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Estudos sobre estimadores de riqueza de espécies, perturbações experimentais e persistência ao longo de cinco anos em comunidades de macroinvertebrados bentônicos em riachos

Este exemplar corresponde à redação final da tese defendida pelo(a) candidato (a) ADRIANO SANCHES MELO.

e aprovada pela Comissão Julgadora.

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para obtenção do título de doutor em Ecologia.

Orientador: Dr. Claudio Gilberto Froehlich

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Primeiro um som
interior
dentro da terra um rio quer nascer
o mato ri
o homem ri
e a terra chora água ao parir
...

Dois rios, Fernando Brant

Aos meus pais, por mostrarem a trilha certa.

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Técnica. Estou satisfeito com os resultados apresentados nesta tese e em parte isto é devido a existência e facilidade no uso de tais recursos financeiros. Os trabalhos sobre colonização e rolagem de pedras em Intervales e o experimento na Nova Zelândia certamente não teriam sido realizados sem tais recursos. Talvez de maior importância ainda do que estes três trabalhos, seja a experiência profissional obtida no estágio de cinco meses na Nova Zelândia. Durante esta viagem, foi gratificante perceber que durante meu doutorado tive melhores condições de trabalho do que os alunos do laboratório na Nova Zelândia. Diferente dos alunos de lá, nunca precisei compartilhar computador, impressora etc, nunca deixei de ir ao campo por falta de diárias e o mais surpreendente, pude fazer um estágio no exterior sem ter que passar por demoradas "burocracias"; apenas enviei à FAPESP um pedido simples de duas paginas! Espero que FAPESP continue com este programa.

Sumário

Resumo

Foram abordados diversos aspectos de estimadores de riqueza de espécies e o papel de perturbações em riachos. Numa avaliação sobre estimadores de riqueza na comunidade, todos estimadores foram dependentes do tamanho amostral (Cap. 1). Tal dependência é em parte devido à não estabilização da curva de acumulação de espécies raras. Concluo que o uso de tais métodos na estimativa de riqueza de espécies numa área não é confiável (Cap. 2). Apesar da ausência de métodos confiáveis para a estimativa de riqueza na comunidade, mostro que outros métodos podem ser usados na estimativa de riqueza em tamanhos amostrais maiores (Cap. 3). Fiz dois experimentos para investigar a resposta de comunidades de macroinvertebrados a perturbações experimentais. No primeiro, feito na Nova Zelândia, comunidades de riachos em pastagens não diferiram daqueles em campos de vegetação nativa (Cap. 4). Houve redução da abundância total dos organismos, mas subsequente recuperação em oito dias. A riqueza de espécies permaneceu estável. Logo após a perturbação houve aumento da agregação da comunidade. No segundo experimento, pedras no leito de três riachos foram perturbadas (Cap. 5). Foi testada a hipótese de que a recuperação dos níveis de abundância e riqueza deveriam ocorrer com maior rapidez no riacho de tamanho intermediário. Os padrões de colonização foram semelhantes entre os três riachos, rejeitando a hipótese de trabalho. Como medida de perturbação causada por enchentes, fiz um experimento com rolagem de pedras marcadas em cinco riachos (Cap. 6). A frequência de rolagem foi semelhante entre os riachos, embora o enterramento de pedras tenha sido mais frequente no riacho menor, de 1ª ordem. Num estudo de persistência da comunidade de invertebrados durante cinco anos, houve maior concordância entre amostras coletadas na época seca do que naquelas coletadas na época chuvosa, quando enchentes são comuns (Cap. 7). Foi testada a hipótese de que a variabilidade da comunidade aumenta com o tempo. Tal hipótese foi confirmada, embora o aumento da variabilidade tenha sido muito maior nas amostras coletadas no verão.

Abstract

In this thesis I address topics of species richness estimation and the role of disturbance in streams. In an evaluation of species richness estimators, all methods were dependent on sample size. Such dependence is in part due to the non-stabilization of the curve of accumulation of rare species. The results indicate that estimates of species richness in an area are not reliable, although they might be useful for comparative purposes. Despite the lack of reliable methods to estimate species richness in an area, in a second evaluation work I show that several methods can be used to estimate species richness in extrapolated sample sizes. The main application of such methods would be in the standardization of different sample sizes. Two experiments were carried out to investigate the response of stream macroinvertebrate communities to experimental disturbance. In the first study, done in New Zealand, response of communities in streams draining pastures did not differ from that observed in streams draining native tussock fields. There was a decrease in abundance following the disturbance event. Eight days after the disturbance event, abundance was similar to control levels. Species richness remained unchanged. Immediately after the disturbance event there was an increase in patchiness of the stream communities. In the second experiment, stones in three streams differing in size were disturbed. I tested the hypothesis that recovery would be fastest in the medium-sized stream. The colonization patterns were similar in all three streams, causing the rejection of the stated hypothesis. In a study of persistence of invertebrate communities inhabiting five streams during five years, it was observed that winter (dry season) samples were more concordant over years than summer (rainy season) samples. The hypothesis that community variability increases with time was tested. The hypothesis was supported, although the increase in variability was much more conspicuous for summer than for winter communities.

Introdução geral

Naturalistas europeus durante o século XIX ficaram perplexos com a diversidade de espécies encontradas em suas viagens aos trópicos. De fato a maior quantidade de espécies em áreas tropicais é um dos padrões mais evidentes em Ecologia e, com poucas exceções, é observado nos mais diferentes grupos animais e vegetais (MacArthur 1972). Até cerca de 30 anos atrás, no entanto, acreditava-se que a fauna de invertebrados em riachos constituísse uma exceção a este padrão geral. Stout e Vandermeer (1975) compararam a fauna de riachos tropicais e temperados usando a mesma metodologia e observaram que a fauna de riachos não era uma exceção ao padrão geral de maior diversidade nos trópicos. Os autores chegaram a tal conclusão usando grandes amostras e um método de estimativa de riqueza de espécies. Em riachos tropicais a maioria das espécies possui baixa densidade, enquanto em regiões temperadas as espécies em geral são abundantes. Com pequenas amostragens, coleta-se um menor número de espécies em riachos tropicais do que em riachos de áreas temperadas. Aumentando o tamanho amostral, rapidamente coleta-se a grande maioria das espécies em um riacho de área temperada. Num riacho tropical, pelo contrário, a curva de acumulação de espécies em função do esforço amostral cresce lenta e indefinidamente, ultrapassando valores normalmente encontrados em regiões temperadas. Quanto maior a coleta, maior o número de espécies e isto não é um fenômeno restrito a riachos (Walther et al. 1995; Walther et al. 1995; Novotný e Basset 2000).

No Parque Estadual Intervales, onde a maioria dos trabalhos desta tese foram feitos, identifiquei desde o mestrado 60.521 indivíduos representando 217 espécies (não inclui Chironomidae e Acari). Destas, 44 (20%) apareceram com apenas um indivíduo e 18 (8%) apareceram com dois indivíduos. É provável que uma boa parte destas espécies raras não pertençam a corredeiras, onde as coletas foram feitas. Talvez elas sejam comuns em outros microambientes dentro do riacho e foram coletadas acidentalmente. Enquadram-se neste grupo várias larvas de Coleoptera e Diptera. Dada a quase inexistência de conhecimento sobre história natural das espécies em estudo, infelizmente não pude simplesmente ignorá-las. Uma outra parte das espécies raras no entanto, certamente vive em corredeiras. Nesta caso, é provável que elas sejam raras nos riachos estudados, mas abundantes em outros. Uma evidência deste último fenômeno é a presença relativamente comum de diversas espécies de Trichoptera em um dos riachos estudados (riacho 6 em Melo e Froehlich 2001 e riacho 3 no Capítulo 7) e ausência ou presença esporádica em outros.

Em escalas espaciais grandes, como províncias biogeográficas e mesmo continentes, fatores históricos relacionados ao aparecimento e posterior radiação de linhagens de espécies fornecem em grande parte explicações para macro-padrões de diversidade. Por exemplo, no sudeste do Brasil temos apenas três gêneros da família Perlidae (Plecoptera), enquanto no Hemisfério Norte existem 15 gêneros. Sabe-se que este grupo originou-se no Hemisfério Norte e lá diversificou-se, colonizando partes da América do Sul em tempos geológicos mais recentes (Illies 1969).

Em escalas espaciais pequenas, como um trecho de riacho, diversos outros fatores concorrem como explicações plausíveis aos padrões de diversidade encontrados (Vinson e Hawkins 1998). Qual deles é o melhor ou mais aplicável é algo muito discutido. Num mesmo local, certamente vários fatores atuam simultaneamente. Em alguns casos, certo fator pode até ter preponderância sobre outros, mas dificilmente ele terá aplicação universal. Entre os diversos fatores que influenciam a determinação do número de espécies num dado trecho de riacho, perturbações causadas por enchentes vêm recebendo cada vez mais atenção de pesquisadores (Resh et al. 1988; Townsend 1989; Death e Winterbourn 1995; Lake 2000).

Nesta tese, apresento resultados sobre dois tópicos relacionados a diversidade e brevemente citados acima: estimativas de riqueza de espécies e o papel de perturbações na estrutura de comunidades. A seguir apresento um breve panorama dos assuntos abordados nesta tese bem como o contexto e as razões que levaram à realização de cada capítulo.

Diversidade, riqueza e estimadores de riqueza

Assim como acontece com a maioria dos termos amplamente usados em Ecologia (Grimm e Wissel 1997), o termo "diversidade" ou "biodiversidade" é usado de forma ampla na literatura ecológica (Magurran 1988). Na maioria das vezes, o termo se refere direta ou indiretamente ao número de espécies (ou riqueza de espécies) numa amostra, local ou comunidade.

Medir a "diversidade" de um local ou de uma comunidade nem sempre é uma tarefa fácil. Além do número de espécies, muitos pesquisadores crêem que se deve levar em consideração a abundância relativa das espécies. Apesar de aparentemente simples, o uso simultâneo das duas métricas tem gerado uma série de problemas de computação e interpretação (Hurlbert 1971). O primeiro reside no fato de não haver consenso sobre o peso de cada métrica no índice. Assim, variando-se o peso relativo de cada métrica pode-se ter uma infinita quantidade de índices (Hill 1973). De fato, isto se reflete na literatura ecológica, onde pode-se encontrar pelo menos uma dezena de índices de diversidade (Peet 1974; Magurran 1988). Como consequência, dependendo do índice escolhido pode-se ter resultados opostos. A escolha de um deles é algo fundamentalmente arbitrário, apesar das inúmeras "razões" encontradas na literatura (Hurlbert 1971). O segundo problema com índices de diversidade é a sua interpretação, visto que não possuem unidades. Assim a utilidade do índice fica restrita a situações comparativas. A comunidade A pode ser mais diversa do que a B, mas o que o valor do índice significa?

Dada a confusão com índices de diversidade, muitos autores têm usado a riqueza de espécies como medida de diversidade. Esta simples métrica têm uma série de vantagens sobre índices de diversidade, incluindo facilidade de interpretação e correlação com várias características da comunidade. Por outro lado, o uso da riqueza de espécies possui um problema já ressaltado pelos primeiros ecólogos no início do século XX e que foi um dos motivos para a criação de índices de diversidade, a dependência do tamanho amostral. Como dito anteriormente, quanto maior o tamanho amostral, maior o número de espécies coletadas. O problema tende a ser menor quando o tamanho amostral é grande e quando se têm poucas espécies raras. Nestes casos, a curva de acumulação de espécies tende à estabilização conforme se aumenta o tamanho amostral.

Para contornar o problema da dependência da riqueza em relação ao tamanho amostral, foram criados diversos métodos para estimar o número total de espécies na comunidade. Tais métodos podem ser agrupados em três categorias de acordo com os pressupostos e formas de cálculo empregados: Métodos Paramétricos, Métodos Não-Paramétricos e Extrapolações da Curva de Acumulação de Espécies (ou ESAC, Extrapolation of Species Accumulation Curve) (Colwell e Coddington 1994).

Métodos Paramétricos utilizam dados de abundância de cada espécie para o ajuste a uma determinada distribuição. A distribuição mais utilizada é a LogNormal. Neste caso, cria-se classes de abundância com base no logaritmo de base 2 no eixo das abcissas (1-2, 2-4, 4-8, 8-16 etc) e registra-se o número de espécies com abundâncias compreendidas em cada classe no eixo das ordenadas. Caso a amostragem seja grande o suficiente, esgota-se o número de espécies na área e portanto a primeira classe de abundância tende a ter frequência zero de espécies. Por outro lado, as classes de abundância intermediárias passam a possuir as maiores frequências de espécies. O histograma final é uma curva normal e o nome da distribuição, LogNormal, vem do fato de o eixo das abcissas estar na escala logarítmica. Caso as distribuições das espécies realmente sejam lognormais, poderíamos estimar o número total de espécies na comunidade a partir do momento em que a amostragem for suficiente para revelar a classe modal (na verdade a classe modal mais uma ou duas classes a esquerda, para que se tenha certeza de que encontramos a classe modal). Neste caso, dado que a distribuição é simétrica, basta estimar a área da distribuição ainda não amostrada. Apesar de certa elegância matemática e a existência de um corpo teórico sobre a distribuição (e.g. Ugland e Gray 1982; mas veja Limpert et al. 2001), o método é muito pouco usado nos trabalhos recentes (Colwell e Coddington 1994; Walther e Morand 1998; Schmit et al. 1999). Creio que isso possa ser devido a três fatores. Dependendo de como se resolve a separação das classes de abundância (por exemplo 1, 2-3, 4-7 etc ou 1, 2-3+1/2 das com 4, 1/2 das com 4 + 5-7 + 1/2 das com 8 etc) pode-se ter resultados muito diferentes (Lobo e Favila 1999). O método também não obteve bons resultados em duas das primeiras avaliações sobre estimadores de riqueza (Palmer 1990; Baltanás 1992). Talvez ainda mais importante do que os dois fatores anteriores, o método não foi incluído no programa de computador EstimateS (Colwell 1997), sem dúvida o mais utilizado por pesquisadores atualmente.

Métodos Não-Paramétricos são bem mais simples e necessitam apenas da informação da riqueza observada na amostra e o número de espécies raras, definidas dependendo do método como aquelas com presença em 1, 2, 3 etc unidades amostrais ou com 1 ou 2 indivíduos. Muitos destes métodos foram criados na década de 1980 e são relativamente bem utilizados hoje em dia.

A terceira classe de estimadores, Extrapolação da Curva de Acumulação de Espécies, é composta por métodos bem intuitivos e precisam apenas da informação de presença/ausência das espécies coletadas em cada unidade amostral. A estimativa é obtida construindo-se uma curva de acumulação de espécies em função de tamanhos amostrais sucessivamente maiores e ajustando-se uma função matemática assintótica aos dados. Em outras palavras, extrapola-se a curva ajustada até o ponto em que a taxa de acréscimo de espécies é zero ou seja quando a curva estabiliza. Teoricamente qualquer função assintótica pode ser usada, embora na prática observamos que algumas funções possuem comportamento problemático, produzindo em alguns casos estimativas menores do que os valores de riqueza observados. Uma vantagem deste tipo de estimadores é que muitas das funções usadas possuem em suas fórmulas parâmetros que podem ser interpretados biologicamente (Flather 1996).

O uso de estimadores de riqueza é relativamente recente na literatura e foi grandemente ampliado a partir da publicação de uma revisão do assunto por Colwell e Coddington (1994). Nesta revisão os autores apresentam os diversos métodos numa linguagem acessível a ecólogos e fazem uma pequena avaliação dos diversos estimadores apresentados usando dados sobre um banco de sementes. Nos últimos anos foram feitas diversas avaliações dos métodos disponíveis usando conjuntos de dados provenientes de variadas comunidades biológicas (Chazdon et al. 1998; Walther e Morand 1998; Schmit et al. 1999; Toti et al. 2000).

Capítulos 1 e 2

No Capítulo 1 desta tese, avalio 13 estimadores da riqueza de espécies usando dados de comunidades de macroinvertebrados em riachos. Para tanto, uso dados coletados em escalas locais (em apenas um riacho) e em escalas regionais (diversos riachos dentro de uma mesma bacia hidrográfica). Os conjuntos coletados em apenas um riacho foram provenientes da Serra do Japi (Jundiai/SP), Parque Estadual Intervales (Iporanga/SP) e Serra da Mantiqueira (Pindamonhangaba/SP). Os dados representando a escala regional foram coletados em diversos afluentes do Rio do Carmo (ou Rio Pilões), no P. E. Intervales.

Os resultados obtidos no Capítulo 1 mostram que os estimadores de riqueza na comunidade não tiveram um desempenho tão bom como o esperado. Isto foi em parte devido ao contínuo aparecimento de novas espécies conforme o esforço amostral era ampliado. A partir de tais resultados, abordo no Capítulo 2 uma crítica ao uso de tais métodos. No capítulo abordo diretamente métodos não-paramétricos, embora os argumentos utilizados possam ser estendidos para outros métodos. Todos os métodos de estimativas de riqueza pressupõem que a riqueza num local seja finita e que a taxa de encontro de espécies raras deve diminuir com o tempo. Usando conjuntos de dados próprios e de outros pesquisadores, mostro que tal pressuposto não acontece na prática, colocando em dúvida a utilidade de tais estimadores.

Capítulo 3

Em parte devido à baixa confiabilidade de estimadores de riqueza na comunidade, decidi estudar a possibilidade de se estimar a riqueza de espécies não para a comunidade como um todo, mas sim para um tamanho amostral maior. Um exemplo. Com 20 unidades amostrais (parcelas, armadilhas, pedras em riachos etc) coleta-se em média 50 espécies. Quantas espécies deveriam ser encontradas caso o tamanho amostral fosse aumentado para 75? Apesar da existência de alguns trabalhos antigos abordando o assunto (Gleason 1922; Evans et al. 1955), pouquíssimos trabalhos têm usado tal tipo de ferramenta hoje em dia. Na literatura recente encontrei apenas um trabalho avaliando tais métodos para um problema análogo (Keating et al. 1998). Apesar da pouca atenção dispensada ao assunto, tais métodos poderiam ser de grande utilidade na padronização de diferentes tamanhos amostrais. Atualmente, resolve-se o problema por meio de interpolações. Quando o esforço amostral é expresso em números de indivíduos, em geral usa-se curvas de rarefação para padronizar o esforço amostral pelo tamanho da menor amostra (Simberloff 1979; Gotelli e Colwell 2001). Usando princípio semelhante, é possível fazer tal tipo de padronização em estudos usando unidades amostrais (veja exemplo em Lewinsohn 1991). Ainda para unidades amostrais, pode-se usar curvas de acumulação de espécies (curvas do coletor) para padronizar tamanhos amostrais diferentes. A grande desvantagem destes métodos é a perda de informação contida nas amostras maiores. No Capítulo 3 avaliei diversos métodos disponíveis na literatura e também um desenvolvido durante o próprio trabalho e usado também no Capítulo 2. As avaliações foram feitas de forma exploratória usando diversos conjuntos de dados, alguns próprios e outros de colegas. Tais conjuntos adicionais abrangeram uma ampla gama de métodos de coleta e organismos envolvidos, proporcionando grande solidez aos resultados obtidos. Diferente dos resultados obtidos no Capítulo 1, quando o objetivo era estimar a riqueza na comunidade, alguns métodos avaliados no Capítulo 3 tiveram um excelente desempenho na estimativa de riqueza em tamanhos amostrais maiores.

Perturbações em comunidades de macroinvertebrados em riachos

Durante muito tempo a idéia que populações, comunidades, bem como interações entre espécies, eram estritamente reguladas, gerando situações de equilíbrio ou "um balanço da natureza", foi dominante no pensamento ecológico (Wu e Loucks 1995). Sob tais idéias, caso não houvesse interferência humana, os tamanhos populacionais das espécies variariam pouco com o tempo. Como consequência, algumas propriedades das comunidades como abundância relativa e riqueza de espécies seriam constantes no tempo. Desvios dos estados de equilíbrio seriam fortemente pressionados pelas interações biológicas, causando o retorno ao estado original. Neste sentido, desvios dos estados de equilíbrio eram vistos como anomalias e não como parte integrante dos sistemas biológicos.

Com o acúmulo de dados empíricos e também com o desenvolvimento de modelos matemáticos para relações intra e interespecíficas (DeAngelis e Waterhouse 1987), a idéia de equilíbrio foi aos poucos perdendo força. A idéia de que perturbações naturais desempenham papel não trivial na determinação de populações e comunidades não é nova (e.g. Hutchinson

1961), mas passou a receber grande atenção de ecólogos a partir de alguns trabalhos empíricos e revisões do assunto (Connell 1978; Sousa 1984; White e Pickett 1985).

Em ecologia de riachos, maior interesse sobre perturbações surgiu depois dos trabalhos de Fisher et al. (1982) com invertebrados bentônicos e Grossman et al. (1982) com peixes. No fim da década de 1980, havia quase um consenso entre ecólogos trabalhando com riachos sobre a grande importância de perturbações, principalmente aquelas causadas por enchentes (Resh et al. 1988). Já durante este período, houve o desenvolvimento de teorias ecológicas centradas em perturbações em riachos (Minshall e Petersen 1985; Townsend 1989). Durante a década de 1990, foram publicados importantes trabalhos empíricos confirmando a importância de perturbações, principalmente por enchentes (Flecker e Feifarek 1994; Death e Winterbourn 1995; Townsend e Scarsbrook 1997).

Durante as duas últimas décadas, a maior atenção dada ao papel de perturbações em riachos resultou num grande volume de publicações. Destas publicações alguns resultados foram observados repetidamente, possibilitando algumas conclusões gerais. Uma delas é que perturbação por enchente é um fenômeno quase universal em riachos. Após uma enchente, a densidade de organismos é grandemente reduzida, em alguns casos chegando a 2-5% da densidade original. O restabelecimento das densidades originais em geral é rápida (30-120 dias), mas depende em parte da intensidade da enchente e da estabilidade do trecho em questão (Mackay 1992). Riachos com pedras grandes e parcialmente enterradas em geral são menos afetados. Durante enchentes, diversos microhabitats dentro dos riachos são usados como refúgios. Entre eles, regiões com baixa força hidrológica (Lancaster 1999), sedimentos sob a superfície (Dole-Olivier et al. 1997), pedras parcialmente enterradas (Matthaei et al. 2000), regiões fora do riacho inundadas durante enchentes (Matthaei e Townsend 2000) e regiões associadas a grandes troncos de árvores mortas (Palmer et al. 1996). A recolonização de áreas afetadas por enchentes parece ser feita principalmente por deriva (Townsend e Hildrew 1976), embora em eventos de baixa magnitude deslocamentos de indivíduos sobre a superfície possam ser importantes (Brooks e Boulton 1991).

Capítulo 4

No capítulo 4 descrevo os resultados de um trabalho feito em colaboração com o Prof. Colin Townsend a o Dr. Dev Niyogi, na Nova Zelândia. O trabalho teve como objetivo testar a hipótese de que riachos em locais de vegetação nativa seriam mais resistentes e mais resilientes a perturbações do que aqueles em regiões de pastagens. A vegetação nativa era composta por campos de touceira (tussock), com fisionomia semelhante aos campos de altitude do Brasil Central. Os riachos em campos de tussock eram bem sombreados pela vegetação marginal e boa parte do leito recoberto por musgos. Tais musgos oferecem resistência ao deslocamento das pedras durante enchentes, sendo portanto indicadores de alta estabilidade do leito (Suren et al. 2000). Por outro lado, riachos em pastagens praticamente não continham musgos nem eram sombreados.

Três riachos em cada vegetação foram usados como réplicas. A perturbação consistiu na passagem de um jato d'água propulsionado por uma bomba. A perturbação foi aplicada em trechos de 60-90 m dependendo do riacho. As coletas foram feitas imediatamente antes da perturbação, logo depois e oito dias após. Em cada ocasião, quatro unidades amostrais foram coletadas usando um amostrador Surber. A coleta feita antes da perturbação foi designada como controle, a coletada imediatamente depois foi usada para medir a resistência da comunidade e a coletada oito dias depois para medir a resiliência. Foi utilizada Análise de Variância com medidas repetidas para avaliar a resistência e resiliência entre os dois grupos de riachos. Para avaliar a agregação da comunidade, o índice de similaridade de Sørensen foi usado para comparar as unidades amostrais dentro de cada riacho e época de coleta.

Capítulo 5

Na literatura existem diversos trabalhos sobre experimentos de colonização de substrato no leito de riachos por macroinvertebrados. Poucos no entanto comparam padrões de colonização em riachos de diferentes tamanhos. No Capítulo 5, descrevo um experimento de colonização feito em três riachos na bacia do Rio do Carmo, P. E. Intervales. Os riachos eram bem diferentes tanto em tamanho como na fauna de peixes residentes. O riacho 1 era de primeira ordem e aparentemente não possuía nenhuma espécie de peixe. O local de trabalho ficava próximo ao ponto onde a água flui durante todo o ano. No riacho 2, a largura do leito era de cerca de 10 m e a fauna de peixes era dominada por cascudos. No riacho 3, a largura era de 20 m e diversas espécies de peixes insetívoros estavam presentes. A hipótese do trabalho era de que no menor riacho, a pequena área a montante do ponto de trabalho deveria limitar a quantidade de colonizadores potenciais. No riacho 3, a grande quantidade e riqueza de espécies de peixes deveriam inibir o comportamento de deriva dos invertebrados, principal mecanismo de colonização apontado na literatura. Por outro lado, o riacho 2 não seria afetado nem pela área a montante nem pela fauna de peixes. A colonização no riacho 2 portanto deveria ser mais rápida do que nos outros dois pontos. O experimento foi feito com pedras medindo cerca de 18 cm do próprio riacho. Escolhi setenta pedras em cada riacho e retirei todos os invertebrados associados. Em seguida, as pedras foram marcadas e colocadas em regiões de corredeira do riacho. Depois de 1, 2, 4, 8, 16, 32 e 64 dias após o início do experimento, 10 pedras foram coletadas com o auxílio de um puçá e todos invertebrados fixados para posterior identificação.

