate types (Table 1; Figure 1c). While rough substrates accumulated on average only 1.2 times more chlorophyll *a* than smooth substrates in exclusion treatments, in control treatments the difference between rough and smooth substrates reached 1.9 times on average (Figure 1c).

Discussion

Our hypothesis that distinct environmental condition of pools and riffles would mediate the interaction strength between grazers and periphyton was not supported, as shown by the non-significant interaction terms between mesohabitat type and grazers for all response variables. This result is unexpected, since pools and riffles have distinct environmental conditions that would be expected to influence the performance of grazers and, consequently, regulate ecological processes like sediment and biomass accrual (e.g., Flecker 1997). However, there are two possible explanations for this result. First, Marco stream is a cold and oligotrophic stream that flows along a grassland plateau, characteristics that have a negative influence on both primary production and allochthonous organic matter input (as shown by the Autotrophic Index and the classification of Lakatos (1989)). Therefore, the scarce availability of food resources for grazers could force them to forage at different mesohabitats. Second, our electrical exclusion treatment excluded organisms larger than 1 cm. This non-selective

removal of all large organisms may have resulted in the exclusion of grazers with different habitat preferences. For instance, Buck & Sazima (1995) observed that four species of armored catfishes occupied different habitats within a stream in southeastern Brazil, from shallow and slow water areas to deep and fast flowing places.

The only significant effect observed for mesohabitat was its positive interaction with substrate roughness in the accrual of total dry mass, reflecting the synergistic effect of higher rates of sediment deposition in pools (Allan & Castillo 2007) and the competence of rough substrates to retain greater amounts of organic matter and sediment than smooth substrates (e.g., Taniguchi & Tokeshi 2004). Most of this mass accumulated on rough substrates in pools was inorganic sediment (the difference between total dry mass and AFDM) which could have imposed negative impacts on algal biomass. However, mesohabitat had no effect on the accrual of algal biomass, as shown by the results on AFDM and chlorophyll *a*.

The evident patterns of higher algal biomass, organic matter and total dry mass in exclusion than in control treatments indicate that grazing fish play a major role in the studied stream by consuming algae and detritus and/or by removing sediment. This strong interaction has been reported in Neotropical streams, in which different organisms, such as a detritivorous fish in Venezuela (Flecker 1996), armored catfishes in Panama (Power 1990), shrimps in Puerto Rico and Brazil (Pringle and Blake 1994, Souza & Moulton 2005), and baetid mayflies in Brazil (Moulton et al. 2004), act as keystone species or ecosystem engineers (*sensu* Jones et al. 1994). In this context, our results add important informations on processes affecting the functioning of high-altitudinal subtropical streams.

A second strong result found in this study was the greater accrual of algal biomass and AFDM on rough than on smooth substrates, a pattern usually attributed to the greater availability of refuges on rough substrates (e.g., Bergey & Weaver 2004, Bergey 2005). In fact, we found that this result was not dependent on the absence or presence of grazing fish.

However, despite not statistically significant, the larger difference in algal biomass between rough and smooth substrates in the presence than in the absence of fish is probably ecologically significant in this stream in which most of the periphytic matrix is inorganic and heterotrophic, allowing us to suggest that refuge crevices may be important at least for part of the algal assemblage. This result is consistent with two previous works in which we suggested the occurrence of a refuge effect on rough substrates (Schneck et al. 2011, Schneck & Melo unpublished manuscript). It is also important to consider that the experiment excluded only large organisms including fish that prey on invertebrates, but did not exclude grazing insects. Grazing insects can contribute in the removal of large amounts of algal biomass. For instance, Barbee (2005) excluded only insects and found that they removed 30% more algal biomass on control than on exclusion treatments, while common grazing fish species did not contribute to differences between treatments. Also, invertebrate grazers could have been benefited by the exclusion of predatory fish, thereby removing a greater amount of biomass and diminishing the difference between exclusion and control treatments. Similar trophic cascades have been suggested to occur in some Neotropical streams, with shrimps inhibiting the activity of grazing baetid mayflies (Moulton et al. 2004) and fishes negatively affecting shrimps and grazing baetid mayflies (Moulton et al. 2010). Unfortunately, we do not have quantitative data on invertebrates to test this hypothesis, but it was evident during the experiment that larvae of caddisflies and midges were more abundant on exclusion than on control treatments (F. Schneck, personal observation).

Our results showed that mesohabitats, grazing fish and substrate roughness are key ecological factors in the studied subtropical stream, determining the accrual and loss of algal biomass and sediment. Their independent and interacting effects are not simple and even subtle changes on biotic interactions and on habitat heterogeneity (here measured at the mesohabitat and substrate roughness scales) may have strong impacts on ecosystem

properties. This study suggests that the loss of grazers may cause the accumulation of large amounts of organic and inorganic mass, which may negatively affect the structure of habitats. On the other hand, the simplification of habitats, even at the small-scale of substrate roughness, may diminish the availability of food resources by diminishing algal biomass accrual and retention of organic matter. Therefore, understanding the effects of these key factors may have important implications for management and conservation efforts.

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Table 1. Summary of the results of split-split-plot ANOVAs for the effects of mesohabitat (pools and riffles), large grazers (exclusion and control), and substrate roughness (smooth and rough) on total dry mass (a), ash free dry mass (AFDM) (b), and chlorophyll *a* (c). *P* values < 0.05 are in bold.

	df	MS	<i>F</i>	<i>P</i>
(a) Total dry mass				
Error (Block)				
Mesohabitat (Me)	1	3.591	5.389	0.049
Residuals	8	0.666		
Error (Block/Grazers)				
Grazers (Gr)	1	5.118	24.702	0.001
Me x Gr	1	0.001	0.005	0.945
Residuals	8	0.207		
Error (Within)				
Substrates (Su)	1	33.475	213.399	<0.001
Me x Su	1	0.809	5.159	0.037
Gr x Su	1	0.170	1.085	0.313
Me x Gr x Su	1	0.041	0.264	0.614
Residuals	16	0.157		
(b) AFDM				
Error (Block)				
Mesohabitat (Me)	1	0.312	0.635	0.449
Residuals	8	0.491		
Error (Block/Grazers)				
Grazers (Gr)	1	5.276	7.875	0.023
Me x Gr	1	0.155	0.232	0.643
Residuals	8	0.670		
Error (Within)				
Substrates (Su)	1	12.511	29.875	<0.001
Me x Su	1	0.102	0.245	0.628
Gr x Su	1	0.261	0.624	0.441
Me x Gr x Su	1	0.016	0.038	0.847
Residuals	16	0.419		
(c) Chlorophyll <i>a</i>				
Error (Block)				
Mesohabitat (Me)	1	0.116	0.128	0.730
Residuals	8	0.907		
Error (Block/Grazers)				
Grazers (Gr)	1	2.962	14.615	0.005
Me x Gr	1	0.301	1.486	0.258
Residuals	8	0.203		
Error (Within)				
Substrates (Su)	1	1.992	9.962	0.006
Me x Su	1	0.001	0.007	0.936
Gr x Su	1	0.593	2.967	0.104
Me x Gr x Su	1	0.101	0.503	0.488
Residuals	16	0.200		