Capítulo 6

Um aspecto fundamental em estudos de perturbação em riachos é a quantificação da perturbação em si (Townsend et al. 1997). Na maioria dos trabalhos sobre o assunto, o impacto de enchentes é estimado a partir de fórmulas que levam em consideração a inclinação do trecho e altura da coluna d'água (e.g. Cobb et al. 1992). Entretanto, nos últimos anos diversos pesquisadores têm usado informações de rolagem de pedras previamente marcadas para medir perturbações por enchentes (Lake e Schreiber 1991; Death e Winterbourn 1995; Downes et al. 1998). No Capítulo 6 apresento um experimento com rolagem de pedras em cinco riachos no P. E. Intervales. O trabalho visou estudar a variação na frequência de rolagem ao longo do ano, o efeito do tamanho de pedra e a estabilidade relativa do leito entre os cinco riachos estudados. O último tópico serviu também como subsídio para análise dos dados do trabalho de persistência da comunidade, descrito a seguir.

Capítulo 7

Saber o quanto a estrutura de certa comunidade de organismos persiste ao longo do tempo é uma questão de extrema importância para a Ecologia. Underwood (1986) sugere que uma das características que certo agrupamento de espécies (assemblage) deve possuir para ser considerada uma "comunidade" é a persistência no tempo. Do ponto de vista aplicado, é fundamental saber a amplitude de variação de uma comunidade em condições naturais (Karr e Chu 1999). Na ausência de tais informações, não se pode dizer que tal mudança na comunidade foi devida a fatores naturais, como enchentes, ou como consequência de alguma fonte poluidora. No Capítulo 7 apresento um estudo de persistência em cinco riachos no P. E. Intervales durante cinco anos. Especificamente, o trabalho aborda quatro questões relacionadas entre si: (1) existem comunidades distintas e recorrentes ao longo dos anos no verão e no inverno?, (2) comunidades na estação de baixa variabilidade ambiental (inverno, a estação seca) são mais constantes e similares entre si ao longo dos anos do que comunidades em estações com alta variabilidade ambiental (verão, época chuvosa)?, (3) a variabilidade das comunidades ao longo dos anos é relacionada a variabilidade ambiental? e (4) a variabilidade da comunidade aumenta com o tempo?

Notas sobre o desenvolvimento da tese

O projeto inicial desta tese enviado a FAPESP (Fundação de Amparo a Pesquisa do Estado de São Paulo) previa apenas os trabalhos apresentados nos Capítulos 1 (Estimadores de riqueza na comunidade) e 7 (Persistência da comunidade). O projeto foi criticado pela assessoria da FAPESP, entre outros aspectos, pela ausência de um enfoque experimental e pela restrita metodologia de coleta e também de análise. Atendendo a tais críticas incluí nos trabalhos de tese o experimento de colonização contido no Capítulo 5.

O experimento com rolagem de pedras apresentado no Capítulo 6 estava previsto no projeto original, mas de forma breve. A previsão é que ele serviria apenas como uma medida de perturbação nos riachos em estudo, auxiliando a interpretação dos dados do trabalho de persistência da comunidade (Capítulo 7). No entanto, a idéia do trabalho foi ampliada no desenvolver da tese e além de subsidiar a interpretação dos dados do Capítulo 7, decidi escrever um artigo curto sobre os resultados do experimento, apresentados no Capítulo 6.

A crítica na utilização dos estimadores de riqueza na comunidade, apresentada no Capítulo 2, surgiu a partir da análise e redação da própria avaliação dos estimadores feita no Capítulo 1. No artigo publicado referente à avaliação dos métodos não havia espaço para aprofundamentos, propiciando portanto a redação de um trabalho curto independente. A oportunidade do desenvolvimento do trabalho apresentado no Capítulo 3 ocorreu também durante as análises do Capítulo 1. Durante os cálculos dos estimadores baseados em extrapolação da curva de acumulação de espécies, observei que os ajustes de algumas funções eram excelentes. Apesar da baixa confiabilidade obtida quando se extrapolava a curva ao infinito, observei que extrapolações de menores magnitudes poderiam produzir bons resultados. Alguns testes iniciais foram animadores e dada a quase ausência de trabalhos abordando tais estimadores, decidi dedicar mais tempo ao assunto. Felizmente, tive a oportunidade de discutir meus resultados com diversos colegas do curso. Isto tornou possível o desenvolvimento de um trabalho em conjunto, que passou a incluir outros conjuntos de dados e outras análises.

Finalmente, o trabalho do Capítulo 4 surgiu após a oportunidade de um estágio na Nova Zelândia. No projeto original estava previsto um estágio no exterior, mas não existia a definição do local nem do desenvolvimento de um trabalho independente. Minha intenção original era apenas analisar e escrever os trabalhos de tese durante tal estágio. No entanto, visto que não estava atrasado com os trabalhos de tese, achei que seria uma boa oportunidade para trabalhar com faunas distintas e também em conjunto com outros pesquisadores.

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Capítulo 1

Evaluation of methods for estimating macroinvertebrate species richness using individual stones in tropical streams[1]

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[1] Melo, A. S. and C. G. Froehlich. 2001. Evaluation of methods for estimating macroinvertebrate species richness using individual stones in tropical streams. Freshwater Biology 46: 711-721.

Abstract

The most straightforward way to assess diversity in a site is the species count. However, a relatively large sample is needed for a reliable result because of the presence of many rare species in rich assemblages. The use of richness estimation methods is pointed by many authors as a solution for this problem in many cases. We examined the performance of 13 methods for estimating richness of stream macroinvertebrates inhabiting riffles both at local (stream) and regional (catchment) scales. The evaluation was based on (1) the smallest sub-sample size needed to estimate total richness in the sample, (2) constancy of this size, (3) lack of erratic behaviour in curve shape, and (4) similarity in curve shape through different data sets. Samples were from three single stream sites (local) and three from several streams within the same catchment basin (regional). All collections were made from protected forest areas in Southeast Brazil. All estimation methods were dependent on sub-sample size, producing higher estimates when using larger sub-sample sizes. The Stout and Vandermeer method estimated total richness in the samples with the smallest sub-sample size, but showed some erratic behavior at small sub-sample sizes, and the estimated curves were not similar among the six samples. The Bootstrap method was the best estimator in relation to constancy of sub-sample sizes, but needed an unacceptably large sub-sample to estimate total richness in the samples. The second order Jackknife method was the second best estimator both for minimum sub-sample size and constancy of this size and we suggest its use in future studies of diversity in tropical streams. Despite the inferior performance of several other methods, some produced acceptable results. Comments are made on the utility of using these estimators for predicting species richness in an area and for comparative purposes in diversity studies.

Introduction

An old and common problem when studying species assemblages is to know how many different kinds of organisms there are in the study area. Two situations where this information is very important are (a) setting priorities in choosing conservation areas and, in a more general sense, (b) when comparing two species assemblages for research purposes (Magurran 1988; Colwell and Coddington 1994). In the first case, knowledge of biological diversity in different areas can optimize the use of scarce resources in choosing areas of high diversity and/or with many rare and endemic species (Prendergast et al. 1993; Pressey et al. 1993). In the second case, one can assess the impact of human disturbance on species loss through comparison with undisturbed areas (Flather 1996; Keddy and Drummond 1996).

To achieve a good estimate of species richness in an area, one must take sufficient samples to include most of the rare species (Cao et al. 1998). For the same sample size a species poor assemblage, but with equally abundant species, might produce a greater richness than another truly rich assemblage, but composed of a large number of rare species. A good example of this problem was presented by Stout and Vandermeer (1975), who found greater richness in temperate streams than in tropical counterparts when using few samples. With an increased sample size, tropical streams were shown clearly to be richer than temperate ones.

The assessment of richness in a given area requires a count of observed species, but the larger the sampling effort, the larger will be the number of observed species (Walther et al. 1995). To circumvent this problem, it is necessary either to collect all the individuals in the area, something that is rarely possible, or to use an estimation method, as suggested by many authors (Burnham and Overton 1979; Smith and van Belle 1984)

Several mathematical models have been developed, mainly in the past 20 years, to allow for such estimation (see reviews in Bunge and Fitzpatrick 1993; Colwell and Coddington 1994). These models are based on different mathematical approaches and can be grouped in three classes: parametric, non-parametric and extrapolations of species accumulation curves. Parametric methods need information on the abundance of each observed species, which are then fitted to a theoretical model of expected frequencies for each class of abundance. Due to the requirement for data on the abundance of each species, laborious computation, and bad performance in some previous evaluations (Palmer 1990; Walther and Morand 1998), they have been little used in recent years and are not considered in the present study. Non-parametric methods are easily computed and most of them do not need information about the abundance of each species (Burnham and Overton 1979; Colwell and Coddington 1994). Most are composed of the number of observed species and a second term related to the number of rare species. The third class of estimators is composed of models of extrapolation of species accumulation curves. Included in this class are the Stout and Vandermeer (1975) method used mainly in stream ecology, and several modifications and transformations of a hyperbole of two parameters known as the Michaelis-Menten equation, first used by biochemists on enzyme kinetics (Clench 1979; Raaijmakers 1987; Lamas et al. 1991; Soberón and Llorente 1993).

The choice of a class of methods or a single method is not an easy task. Few evaluations have used either simulated or real data, while a good model for one kind of data might be poor for another. For example, the Bootstrap non-parametric method of resampling was one of the best evaluated in a parasite richness study (Walther and Morand 1998), but was one of the worst in another evaluation using plants in a tropical forest (Chazdon et al. 1998). This discrepancy occurs because of differing characteristics of the data sets, such as the patchy distribution of species in the area (Chazdon et al. 1998), size of sampling units, and the relative number of the sampled species to the available pool (Walther and Morand 1998).

The best way to evaluate different methods of estimating richness is to compare the estimates to the true richness, something that is usually not possible. In species rich assemblages such as macroinvertebrates in tropical streams, species accumulation curves rarely reach an asymptote, reflecting the continuous collection of rare, previously unseen species. Many of these rare species are from different habitats and hence are collected accidentally. A good way to solve this problem is to remove from analyses those species considered accidental, based on knowledge of their natural history. However, at the moment, this is practically impossible for stream macroinvertebrates in most tropical regions. Thus, our evaluation approach was to estimate total richness in unusually large sample sizes by using different sub-sample sizes.

We evaluated 13 available methods for richness estimation of macroinvertebrates inhabiting riffles at local (single stream) and regional (several streams within the same catchment basin) scales. Evaluation was mainly done observing the behaviour of each method and its ability to estimate the richness in a large sample using different sub-sample sizes.

Methods

Study sites

Evaluation was done using six macroinvertebrate data sets from quantitative collections in streams located in protected forest areas in São Paulo State, Brazil. Three data sets were from single streams and are here called local samples. The other three, termed regional samples, were from several stream sites within the same catchment basin.

The first local sample was collected in the Ermida Stream (23° 14'S, 46° 56'W), a third order stream at an elevation of 860 m located in the Serra do Japi, municipality of Jundiai. The mean annual precipitation in the area is about 1400 mm and the vegetation is Tropical Semi-Deciduous Montane Forest. The area was impacted recently by fire and at the collection site the riparian vegetation was an old secondary growth that partially shaded the stream. At the time of the collection the stream bed was not covered with sediment and the water was clear. Collections were made from September through mid November 1996, comprising the end of the dry and beginning of the rainy seasons. Further information about physical and biological aspects of the area can be found in Morellato (1992). The second collecting site was the Carmo River (24° 18'S, 48° 25'W), a fourth order stream at an elevation of 520 m, that drains a well conserved and protected area, the Parque Estadual Intervales. The vegetation is Tropical Ombrophilous Submontane-Montane Forest, commonly known as Tropical Rain Forest (Mueller-Dombois and Ellenberg 1974). The mean annual rainfall is 1696 mm (8 y record) and the collection was done in July 1997, in the middle of the dry season. The third local sample was collected in the Cedro Stream (22° 45'S, 45° 28'W) at an elevation of 950 m in the Serra da Mantiqueira, municipality of Pindamonhangaba. The vegetation is Tropical Evergreen Seasonal Submontane Forest and at the collecting site we did not observe any major human disturbance. The stream has many waterfalls, and in the stream bed large boulders (> 0.5 m) are common. Sampling was done in December 1998 and January 1999 and although these months were in the middle of the rainy season, we did not observe any spate during the collecting period.

Regional samples were from the Carmo River Basin located in the Parque Estadual Intervales, the same area as the second local sample. The first regional sample was collected in nine stream sites in summer and the second one in the same previous nine stream sites plus one in winter. The third one was composed by the sum of the previous summer and winter samples. This last sample does not present any new information, but was used because it represents a very large sample and so allowed us to evaluate the performance of the estimators on unusually large samples. The streams were first through fifth order and ranged from 1 to 21 m in width. Summer collections were made in the rainy period of February and March and the winter collections in the dry months of July and August, both in 1997.

Sampling and sorting of macroinvertebrates

The sampling and sorting procedures were the same for all samples. They consisted of sampling individual stones 15-20 cm maximum diameter in riffles using an U-net with a 250 µm-mesh, designed to avoid the loss of active swimmers (Scrimgeour et al. 1993). After taking the stones from the stream bed, they were immediately examined for attached individuals. The contents of the U-net were transferred to a white tray and all visible invertebrates fixed in 80% ethanol.

Seventy-five stones were collected in each of the two first local samples, hereafter called Japi and Carmo samples. Because of the non-stabilization of the species accumulation curves in Japi and Carmo samples and given our intention to explore the effects of sample size on the performance of the estimators, we collected 150 stones in the third local sample, termed here Pinda. For the regional samples, 25 stones were collected in each stream totalling 225, 250 and 475 stones respectively for the Summer, Winter and the pooled third sample, here called Intervales. These sample sizes are remarkably higher than that used in other studies. As examples, we have used with success, 25 stones to access richness in single streams (Melo and Froehlich 2001) while Lake et al. (1994) sampled 28 stones in a tropical stream in Australia. The only diversity study using stones as sampling units along a catchment we are aware is that of Minshall et al. (1985), who used 60-110 stones per season. Thus, despite none of our samples reach an asymptote, we reason that the six samples represent unusual large efforts and the observed richness found in each one should be close to the true richness in the studied assemblages in the sampling period.

Because of the poor knowledge of the macroinvertebrate fauna in Southeast Brazil, individuals were identified to the lower possible taxonomic level and then sorted as morphospecies. Due to difficulties in separation, even as morphospecies, for mites and chironomid larvae, these were not included in the analysis.

Evaluation of estimators

Thirteen estimators were evaluated, comprising seven non-parametric (NP) and six extrapolation of species accumulation curve (ESAC) methods (Table 1). Computation of the NP methods, MMMean, and MMRuns were done using the software EstimateS v. 5.0.1 (Colwell 1997). The estimators MMClench, Expo, and SV were computed by non-linear regression (see Keating and Quinn 1998) and the estimator MMLamas by solving the function using a spreadsheet software. The fitting of the ESAC methods was done using a species accumulation curve produced by 200 randomizations of the order of sampling units appearances computed in EstimateS. For the estimators calculated by EstimateS, we present estimations for most of the sub-sample sizes while only a sufficient number of points to construct a curve were calculated for the others, because of the necessity of independent calculation for each sub-sample size.

Common approaches used to evaluate estimation methods includes some measure of bias and accuracy of the estimated richness in relation to the true richness using an a priori chosen sub-sample size. However, in rich assemblages as used here, the estimated richness is strongly dependent on sample size. Thus, for a given estimation method, different sub-samples sizes will produce different bias and accuracy values (Hellmann and Fowler 1999). As we do not have any reason to choose a specific sub-sample size a priori, we opted for not using such bias and accuracy statistics. Instead, we used 4 criteria we argue are more practical and realistic. The 4 criteria were: (1) the smallest sub-sample size required to estimate the observed richness in the total sample, (2) constancy of the sub-sample size needed to estimate the observed richness in the total sample, measured as one standard deviation of the previous criterion, (3) lack of erratic behaviour in curve shape, specifically large variations of estimates for closely similar sub-sample sizes, and (4) similarity in curve shape through the six sample data sets. In order to apply the first two criteria and to allow future practical uses, the results are presented as percentages instead of the absolute number of stones.

Table 1. Summary of the 13 evaluated estimators comprising seven non-parametric (NP) methods and six estimators based on the extrapolation of species accumulation curves (ESAC).

Class	Abbreviation	Estimator	References
NP	ACE	Abundance-based Coverage Estimator	Chao et al. (1993); Colwell (1997)
NP	ICE	Incidence-based Coverage Estimator	Lee and Chao (1994); Colwell (1997)
NP	Chao 1	Chao 1	Chao (1984); Colwell (1997)
NP	Chao 2	Chao 2	Chao (1987); Colwell (1997)
NP	Jack1	First order Jackknife	Burnham and Overton (1978; 1979)
NP	Jack 2	Second order Jackknife	Burnham and Overton (1978; 1979)
NP	Boot	Bootstrap	Smith and van Belle (1984)
ESAC	MMRuns	Transformation of Michaelis-Menten hyperbole by Raaijmakers. Estimate curves averaged over randomizations (runs)	Raaijmakers (1987); Colwell (1997)
ESAC	MMMean	Transformation of Michaelis-Menten hyperbole by Raaijmakers. Estimate curve computed once for mean species accumulation curve	Raaijmakers (1987); Colwell (1997)
ESAC	MMClench	Michaelis-Menten hyperbole	Clench (1979)
ESAC	MMLamas	Transformation of Michaelis-Menten hyperbole. The curve is adjusted in order to pass through the last point of the species accumulation curve	Lamas et al. (1991)
ESAC	Expo	Negative exponential function	Soberón and Llorente (1993); Colwell and Coddington (1994)
ESAC	SV	Derivation from the equations of Island Biogeography Theory	Stout and Vandermeer (1975)

Results

The six data sets used were very different from each other (Table 2). The Pinda sample was richer than the two other local samples. Constructing a species accumulation curve, the Pinda sample presented 101 observed species on 75 stones, on average, contrasting to 66 for Japi and 71 in Carmo, for the same sample size. Also, the number of species and individuals per stone in Pinda was about twice the number found in Carmo. Finally, the Pinda sample was composed of twice the number of stones sampled in the other two local samples. Regional samples also presented obvious differences to each other, either in observed richness or in sample size (Table 2). Despite these structural differences in the sampled assemblages, results presented by the different estimators were very similar in all six used data sets (Figs 1 and 2).

The estimators MMRuns, MMMean and Expo estimated values only slightly larger than the observed richness at small sub-sample sizes. Increasing the sub-sample sizes, they tended to produce values smaller than the observed richness (Figs 1 and 2). Additionally, MMRuns produced erratic behaviour at small sub-samples sizes.

The estimator Boot produced values only slightly larger than the observed richness, but in contrast to the three previous estimators, they were consistent for all sub-sample sizes. MMLamas and MMClench showed similar behaviours to Boot except that they estimated larger values at small sub-sample sizes. The mean sub-sample sizes required to estimate the total richness in the samples were 64, 65.3 and 70.58%, respectively for MMLamas, Boot and MMClench (Table 3).

SV method estimated the total richness in the sample with the smallest sub-sample size (Mean 15.58%) and was rather constant through the six samples (SD 4.43%) (Table 3). However, it produced erratic behaviour in Summer and Intervales samples at small sub-sample sizes. Also, the curve shape was not constant along all samples. SV curves were similar to NP methods for Japi, Winter and Intervales samples. However, in Carmo and Pinda samples the estimates were too high at large sub-sample sizes (Figs 1 and 2).

Table 2. Summary of observed richness and sample size of the three local samples (Japi, Carmo and Pinda) and the three regional samples (Summer, Winter and Intervales) used in the evaluations.

Sample	Observed Richness	Sample size (stones)	Total individuals	Mean species per stone	Mean individuals per stone
Japi	66	75	3759	12.67	50.12
Carmo	71	75	2673	9.91	35.64
Pinda	117	150	10,339	18.46	68.93
Summer	119	225	6831	9.68	30.36
Winter	142	250	10,767	10.99	43.07
Intervales	162	475	17,598	10.62	37.05

Unlike the ESAC methods, NP estimators (except Boot) presented more similar behaviours to each other. ICE produced erratic behaviour at small sub-sample sizes. In Japi, Carmo and Pinda samples, Jack 2 was similar to Chao 1 and Chao 2 methods, but produced larger estimated values for the other three samples (Figs 1 and 2). Jack 2 estimated total richness with the smallest sub-sample size among the NP methods and the constancy of this estimate was inferior only to Boot method (Table 3).

The best estimator with respect to minimum sub-sample size was the SV method, but it did not present good results in relation to two other criteria. Boot estimator had the best performance in relation to the constancy of minimum sub-sample size, but needed an unacceptably large subsample size to estimate total richness in the sample (Table 3). On the other hand, Jack 2 was the second best both in minimum sub-sample size and in constancy and did not present problems in relation to the other two criteria (Table 4). Although Chao 1, Chao 2 and Jack 1 scored lower when compared to Jack 2, they presented good results in relation to all 4 criteria. ACE and ICE methods followed in an inferior level. Boot, MMLamas and MMClench estimators performed poorly with regard to observed richness. The worst of the evaluated methods were MMRuns, MMMean and Expo, which underestimated the observed richness at many sub-sample sizes, including when using the total sample (Table 3).

Table 3. Minimum sub-sample size expressed as percentage required by each method to estimate the observed richness in the three local and three regional samples. MMRuns, MMMean and Expo estimators underestimated observed richness in the total sample even when using all sampling units.

Estimator	Japi	Carmo	Pinda	Summer	Winter	Intervales	Mean	SD
ACE	48.0	32.0	47.3	54.7	45.2	44.0	45.20	7.46
ICE	46.7	34.7	44.7	50.7	48.8	47.6	45.53	5.67
Chao 1	28.0	24.0	34.0	40.9	31.6	31.4	31.65	5.71
Chao 2	30.7	24.0	33.3	38.7	32.8	31.8	31.88	4.75
Jack 1	41.3	41.3	40.0	37.3	35.6	38.5	39.00	2.29
Jack 2	26.7	26.7	25.3	23.6	22.4	24.2	24.81	1.73
Boot	66.7	66.7	65.3	64.9	63.6	64.6	65.30	1.22
MMRuns	--	--	--	--	--	--	--	--
MMMean	--	--	--	--	--	--	--	--
MMClench	72.0	66.7	80.7	58.2	64.0	81.9	70.58	9.42
MMLamas	62.7	62.7	70.7	57.8	59.2	70.9	64.00	5.61
Expo	--	--	--	--	--	--	--	--
SV	16.0	21.3	16.0	10.2	10.8	19.2	15.58	4.43

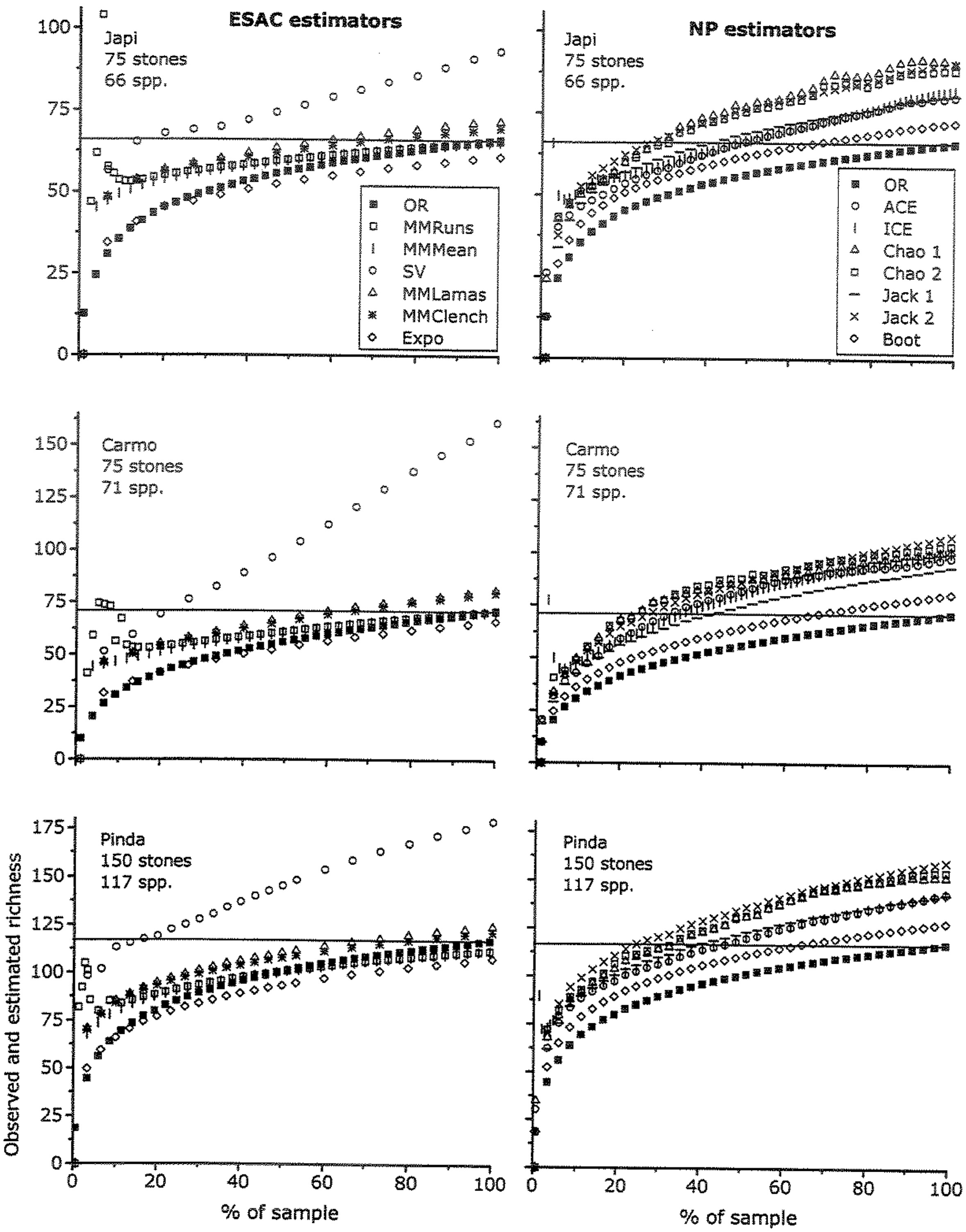

Fig. 1 Curves of accumulation of observed species and estimates for local samples. The horizontal line indicates the total number of species observed in the sample. OR = observed richness. See Table 1 for definitions of the estimators.

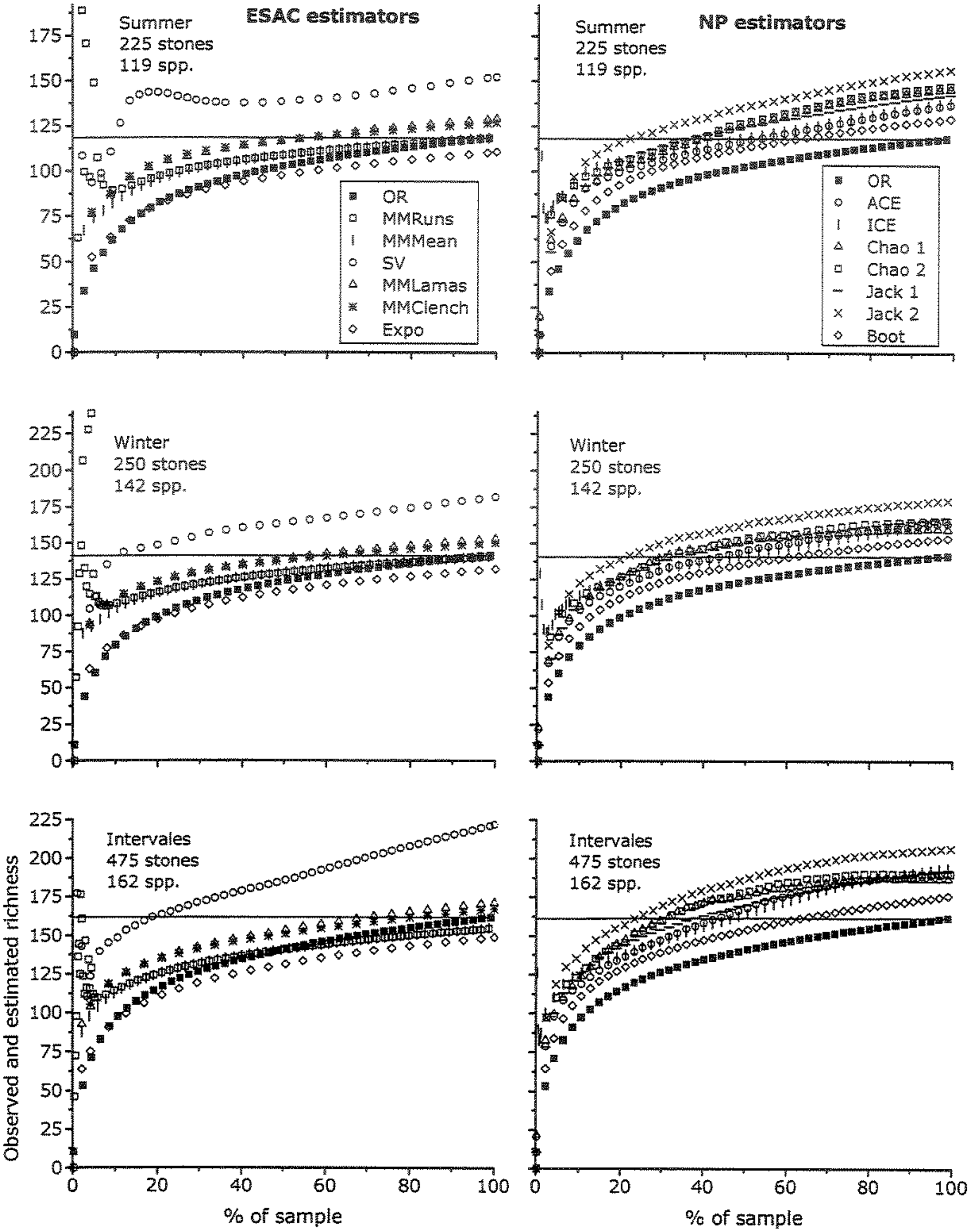

Fig. 2 Curves of accumulation of observed species and estimates for regional samples. The horizontal line indicates the total number of species observed in the sample. OR = observed richness. See Table 1 for definitions of the estimators

Table 4. Performance of the estimators scored by four criteria. MSS, minimum sample size required to estimate richness in total sample; CSS, constancy of required sub-sample size (1 SD of MSS); EB, erratic behaviour; SCS, similarity in curve shape. MSS and CSS ordered by performance.

Estimator	MSS	CSS	EB	SCS
ACE	6	9	good	good
ICE	7	7	bad	good
Chao 1	3	8	good	good
Chao 2	4	5	good	good
Jack 1	5	3	good	good
Jack 2	2	2	good	good
Boot	9	1	good	good
MMRuns	11	11	bad	good
MMMean	11	11	good	good
MMClench	10	10	good	good
MMLamas	8	6	good	good
Expo	11	11	good	good
SV	1	4	bad	bad

Discussion

Non-parametric methods were, overall, better than extrapolation of species accumulation curves. Expo, Boot and all four transformations or modifications of the Michael-Menten hyperbole presented the poorest results, and should not be used for species rich data with many rare species. ACE and ICE methods performed at an intermediate level. Jack 2 was the best of the evaluated methods based on the four previously chosen criteria and we recommend its use in future studies of macroinvertebrate diversity in tropical streams. Jack 1, Chao 1 and Chao 2 presented good results and were inferior to Jack 2 only because of the requirement for larger sub-sample sizes.

In spite of the use of different approaches and different data sets by different authors to evaluate estimation methods, there is some congruence in results. The bad performance of

Expo estimator was also found by Peterson and Slade (1998). The Boot estimator, which needed an unacceptably large sub-sample to estimate sample richness (65.3%), was also a poor estimator in the studies of Colwell and Coddington (1994) and Chazdon et al. (1998). On the other hand, Boot was considered a good estimator when utilised in species poor samples with few rare species, such as parasite species richness (Walther and Morand 1998), and this is also in accord with Smith and van Belle (1984) who suggested the Boot method for well sampled assemblages. MMClench produced poor estimates of richness in our samples and was also considered inadequate by Keating and Quinn (1998) on simulated and real data sets. The SV method, mainly used in stream ecology (Stout and Vandermeer 1975; Minshall et al. 1985; Haynes 1987), which has only been evaluated once on a simulated data set, produced a poor result when compared to the other two methods tested, Jack 1 and the parametric Cohen method (Baltanás 1992). The SV estimator was the best in relation to minimum sub-sample size required (Table 3 and 4), but the curve shape was not constant among the six data sets and hence cannot be used with confidence (Figs 1 and 2). We observed that when fitting SV to observed richness at different sub-sample sizes, the fitted curve was very similar to the observed points. However, the extrapolated part of the fitted curves was too sensitive to small differences in the shape of the observed richness curve, resulting in very different values of the extrapolated asymptote.

The recently developed ICE and ACE methods received intermediate scores in our analyses and have only been evaluated twice before, producing conflicting results (Chazdon et al. 1998; Walther and Morand 1998). In the species poor assemblage of parasites per host they were incapable of producing any estimate for many sub-sample sizes and were considered inadequate for this kind of data set (Walther and Morand 1998). On the other hand, Chazdon et al. (1998) evaluated eight methods for predicting species richness of seedlings and saplings in tropical forests in Costa Rica and concluded that the ICE method was the best. They found the method to be robust to sample size and patchiness and suggest its use in sites with high species richness. One problem stated by Walther and Morand (1998) is that ACE and ICE methods need definition, a priori, of a parameter related to the definition of rare species. We used the default value 10 proposed by Colwell (1997) in EstimateS and also used by Chazdon et al. (1998). Increasing this value gave higher estimated values, reaching values close (ICE) or superior (ACE) to those estimated by Jack 2, when the parameter was set to the number of stones in the sample (results not shown here).

The best of the evaluated methods, Jack 2, Jack 1, Chao 1 and Chao 2 were also considered the best or among the best estimators in other evaluations and their use has been suggested by many authors. Colwell and Coddington (1994) evaluated the performance of eight methods on a seed-bank data set and suggested the use of Chao 2 and Jack 2. Chazdon et al. (1998) in a study of seedling and sapling diversity found that ICE and Chao 2 were robust to sample size and patchiness. Although Palmer (1990; 1991) used a different approach to evaluate estimation methods, he proposed the use of Jack 1 and Jack 2, while Walther and Morand's (1998) evaluation of parasites per host data sets recommended the use of Chao 2 and Jack 1. Peterson and Slade (1998) tested seven methods for their ability to estimate the number of states in Mexico and United States by using records of automobile license plates taken in two cities along several days and found the Chao 2 method was one of the best (they did not evaluate Jack 1, Jack 2, and Chao 1).

An ideal situation for evaluating richness estimators is to compare the estimated value to total species richness in an area. However, to know the total richness in rich communities with many rare species, such as tropical stream macroinvertebrates, an unfeasible large sample may be needed. As many species exist at larger space and time scales (regional pool), we will practically never know how many species there are in a given place, because of the continuous appearance of rare species over time and with increasing catch effort.

Another problem of evaluating richness estimators are the criteria used to score the different methods. Palmer (1990; 1991) and Baltanás (1992) chose one fixed sub-sample size and applied several statistics to decide which method could estimate the richness in total samples with low bias and high precision. They did not present any explanation for choice of sub-sample size. A quick look at Figs 1 and 2 and Table 3 reveals that except for the Expo, MMMean and MMRuns methods, all other methods were capable of estimating the total sample richness, but at different sub-sample sizes. If we opt for an a priori sub-sample size of say 65% of samples, we would conclude that Boot was the best estimator.

An important characteristic expected from a estimation method, is that it should estimate total richness independently of sample size. However, as Figs 1 and 2 show, all evaluated methods in our study were strongly dependent of sub-sample size. In practice, this means that the richness estimate for a given sample size is not a reliable estimate of the true richness in the studied assemblage. It is likely that a different sub-sample size would produce a different estimated richness. Thus, agreeing to Baltanás (1992) and Schmit et al. (1999), caution should be taken when using the absolute values produced by some estimation method from species rich assemblages to assess total richness in an area.

In other hand, the very close sub-sample sizes needed for some methods to estimate the observed richness in the total sample (Table 3), even through rather different data sets as the 6 used samples (Table 2), reinforces the suggestions of Palmer (1990) and Baltanás (1992) about the good reliability of using such methods for comparison purposes. In the case of Jack 2, Jack 1 and Boot estimators, the range of sub-sample sizes needed to estimate richness in total samples were respectively 22.4-26.7, 35.6-41.3 and 63.6-66.7 percent of total samples. In a similar study, Hellmann and Fowler (1999) used five data sets of plants with different species richness and proportions of rare species and found that for Jack 1, Jack 2 and Boot, the sub-sample size needed to estimate richness in the total sample were respectively, 22.6-29.1, 36.8-43.9 and 63.1-69.0 percent of total samples, values very close to those found in our study. Thus, it seems that these estimation methods are not only strongly dependent on sub-sample size but also it seems that this dependence occurs in a predictable way. In other words, at least for assemblages where species accumulation curves are not approaching an asymptote, it is possible to estimate the richness in a sample of a given size by using a sub-sample of a constant proportion. Using the Jack 2 method, the richness expected in a sample of 40 sampling units can be estimated by using only 10 sampling units. As highlighted before, in many cases there is a reason for using an estimated richness instead of the observed richness. If samples do not have a proper size, potential erroneous conclusions can be obtained due to differences in equitability and proportion of rare species between data sets. For example, despite the Carmo sample being richer than the Japi sample (Table 2), this was apparent in the curve of accumulated observed richness only after 38 stones had been pooled. Using the estimated values of the Jack 2, this was apparent after the pooling of 14 stones (see Lande et al. 2000 for a more comprehensive statement of the problem and an alternative solution using a diversity index). Anyway, further studies addressing specifically this question are necessary for a reliable conclusion.

Despite the structural differences among sampled assemblages highlighted previously, Jack 2 and, to a lesser extent, Jack 1, Chao 1 and Chao 2 presented very similar results across all six data sets. Taking into account their good performance in other evaluations cited above, these methods seen robust to variation in data structure and hence should be used for comparative purposes in assemblages similar to those tested here and in those kinds of assemblages for which no previous evaluation is available.

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Capítulo 2

On the use of jackknife and related non-parametric techniques to estimate species richness in an area[1]

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[1] Melo, A. S. On the use of jackknife and related non-parametric techniques to estimate species richness in an area. Unpublished manuscript.

Abstract

Species richness in an assemblage is frequently used as a measure of biological diversity. However, observed species richness is strongly dependent on sample size. If more samples are collected, more species are observed. Non-parametric species richness estimators, such as the Jackknife 1 and 2 and the Chao 1 and 2, are indicated in the literature as potential solutions to the problem of dependence of observed species richness on sample effort. These methods are intended to estimate the total species richness in an area using a small sample effort. Non-parametric estimators are based on the number of species observed, and the number of rare species in a sample, i.e., that occurred in one and or two sampling units, or with one and or two individuals. High estimates are produced when samples contain large proportions of rare species. Using a range of real data sets, I show that estimates produced by non-parametric methods are just a linear function of the observed species richness. An implicit assumption of these non-parametric techniques is that the rare species curve should present high values at small sample sizes and decreasing values as sampling effort is increased. This assumption was not observed in the six data sets presented. Instead, the rare species curve flattens off around a constant value as sampling effort increases. I conclude that non-parametric estimators are not reliable to estimate species richness in an area. Comments are made on the potentiality of using non-parametric estimators in the comparisons of species assemblages.

Introduction

Researchers are increasingly looking for new tools in order to improve conservation efforts to save biological diversity. Included in these tools are the protocols for rapid assessment of biodiversity (Coddington et al. 1991), the use of morphospecies as a surrogate for biological species (Oliver and Beattie 1996), the selection of indicator taxa (Brown and Freitas 2000), and techniques to estimate species richness in a given area (Colwell and Coddington 1994). All these techniques are intended to guide the selection of reserves, by optimizing the use of scarce funds to save the greatest number of species, endemic or threatened taxa, key taxa in the ecosystem functioning, and unique ecosystems.

In this sense, species richness estimators are thought to be a valuable technique, as they would estimate diversity in a given area using small sampling efforts (Colwell and Coddington 1994). Differently from the number of observed species in a survey, which is generally dependent on sample size, estimated species richness are expected to be quite independent of sample size. They would produce good estimates of species richness in an area using fewer samples than what would be required by counting observed species (Gotelli and Colwell 2001), thus saving time and money. Fig. 1 depicts an estimated curve that would be produced by an ideal method as well as the corresponding observed species accumulation curve in function of increasing sample size. While the curve of accumulated species richness observed increases slowly with sampling effort, the ideal estimator would produce values close to the actual species richness in the area using small sample sizes and then flatten off.

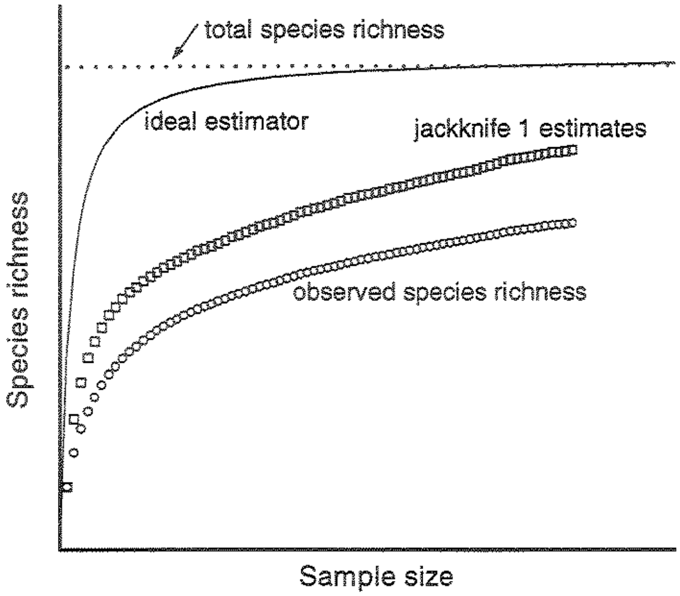

Fig. 1. Observed species accumulation curve, the respective jackknife 1 estimate curve, and a hypothetical ideal estimate curve. The jackknife 1 curve follows the observed species curve in a regular way, increasing slowly with sample size. On the other hand, the hypothetical ideal estimator produce richness estimates around the total number of species in the area using small sample size and then flatten off.

Species richness estimators were reviewed by Colwell and Coddington (1994), who present the several techniques currently available in an easy way to biologists. They distinguished three classes of estimators, namely Extrapolations of Species Accumulation Curves (ESAC), Parametric Estimators, and Non-parametric Estimators. Included in the first class are several asymptotic functions, such as the Michaelis-Menten hyperbole of enzyme kinetics used by biochemists (Keating and Quinn 1998). Parametric estimators are based in the assumption that biological data follow a specific distribution, such as the lognormal. Besides having no assumption regarding data distribution, non-parametric methods are easily computed and include Jackknife 1, Jackknife 2, Chao 1, and Chao 2 (Table 1). They are obtained by summing the number of species already observed in a sample and a second term related to the proportion of observed species that were rare in the sample (Table 1). High species richness estimates are obtained when non-parametric techniques are employed on samples with high proportion of rare species.

Table 1. Non-parametric estimators of species richness in an area. Q_1 = number of species which occurred in exactly one sampling unit. Q_2 = number of species which occurred in exactly two sampling units. n = number of sampling units. F_1 = number of species which occurred with exactly one individual. F_2 = number of species which occurred with exactly two individuals.

Estimators	Formulae	References
Jackknife 1 (Jack 1)	$S_{Jack1} = S_{obs} + Q_1\left(\frac{n-1}{n}\right)$	Burnham and Overton (1978), Colwell and Coddington 1994
Jackknife 2 (Jack 2)	$S_{Jack\,2} = S_{obs} + \left(\frac{Q_1(2n-3)}{n} - \frac{Q_2(n-2)^2}{n(n-1)}\right)$	Burnham and Overton 1978, Colwell and Coddington 1994
Chao 1	$S_{Chao1} = S_{obs} + \frac{F_1^2}{2F_2}$	Chao 1984 (1984), Colwell and Coddington 1994
Chao 2	$S_{Chao2} = S_{obs} + \frac{Q_1^2}{2Q_2}$	Chao 1987 (1987), Colwell and Coddington 1994

As previously outlined, a desired trait of species richness estimators is the independence of sample size, thus able to produce reliable values even when using small sampling efforts. However, Colwell and Coddington (1994) discuss limitations of using non-parametric estimators on small sample sizes. They point out that Jackknife estimators attain their maximum values at approximately twice the observed richness, while Chao estimators at about half the square of the observed number of species. As a consequence, Colwell and Coddington (1994) predict that, "...these estimators should correlate strongly with sample size until half (or the square root of twice) the total fauna is observed and thereafter become gradually independent of sample size until finally the observed richness and the estimate converge." In fact, in a recent evaluation of species richness estimators, we show that estimated richness curves do not attain an asymptote early (Melo and Froehlich 2001a). Instead, they follow the observed species accumulation curve in a quite regular way, estimating values in a fixed proportion above the observed richness along most of the increasing sampling effort (Fig. 1).

Here, I empirically explore how strong is the dependence of estimators on observed species richness. Comments are made on the usefulness of using non-parametric estimators to predict species richness in an area and in the comparisons of species assemblages.

An empirical relationship

A striking relationship of dependence of richness estimates with observed values was obtained by plotting the number of sampling units needed to observe a given richness value when constructing a species accumulation curve, and the number of sampling units needed to estimate the same value using a non-parametric estimator. Fig. 2 shows such a relationship using the Jackknife 1 estimator and a data set of macroinvertebrates living on stream stones (Table 2, local data set) (Melo and Froehlich 2001a). Notice that dots in Fig. 2 do not depict richness values. Richness values were used only to match the corresponding number of sampling units in which the same number of species can be obtained from the cumulative observed list and from estimates of the first order Jackknife. As the Jackknife estimates may be non-integer values, a precise match between the two metrics was achieved by using the species richness estimated by the first order Jackknife for each cumulative number of sampling units (y-axis) and the corresponding interpolated x-value (Fig. 2).

The coefficient of determination obtained from Figure 2 is very high (r^2=0.997), demonstrating that richness estimates values were just a linear function of the observed values. The relationship is so strongly linear that the extrapolation of the fitted linear regression in Figure 2 can be used as richness estimator in a larger sample size. In fact, such estimates of species richness for larger sample sizes are very reliable (A. S. Melo et al. unpublished manuscript, [Capítulo 3]).

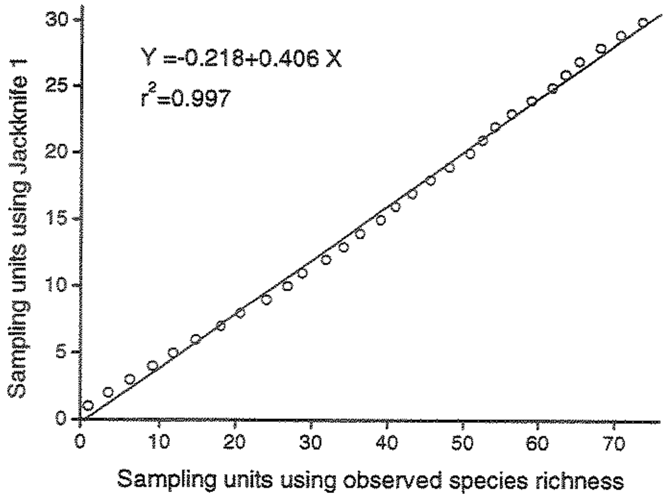

Fig. 2. Relationship between the number of sampling units required to observe a given species richness and the number of sampling units required to estimate the same species richness using the jackknife 1 estimator. A precise match was obtained by using jackknife 1 species richness estimates for each cumulative number of sampling units and the corresponding interpolated value needed to observe the same richness in a species accumulation curve. Observed species richness in a given sample size can be obtained using the jackknife 1 estimator over a nearly fixed proportion of the same sample size, represented by the inclination of the linear fit. For the used data set, this proportion is around 40%. Used data are from stream macroinvertebrates occurring in 75 sampling units (stones) collected in a stream site (local data set in Table 2).

The relationship shown in Fig. 2 is easily extended to other non-parametric estimators. Figure 3 shows the relationship using Jackknife 2, Chao 1, and Chao 2 for the same stream macroinvertebrates data set. Notice that, when using different non-parametric estimators, differences are greatly restricted to the inclination of the linear relationship. For the used data set, Jackknife 2 estimator is able to produce a given observed richness value using the smallest sample size, while the Jackknife 1 using the largest sample size.

I further construct the relationship depicted in Fig. 2 to a range of other data sets (Table 2). The data sets comprise different taxa, species richness, sampling effort, and data structure. As seen using the stream invertebrate data set, there were strong correlations between the number of sampling units required to estimate and to observe a given species richness value (Table 3). Better correlations were observed for Jackknife 1 and 2 estimators rather than for Chao 1 and 2 estimators.

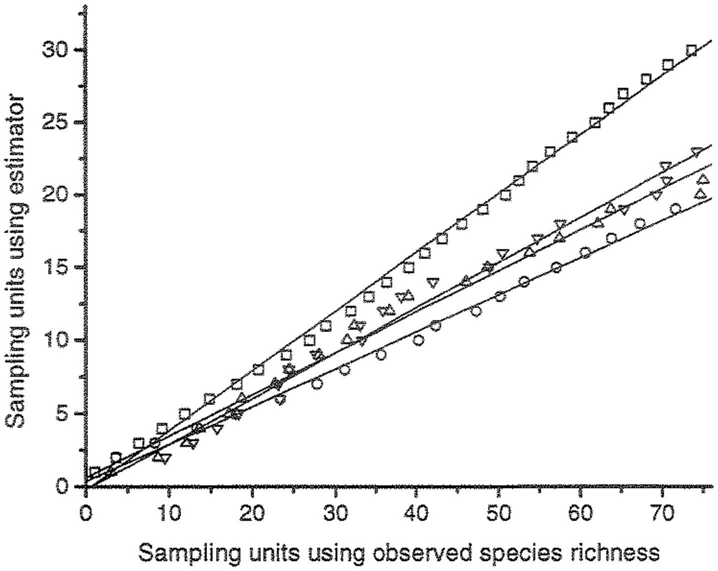

Fig. 3. The same relationship shown in Fig. 2 extended to other non-parametric estimators. □ = Jackknife 1 . ○ = Jackknife2. Δ = Chao 1. ∇ = Chao 2. Jackknife 2 estimate a given richness value using the smallest proportion of the sample size needed to observe the same given richness value, while the Jackknife 1 using the largest proportion. Used data are from stream macroinvertebrates occurring in 75 sampling units (stones) collected in a stream site (local data set in Table 2). Parameters of the linear fit and the determination coefficient are shown in Table 3.