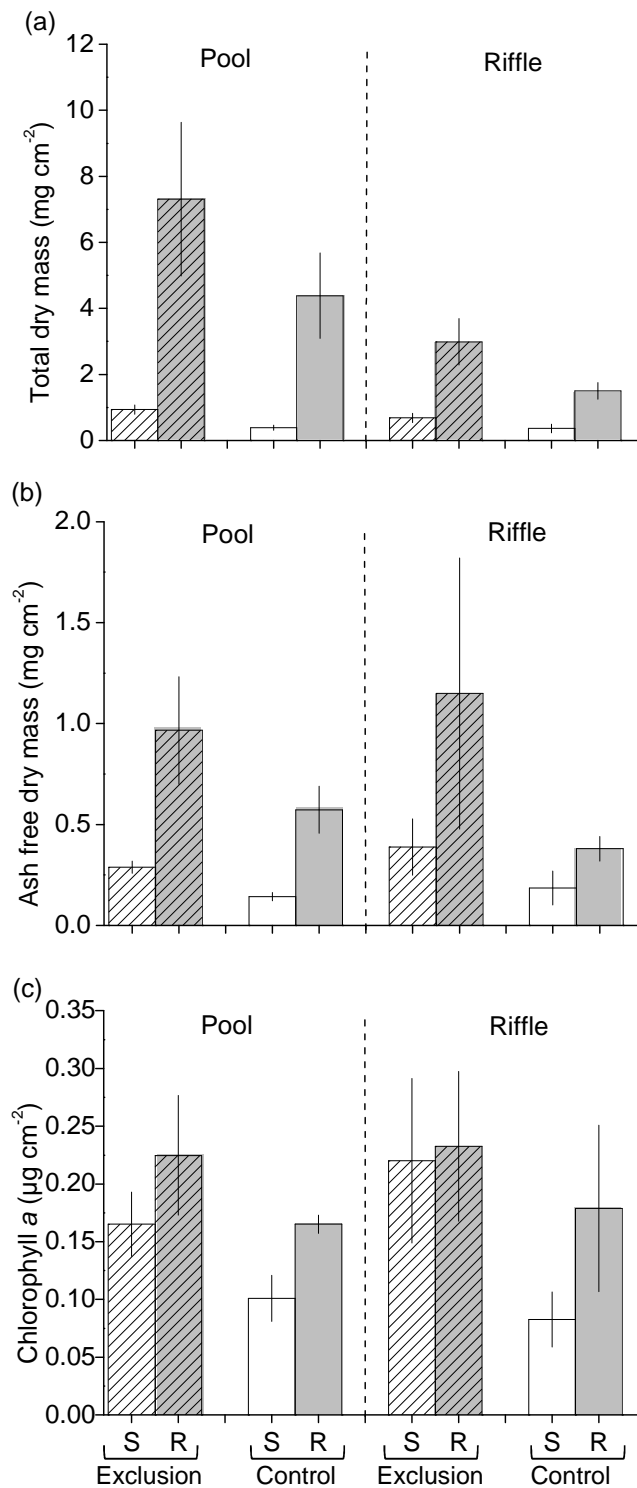


Figure 1. Mean (\pm standard error) of total dry mass (a), ash free dry mass (b), and chlorophyll *a* (c) in pools and riffles (separated by a dashed line), in exclusion (hatched bars) and control (non-hatched bars) treatments for large grazers, and on smooth (S, white) and rough (R, grey) substrates.

Considerações finais

Na presente tese abordei especificamente a importância da heterogeneidade de habitat na escala de rugosidade de substratos, denominada por Bergey & Resh (2006) como *small-scale spatial heterogeneity*, escala esta apropriada para a utilização de algas bênticas como organismos modelo (ver Apêndice 2 para a lista de algas encontradas). Em linhas gerais, os resultados foram condizentes com as hipóteses que formulei para cada experimento. Mais importante, os resultados obtidos nos diferentes capítulos desta tese estão interligados e se complementam, gerando um panorama geral dos efeitos da rugosidade de substratos na estruturação de assembleias de algas bênticas em riachos.

Os resultados do Capítulo 1 mostram que a assembleia de algas responde positivamente à rugosidade, com um aumento pronunciado na riqueza de espécies. Por outro lado, os efeitos positivos na riqueza e densidade são dependentes da forma de vida de diferentes grupos de algas, sendo que grupos mais suscetíveis a distúrbios foram exatamente os mais beneficiados pela ocorrência de fendas nos substratos rugosos. O primeiro resultado corrobora a hipótese de uma relação positiva entre heterogeneidade de habitats e diversidade mesmo em uma escala fina. Já o segundo resultado vai mais adiante ao demonstrar que o efeito da heterogeneidade de habitats pode variar de acordo com os atributos biológicos dos organismos estudados. Este segundo resultado tem consequências práticas importantes frente às crescentes perturbações antrópicas em riachos, uma vez que existe, por exemplo, a partição de recursos por grupos funcionais de invertebrados, que se alimentam de diferentes estratos do biofilme perifítico, selecionando assim algas com diferentes formas de vida (e.g., Tall et al. 2006). Desta forma, alterações na composição de algas no biofilme perifítico poderiam vir a alterar toda a cadeia trófica do ecossistema. Outro resultado inédito que apresento nesta

tese é o efeito da rugosidade de substratos na distribuição de espécies, com a ocorrência simultânea de dois mecanismos determinando a distribuição de espécies em substratos lisos e rugosos: *turnover* e aninhamento.