A practical observation of the relationship depicted in Fig. 2 is obtained by comparing results of Hellmann and Fowler (1999), and Melo and Froehlich (2001a). Hellmann and Fowler (1999) used five data sets of plants with different species richness and proportions of rare species. They found that for the Jackknife1 and the Jackknife 2, the sub-sample sizes needed to estimate actual species richness in the total samples were respectively, 22.6-29.1% and 36.8-43.9% of total samples. Melo and Froehlich (2001a) used six data sets of stream macroinvertebrates comprising different locations and spatial scales (local = one stream site, and regional = several sites inside a same catchment basin). They observed that Jackknife 1 and Jackknife 2 estimated actual richness in the total samples using subsamples of sizes 22.4-26.7% and 35.6-41.3% of the total samples, respectively.

Table 2. Summary of the data sets used to investigate the dependency of non-parametric richness estimates on observed species richness. All localities in Brazil.

	Stream macroinvertebrates local	Stream macroinvertebrates regional	Spiders	Trees	*Drosophila* spp.	Harvestmen	Frogs
Locality	Jundiai, São Paulo	Iporanga, São Paulo	Linhares, Espirito Santo	Campinas, São Paulo	Barreiro Rico, São Paulo	Ubatuba, São Paulo	Ilha de São Sebastião, São Paulo
Geographical Coordinates	23° 14'S, 46° 56'W	24° 18'S, 48° 25W	19° 10'S, 40° 05'W	22° 49'S, 47° 07'W	22° 40'S, 48° 10'W	23° 26'S, 45° 04'W	23° 47'S, 45° 24'W
Vegetation	Tropical Semi-Deciduous Montane Forest	Atlantic Rain Forest	Atlantic Rain Forest	Tropical Semi-Deciduous Forest	Tropical Semi-Deciduous Forest	Atlantic Rain Forest	Atlantic Rain Forest
Sampling units	stones (15-20 cm. diam.) in stream riffles	stones (15-20 cm. diam.) in stream riffles	time intervals	10 x 10 m contiguous plots	traps using fermented bananas	8 x 8 m plots on litter	8 x 8 m plots on litter
Sample size	75 (1 stream site)	475 (10 streams inside a catchment)	243	100	180	63	92
Species richness	66	162	287	101	57	40	15
Individuals	3759	17,598	1982	1465	8166	764	846

Table 3. Parameters of the linear model and the determination coefficient obtained by fitting the relationship of the number of sampling units needed to observe a given richness value and the number of sampling units needed to estimate the same value using a non-parametric estimator.

	Jackknife1			Jackknife 2			Chao 1			Chao 2		
Data set	a	b	r^2	a	b	r^2	a	b	r^2	a	b	r^2
Stream macroinvertebrates regional	-2.876	0.388	0.998	-2.891	0.244	0.995	6.970	0.305	0.994	-1.038	0.338	0.993
Spiders	1.899	0.393	0.999	2.168	0.239	0.999	-4.008	0.342	0.998	-19.698	0.378	0.899
Trees	2.400	0.329	0.992	2.391	0.200	0.990	-3.103	0.338	0.981	-6.200	0.310	0.931
Stream macroinvertebrates local	-0.218	0.406	0.997	0.374	0.255	0.994	0.643	0.283	0.984	-0.203	0.311	0.985
Drosophila spp.	0.833	0.400	0.999	1.658	0.244	0.996	3.885	0.163	0.967	3.457	0.168	0.969
Harvestmen	1.505	0.299	0.993	2.226	0.154	0.983	1.200	0.212	0.981	0.912	0.089	0.929
Frogs	-1.134	0.430	0.987	-1.043	0.288	0.949	0.051	0.436	0.989	-4.400	0.390	0.932

Non-parametric estimates and the number of rare species in a sample

An implicit assumption to obtain the ideal estimated curve in Fig. 1 is that the number of rare species (i.e. those occurring in 1 and or 2 sampling units/individuals) is high in small samples and decreases linearly as sampling effort increases. Figure 4a illustrates a hypothetical example using a Jackknife 1 estimate curve and its three components, (1) the observed species richness curve, (2) the rare species curve (i.e. the number of species observed in 1 sampling unit), and (3) the correction factor for sample size (i.e. [n-1]/n, where n is the number of sampling units). For each sample size, Jackknife 1 estimates is obtained by multiplying the number of rare species by the correction factor and then summing the obtained value with the observed species richness (Table 1). Observed species richness curve in Fig. 4a was obtained from the stream macroinvertebrates data set (Table 2, local data set), while the rare species curve was obtained mathematically in order to produce the hypothetical Jackknife 1 estimated curve. Notice that this hypothetical rare species curve required in order to produce the ideal estimator is unreal, as at small sample sizes the number of rare species is higher than the number of observed species. The asymptote of the hypothetical Jackknife 1 curve was chosen arbitrarily, but it is in the range of species richness commonly found in other similar streams in the region (Melo and Froehlich 2001a; 2001b).

For the same data set from which the observed species richness curve in Figure 4a was obtained, Figure 4b shows the estimated Jackknife 1 curve using the actual rare species curve. The actual rare species curve differs from the ideal rare species curve in two ways. The actual rare species curve does not decrease linearly as sample size increases and the absolute number of rare species is low when compared to the ideal rare species curve. Thus, after around 10 sampling units are collected, the correction factor of sample size tends to flatten off close to the unity and the Jackknife 1 estimated curve becomes mostly the sum of the observed species richness with a practically constant number of rare species. Figure 5 shows curves for number of rare species (occurring in only one sampling unit), observed species richness, and the corresponding Jackknife 1 estimates to other six data sets. Despite the range of assemblage types, sample effort, and taxa, the curves of the numbers of rare species in all six data sets do not present a clear trend of decrease as sample size increase. Instead, the curves tend to flatten off around a constant number of rare species.

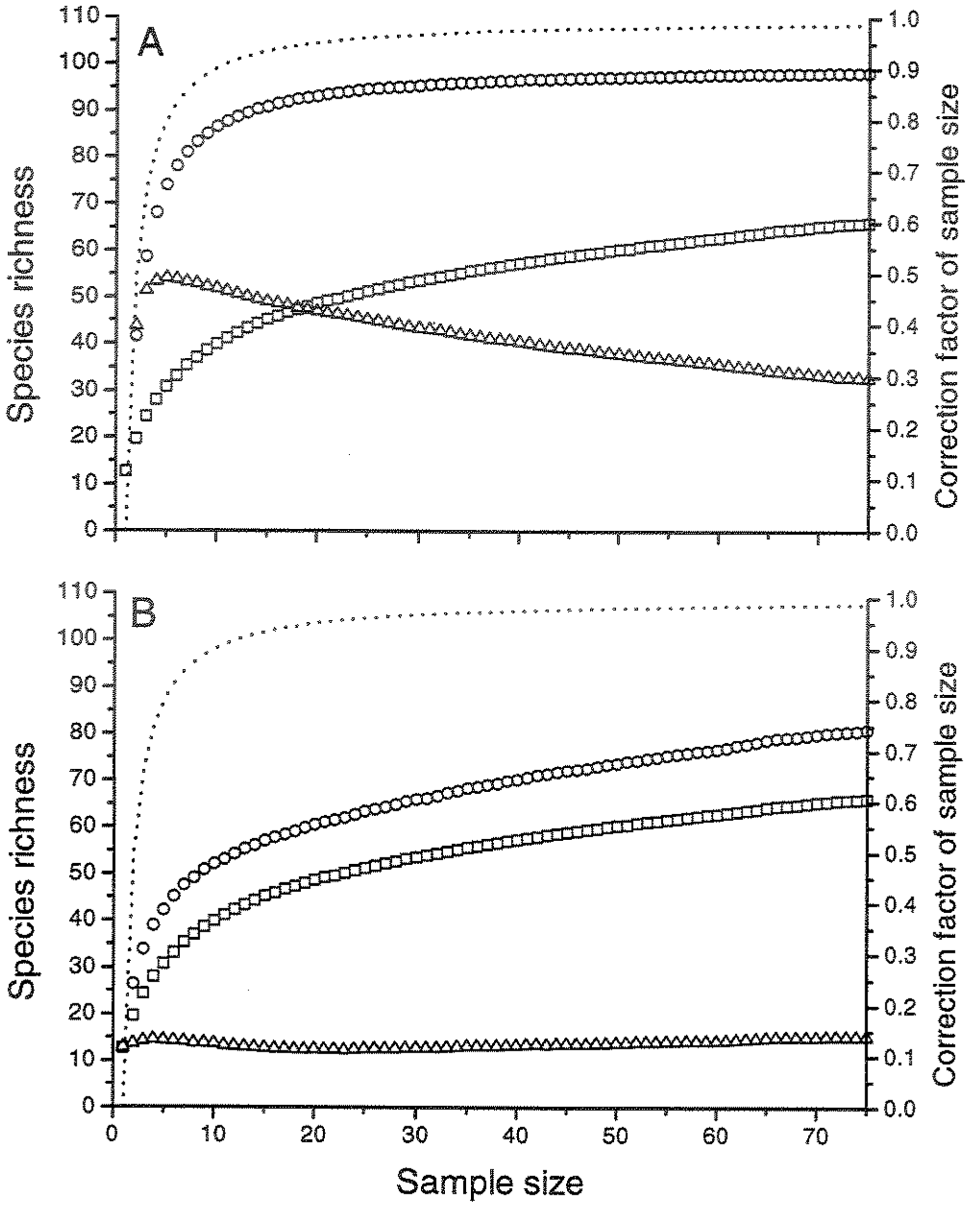

Fig. 4. Jackknife 1 curve estimates and its three components in desired hypothetical (A) and real (B) situations. ○ = Jackknife 1 estimate curves. □ = Observed species accumulation curves. Δ = Rare species curves represented by the number of species which occurred in exactly one sampling unit. --- = Correction factor of sample size ([n-1]/n, where n is sample size). Jackknife 1 estimates are obtained by multiplying the number of rare species by the correction factor and then summing the obtained value with the observed species richness. Observed species richness curves were obtained from the stream macroinvertebrates data set (Table 2, local data set). Rare curves were obtained mathematically in order to produce the hypothetical Jackknife 1 estimated curve (A) and from the stream macroinvertebrates data set (B). The real rare species curve (B) is low and does not decrease with sample size, as would be expected in order to produce the jackknife 1 estimates in the hypothetical situation (A). Notice that the ideal rare species curve in (A) is unreal at small sample sizes, as the number of rare species is higher than the number of observed species.

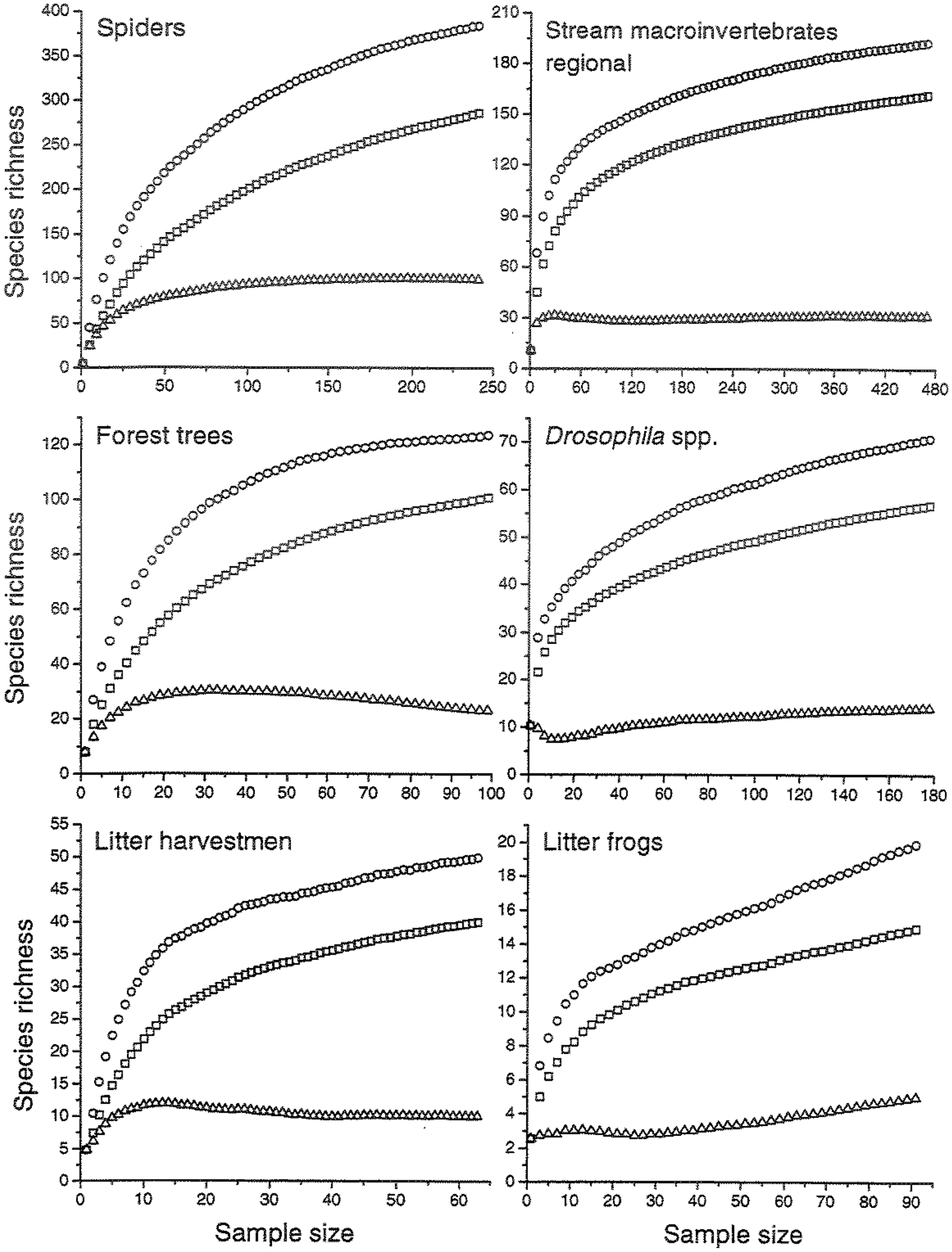

Fig. 5. Observed species accumulation curves, rare species curves (species occurring in one sampling unit), and the corresponding jackknife 1 estimate curves for the range of data sets described in Table 2. Rare species curves do not decrease with sample size, as would be expected in order to produce the ideal situation depicted in Fig. 4A. ○ = Jackknife 1 estimate curves. □ = Observed species accumulation curves. Δ = Rare species curves.

Discussion

The assumption that the number of rare species decreases as sampling size increases was only weakly supported in the tree data set, and clearly not supported in the remaining six data sets presented. Instead, the number of rare species increased at small sample sizes and then tended to flatten off. This finding is not completely unexpected, as it is predicted in the Log-Series distribution of Fisher et al. (1943). In this distribution, the expected number of species with one individual is given by multiplying the parameters x and α, where x varies from 0 to 1 and α is known as the diversity index of the distribution. When the ratio number of individuals by the number of species (N/S) is large, x tends to the unity and if for instance the ratio is 20, x is around 0.99. Thus, for large N/S ratios the expected number of species with one individual is nearly equal to the α value. It has been found in a number of studies that the α value is very constant as sample size increases (Taylor et al. 1976), and this independence of sample size is considered a good trait of this diversity index (Southwood 1978; Magurran 1988). Further support to the constancy of the number of rare species as sample size increases is observed in the extensive collections of herbivorous insects in host plants presented by Novotný and Basset (2000), and spiders (Toti et al. 2000).

The above results about the constant number of rare species in a sample imply that nonparametric estimates will be simply the sum of observed species richness and a nearly constant value. The goal of estimating the number of species in an area is illusive unless sample size is so large that the rare species curve starts to decrease. In this situation, usefulness of nonparametric estimators becomes doubtful, as the researcher will have a good estimate of species richness in the area by simply using the number of species already sampled. Thus, the original goal of estimating species richness in an area using a small sample is not attained by nonparametric estimators. It is noteworthy to observe that sample sizes in most data sets in

Table 2 are not small. For example, the sample size of the stream macroinvertebrates data set (local) is 2-3 times larger than the sample size usually employed to assess diversity in a stream site (Stout and Vandermeer 1975; Minshall et al. 1985; Melo and Froehlich 2001b).

It is likely that many rare species in a sample are actually vagrant species, collected accidentally. Removing these species from data sets, it is possible that as sampling effort is increased the rare species curve would tend to decrease, improving non-parametric richness estimates. However, given the current poor knowledge of the natural history in many species rich assemblages, especially in the tropics, this would be arbitrary in most cases.

A potential usefulness of non-parametric species richness estimators, yet to be evaluated, is in comparisons of different assemblages. Stout and Vandermeer (1975) showed that previous beliefs that temperate streams were richer in insect species than their tropical counterparts were due to insufficient sampling. Extrapolating a species accumulation curve with an asymptotic function, they showed that tropical streams are in fact richer than temperate streams. However, this becomes clear only after a large sample size have been collected. This is because species rich assemblages generally comprise a large number of species with patchy distribution and a great number of rare species. In this sense, Stout and Vandermeer (1975) argue that methods of estimating the species pool could potentially provide better comparative grounds, as they would produce high values for assemblages with high number of rare species.

While this work is restricted to non-parametric estimators, it is possible that other currently available estimators of species richness in an area are also strongly dependent on the observed richness. This speculation is based on the similarity in behavior of non-parametric and other estimators in recent evaluations (Melo and Froehlich 2001a). In case this speculation is found to be true in future evaluations, the question remains on the feasibility of estimating species richness in an area using small samples.

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Capítulo 3

Comparing species richness among assemblages using sample units: why not use extrapolation methods to standardize different sample sizes? [1]

[1] Melo, A. S., R. A. S. Pereira, A. J. Santos, G. J. Shepherd, G. Machado, H. F. Medeiros, and R. J. Sawaya. in press. Comparing species richness among assemblages using sample units: why not use extrapolation methods to standardize different sample sizes? Oikos.

Abstract

Comparisons of species richness among assemblages using different sample sizes may produce erroneous conclusions due to the strong positive relationship between richness and sample size. A current way of handling the problem is to standardize sample sizes to the size of the smallest sample in the study. A major criticism about this approach is the loss of information contained in the larger samples. A potential way of solving the problem is to apply extrapolation techniques to smaller samples, and produce an estimated species richness expected to occur if sample size were increased to the same size of the largest sample. We evaluated the reliability of 11 potential extrapolation methods over a range of different data sets and magnitudes of extrapolation. The basic approach adopted in the evaluation process was a comparison between the observed richness in a sample and the estimated richness produced by estimators using a subsample of the same sample. The Log-Series estimator was the most robust for the range of data sets and subsample sizes used, followed closely by Negative Binomial, SO-J1 (an empirical relationship between number of species observed and estimated by the Jackknife 1), Logarithmic, Stout and Vandermeer, and Weibull estimators. Performance of the estimator Log-Linear was dependent on data sets used, while estimators Exponential, Clench, Power, and the formulae developed by Evans et al. presented bad performance in most of the data sets used. When applied to a set of independently replicated samples from a species-rich assemblage, standard deviations and 95% confidence intervals of estimates produced by the six best evaluated methods were comparable to those of observed richness in the samples. Performance of estimators tended to be better for species-rich data sets (> 30-40 spp.) rather than for those which contained few species (< 30 spp.). Good estimates were found when extrapolating up to 1.8-2.0 times the size of the sample. We suggest that the use of the best evaluated methods within the range of indicated conditions provides a safe solution to the problem of losing information when standardizing different sample sizes to the size of the smallest sample.

Introduction

A straightforward way to compare diversity among assemblages is the use of species richness. Advantages of using this metric are its great intuitive appeal, simple computation, and avoidance of pitfalls in choosing one among several diversity indices available (James and Rathbun 1981; Magurran 1988). It is well known, however, that species richness is strongly dependent on sample size. As more sample units or individuals are collected, more species are found (Walther et al. 1995; Condit et al. 1996; Gotelli and Colwell 2001). Thus, comparing assemblages using different sample sizes may produce erroneous conclusions (Stout and Vandermeer 1975).

Traditionally, there are two forms of standardization of sample sizes when comparing assemblages: the collection/observation of a given number of individuals or of sampling units (e.g. plots, transects or traps) (Gotelli and Colwell 2001). Whether individuals or sampling units are better, is a currently disputed question (see discussion in Barbour and Gerritsen 1996; Courtemanch 1996; Vinson and Hawkins 1996) and in most cases researchers opt for the form most frequently used in their research fields. In studies using number of individuals as samples, it is possible to standardize different sample sizes by applying the rarefaction technique to the larger samples to obtain the expected richness in a sample of the same size of the smallest one (Sanders 1968; Hurlbert 1971; Simberloff 1979). Similarly, in studies using sampling units, it is possible to construct a species accumulation curve of the largest sample, remove the additional unit samples, and then record the species richness observed in the standardized sample size. The major criticism of these two approaches is the loss of information represented by the deleted additional individuals or sampling units in the largest samples (Williamson 1973; Magurran 1988; Elphick 1997).

A potential way to circumvent this loss of information is to use a richness estimate for the less sampled assemblages expected to occur if sample size were the same of the largest sample. This approach is not new (Gleason 1922; Evans et al. 1955), but has received little attention from ecologists over the last decades. Solow and Polasky (1999) presented an estimator to be used when sample size is expressed as number of individuals. Tackaberry et al. (1997) suggested a simple extrapolation technique using sampling units, but based on the knowledge of the physical location of each sampling unit. When large and structurally similar data sets are available, a promising extrapolation technique is presented by Plotkin et al. (2000). The technique uses a calibrated parameter obtained from a similar data set to extrapolate species richness in the data set in study.

A second potential situation where extrapolation of species richness to larger sample sizes might be useful is in the comparisons of assemblages differing in the proportion of rare species and heterogeneity. A species-poor assemblage, but with species distributed homogenously in the sampled area, might produce a species richness value higher than one observed in a species-rich assemblages with a large proportion of rare species and with patchy distribution in the area. In these cases, the species accumulation curves intersect and reliable comparisons can only be done using large sample sizes. An example of the problem is presented by Stout and Vandermeer (1975), who compared stream insect assemblages in tropical and temperate areas. Lande et al. (2000) suggest the use of the Simpson diversity index as a solution to ranking assemblages when using small sample sizes. Alternatively, if interest relies specifically in species richness, a second potential solution yet to be evaluated is to compare assemblages using extrapolated sample sizes. If a reliable extrapolation technique is available, one may choose to compare assemblages using for instance richness values estimated to occur if sample sizes were doubled.

Most of the potential methods that can be used to produce richness estimates for a defined sample size when using sampling units are extrapolations of species accumulation curves (e.g. Soberón and Llorente 1993). The parameters obtained in the adjusted equation using the species accumulation curve of the less sampled assemblage, are used to extrapolate to a larger sample size. Although simple, only a few models in a small number of papers have evaluated the closeness of estimates in relation to *a priori* known actual richness (Arrhenius 1923; Palmer 1990; Tackaberry et al. 1997; Keating et al. 1998).

Here we assessed the reliability of 10 currently available estimators plus one here described. We evaluated the accuracy, precision, and bias, and compared their performance in relation to the known actual richness in replicated data sets from two different assemblages. Additionally, in order to investigate the robustness of the evaluated estimators, we applied these 11 methods to estimate the known richness in six data sets using a range of subsample sizes. These data sets correspond to different taxa, assemblage structure, and were obtained through disparate sampling methods.

Keating et al. (1998) evaluated several extrapolation techniques to the analogous problem of effectiveness of further sampling in species inventories. They used data sets from beetles, vascular plants, and nine model communities with 10, 100, and 1000 species and high, medium, and low evenness. Here we expand the results of Keating et al. (1998) by (1) focusing in the specific problem of standardization of different sample sizes to compare species richness among assemblages, (2) including data from a large range of real assemblages, (3) including previously unevaluated estimators, and (4) using replicated data sets in order to compare the variability of estimates to the natural variability of observed richness among data sets derived from a same assemblage.

We were mainly concerned with the reliability of using such methods in practical situations. Thus, the approach used in the evaluation process was kept as practical and simple as possible, in order to allow a wide range of potential users to grasp and apply them in their ecological or conservation studies.

Methods

The estimators

The simplest way of estimating species richness in a sample is to count observed species in a subsample. Except for the cases in which the subsample has already included all species occurring in the sample, this estimate will be a negatively biased estimator of the richness in the sample and its accuracy will depend on the difference between the sample and the subsample sizes. We included the observed richness in a subsample as an estimate of the richness in the sample from which the subsample was drawn in order to examine how much the 11 evaluated estimators are able to improve the reduction in bias and increase in accuracy in relation to this simple estimate (Palmer 1990). From the 11 estimation methods evaluated in our study, seven were extrapolations of functions fitted to species accumulation curves. In these cases, we constructed a species accumulation curve for a subsample and fitted one of the models. The fitted parameters obtained using this subsample were then used to estimate the species richness in the total sample from which the subsample was drawn. Three of these estimators, the Logarithmic (Log), Exponential (Expo), and Clench models, are presented in Soberón and Llorente (1993) and differ from each other in the probabilities of adding new species as more sample units are collected. Stout and Vandermeer (1975) presented a model (hereafter SV) derived from the Island Biogeography theory and, like the Exponential and Clench models, it can be used to estimate total species richness of the species pool. In other words, the number of species expected to be obtained when sample size increases to infinite (Melo and Froehlich 2001a). The Weibull model has been used in several research fields, and was selected because of its good performance on fitting several species accumulation curves of bird data sets recorded from different human land use developments (Flather 1996). Two models traditionally used in the species-area literature were also evaluated, the LogLin (Gleason 1922; Palmer 1990) and the Power model (Arrhenius 1921; Flather 1996). Equations of each of these curve fitting models are given in Table 1.

Table 1. Curve fitting models used to extrapolate species accumulation curves.

Name	Model	References
Logarithmic (Log)	$S = \frac{1}{z}\ln(1 + zax)$	Soberón and Llorente (1993)
Exponential (Expo)	$S = ab\left(1 - e^{-bx}\right)$	Soberón and Llorente (1993)
Clench	$S = \frac{ax}{1 + bx}$	Soberón and Llorente (1993)
Stout and Vandermeer (SV)	$S = \frac{a}{x^{-z} + \frac{a}{T_\infty}}$	Stout and Vandermeer (1975)
Weibull	$S = a\left\{1 - e^{-[b(x-c)]^d}\right\}$	Flather (1996)
LogLinear	$S = a + b\log(x)$	Gleason (1922), Palmer (1990)
Power	$S = ax^b$	Arrhenius (1921), Flather (1996)

The remaining four estimators evaluated are based on different rationales and computations. Evans et al. (1955) proposed a simple estimator (called here as ECB) obtained by solving a formula which takes into account only the number of sampling units collected and the respective number of species found,

$$S = \frac{s\log(N+1)}{\log(n+1)}$$

where S is the estimated species richness expected to occur in N unit samples and s is the number of species observed in n unit samples.

We also used the estimators Negative Binomial (NB) and Logarithmic Series (LS) that have recently been well evaluated by Keating et al. (1998) for a slightly different problem, the estimation of the effectiveness of further sampling in species inventories. Estimators NB and

LS are given in Efron and Thisted (1976), following Fisher et al. (1943). They were originally based on the information of numbers of species that occurred with 1, 2, 3 etc individuals in a sample. However, in order to standardize all estimators in the study, we opted to use the information of numbers of species which occurred in 1, 2, 3 etc sampling units instead of those occurring with 1, 2, 3 etc individuals. The NB estimator is given by Efron and Thisted (1976) as,

$$\Delta_{\alpha\gamma}(t) = \frac{-\eta_1\left\{(1+\gamma t)^{-\alpha} - 1\right\}}{(\gamma\alpha)}$$

where $\Delta_{\alpha\gamma}(t)$ is the estimated number of species expected to be found in the additional sample size t, which is expressed as the proportion of the sample already collected. Thus, for the problem of estimating the number of species in a larger sample size, the estimate value is obtained by summing the number of species observed in the sample and $\Delta_{\alpha\gamma}(t)$. η_1 is the statistical expectation of the number of species occurring in only 1 sampling unit (or 1 word in the work of Efron and Thisted 1976) and is estimated here as the number of species occurring in only 1 unit sample in the subsample. The parameters α and γ are obtained by fitting a nonlinear regression to the equation,

$$\eta_x = \frac{\eta_1\{\Gamma(x+\alpha)\}}{\{x!\Gamma(1+\alpha)\}\gamma^{x-1}}$$

where η_x is the number of species occurring in exactly x unit samples and Γ represents the gamma function.

The LS estimator is obtained when we set $\alpha = 0$ and, following Efron and Thisted (1976), it is given as,

$$\Delta_{0\gamma}(t) = \left(\frac{\eta_1}{\gamma}\right)\log(1+\gamma)$$

The last evaluated estimator is an empirical method (A. S. Melo, unpublished manuscript, [Capítulo 2]) first evaluated here that relates the number of sampling units needed to collect a given number of species and the number of sampling units needed to estimate the same given number of species using a non-parametric richness estimator, such as the first order Jackknife (see a review of non-parametric estimators in Colwell and Coddington 1994). Jackknife estimates of species richness were developed in order to predict the number of species occurring in a given area based on the number of observed species in a sample and the number of these species that were rare, i.e., that occurred in only 1, 2, 3 etc sampling units (Burnham and Overton 1978; Colwell and Coddington 1994). Previous studies have shown, however, that such estimates are dependent on sample size (e.g. Schmit et al. 1999; Melo and Froehlich 2001a), and in most cases this dependence is so strong that it can be useful as a predictive tool. Fig. 1 shows the relationship between the number of sampling units needed to estimate a given species richness by the first order Jackknife and the number of sampling units necessary to observe the same number of species when constructing a species accumulation curve. Note that the points in Fig. 1 do not depict species richness. Species richness was used only to match the corresponding number of sampling units in which the same number of species can be obtained from the cumulative observed list and from estimates of the first order Jackknife. As the Jackknife estimates are continuous values, a precise match between the two axes was achieved by using the species richness estimated by the first order Jackknife for each cumulative number of sampling units (y-axis) and the corresponding interpolated x-value needed to observe each of such first order Jackknife estimates. Thus, for our proposed method (hereafter SO-J1), (1) we constructed the relationship shown in Fig. 1, (2) fitted a linear model, (3) extrapolated the fitted linear model to the x-value corresponding to the sample size we intended to estimate, (4) and recorded the species richness estimated by the first order

Jackknife using the corresponding number of sampling units obtained in (3). A limitation of the method is the inability to produce estimate values when the intended extrapolated sample size is much larger than the sample size of the available sample, usually more than 2-3 times. In these cases, the number of sampling units needed to be used by the first order Jackknife to estimate the extrapolated value (step 3 above) is larger than the number currently available in the sample in use.

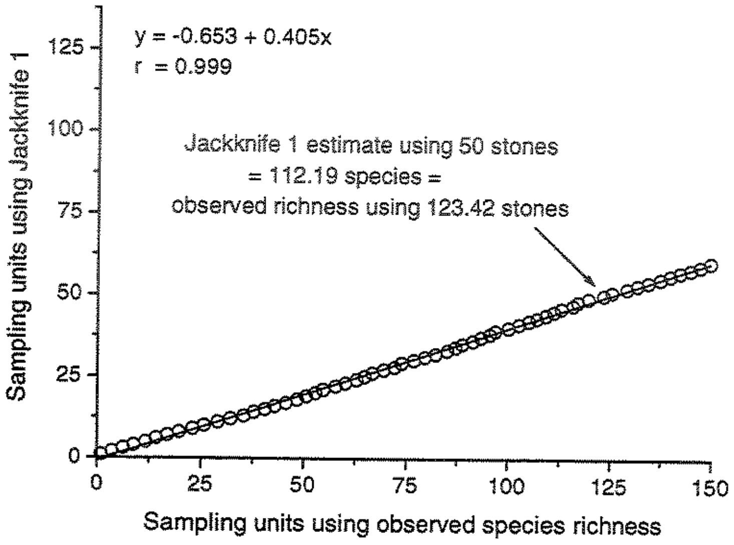

Fig. 1. Relationship between the number of sampling units needed to observe a given number of species and the number of sampling units needed to estimate the same species richness using the Jackknife 1 estimator. A precise match was obtained by using Jackknife 1 species richness estimates for each cumulative number of sampling units and the corresponding interpolated value needed to observe the same species richness in a species accumulation curve. Estimator SO-J1 is obtained by extrapolating the linear fitted model to a larger sample size and recording the corresponding species richness estimate produced by Jackknife 1 estimator. Data are from stream macroinvertebrates occurring in the 150 sampling units (stones) data set Pinda used in the replicated study.

Replicated study

In the first part of the study, we assessed accuracy, precision and bias of the 11 evaluated methods by comparing the richness estimate obtained using a subsample to the actual observed richness in the sample from which the subsample was obtained. The replicate samples were from two large and homogenous data sets of macroinvertebrates living on stones in stream (Melo and Froehlich 2001a) and fig wasps associated with fig fruits of *Ficus eximia* Schott (Moraceae) (Pereira et al. 2000).

The stream macroinvertebrates data set (here called Pinda) consisted of 10,339 individuals belonging to 117 morphospecies occurring in 150 sampling units (stones) collected from a stream reach in Pindamonhangaba, São Paulo state, Brazil (22° 45'S, 45° 28'W). We divided this data set randomly in 6 samples of 25 stones each. We opted for this sample size as it had been used with success in a previous study of diversity (Melo and Froehlich 2001b). From each 25-stone sample, we randomly selected two distinct subsamples of 12 stones each, which were used to estimate the richness in the 25-stone sample.

The fig wasp data set consisted of 13,582 individuals distributed in 13 species and 300 fig fruits collected in Londrina, Paraná state, Brazil (23° 18'S, 51° 09'W). To determine a meaningful sample size for the fig wasp data set, we constructed a species accumulation curve to obtain the minimum sample size in which we could have a good representation of the fig wasp assemblage occurring in *Ficus eximia* fig fruits. After this visual analysis, we concluded that 25 fig fruits was a good sample size; thus the 300 fig fruit data set gave us 12 distinct samples. Following the macroinvertebrate samples, we opted for using a subsample of 12 fig fruits. However, different from the macroinvertebrates samples, only one subsample of 12 units was drawn from each sample.

We used the software EstimateS version 5.0.1 (Colwell 1997) to construct species accumulation curves (100 runs) for all 24 subsamples from the stream macroinvertebrates and fig wasp data sets. Curves were fit using the software Origin version 4.1 (Microcal Software, Northampton, MA, USA). Parameters γ and α used in NB and LS estimators were obtained by writing a specific routine in S-Plus 2000 software (MathSoft, Inc., Cambridge, Ma, USA).

Unreplicated study

In the second part of the study, we assessed the robustness of the different estimators over a range of different subsample sizes and assemblage structures. We used data from six different diversity studies on spiders, trees, *Drosophila* spp., stream macroinvertebrates (Melo and Froehlich 2001a), litter harvestmen, and litter frogs. The six assemblages were very different from each other, comprised representatives from different taxa and habitats, and were obtained through distinct collection methods (Table 2).

For each assemblage, we randomly selected four sets of 30 subsamples, representing subsamples of sizes 40, 55, 70, and 85 percent of the total number of sample units. Each subsample was then used to estimate the species richness in the total sample from which it was derived. Contrary to the replicated study, these subsamples obviously were not independent from each other.

Table 2. Summary of the six data sets used in the unreplicated study. All localities in Brazil.

	Spiders	Trees	Stream macroinvertebrates	*Drosophila* spp.	Harvestmen	Frogs
Locality	Linhares, Espirito Santo	Campinas, São Paulo	Jundiai, São Paulo	Barreiro Rico, São Paulo	Ubatuba, São Paulo	Ilha de São Sebastião, São Paulo
Geographical Coordinates	19° 10'S, 40° 05'W	22° 49'S, 47° 07'W	23° 14'S, 46° 56'W	22° 40'S, 48° 10'W	23° 26'S, 45° 04'W	23° 47'S, 45° 24'W
Vegetation	Atlantic Rain Forest	Tropical Semi-Deciduous Forest	Tropical Semi-Deciduous Montane Forest	Tropical Semi-Deciduous Forest	Atlantic Rain Forest	Atlantic Rain Forest
Sampling units	time intervals	10 x 10 m contiguous plots	single stones (15-20 cm. diameter) in stream riffles	traps using fermented bananas	8 x 8 m plots on litter	8 x 8 m plots on litter
Sample size	243	100	75	180	63	92
Species richness	287	101	66	57	40	15
Individuals	1982	1465	3759	8166	764	846

Estimates for the seven curve fitting methods were obtained by constructing a species accumulation curve (100 runs) for each subsample and fitting each function using non-linear regression. The random draw of the subsamples, construction of the species accumulation curves, and fit of the seven functions were done by using a routine written in S-Plus 2000. We had difficulties in fitting the Weibull model to some subsample sizes in some data sets, because the parameter a which denotes the asymptote of the fitted equation tended to increase indefinitely. For these cases, we fitted each subsample individually in Origin software setting the parameter a as 500. Parameters γ and α used in NB and LS estimators and the computation of the SO-J1 method were obtained by writing specific routines in S-Plus 2000 software (S-Plus routines are available on request from the first and second authors).

Evaluation of estimation methods

In order to make results comparable among all assemblage data, we used the percentage of error in relation to the actual richness, calculated as the difference between the estimated and actual richness in the total sample, divided by the actual richness in the total sample.

For the replicated study, we explored the results produced by the different estimators in two ways. In the first, we plotted the estimated values using 12 sampling units and the known actual richness in 25-sampling unit samples. This plot provides a simple way of assessing visually accuracy, precision, and bias of the estimates in relation to the actual richness in the respectively sample from which it was drawn. Moreover, the plot shows to what extent the estimated values are correlated to the actual richness values.

We also compared the variability of the estimated values with the variability of the actual richness values observed among the 25-sampling unit samples. This was achieved by calculating percentage of errors for each estimation method and also for the values of richness observed in each 25-sampling unit sample. To compute the percentage of errors we used as actual richness the mean richness value observed in 25-sampling unit samples, obtained from a species accumulation curve constructed using the entire data set (150 stones or 300 fig fruits). Accuracy and precision of all estimation methods and also of the observed richness in 25 sampling units were measured as the mean and the standard deviation of the percentage of error values. Bias was measured as the percentage of values that overestimated actual richness minus 50%. A good estimator method should produce values close to zero for accuracy, precision, and bias.

Results

Replicated study

Estimates for Pinda samples were positively correlated with the actual richness for all evaluated methods, and except for the LogLin estimator, all other methods produced estimates more correlated with the actual richness than the observed species richness in 12 sampling units (Fig. 2). However, correlations between estimates and the actual richness for fig wasps were variable, and even a strongly negative correlation was observed for the NB estimator (Fig. 3). Moreover, estimate values for fig samples were much more variable through all methods than for Pinda samples (Figs 2 and 3).

Despite the differences in correlation between estimated and observed richness in the two data sets, there were agreements in bias for some estimation methods. Methods that overestimated or underestimated the actual richness in Pinda samples, in general also overestimated or underestimated richness in fig wasp samples (Figs 2 and 3). As would be expected, the observed richness in 12 sampling units (SO-12) strongly underestimated the actual richness in 25-sampling unit samples. For fig wasps, however, the observed richness in 12 fig fruits (SO-12) produced values more accurate than in Pinda samples. Moreover, in three cases the observed richness in 12 fig fruits were the same as those observed in the 25-fig fruit samples. The Expo model produced estimates very similar to the observed richness in 12 sampling units and in all cases underestimated the actual richness. Following the Expo model, the Clench model also underestimated the actual richness in all but five cases of fig wasps. The Power model produced overestimates for all Pinda samples and 9 of the 12 samples of fig wasps. The ECB estimator also overestimated the actual richness in the two data sets, although with higher accuracy and precision for Pinda than for fig wasp sample. The LogLin estimator tended to underestimate the actual richness in Pinda samples, but was slightly positively biased for fig wasp samples. The estimators Log, SV, Weibull, ECB, SO-J1, NB, and LS produced very similar estimates to each other for Pinda samples and in all cases the 95% confidence limit included the expected actual richness (depicted in Fig. 2 as the diagonal dotted line inside the boxes). For fig wasps, only the estimators Log, LogLin and LS produced 95% confidence limits that included all the expected actual richness range, although the confidence limit lines for methods SV, Weibull, and SO-J1 crossed the expected actual richness (diagonal dotted lines) close to the tails (Fig. 3).

The mean percentage error of each estimation method in relation to the average observed richness in 25 sampling units, obtained from a species accumulation curve using all sampling units in each data set, were consistent with the results described above (Table 3). For Pinda samples, the mean percentage of error of estimators Log, SV, Weibull, LogLin, ECB, SO-J1, NB, and LS were low and in some cases close to zero. For these estimators, the 95% Confidence Interval (CI) included the zero value (Table 3, Fig. 4). Moreover, the mean error value and the 95% CI of these estimators were very similar to that produced by the observed richness in the six 25-stone samples (SO-25) (Fig. 4). The same estimators that produced mean errors close to zero for Pinda samples, also produced mean errors close to zero for fig wasp samples, except for the methods ECB and NB. The estimator LogLin, that produced a negative mean error for Pinda samples, produced a slightly positive error for fig wasp samples, and the absolute error value was comparable to the other well-evaluated estimators for this data set. However, despite the similarities between the two data sets in rank performance in relation to mean error, the 95% CI of estimates for fig wasp samples were in general much wider than in Pinda samples. Furthermore, differently from results obtained from Pinda samples, the 95% CI of estimates for fig wasp samples were much wider in relation to that produced by the observed richness in the 12 25-fig fruit samples (Fig. 4).

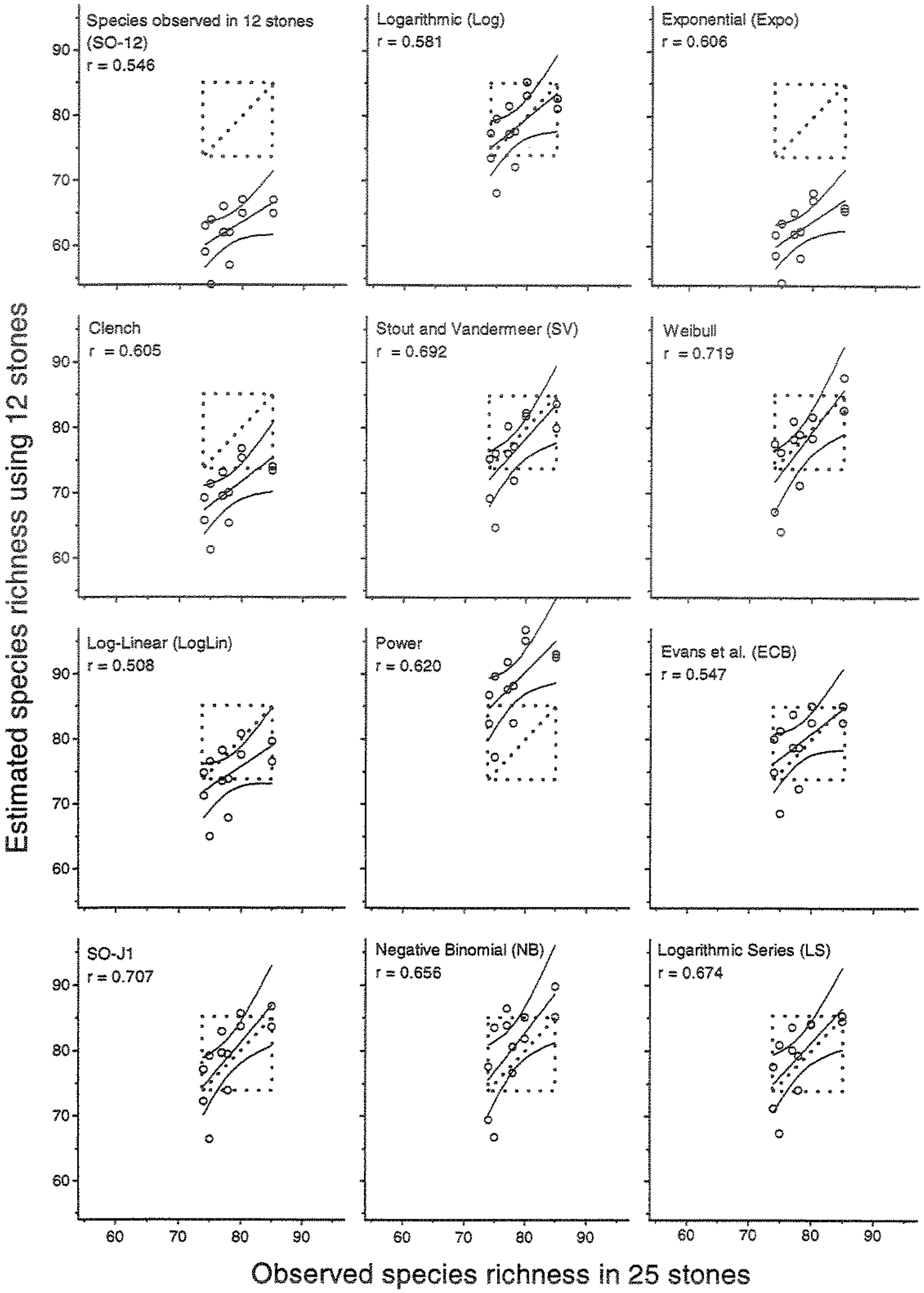

Fig. 2. Relationship between stream macroinvertebrates species richness (Pinda data set) observed in 25 sampling units (stones) and the estimated species richness by 12 methods using subsamples of 12 stones. Two distinct random subsamples were taken from each of six 25-stone samples. The dotted square indicates the range of the six observed richness values in 25-stone samples and the dotted diagonal line the expected estimated richness if estimators produce the same value of observed species richness. Solid lines represent the linear fit to data and 95% confidence limits.

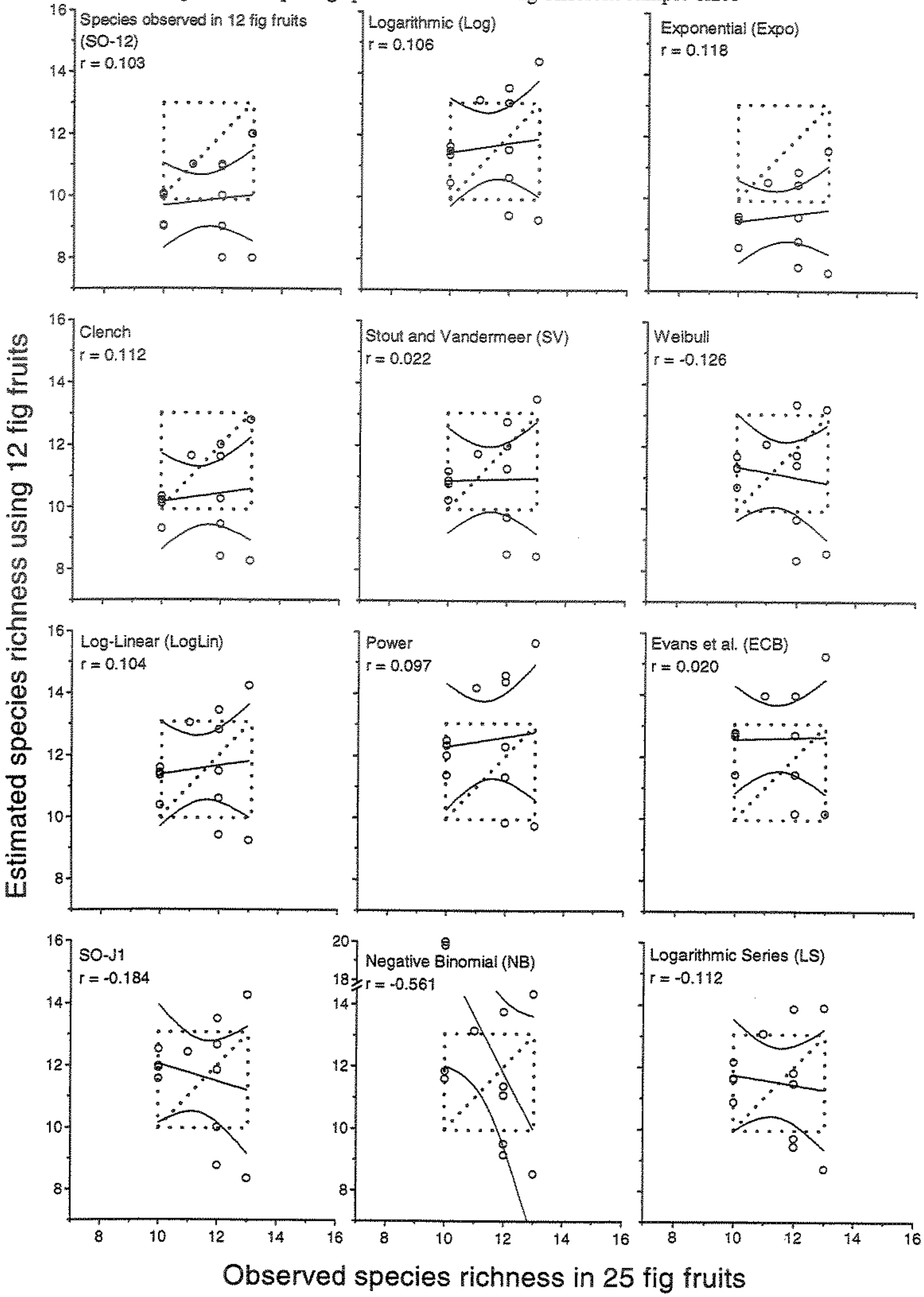

Fig. 3. Relationship between fig wasp species richness observed in 25 sampling units (fig fruits) and the estimated species richness by 12 methods using subsamples of 12 fig fruits. One subsample was taken randomly from each of 12 25-fig fruit samples. The dotted square indicates the range of the 12 observed richness values in 25-fig fruit samples and the dotted diagonal line the expected estimated richness if estimators produce the same value of observed species richness. Solid lines represent the linear fit to data and 95% confidence limits.