Além de aumentar a coexistência de espécies e afetar a composição e distribuição de espécies, a heterogeneidade de habitats pode também diminuir a variabilidade temporal de assembleias, como demonstrado no Capítulo 2. O ponto fundamental aqui é que, diferentemente de estudos anteriores, foi possível atribuir o resultado à rugosidade *per se*, uma vez que foram excluídos outros mecanismos que também poderiam ser responsáveis por esse efeito, como a estabilidade física do habitat e a maior riqueza de espécies em substratos rugosos. Este resultado, conjuntamente com os resultados encontrados no Capítulo 1, permitiu sugerir que as fendas nos substratos rugosos serviram efetivamente como refúgios para algumas populações de algas bênticas, estendendo para escalas finas e para algas bênticas a ideia de que habitats heterogêneos protegem um maior número de organismos contra distúrbios e predação/herbivoria em função da maior disponibilidade de refúgios.

Mas será que o efeito ‘protetor’ dos refúgios sobre a assembleia algal ocorre também em situações de distúrbios severos? Levando em conta os resultados apresentados nos Capítulos 1 e 2, poderíamos esperar que assembleias ocorrentes em substratos rugosos fossem mais resistentes e resilientes que assembleias ocorrentes em substratos lisos, pois no primeiro caso um maior número de organismos estaria protegido em refúgios. Porém, a interação entre distúrbios e heterogeneidade de habitats não se mostrou tão clara e simples, uma vez que no estudo apresentado no Capítulo 3 as assembleias ocorrentes em substratos lisos e rugosos não diferiram na resistência e resiliência frente a um distúrbio hidrológico experimental. Ou seja, a resposta para a pergunta no início deste parágrafo é negativa. Os resultados obtidos permitiram demonstrar que habitats heterogêneos podem não ser efetivos na proteção de organismos, contrariando em parte as evidências existentes na literatura de que refúgios

protegem organismos contra distúrbios hidrológicos. As evidências obtidas nos trabalhos apresentados nesta tese (Capítulos 2 e 3) e em alguns estudos de outros autores (e.g., Melo et al. 2003; Brown 2007), permitem levantar a hipótese de que o efeito positivo da heterogeneidade de habitats sobre a persistência de assembleias bênticas é dependente da intensidade do distúrbio. Durante períodos em que não há distúrbios hidráulicos severos, assembleias em substratos rugosos foram mais persistentes, provavelmente em decorrência do maior número de refúgios que protegeram os organismos frente a distúrbios de menor magnitude, sejam estes hidráulicos ou de pastejo (Capítulo 2). Já em uma situação de um distúrbio hidrológico de maior magnitude, como o distúrbio experimental aplicado no estudo do Capítulo 3, as fendas nos substratos rugosos não foram eficazes como refúgios.

A rugosidade de substratos exerce efeito não somente na composição e estrutura das assembleias de algas bênticas, mas também nas interações bióticas e em propriedades dos ecossistemas, através, por exemplo, de alterações na biomassa algal e no acúmulo de sedimentos, como demonstrado no Capítulo 4. Os resultados mostraram que peixes pastejadores exercem papel-chave no ecossistema estudado, removendo biomassa algal e sedimentos, e assim, modificando o habitat e provavelmente influenciando a estruturação de assembleias de algas e também de invertebrados bênticos. Além disso, substratos rugosos acumularam maiores quantidades de biomassa algal e matéria orgânica que substratos lisos tanto em remansos como em corredeiras, porém, uma maior quantidade de massa seca total (orgânica e inorgânica) em substratos rugosos foi observada somente em remansos, não havendo diferença entre substratos em corredeiras. Este resultado evidencia a importância da heterogeneidade de habitats tanto na escala da rugosidade de substratos como na escala de mesohabitats, sugerindo que a homogeneização de habitats pode diminuir a disponibilidade e variabilidade espacial de recursos alimentares para organismos pastejadores. Por fim, sugiro a ocorrência de um efeito de refúgio em substratos rugosos, uma vez que a diferença de

biomassa algal entre os tipos de substratos apresentou uma tendência a ser maior na presença que na ausência de peixes. Esta afirmação é consistente com os resultados apresentados nos Capítulos 1 e 2 e agrega importante informação aos resultados obtidos no Capítulo 3.