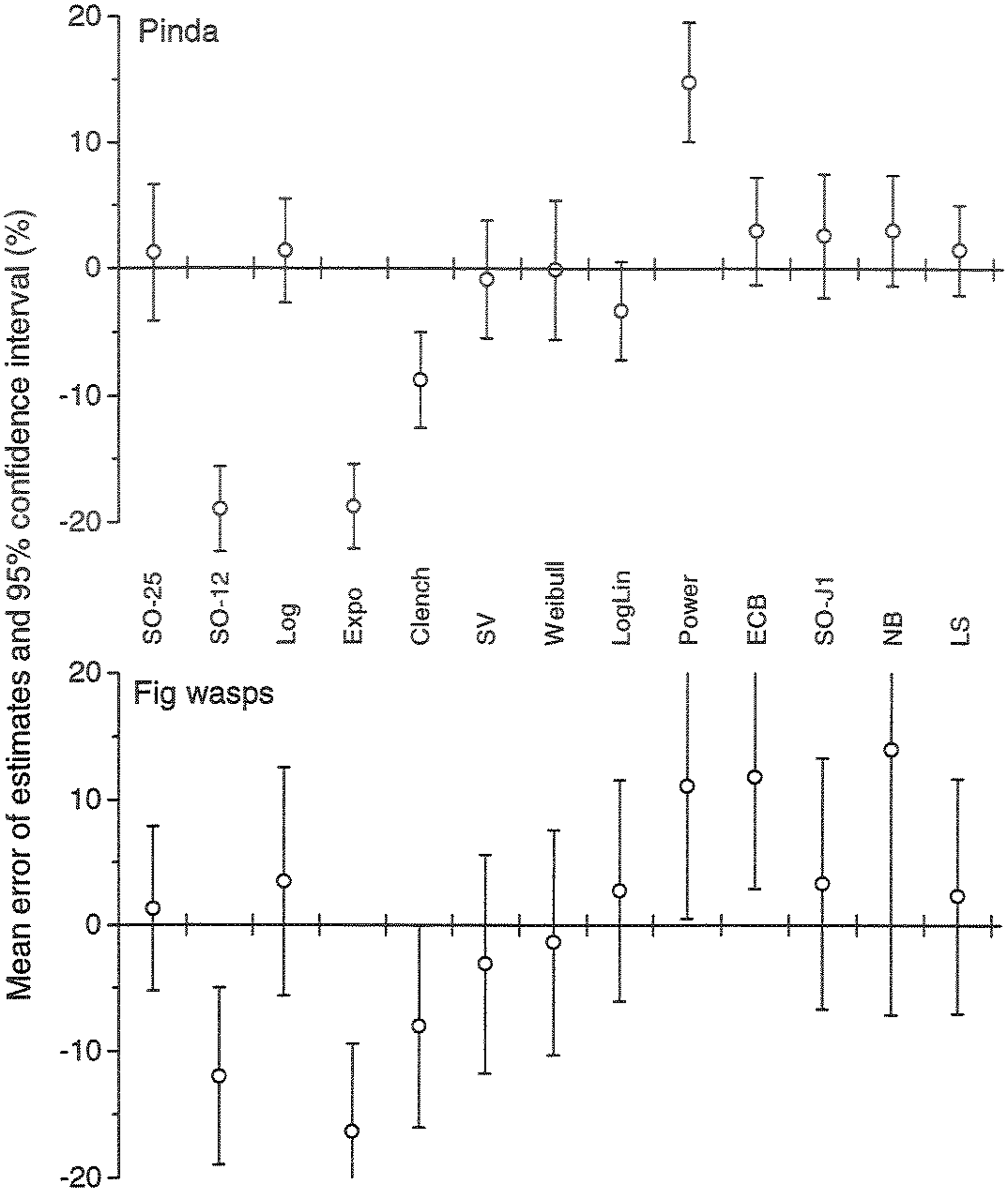

Fig. 4.Error of estimates produced by the 12 methods evaluated and the observed richness in 25 sampling units for Pinda and fig fruit samples. Errors were computed as the difference between estimated and average observed richness, divided by average observed richness. Average observed richness for Pinda samples was 77.2 and for fig wasps 11.04 species and were obtained from species accumulation curves using the entire data sets (150 stones or 300 fig fruits). See Table 3 for abbreviations of estimators.

Table 3. Summary of percentage of errors of estimates and observed richness in 25-sampling unit samples in relation to the average observed species richness in 25 sampling units, obtained from the species accumulation curve using the entire data set (150 stones or 300 fig fruits). Mean and standard deviation (SD) measure respectively accuracy and precision of estimators. Bias is expressed as percentage of overestimates minus 50%. A good estimator should produce mean, standard deviation, and bias close to zero. SO-25 = Observed species richness in 25-sampling unit samples. SO-12 = Observed species richness in 12-sampling unit subsamples. SO-12 is included in the table in order to allow the assessment of how much the evaluated estimation methods are able to improve accuracy, precision, and bias in relation to this simple estimate. Estimates for all methods were obtained using 12-sampling unit subsamples. n = 12, except Pinda SO-25 n = 6.

Estimators	Pinda			Fig wasps		
	Mean	SD	Bias	Mean	SD	Bias
SO-25	1.25	5.14	0	1.30	10.33	8.3
SO-12	-18.93	5.28	-50	-12.01	11.00	-41.7
Logarithmic (Log)	1.40	6.47	25	3.45	14.30	16.7
Exponential (Expo)	-18.74	5.27	-50	-16.35	10.91	-41.7
Clench	-8.76	5.90	-50	-8.03	12.59	-16.7
Stout and Vandermeer (SV)	-0.85	7.34	-8.3	-3.09	13.66	-16.7
Weibull	-0.09	8.65	16.7	-1.37	14.09	16.7
Log-Linear (Log-Lin)	-3.33	6.10	-16.7	2.72	13.88	16.7
Power	14.86	7.50	50	11.07	16.58	33.3
Evans et al. (ECB)	2.97	6.71	25	11.77	13.98	33.3
SO-J1	2.60	7.77	16.7	3.30	15.74	25
Negative Binomial (NB)	3.05	6.88	25	13.97	33.21	16.7
Logarithmic Series (LS)	1.50	5.63	16.7	2.31	14.69	16.7

Unreplicated study

As in the replicate study, the Expo and Clench estimators underestimated the actual richness using all four subsample sizes and also in all six data sets (Fig. 5). The Power model tended to overestimate the actual richness, except in the *Drosophila* spp. and frog samples. The LogLin model that yielded underestimates in Pinda samples and slight overestimates in fig wasp samples, underestimated the total richness in all data sets. The ECB estimator presented low accuracy and did not yield a consistent bias for all six data sets. The SV and Weibull estimators tended to produce negative errors, but of low magnitude. The NB and LS estimators, followed by the Log model, were robust for different data sets and presented low negative and positive errors. The estimator SO-J1 produced results comparable to NB and LS estimators, but failed to produce values for the 40% subsample size.

Except for the ECB method, which showed no clear trend over the increasing subsample sizes, all the remaining estimators increased in accuracy when using larger subsample sizes. However, this increase was not conspicuous for NB and LS methods, which produced low errors even when using the 40% subsample size (Fig. 5). At least for the overall good estimators NB, LS, SO-J1, Log, SV, and Weibull, better accuracy and precision were found when using species-rich than the species-poor frog assemblage.

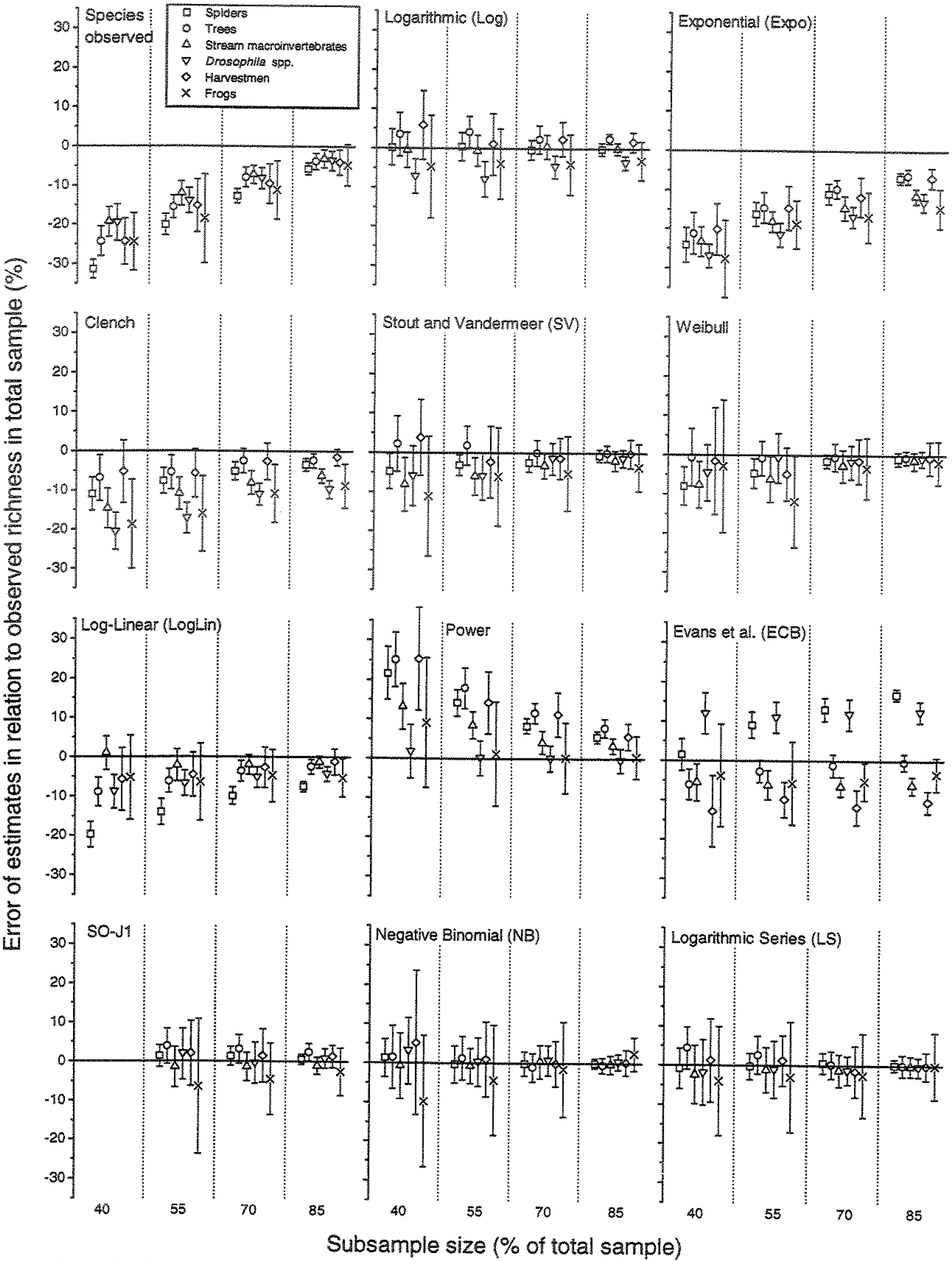

Fig 5. Error of estimate in relation to the observed richness in the data set produced by the 12 methods evaluated using subsamples of different sizes, and from different data sets. Mean and standard deviation for each subsample size were computed from 30 subsamples randomly draw from each data set.

Discussion

Expo, Clench, and LogLin models tended to underestimate the actual richness, while the Power model tended to overestimate it. The ECB estimator produced biased and inaccurate estimates depending on the data set used. In some data sets, the ECB produced consistently negatively biased estimates, while in others the method produced positively biased estimates. The SV and Weibull models performed well in the replicated study, but slightly underestimated the actual richness in the unreplicated study. The NB estimator was in general unbiased and very accurate, except in the species-poor fig wasp samples. The Log, SO-J1 and LS estimators also performed well in the replicated study, but the Log model was less accurate than SO-J1 and LS methods in the unreplicated study.

Our results closely agree with the findings of Keating et al. (1998), who evaluated the bias of estimators Log, Expo, Clench, LogLin, Power, NB, and LS for the analogous problem of effectiveness of further sampling in species inventories. The Expo and Clench models that underestimated actual richness here, were also shown to be negatively biased by Keating et al. (1998). The generally biased LogLin and Power estimators, but that produced good results in some data sets (LogLin with fig wasps and Power with *Drosophila* spp. and frogs), also tended to be biased in Keating et al.'s study, but again produced good unbiased results in some particular data sets. Keating et al. reported biased results for the Log model, although the bias sign was dependent on the data sets evaluated. Also agreeing with our results, LS and NB estimators were well evaluated by Keating et al., and those authors suggested the use of NB estimator as the most robust and generally unbiased estimator.

Poor performance of Clench model is further supported by Keating and Quinn (1998). Palmer (1990) and Tackaberry et al. (1997) evaluated the models LogLin and a method analogous to the Power model in predicting plant species richness. They found that the Power model overestimated the actual richness known to occur in study plots. LogLin overestimated actual richness in most samples of Palmer's (1990) study, but tended to underestimate actual richness in the study of Tackaberry et al. (1997). In contrast to the result presented by Palmer (1990) and in accordance to Tackaberry et al. (1997), the LogLin model tended to underestimate actual richness in our study, suggesting that its performance is dependent on data set in study. Models Expo, Clench, LogLin, and Power have been used to fit species accumulation curves in a number of studies (e.g. Condit et al. 1996; León-Cortés et al. 1998; Moreno and Halffter 2000). However, despite good fit of these models to species accumulation curves in these studies, and consequently the usefulness of the fitted parameters, we do not recommend the use of these models for predicting extrapolated species richness.

At least for the better-performing estimation methods, NB, LS, SO-J1, Log, SV, and Weibull, estimators produced better results in species-rich than in species-poor data sets. Clear evidence of this can be seen comparing results of Pinda (74-85 species) versus fig wasp samples (10-13 species) in the replicated study and the 5 species-rich data sets (40-287 species) versus the species-poor frog data set (15 species) in the unreplicated study. When calculating estimates for fig wasp samples, we noted that this bad performance is due to the high heterogeneity among subsamples of species-poor data sets. Given the low number of species in fig wasp subsamples, the inclusion or not of 1-2 rare species due to chance can produce very different estimates. In the unreplicated study, this is evident from the relatively large error bars of frog subsamples in Fig. 5. Bad performance of estimators in species-poor assemblages was also found by Keating et al. (1998), who reported biased estimates in their medium-evenness and low-richness (random-fraction, 10 species) model communities.

As would be expected, there was an increase in accuracy and precision of estimates in the unreplicated study with increased subsample sizes. An exception was the ECB estimator, that produced even worse estimates on increased subsample sizes for the spider data set. For estimators NB and LS, good accuracy were obtained for species-rich data sets even when using subsamples of only 40% of the total sample size, although with a low precision, shown by the large error bars. Using subsamples of 55%, both accuracy and precision were considerably improved for these two estimators, except in the frog data set. In the replicated study, subsamples of 48% (12 from 25 sampling units) produced good results when using the six best estimators (NB, LS, SO-J1 Log, SV, and Weibull), at least for species-rich Pinda samples. For these data, 8-10 of 12 estimated values were included in the range of the observed richness in 25 stones (the dotted squares in Fig. 2). Thus, we suggest that at least for species-rich data sets, extrapolations are safe up to sample sizes 1.8-2.0 times the size of the sample in study.

Although we found that NB and LS, followed by SO-J1, Log, SV, and Weibull estimators, were in general accurate and not strongly biased, this finding was based on averages of several subsamples. An estimator can be very accurate when averaging several estimates, despite a high variance among estimate values (low precision). In actual use, the researcher commonly will have only one sample, and it is of interest to know how reliable a single estimate produced by an estimation method is. On the other hand, we should recall that an observed richness value in a given sample is only an estimate of the mean actual richness value for that particular sample size in the assemblage under study. Reliability of an estimation method can be assessed comparing the variation of its estimates with the variation of the observed richness values among several independent samples collected from the assemblage in study. From Table 3 and Fig. 4 we can observe that the SD and the 95% CI for the 12 estimate errors produced by NB, LS, SO-J1, Log, SV, and Weibull estimators are slightly higher but comparable to those produced by the six observed richness values in Pinda samples. However, for fig wasp samples, estimates of LS, SO-J1, Log, SV, and Weibull methods were reasonably more variable than the observed richness in 12 25-fig fruit samples. Furthermore, the NB method produced a SD three times higher than that produced by the observed richness in the 12 25-fig fruit samples, due to two outlier values (Fig. 3). As commented earlier, more reliable estimates are likely to be obtained when working with species-rich samples.

Three issues should be considered when choosing and using species richness estimators. The first is the ease of computation. Estimators Log, SV, and Weibull are extrapolations of species accumulation curves. The construction of such curves is easily done using available free software (e.g. EstimateS, Colwell 1997). The nonlinear fitting of curves is available in most of the statistical and graphical softwares available. The methods NB and LS on other hand are based on fitting a model to the number of species occurring in 1, 2, 3 etc sampling units. Also, the model includes in its computations the gamma function, which might not be available in some statistical softwares. A further advantage of using the models Log, SV, and Weibull is the interpretability of the fitted parameter, which might be useful in diversity studies (see respectively Soberón and Llorente 1993; Stout and Vandermeer 1975; and Flather 1996). The estimator SO-J1 is not usually able to extrapolate to sample sizes larger than twice the sample in study. For these cases, when extrapolating the linear function in Fig. 1, the obtained y-value is higher than the number of sampling units in the sample. A second issue specific to the estimator SO-J1 is the assumption of the linear relationship depicted in Fig. 1. The relationship is likely to be found in assemblages containing a high proportion of rare species and or when sample size is small. For assemblages well sampled and or with few rare species, the Jackknife 1 estimates will attain an asymptote and the consequent linear relationship in Fig. 1 will not be found, invalidating thus the method SO-J1. The last issue to be considered is that as any statistical estimation method, extrapolation assumes that additional sample sizes come from the same universe from which current samples were collected. If samples were collected in random locations inside a 10 ha plot, extrapolated richness values will be valid only for the same 10 ha area.

Recently, Walther and Martin (2001) suggested the use of methods that estimate species richness in the area as a way of standardizing different sample sizes (see review of these methods in Colwell and Coddington 1994). In this case, comparisons would be made using the estimated richness expected to occur in the total area of study. A practical problem of this approach is the strong dependence of richness estimates produced by these methods on the observed richness. In fact, the well-behaved estimator SO-J1 is based on this dependence (Fig. 1). This dependence is very strong until sample size is increased enough to collect most of the species in the study area (Colwell and Coddington 1994; Melo and Froehlich 2001a, A. S. Melo unpublished manuscript). While this can be feasible in some species-poor assemblages as the one used by Walther and Martin (2001), it is not usually feasible for species-rich assemblages (Schmit et al. 1999; Gotelli and Colwell 2001; Melo and Froehlich 2001a).

We argue that a good estimator must produce reliable estimates independent of the structure of the data being used. Methods that perform very well with some data structures but not with others should be avoided, as a researcher does not know, *a priori*, which one is the best for a specific problem. As a first option, we suggest the use of LS estimator, followed by any one of the NB, SO-J1, Log, SV, or Weibull methods. Performance of LS method was consistently good with the replicated and the unreplicated studies and the several data sets used. Despite the generally unbiased and accurate average estimates produced by these six estimators throughout the range of situations evaluated, precision was too low in species-poor assemblages (less than 15-20 species) and for extrapolations greater than 1.8-2.0 times the sample in study. The first restriction should not be a major problem in most diversity studies as interest often centers on species-rich assemblages, which can potentially reflect fine environmental differences (e.g. Kremen 1992; Brown and Freitas 2000). The restriction on magnitude of extrapolation should be enough for most of the studies where different sample sizes are caused by loss of samples, destruction of traps by animals or bad weather, and shortage of time or money. The great robustness of the six methods suggested to different data structures provides a safe solution to the problem of losing information by standardizing different samples sizes to the size of the smallest sample.

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Capítulo 4

Resistance, resilience and patchiness of invertebrate assemblages in native tussock and pasture streams after a hydrological disturbance[1]

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[1] Melo, A. S., D. K. Niyogi and C. R. Townsend. Resistance, resilience and patchiness of invertebrate assemblages in native tussock and pasture streams after a hydrological disturbance. Submitted for publication in June 2002.

Abstract

Disturbance caused by a high discharge event is perhaps the most important driving force affecting invertebrates in streams, causing reduction in abundance and redistribution of individuals among habitats and patches. We generated standard hydrological disturbances to investigate the role of disturbance in streams in two landscapes differing in land uses: native tussock grasslands and exotic pasture catchments. We tested whether physical differences in streambed structure confer higher resistance and resilience in tussock rather than pasture sites. We also investigated changes in patchiness in riffles in the streams caused by the disturbance. Invertebrate abundance decreased immediately after the disturbance, but species density and species richness (rarefied) remained unchanged. Eight days after the disturbance event, abundance was similar to samples collected immediately before the disturbance. Resistance (measured as decrease in total abundance) and resilience (measured as recovery of total abundance within eight days) did not differ significantly between the land uses. Patchiness increased in both stream types immediately after the disturbance, but decreased to pre-disturbance levels after eight days. Disturbance caused a redistribution of individuals among patches, some receiving individuals, others losing individuals, and some remaining unchanged.

Introduction

Natural physical disturbances have been recognized by ecologists as an important factor determining the structure of communities (Sousa 1984; Resh et al. 1988). In streams, high discharge events are thought to be the main disturbance structuring invertebrate communities (Townsend 1989; Reice et al. 1990; Lake 2000), reducing abundances of most species (Scrimgeour et al. 1988; Hax and Golladay 1998) and redistributing individuals among habitats (Palmer et al. 1996). Recovery following a flood is usually fast and original abundances are generally regained within 30-120 days depending on disturbance intensity (Mackay 1992; Flecker and Feifarek 1994).

High discharge events can affect stream invertebrates in two ways: by detachment from the substrate or as a result of movement of stones with the potential to crush or bury individuals. The first mechanism is indicated by the dislodgement of increasing numbers of invertebrates as discharge in experimental channels is increased (Holomuzki and Biggs 1999) and by the concentration in hydraulic refuges, after floods, of species that are vulnerable to flow detachment (Lancaster 2000). The second mechanism is indicated by higher post-flood densities on embedded stones than on loose stones (Matthaei et al. 2000), weak effects of floods in stream reaches with high sediment stability (Cobb et al. 1992) and high mortality in experimental channels containing large, mobile substrate particles (Holomuzki and Biggs 1999). Additionally, disturbance can differentially affect patches that differ in physical structure and stability (Downes et al. 1997); for example, sites with high physical heterogeneity offer a range of hydraulic refuges (dead zones)(Downes et al. 1998).

Natural physical disturbance is known to induce patchiness in the distribution of organisms in terrestrial (Segura et al. 1998) and marine systems (Underwood 1998), but few studies have specifically addressed this issue in stream systems (Lake 2000). Lancaster and

Hildrew (1993) showed that some insect species respond to floods by concentrating in patches where shear stress does not increase with an increase in discharge, while Palmer et al. (1995) observed that larval chironomids and copepods were redistributed among habitats following a disturbance. The Patch Dynamics Concept of stream communities (Townsend 1989) suggests that disturbances cause a mosaic of patches, some losing individuals, some not affected, and some receiving individuals. Fast recolonization of depleted patches is possible due to the existence of refuges. Given a long recurrence interval, communities would be expected to have greatest patchiness immediately following a disturbance, and then tend toward a more even distribution of individuals among patches until a new disturbance resets the system.

The study of physical disturbance in streams can be assisted by the use of manipulative experiments to simulate discharge disturbance (Matthaei et al. 1996). In contrast to previous studies, which were generally concerned with small-scale impacts (single stones up to 10 m^2) and located in a single stream, we applied a hydrological disturbance to reaches 60-90 m long in replicated stream sites. We examined resistance, resilience and patchiness of macroinvertebrate assemblages following disturbance in streams draining exotic pasture or native tussock grassland. In contrast to pasture sites, streambeds in tussock sites are more heterogeneous (C.R.Townsend, unpublished data), which could provide a greater range of hydraulic refuges, and are covered by moss, indicating higher bed stability (Englund 1991).

Materials and methods

Study Area

The study was carried out in second and third order tributaries of the Taieri and Waipori Rivers (45° 43' S, 169° 51' E, total area encompassing all sites = 132 km^2), in the Otago province of the South Island of New Zealand. Studied streams located in tussock catchments were Clarks, Anchor, and Stone. Streams located in pasture were Broad, Bush, and M. Road. Width and discharge were similar at all sites. All study streams contained brown trout (*Salmo trutta* L.), an introduced species that can affect invertebrate behaviour and community composition (Townsend 1996).

The streambed at all sites was composed mainly of cobbles and gravels. Streams in tussock catchments were partially shaded by overhanging grasses, especially *Chionochloa* and *Festuca* species. Mosses were abundant at tussock sites and covered about half of the substrate. Grazing by sheep occurred in tussock catchments historically, but only at low intensity and only during some periods in the year. Grazing had not occurred in our tussock catchments for at least two years. Streams in pasture catchments were unshaded and subjected to direct sunlight. Mosses covered less than 5% of the substrate in these streams.

Invertebrate assemblages differ between streams in pasture and tussock catchments (R.H. Riley and C.R. Townsend, unpublished data). The most conspicuous difference is the high abundance of *Deleatidium* spp. mayflies in pasture sites. Invertebrate assemblages in streams in tussock catchments were dominated by *Potamopyrgus* snails and *Paracalliope* amphipods. Appendix 1 shows the taxa identified in each of our study streams.

Experimental Disturbance

The experiments were done using a repeated measure design. Samples collected immediately before the disturbance treatments were considered controls, while samples collected two hours and eight days after the experimental disturbance were used to measure resistance and resilience, respectively. Three replicates of each stream type were used, totaling 18 samples. Four sampling units, collected in similar riffle areas using a Surber sampler (area = 0.062 m^2), composed each sample.

An experimental hydrological disturbance was applied to the streams by means of a water compressor and hose to simulate the high velocities and shear stresses that occur during a

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flood. In each stream we systematically hosed a 60-90 m reach for about one hour. The operator moved downstream, "washing" the streambed and taking care to apply the water jet to all portions of the bed, including riffles, pools and areas under overhanging vegetation and banks. The experimental disturbance was enough to turn the water completely turbid, move stones, and cause the detachment of portions of moss in tussock sites. As a measure of disturbance intensity, shear stress caused by the water jet was sufficient to move even the heaviest standard hemisphere described by Statzner and Müller (1989).

Sampling units collected using Surber samples were preserved individually in the field. In the laboratory, we split each sampling unit into two equal halves and sorted all individuals from one of them. Invertebrates were identified to the lowest taxonomic level possible. In cases in which species identification was not possible, we separated individuals into morphospecies.

Analysis

Resistance and resilience in pasture vs. tussock streams

Resistance and resilience of the invertebrate assemblage were assessed using Two Way Repeated Measures Analysis of Variance (ANOVA), with land use (pasture, tussock) as the between-subjects factor and time (before, after, and eight days after) as the within-subject factor. Due to the non-independent levels of the time factor and the consequent potential problems in the variance-covariance matrix, p-values were corrected for time and interaction factors by the Huinh-Feldt method (Gurevitch and Chester Jr. 1986; Looney and Stanley 1989). Significance level was set at 0.05. We set two *a priori* contrasts in case the within-subjects factor (Time) was significant, before vs. after and before vs. eight days after sampling times.

Three metrics were employed in the ANOVA analyses: (1) $\log_{10}$ abundance, (2) species density (number of species in samples), and (3) species richness (expected values derived by rarefaction; see Gotelli and Colwell 2001). The reason for including rarefaction estimates of species richness was the report of McCabe and Gotelli (2000) that a decrease in species density in a small-scale disturbance experiment was a consequence of reduced abundance in disturbed treatments. Our original predictions were that tussock sites would be more resistant and resilient than pasture sites, reflecting respectively higher overall bed stability and more refuges.

Patchiness following the disturbance

We investigated changes in patchiness within a stream site in an exploratory way using nonmetric multidimensional scaling (NMS) ordination of the four sampling units collected in each stream site and sampling time. We used Sørensen distances for $\log_{10}$-transformed abundances and performed the ordination in two dimensions. The analysis was carried out in PC-ORD software (version 4.10, MjM Software, Gleneden Beach, Oregon, USA) using all 72 sampling units (2 stream types x 3 replicated stream sites x 3 sampling times x 4 sampling units). However, we plotted results separately for each stream site due to the large number of sampling units. We expected that sampling units collected in the same site before the disturbance would be positioned close to each other in ordination space, while sampling units collected after the disturbance would be scored far from each other.

We also tested the hypothesis of increased patchiness (or heterogeneity) following the disturbance, and a subsequent decline, using the same ANOVA design as employed in the analysis of resistance and resilience. However, the metric we used was the mean of the squared Sorensen similarities (1 - Sørensen distance) from all six possible pairwise comparisons among the four sampling units collected at each stream site and sampling time. Sorensen similarities were calculated using $\log_{10}$-transformed abundance. Our prediction was that similarity among sampling units would be high before the disturbance (low patchiness), decrease immediately after, and then increase toward original levels after eight days.

Results

Resistance and resilience in tussock vs. pasture streams

Average abundance decreased significantly in streams from both land uses immediately after the experimental disturbance (Fig. 1; Table 1a; *a priori* contrast before vs. after, $p = 0.009$). By eight days after the disturbance, abundance of invertebrates had recovered to levels similar to control samples (Fig. 1; Table 1a; *a priori* contrast before vs. eight days after, $p = 0.260$). Resistance tended to be higher in the tussock streams while resilience was similar for tussock and pasture streams (Fig. 2). However, overall there was no significant interaction between land use and time ($p = 0.451$; Table 1a), indicating that both stream types responded in a statistically indistinguishable manner to the disturbance.

Species density and species richness responded in a similar manner to the disturbance. For both metrics, the effects of land use, time, and the interaction between them were not significant (for all cases $p > 0.3$; Table 1b and 1c). The number of species did not change as a result of the disturbance, and the pattern was similar for the two land uses (Fig. 1).

Patchiness following the disturbance

Exploratory NMS ordination separated the two land uses weakly, producing higher scores for tussock sites and lower scores for pasture sites along the second axis (Fig. 3). Sampling units collected before the disturbance were scored closer to each other than those collected immediately afterwards in all three tussock sites and, to a lesser extent, in one stream draining pasture (Bush Stream). With the exception of Broad Stream, sampling units collected eight days after the disturbance tended to be scored along the first axis at positions intermediate between sampling units collected before and immediately after the disturbance. For all six stream sites, the first ordination axis was strongly correlated to $\log_{10}$ abundance ($r > 0.89$, $p < 0.0001$).

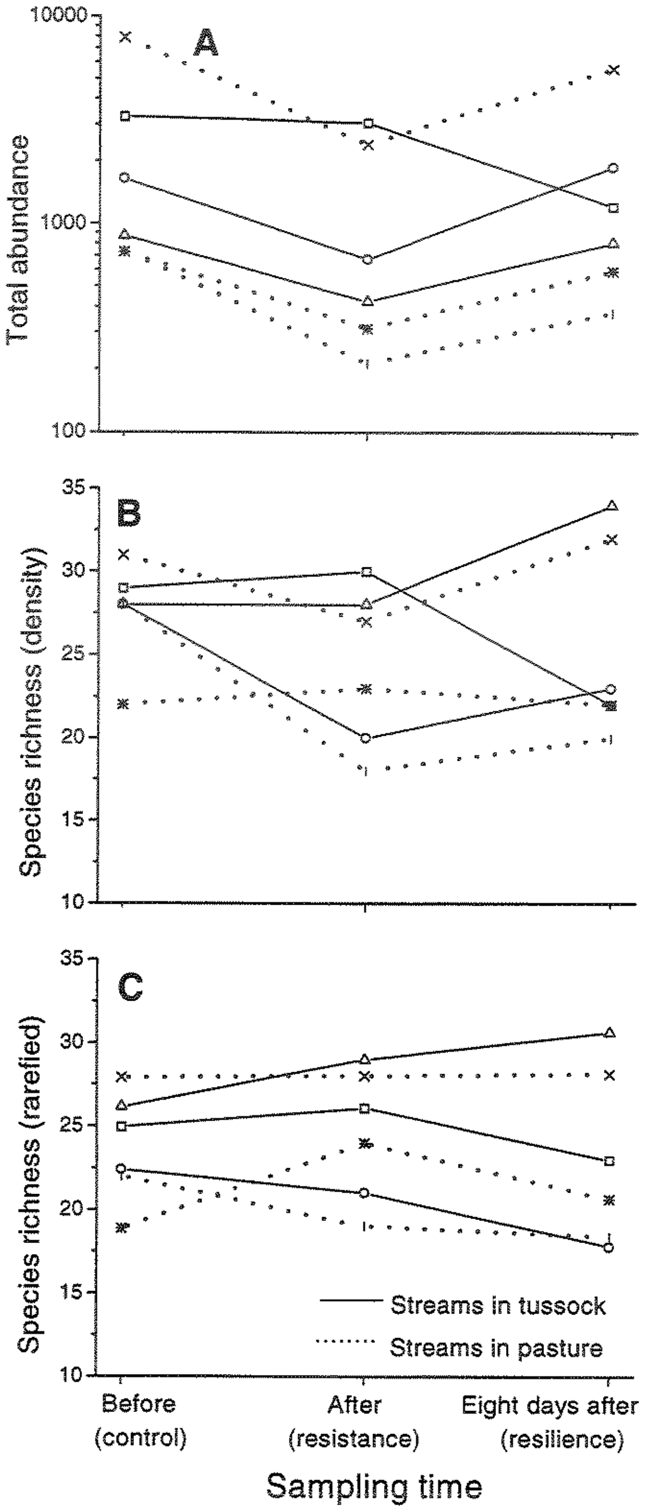

Fig. 1. Total invertebrate abundance (A), species density (B), and species richness (rarefied) (C) in six stream sites for three sampling times. Streams in tussock: □ = Clarks, ○ = Anchor, and Δ = Stone. Streams in pasture: x = Broad, ı = Bush, and * = M. Road.

Table 1. Repeated measures ANOVA of effects of land use (tussock vs. pasture) on (a) $\log_{10}$ total abundance, (b) species density and (c) species richness (rarefied). p-values for within-subjects analysis were corrected using Huynh-Feldt method for repeated measures ANOVA.

(a) $\log_{10}$ total abundance

	df	MS	F	p
Between subjects				
Land Use (LU)	1	0.049	0.079	0.795
Error	4	0.639		
Within subjects				
Time (T)	2	0.193	7.012	0.027
T x LU	2	0.023	0.835	0.451
Error	8	0.027		

(b) species density

	df	MS	F	p
Between subjects				
Land Use (LU)	1	20.056	0.438	0.544
Error	4	45.778		
Within subjects				
Time (T)	2	17.167	1.077	0.385
T x LU	2	1.722	0.108	0.899
Error	8	15.944		

(c) species richness (rarefied)

	df	MS	F	p
Between subjects				
Land Use (LU)	1	11.119	0.205	0.674
Error	4	54.351		
Within subjects				
Time (T)	2	3.058	0.596	0.574
T x LU	2	0.026	0.005	0.995
Error	8	5.133		

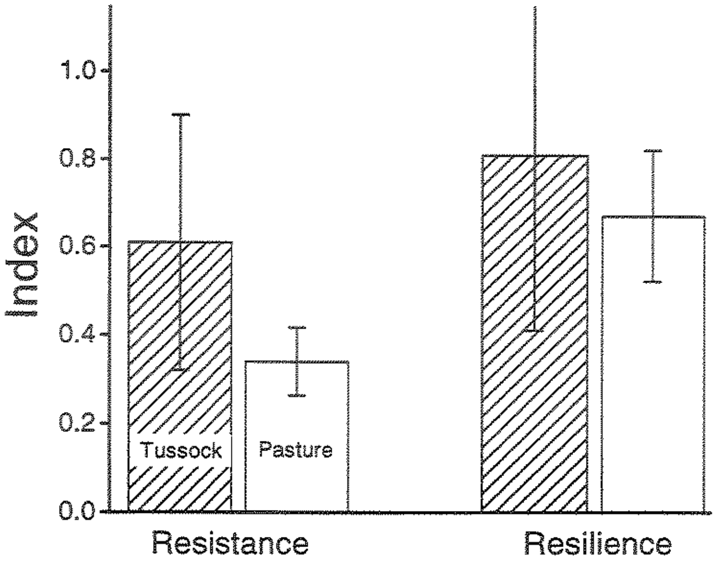

Fig. 2 Average resistance and resilience of the macroinvertebrate assemblage in the face of a discharge disturbance in tussock and pasture streams. Resistance was obtained as abundance immediately after the disturbance divided by abundance immediately before it. Resilience was obtained as abundance eight days after the disturbance divided by abundance immediately before the disturbance. Standard deviations shown. Statistical assessment of differences in resistance and resilience between the two land uses are provided in Table 1a.

Despite the significant overall reduction in abundance detected by the ANOVA analysis, some sampling units were not affected negatively by the disturbance treatment. Except for Bush Stream, all streams had sampling units immediately after the disturbance with similar densities to control sampling units (scored close to the control sampling units in Fig. 3). In the stream Clarks one sample unit had total abundance higher than the controls (scoring to the right of the reference units in the NMS), probably reflecting the accumulation of individuals dislodged from patches affected negatively by the disturbance. We checked whether sample units with high abundance after the disturbance were located downstream in the study sites, in which case high abundance would just be an artificial consequence of dislodged individuals from upstream, disturbed areas. There was no evidence of such an artifact; density of invertebrates after the disturbance was not significantly related to location in the stream reach.

Results of the ANOVA analysis of mean Sørensen similarities were similar to those obtained using abundance (Table 2). The interaction between land use and time was not significant (p = 0.220; Table 2). There was no difference between land uses (p = 0.418) but a significant difference was found among sampling times (p = 0.008). Mean similarity among sampling units was high before, decreased immediately after (*a priori* contrast before vs. after, p = 0.017), and recovered to original levels by eight days after the disturbance (*a priori* contrast before vs. eight days after, p = 0.201; Fig. 4).

Table 2. Repeated measures ANOVA of effects of land use (tussock vs. pasture) on mean Sørensen similarity among each of four sampling units collected at each stream site and sampling time. p-values for within-subjects analysis were corrected using the Huynh-Feldt method for repeated measures ANOVA.

	df	MS	F	p
Between subjects				
Land Use (LU)	1	0.0026	0.812	0.418
Error	4	0.0032		
Within subjects				
Time (T)	2	0.0121	11.026	0.008
T x LU	2	0.0021	1.893	0.220
Error	8	0.0011		

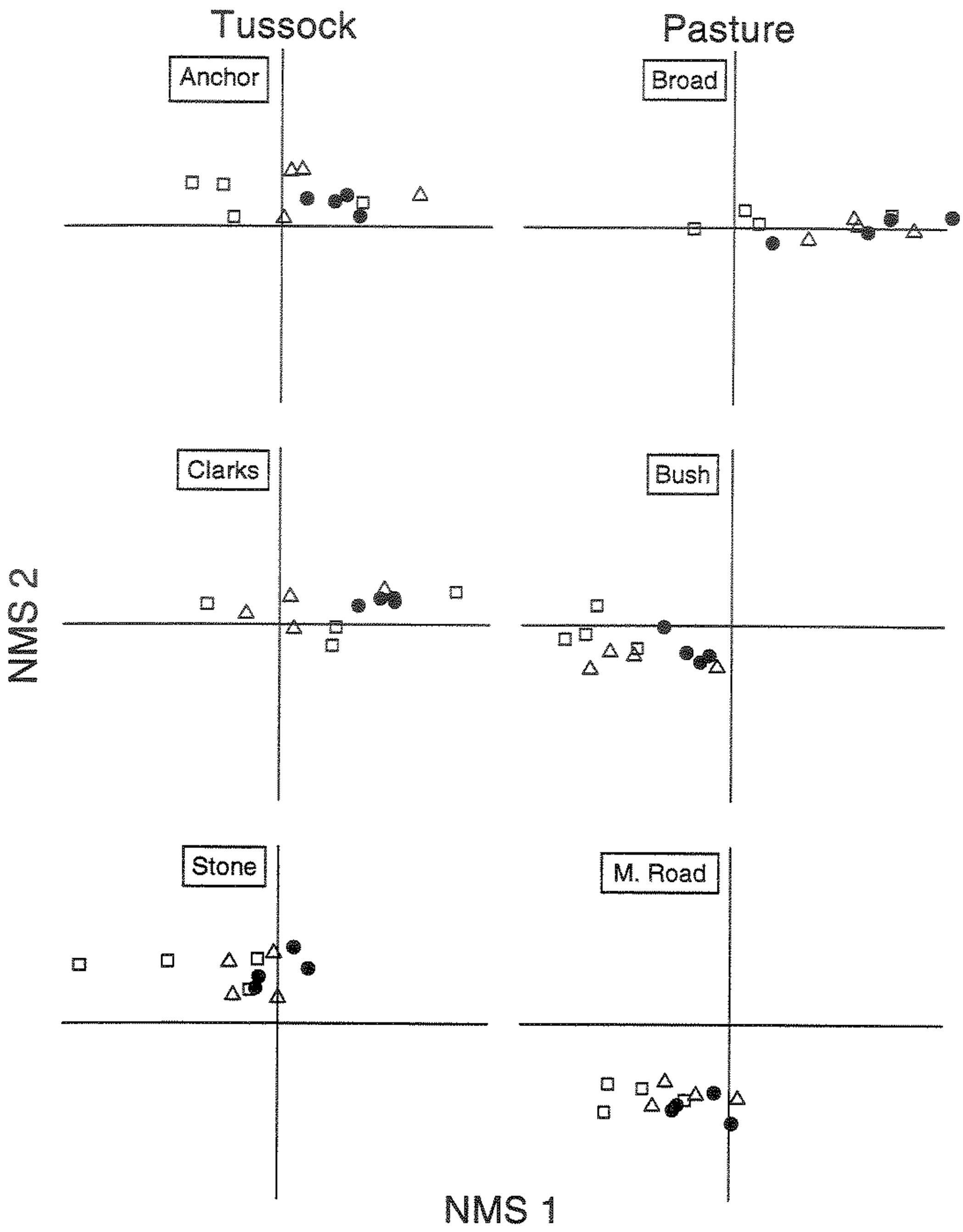

Fig. 3. NMS ordination of all 72 samples collected immediately before, immediately after, and eight days after the experimental disturbance. The analysis was performed once for all samples together, but plotted separately for each stream site. For both axes, lengths are the same and were scaled to the maximum score range of the 1st axis of the analysis. Stress value is 12.2%. Sites on the left are from tussock catchments and those on the right from pasture catchments. ● = Before, □ = After, and △ = eight days after the disturbance.

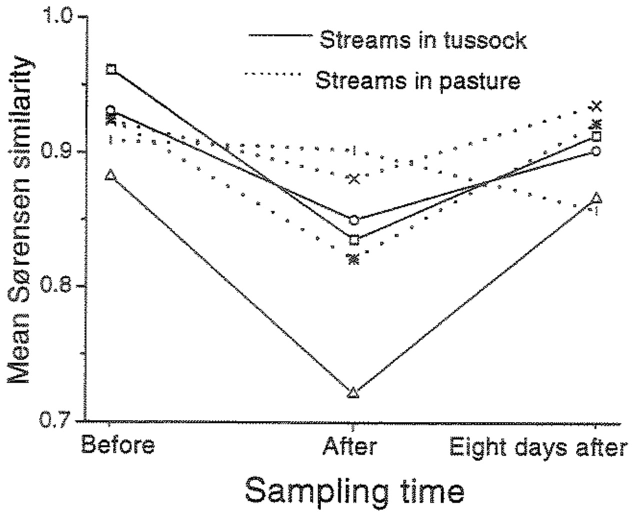

Fig. 4. Mean Sorensen similarity among the four sampling units collected at each stream site and sampling time. □ = Clarks. ○ = Anchor. Δ = Stone. X = Broad. I = Bush. * = M. Road.

Discussion

Several experimental studies have examined the effects of disturbance on stream invertebrate communities. However, these have generally been concerned with very small scales, such as individual stones (Lake and Schreiber 1991), artificial substrates (Malmqvist and Otto 1987; McCabe and Gotelli 2000), baskets filled with stones (Reice 1985; Death 1996) or small areas up to 9 m^2 (Matthaei et al. 1996). Furthermore, most of them were unreplicated (Mackay 1992; but see Death 1996). While small scale studies have shown some of the ways that disturbance can influence stream communities, it is likely that experiments incorporating large areas and replicated in a number of streams will produce more realistic and general results (Death 1996). For example, recovery in a stream patch is likely to be influenced by the surrounding patches. In studies where the disturbance effect was applied to small patches, resilience is likely to be greater than what would be observed in natural floods, where surrounding patches are generally also affected and fewer colonists are available (Brooks and Boulton 1991; but see agreements in response of invertebrates to natural and experimental disturbance events in Matthaei et al. 1997).

Resistance and resilience in pasture and tussock sites

The reduction in invertebrate abundance immediately after the disturbance is in accordance with previous studies on natural and experimental hydrological disturbance. We did not observe changes in species density in our study and this concurs with some previous experiments, but not others (reviewed in McCabe and Gotelli 2000). The study of McCabe and Gotelli (2000) is the only one so far to use species richness (rarefied) to measure changes in stream invertebrate diversity. They found an increase in species richness as a result of the disturbance treatment, in contrast to our results where no changes were observed.

Our original hypotheses that tussock streams would be more resistant and resilient in the face of a disturbance in terms of abundance, species density and species richness, were not supported, as assessed by the non-significance of the interaction factor in the ANOVA analyses. This result partially disagrees with Death (1996), who found differences in species density, but not abundance, when comparing recovery in physically stable and unstable stream sites. In his study, recovery of species density in stable streams was faster than in unstable streams.

While the lack of statistically significant differences between the land use types in our study might represent the actual situation, we do not exclude completely the original hypothesis that invertebrates in tussock streams would be more stable in the face of a disturbance. Our experimental disturbance was designed to mimic a natural flood. Indeed, abundance reductions in our study (59, 6, 51, 70, 71, and 57%, respectively for Anchor, Clarks, Stone, Broad, Bush, and M. Road) were in the range generally found in other studies (e.g. 70%

in Brooks and Boulton 1991; 90% in Matthaei et al. 1996). However, as in other experimental studies, this disturbance was not completely realistic. For example, the disturbance scoured large patches of moss in tussock sites (personal observations), which is unlikely to happen in real floods. In fact we found evidence of previous floods in the riparian vegetation of tussock sites, but no scoured patches of moss in the stream bottom of these streams. Furthermore, the experimental disturbance was able to move even the heaviest hemisphere used by Statzner and Müller (1989) to measure shear stress, indicating that shear stresses were much higher than usually recorded in floods. Average resistance and, to a lesser extent, resilience were somewhat higher in tussock than pasture streams, but the differences were not significant. A natural flood, large enough to cause stone movements but not detachments of moss, might have led to significant differences in resistance and resilience between pasture and tussock sites if greater substrate heterogeneity and stability provided more refugia for invertebrates in tussock streams. This would be analogous to the detection of seed viability of plant species in areas impacted by fire; when fire is of low intensity, it is possible to detect differential survival of seed species, but when fire intensity is high all seeds are killed regardless of species identity (Segura et al. 1998).

Nearly complete recovery was observed within eight days in our study. This is in agreement with reports in the literature regarding natural spates and small-scale disturbance experiments (Brooks and Boulton 1991; Mackay 1992).

Disturbance generating patchiness

Our hypothesis, that disturbance would cause a transient increase in patchiness, was supported by the ANOVA analysis. Similarity among sampling units collected at a site was high before the disturbance, decreased immediately following the disturbance, and then recovered to original levels in eight days. No differences between tussock and pasture sites were detected by the ANOVA analysis. However, the increase in patchiness following disturbance was more conspicuous for tussock than for pasture sites in the NMS analysis.

An increase in habitat heterogeneity after disturbance is predicted by the Patch Dynamics concept (Townsend 1989). Matthaei et al. (2000) found an increase in the number of invertebrates associated with embedded stable stones immediately after a spate and a corresponding decrease in abundance on loose, unstable stones. Similarly, spates may cause an accumulation of individuals in patches where shear stress does not increase during spates (Lancaster and Hildrew 1993). In both cases, increases in heterogeneity after the disturbance were clear, with two distinct patch types: refuges and non-refuges. We collected sample units in similar areas in riffles so any (unmeasured) characteristic of the streambed responsible for the increase in heterogeneity is likely to be subtle.

Palmer et al. (1995; 1996) noted a redistribution of benthic copepods following disturbance, but among rather than within distinct stream habitats (sandy mid-channel, fine sediments around dams, coarse sediments around dams, and dam debris). Species found in specific habitats in the absence of disturbance were found in other habitats after a spate. As a consequence, heterogeneity among habitats was high before a disturbance (i.e. each habitat contained a specific pool of species), and tended to decrease after the disturbance. However, not all patches inside the habitats that acted as refuges (vegetation debris and fine sediments around dams) actually received individuals. Patch-specific characteristics (mainly near-bed flow and water flux) were important in determining whether or not a patch acted as a refuge during a flood.

The picture emerging from our results and previous studies is that floods cause a predictable redistribution of invertebrates. Some patches within habitats (e.g. sample units to the left in Fig. 3; loose stones in riffles, Matthaei et al. [2000]) and some entire habitats (e.g. mid-channel sediments, Palmer et al. [1996]) lose individuals during spates, while some patches within habitats or entire habitats retain or receive individuals, acting as refuges (e.g. right-scored sample units in Fig. 3; embedded stones in riffles, Matthaei et al. [2000]; fine sediments with low bed flow and water flux in dams, Palmer et al. [1996]).

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Appendix 1. Species collected in each stream site presented in four abundance classes. X = 1-9; XX = 10-99; XXX = 100-999; XXXX = 1000-9999 individuals. Tussock stream sites: Anchor, Clarks, and Stone. Pasture sites: Broad, Bush, and M. Road.