Os resultados obtidos nesta tese indicam que a rugosidade de substratos é um fator-chave na organização de assembleias de algas bênticas, influenciando uma série de atributos, como biomassa, riqueza de espécies, composição, padrões de distribuição, variabilidade temporal e resistência e resiliência frente a distúrbios hidrológicos e bióticos. O mecanismo mais plausível para explicar os resultados obtidos é o efeito de refúgio das fendas em substratos rugosos. Há uma tendência dos refúgios protegerem parte da assembleia algal frente a distúrbios hidrológicos e ao pastejo por peixes (Capítulos 3 e 4). Já em situações com fluxo basal e com a presença natural de peixes, as evidências claras de maior riqueza de espécies, maior densidade de grupos suscetíveis a distúrbios, maior persistência e maior biomassa algal (Capítulos 1, 2 e 4) em substratos rugosos também são fortes indicadoras de que a maior disponibilidade de refúgios nestes substratos é responsável pelos resultados observados.

As informações contidas nos trabalhos que compõem esta tese contribuem tanto sob o ponto de vista teórico como prático. No primeiro caso, foram apresentados resultados inéditos, levantadas novas hipóteses e preenchidas lacunas quanto ao conhecimento dos efeitos da heterogeneidade de habitats na escala da rugosidade de substratos sobre um importante componente da comunidade de ambientes lóticos, as algas bênticas. Sob o ponto de vista prático, os resultados desta tese contribuem para o entendimento dos efeitos da crescente simplificação de habitats e perda de espécies em ecossistemas aquáticos, com implicações não somente sobre a diversidade, mas também sobre a dinâmica temporal de assembleias aquáticas e sobre interações bióticas. A manutenção da heterogeneidade de habitats nas mais

diversas escalas, incluindo escalas finas como a rugosidade de substratos, poderá contribuir na manutenção da diversidade e da variabilidade natural das assembleias de ambientes lóticos.

Finalizando, é importante ressaltar que muitas questões permanecem em aberto, como por exemplo: Quais os efeitos da homogeneização de habitats na mesma escala do presente estudo (i.e., rugosidade de substratos) sobre a cadeia trófica? Outros grupos de organismos respondem de forma semelhante à heterogeneidade de habitats quanto à variabilidade temporal? Como se dá a interação entre diferentes tipos de distúrbios, tamanho de fendas e grupos de algas com distintas formas de vida? A partir de que ponto um distúrbio passa a ser suficientemente severo para anular o efeito de refúgios? Será que em situações de distúrbios severos a estabilidade física do habitat (ou de substratos) é mais importante que a heterogeneidade (ou rugosidade)? Desta forma, os estudos apresentados nesta tese de forma alguma esgotam as questões referentes aos efeitos que a heterogeneidade de habitat exerce sobre a biota nas mais diversas escalas.

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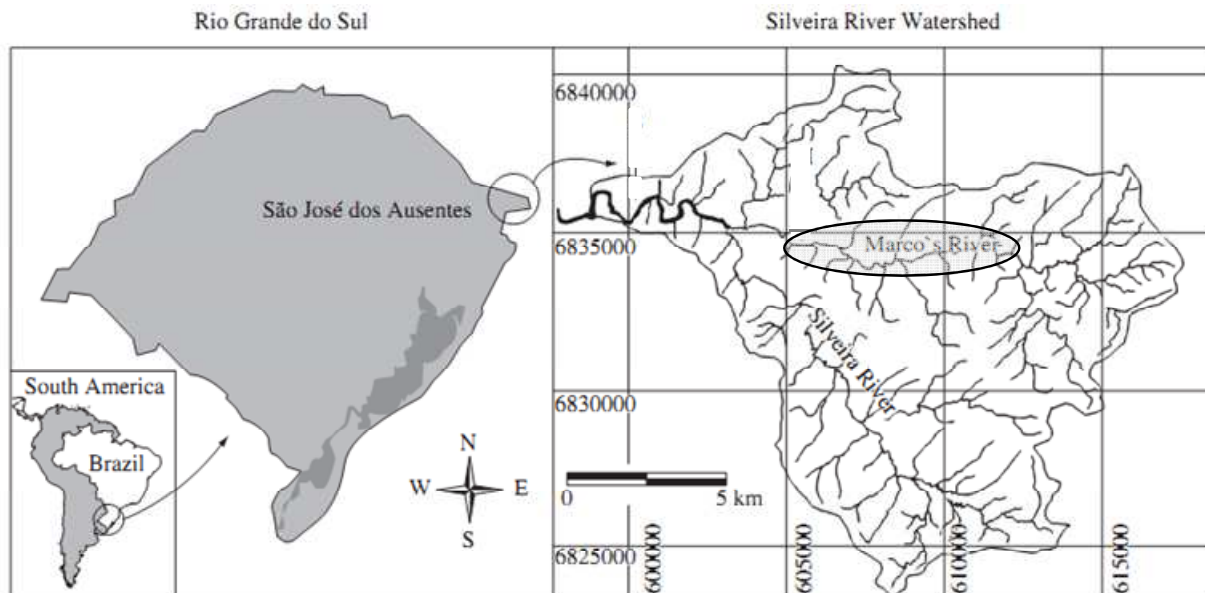
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Apêndice 1

Mapa da área de estudo. Rio do Marco, São José dos Ausentes, Brasil.



Fonte: Winckler-Sosinski, L. T., Silveira, T. C. L., Schulz, U. H. & Schwarzbald, A. 2008. Interactions between benthic macroinvertebrates and fishes in a low order stream in Campos de Cima da Serra, RS, Brazil. *Brazilian Journal of Biology*, 68: 695-701.

Continuação.

Taxa	Exp. 1 (Caps. 1 e 2)		Exp. 2 (Cap. 3)							
	Substrato liso	Substrato rugoso	Substrato liso				Substrato rugoso			
			C	Após	7d	15d	C	Após	7d	15d
NAVICULALES										
AMPHIPLURACEAE										
<i>Frustulia crassinervia</i> (Brèbisson) Lange-Bertalot & Krammer	X	X	X		X			X		X
<i>Frustulia</i> sp.	X	X	X	X	X			X	X	X
DIADESMIDACEAE										
<i>Luticola costei</i> Metzeltin & Lange-Bertalot	X	X	X							
<i>Luticola</i> sp.1										X
NAVICULACEAE										
<i>Navicula angusta</i> Grunow	X	X			X	X		X	X	X
<i>Navicula cryptocephala</i> Kützing	X	X	X	X	X	X		X	X	X
<i>Navicula cryptotenella</i> Lange- Bertalot	X	X	X		X	X		X	X	X
<i>Navicula</i> sp.1	X	X							X	X
<i>Navicula</i> sp.2	X	X	X	X	X	X		X	X	X
<i>Navicula</i> sp.3	X	X							X	
<i>Navicula</i> sp.4	X	X	X	X	X			X	X	X
NEIDIACEAE										
<i>Neidium</i> sp.1									X	X
<i>Neidium</i> sp.2	X	X						X		
PINNULARIACEAE										
<i>Pinnularia</i> cf. <i>microstauron</i> (Ehrenberg) Cleve	X	X	X					X		X
<i>Pinnularia</i> sp.1	X				X					
<i>Pinnularia</i> sp.2		X								
<i>Pinnularia subcapitata</i> Gregory		X								
SELLAPHORACEAE										
<i>Sellaphora</i> sp.					X					
STAURONEIDACEAE										
<i>Stauroneis</i> sp.	X	X	X						X	
RHOPALODIALES										
RHOPALODIACEAE										
<i>Epithemia</i> sp.	X	X							X	
<i>Rhopalodia operculata</i> (Agardh) Håkanasson										X
SURIRELLALES										
SURIRELLACEAE										
<i>Surirella angusta</i> Kützing	X	X								
<i>Surirella</i> sp.1		X						X		
<i>Surirella</i> sp.2	X	X							X	X
<i>Surirella tenera</i> Gregory	X	X						X		

Apêndice 3

Principais bibliografias utilizadas para a identificação das algas.

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