	Anchor	Clarks	Stone	Broad	Bush	M. Road
ODONATA						
Zygoptera sp. 1						X
EPHEMEROPTERA						
Coloburiscidae						
Coloburiscus humeralis Walker	X	XX	XX	XX	XX	
Leptophlebiidae						
Austroclima spp.	XXX	XX	XX	X		
Deleatidium spp.	XX	X	XX	XXX	XXX	XXX
Nesameletidae						
Nesameletus sp.			X			
PLECOPTERA						
Austroperlidae						
Austroperla cyrene (Newman)	X	XX	X		X	
Gripopterygidae						
Megaleptoperla cf. *diminuta* Kimmins	X		X	XX	X	XX
Taraperla ancilis (Harding & Chadderton)				X		
Zelandobius sp.	X	XX	XX	X	X	X
Zelandoperla sp.				X	X	X
Notonemouridae						
Cristaperla sp.		X		X	X	
TRICHOPTERA						
Unidentified Trichoptera						X
Conoesucidae						
Pycnocentria cf. *evecta* McLachlan	XXX	XXX	XXX	XXX	XXX	X
Pycnocentrodes sp.				XX	XX	
Helicophidae						
Zelolessica cf. *cheira* McFarlane	X	XX	XX	XX	XX	
Helicopsychidae						
Helicopsychidae sp. 1			X	XXX		X
Hydrobiosidae						
Hydrobiosidae sp.1	X	X	XX	XX	X	X
Hydrobiosidae sp.2	X					X
Edpercivalia sp.	X					
Psilochorema sp.	X	XX	X	XX	X	XX

	Anchor	Clarks	Stone	Broad	Bush	M. Road
Hydropsychidae						
Aoteapsyche sp.		X	X		XX	X
Hydroptilidae						
Oxyethira cf. *albiceps* McLachlan	XX	XX	X	XX	X	XX
Leptoceridae						
Hudsonema alienum (McLachlan)	XX	XX	XX	XXX	X	X
Philopotamidae						
Hydrobiosella sp.		X	X			
Philorheithridae						
Philorheithrus sp.	X			XX	X	
Polycentropodidae						
Plectrocnemia maclachlani Mosely		X	X			
Oeconesidae						
Pseudoeconesus sp.	X	X	X		X	
MEGALOPTERA						
Corydalidae (Chauliodinae)						
Archichauliodes diversus (Walker)				X		X
DIPTERA						
Ceratopogonidae						
Ceratopogoninae sp. 1	X	X	X			
Ceratopogoninae sp. 2		X	X	X		X
Dixidae						
Paradixa sp. 1		X		X		
Empididae						
Empididae sp. 1	X	X	X	X		
Simuliidae						
Austrosimulium sp.	XX	XX	XX	XX	X	X
Muscidae						
Muscidae sp. 1						X
Muscidae sp. 2	X					
Tipulidae						
Eriopterini sp. 1					X	
Eriopterini sp. 2		X				
Hexatomini sp. 1		X	X			X
Hexatomini sp. 2		X	X			
Aphrophila sp.	X	XX	XX	XX	XX	X
Limonia sp.			X			

	Anchor	Clarks	Stone	Broad	Bush	M. Road
COLEOPTERA						
Unidentified Coleoptera sp. 1			X			
Unidentified Coleoptera sp. 2	X	X				
Unidentified Coleoptera sp. 3				X		
Elmidae						
Hydora sp.	X	XX	XXX	XXX	XXX	XX
Scirtidae						
Scirtidae sp. 1	X	XX	XX	XX	X	X
Scirtidae sp. 2			X			
Scirtidae sp. 3			X			X
Scirtidae sp. 4			X			
MECOPTERA						
Nannochoristidae						
Nannochorista philpotti (Tillyard)	X	X			X	XX
ACARI (more than 1sp.)	XX	XX	XX	XXX	XX	XX
CRUSTACEA						
Amphipoda						
Amphipoda sp. 1	XXX	XXX	XX	XXX	X	X
Amphipoda sp. 2	XXXX	XXXX	XXX	XXXX	XX	XX
Decapoda						
Paranephrops zealandicus White						X
Ostracoda (likely more than 1sp.)	XX	XX	XX	XXX	XX	XX
MOLLUSCA						
Gastropoda						
Potamopyrgus antipodarum Gray	XXXX	XXXX	XX	XXXX	XXX	XXX
Gastropoda sp. 1	X					
Bivalvia				X		
PLATYHELMINTHES						
Turbellaria	XX	XX	XX	XXX	X	
OLIGOCHAETA (likely more than 1sp.)	XXX	XXX	XXX	XXXX	XXX	XXX

Capítulo 5

Colonization by macroinvertebrates of experimentally disturbed stones in three streams differing in size[1]

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[1] Melo, A. S. and C. G. Froehlich. Colonization by macroinvertebrates of experimentally disturbed stones in three streams differing in size. Unpublished manuscript.

Abstract

We experimentally disturbed stones in three contrasting streams and followed the colonization process during 64 days. The three streams were 0.5-1, 10 and 20 m wide. The smallest stream had a small discharge and the studied area was close to its source. The biggest stream held a diversified assemblage of fishes, including benthonic and nektonic insectivorous species. The medium-sized stream site was far from the source and the fish assemblage was composed mainly of detritivorous armored catfish. We hypothesized that colonization of new patches both in the smallest and the biggest streams would be slower than in the medium-sized stream. In the smallest, the small area upstream could restrict the number of potential colonizers, especially those dispersed by drift. Presence of predaceous fishes in the biggest site would inhibit drift behaviour. The medium-sized stream would not be constrained by neither of the two factors. We assessed data on abundance, species richness and similarity of samples collected 1, 2, 4, 8, 16, 32, and 64 days after the start of the experiment. Colonization patterns for the streams were quite similar to each other, causing the rejection of the stated hypothesis. Colonization was fast, and for the smallest and the medium-sized streams, undisturbed control levels of abundance and species richness were attained in 8-16 days. Recovery of species richness in the biggest stream was similar to the two other streams. However, abundance in the biggest stream increased continually until the end of the experiment, attaining values similar to control samples in day 4 and values significantly higher than those observed in the control sample after day 32. Similarity among colonization days and controls increased until day 16, when curves tended to flatten off at values lower than those observed between control samples. Recovery in the studied streams was fast, and agrees with results from other studies. Lack of support of the original hypothesis is discussed in terms of the validity of the stated assumptions and more generally in relation to the relative importance of drift in colonization of small patches.

Introduction

Disturbance caused by high flow plays a major role in the structure of stream macroinvertebrate assemblages (Resh et al. 1988). During floods, substrates used by benthic organisms such as stones, leaf packs, and wood debris are tumbled and dislocated downstream. Small waterfalls formed by fallen trees and accumulated leaves, twigs, and sand are ruptured, causing movements of substrate located upstream and the consequent burying of patches downstream (Capítulo 6). Following a high flow event, densities of individuals are often decreased. Depending on the disturbance event, the total number of individuals can be reduced up to nearly 100% (Scrimgeour et al. 1988; Matthaei et al. 1997).

Recovery is generally fast and original abundance levels can be attained within 30-120 days (Mackay 1992; Flecker and Feifarek 1994). It is thought that fast recovery is due to presence of unaffected areas or refuges, where individuals accumulate during floods and from which they can disperse to colonize affected patches after high flow resumes (Lancaster and Hildrew 1993; Dole-Olivier et al. 1997). Recovery is affected by a number of factors, including distance from pool of colonists (Gore 1982), small-scale bed characteristics (Williams and Smith 1996), and biotic interactions (Diamond 1986).

Williams and Hynes (1976) assessed the relative importance of drift, aerial sources, upstream migration, and movement up from the substrate during the recolonization process. They concluded that drift was the most important mechanism, accounting for 41.4% of the total number of individuals settled in experimental traps. Further support for the importance of drift during the recovery process is provided by Townsend and Hildrew (1976) and Moser and Minshall (1996).

In this study we tested whether recovery following an experimental small-scale disturbance were different in three contrasting stream sites within a catchment. Site 1 was a first-order stream located around 200-300 m downstream from the area where water flows continually through the year. Site 3 was a fifth-order stream and contained a diversified fish assemblage, including benthonic and nektonic insectivorous species that feed either in riffles or pools. We predicted that recovery in these two sites would be slow. In site 1 because the small area located upstream could limit the number of drifting individuals, and in site 3 because insectivorous fishes could reduce drift rates or at least restrict it (Flecker 1992; Huhta et al. 2000). Site 2, a fourth-order stream, was neither constrained by size of the area upstream nor presence of insectivorous fishes. As a consequence, we predicted that recovery to original levels in site 2 would be relatively faster than in sites 1 and 3.

Material and methods

Study sites

The study was carried out in the Carmo River catchment, at Parque Estadual Intervales (24°18'S, 48°25'W), São Paulo State, Brazil. The vegetation is tropical ombrophilous submontane-montane forest, commonly known as tropical rain forest. The mean annual precipitation in the area is 2040 mm (25 y record). Rainfall is unevenly distributed across two seasons: one rainy (150-400 mm/mo) and warm (15-30 °C) from September through March and another dry (60-150 mm/mo) and cold (0-25 °C) from April to August. The experiment was carried out from July through September 1999, during the dry season. No flood was observed during the period.

Site 1 had 0.5-1 m in width and the streambed composed mainly by sand and stones (10-40 cm long). The studied stream reach had several pools and small waterfalls formed by fallen trees and accumulated twigs, leaves, and sand. Discharge is very reduced in the dry season, and during the experiment it was 0.0034 m^3/s. No fish was observed during five years of study in site 1, although small mailed (or armored) catfish (Loricariidae) may occur. Site 2 had 10 m in width, discharge of 0.408 m^3/s, and the streambed composed of stones and boulders up to 2 m long. Small mailed catfish up to 10 cm that feed on periphyton (Buck and Sazima 1995) are common and are often collected together with invertebrates in nets. Site 3 had the streambed similar to site 2, but width was 20 m and discharge 5.977 m^3/s. The fish assemblage in site 3 is quite diversified, and includes species that feed on benthic and drifting invertebrates (Sabino and Castro 1990; Castro and Casatti 1997). Macroinvertebrate assemblages in sites 1 and 2 are partially similar, but quite distinct from site 3. Sites 1, 2, and 3 represent sites 1, 8, and 10, respectively, in Fig. 1 of Melo and Froehlich (2001), where additional information on studied streams and macroinvertebrate composition can be found.

Experimental procedure

In each stream site, 70 stones (18 cm long) were taken out from the studied stream reach and cleaned of invertebrates. Each stone was labeled with a small non-toxic ink mark and/ or with a small plastic tag attached by angling line. Labeled stones were then placed in riffles in a stream reach of at least 100 m long. After 1, 2, 4, 8, 16, 32, and 64 days of the start of the experiment, 10 stones were sampled. Invertebrates on undisturbed (control) stones located in adjacent riffles were collected in sites 1 and 2 respectively one day before and two-three days after the start of the experiment. Control stones were not collected in site 3 during the experiment and thus we used data from stones collected in the same time of the year, but two years before as reference. Differently from the colonization sampling, 25 control stones were collected at each site, as they were part of concurrent studies in the area (Melo and Froehlich 2001; Capítulo 7).

Stones were sampled using an U-net sampler (Scrimgeour et al. 1993). Macroinvertebrates were removed alive from detritus and fixed in ethanol. In the laboratory, individuals were identified to the lowest taxonomic level possible and then assigned to morphospecies.

Data analysis

We constructed curves of colonization using mean number of individuals and species per stone. Pooled abundance of all species in each stone were $\log_{10}(x+1)$ transformed. We used one-way Analysis of Variance (ANOVA) to test for differences among days of colonization, including the control sample. Ten stones randomly chosen from the 25-stones set composed the control samples in these cases. In case a significant difference was found at $p = 0.05$, we compared each treatment level (day of colonization) with the control sample using Dunnett's test (Day and Quinn 1989). Our intention was to assess the time after the start of the experiment when streams first recovered to control levels.

In order to evaluate recovery of species composition and relative abundances, we computed Sørensen similarity values between the control sample and the seven colonization samples. Each colonization sample was composed by pooling data from the 10 stones collected at each combination of stream and date. Two control samples for each stream were used, each one composed of 10 stones randomly chosen from the 25 stones set available. We evaluated thus the overall similarity using all sampling units collected at a given time of colonization with the controls. We did so because the species composition on each single stone represents too small a fraction of the species in the community. Similarity among single stones would be thus too low, even among control stones. Reported values are mean similarity between a given treatment sample with each of the control samples. Similarity was also calculated between the two control samples. We used $\log_{10}(x+1)$ transformed abundances relativized by total abundance in the sample. This prevented the effects of the low abundance in samples collected early in the colonization process and allowed the calculation of similarity values based only on species composition and relative abundance. Results are presented graphically.

Results

Abundance

Colonization curves for the small- and the medium-sized streams, sites 1 and 2 respectively, were very similar to each other (Fig. 1). Recovery to control level of abundance for these two streams occurred in day 8 and persisted greatly unchanged until the end of the experiment (Table 1). Abundance in stream 3 was consistently higher than in streams 1 and 2. For stream 3, the control level of abundance was attained very soon on day 4. However, the abundance values increased continually until the end of the experiment, attaining values significantly higher than those observed in the control sample after day 32 (Fig. 1; Table 1).

Species richness

Recovery of species richness in stream 1 and 2 were quite similar to each other, as they were for abundance (Fig. 1). However, control level of species richness was attained in day 8 for stream 1 and day 16 for stream 2 (Table 1). There was a decrease in richness in stream 2 on day 32. However, given the great 95% confidence interval exhibited by samples collected on days 16, 64 and in the control, it is likely that this decrease was due to chance. Recovery in stream 3 took place on day 32, and different from abundance, values on days 32 and 64 were not higher than values observed for the control sample (Fig. 1; Table 1).

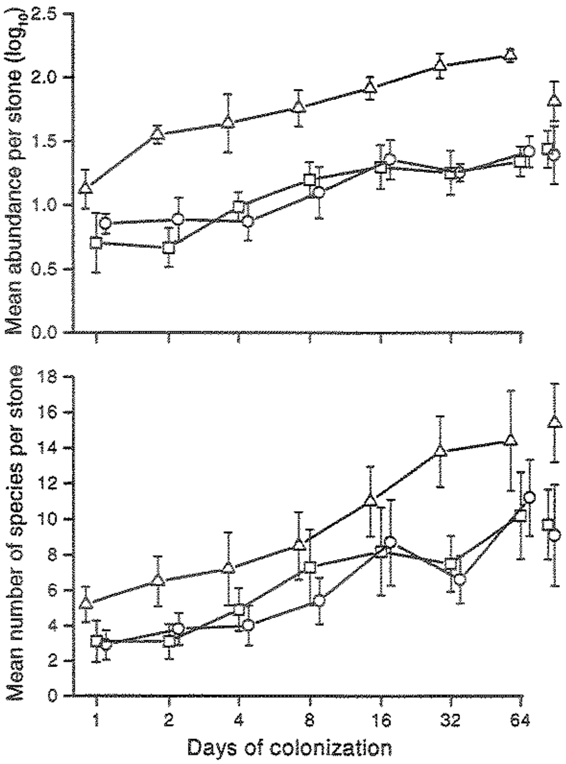

Fig. 1. Colonization curves for abundance and species richness (mean and 95% CI). Some symbols are slightly dislocated around sampling days in order to avoid overlap. □ = site 1, ○ = site 2, △ = site 3. Symbols placed at the right of the graph and not connected by lines represent control samples.

Table 1. Effects of colonization time on abundance and species richness per stone in the three stream sites studied. One-way analysis of variance includes control sample and seven colonization dates (1, 2, 4, 8, 16, 32, and 64 days after the start of the experiment). For all levels n = 10. Dunnett's test is employed to compare each treatment with the control level.

		Analysis of variance		*p*-value of Dunnett's test – treatments versus control						
Stream	Metric	$F_{7,72}$	p	1	2	4	8	16	32	64
1	abundance	12.97	0.000	0.000	0.000	0.001	0.190	0.722	0.421	0.925
2	abundance	9.65	0.000	0.000	0.000	0.000	0.058	1.000	0.705	1.000
3	abundance	23.26	0.000	0.000	0.047	0.332	0.994	0.807	0.032	0.003
1	richness	8.78	0.000	0.000	0.000	0.003	0.312	0.773	0.400	0.999
2	richness	10.85	0.000	0.000	0.001	0.001	0.027	1.000	0.238	0.409
3	richness	15.20	0.000	0.000	0.000	0.000	0.000	0.017	0.784	0.972

Community similarity

Similarity among colonization samples and the control samples tended to flatten off around day 16 for the three streams studied. However, in all three streams similarity values on day 64 were lower than those observed between the two control samples. The difference for stream 1 was small, but was quite large for streams 2 and 3.

We observed that one species of caddisfly (*Leucotrichia* sp.) was abundant in control samples of stream 2, but not in the experimentally disturbed samples. In order to check if differences in similarity between day 64 and controls were due to the absence of this species, we re-ran the similarity analysis excluding this species from the dataset. New similarity values are plotted as unconnected filled circles in Fig. 2. There was a decrease in the difference between day 64 and the control samples, but of very low magnitude.

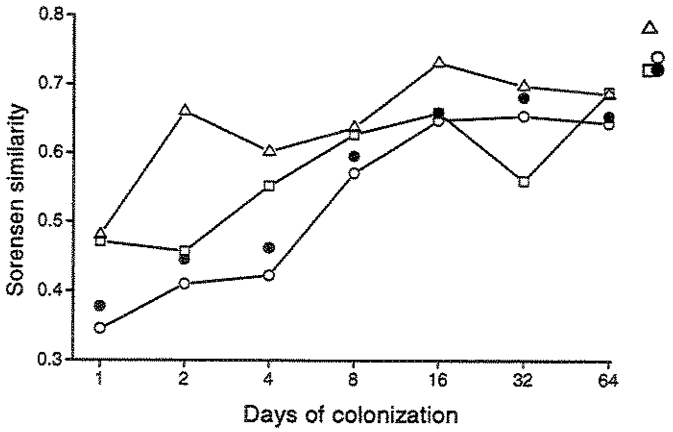

Fig. 2. Sorensen similarity between samples collected at different days after disturbance and two control samples. Values for each day are means from the two comparisons with controls. Symbols place at the right of the graph and not connected by lines represent similarity between the two control samples for each stream site. Some symbols are slightly dislocated around sampling days in order to avoid overlap. □ = site 1, ○ = site 2, ● = site 2 without *Leucotrichia* sp. (see text for explanations), △ = site 3.

Discussion

Colonization patterns did not differ between stream sites 1 and 2, in terms of abundance, species richness and similarity from undisturbed stones. Recovery in stream 3 was similar to streams 1 and 2 in terms of species richness and similarity, but differed in relation to abundance. Mean abundance per stone in stream 3 recovered to control levels after only four days of the start of the experiment, attaining values higher than those of the control after 32 days. It is possible that the density of organisms at the time of the collection of control sample in 1997 was higher than the density during the experiment in 1999, although we have no data to assess this suggestion. The original hypothesis that medium-sized stream 2 would recover to original levels faster than streams 1 and 3 was rejected.

Lack of support for the stated hypothesis might be due to the weakness of the two mechanisms that would generate the expected pattern. It seems that fish presence in stream 3 was not enough to reduce drift to a level low enough to affect colonization of new patches. Also, the upstream pool of colonists in stream 1 does not seem to limit recovery. Additionally, colonization by crawling of nearby individuals might be of significant importance to colonization of small-scale point disturbance (Brooks and Boulton 1991; Marchant et al. 1991). In fact, we observed that some species of crawling caddisfly larvae colonized stones as fast as drift-prone species. This was the case for species of Helicopsychidae and Glosossomatidae. These caddisflies build houses of mineral sediment that prevent them to drift. Nevertheless, they colonized disturbed stones as quickly as mayfly nymphs of the family Baetidae and larvae of Simuliidae (blackflies), known to be common in drift samples elsewhere (e.g. Ramírez and Pringle 1998) (Fig. 3).

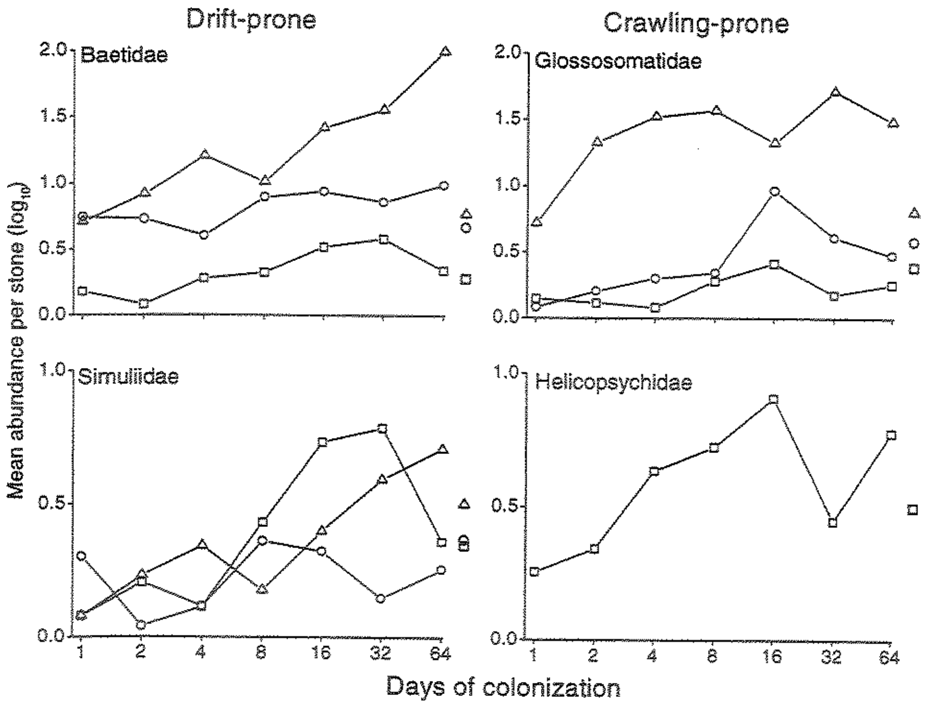

Fig. 3. Mean abundance of selected taxa in relation to main mode of dispersion. Note different scales. □ = site 1, ○ = site 2, Δ = site 3. Symbols placed at the right of the graph and not connected by lines represent control samples. Helicopsychidae larvae occurred at very low abundance (< 0.1 individuals per stone) in streams 2 and 3, preventing a reliable estimation of recovery.

Few studies have compared recovery of disturbed stones among streams of different size. Marchant et al. (1991) did not find differences in colonization rates in five points along a river (stream orders 2-6) in Australia. Lake and Schreiber (1991) found that colonization rates differed among eight streams in the Acheron River, Australia, but these differences were not related to stream size. Rosser and Pearson (1995) compared colonization on experimentally disturbed stones in two streams located at different altitudes and containing different community structure. They found that recovery in terms of species richness did not differ between the two stream sites, but did so for abundance. In the upland site, abundance levels flattened off around day 21 and remained constant thereafter, while in the lowland site abundance increased indefinitely, not attaining a plateau even after 70 days. In the Brazilian

Amazon basin, Freitas (1998) compared colonization of artificial substrate in the Urubui river and two of its tributaries. Colonization rates were low in his study, assessed using total abundance and number of families. He did not find differences in recovery of abundance among the three areas, but he did so for number of families. In the small tributaries, colonization was lower than in the main channel.

Although abundance and species richness per stone recovered to original levels in 8-16 days, the similarity between samples collected on day 64 and the controls were lower than similarity between controls (Fig. 2). This suggests that species composition and/ or relative abundance on samples collected on day 64 were different from the undisturbed stream community. An example of species causing such difference is the caddisfly larvae *Leucotrichia* sp.. This species was very abundant in control samples of stream 2, but was absent in the colonization samples. It seems that this species colonizes stones only by adult oviposition and/ or dispersion of first instar larvae. In fact, *Leucotrichia* sp. build or occupy unused houses made of silk attached to the stone surface. The high cost involved in building or finding an empty case probably prevents larvae from further dispersing after they have settled in a given stone.

In summary, this study did not support the hypothesis that recovery in medium-sized streams would be faster than in small or large streams. Nevertheless, recovery in the three streams studied was rapid and agrees with results found in the literature (Doeg et al. 1989; Mackay 1992; Capítulo 4), supporting the view that stream communities are highly resilient.

Acknowledgements

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Capítulo 6

Disturbance in streams by flood-caused moving and burying of stones: variation among stream sites along a catchment[1]

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[1] Melo, A. S. and C. G. Froehlich. Disturbance in streams by flood-caused moving and burying of stones: variation among stream sites along a catchment. Unpublished manuscript.

Abstract

Surface movement and burying of rocks are indicated in the literature as mechanisms by which floods disturb stream invertebrate assemblages. We designed an experiment to test whether stream site was important in the frequency of stone movement and burying. Five stream sites, from orders 1-4 and differing from each other in relation to substrate composition and presence of debris dams were studied. Labeled stones were placed in the streambed and checked every two months during one year. There was a significant interaction among time of the year and stream site, indicating that periods of highest frequency of movements were not constant through all stream sites. There was no clear trend of movement frequency and stream size. Frequency of burying was much lower than that of stone movements, except in the smallest stream site during the peak of the rainy season where 57% of the stones were buried to some degree. During periods of high rainfall several of the debris dams present in the smallest stream were broken, causing movements of particles located upstream and burying of particles in downstream areas. In a second survey one year after the end of the experiment, 67-100% of the stones were dislodged.

Introduction

Disturbance caused by flood is an important process acting on benthic assemblages occurring in hard bottom running water systems (Resh et al. 1988; Townsend 1989). After flood events, abundance of organisms living on surface stones often decreases (Scrimgeour et al. 1988; Flecker and Feifarek 1994; Rosser and Pearson 1995). This decrease in abundance might be the result of passive detachment from the stone surface, active search for refuge, or even mortality (Dole-Olivier et al. 1997; Holomuzki and Biggs 1999). The exact way in which floods act depressing organisms abundance in streams is not yet fully understood, although turning and burying of particles are likely to be important mechanisms (Englund 1991; Matthaei et al. 1999b; Matthaei et al. 2000; but see Bond and Downes 2000).

Particle movements are affected by a number of factors including size, embedding, and specific place on the stream bed (Downes et al. 1998; Matthaei et al. 1999b; Matthaei et al. 1999a). Thus, it is likely that particles will differ in suitability for organisms, depending on their physical characteristics and position in the streambed. Douglas and Lake (1994) studied the relationship between species richness and stone area and found that small stones have lower species richness than large stones. Similarly, Matthaei et al. (2000) found that after a flood event, abundance of species were higher on embedded stones than on those lying loose over the substratum. In both cases, the authors pointed to the high susceptibility to dislocation of small and loose stones during floods as the causing factor of the decrease in abundance.

Along a catchment, channel morphology differs in several physical features (Church 1996). Headwater streams usually have higher frequency of riffle-pool sequences than larger, downstream sites. Particle sizes in general are dependent on channel gradient, which in turn tend to decrease in downstream sites. In seasonal environments, floods resulting from high rainfall events tend to last longer in downstream sites than in small streams, where the rise and fall of the water level can occur within a few hours. All these differences might result in different disturbance intensity on stream assemblages subjected to the same rainfall regime, but occurring in different places inside a catchment.

Traditionally, assessment of proportions of particles of a given size that moved during a flood event has been made indirectly by calculating the critical force required to cause its dislocation (e.g. Cobb et al. 1992). Recently, river ecologists have assessed particle movements directly, observing the presence/absence of labeled particles in previously mapped places after a flood event (Death and Winterbourn 1994; Townsend et al. 1997; Downes et al. 1998) or measuring the force need to move stones (Downes et al. 1997). Burying and posterior exposing of particles can be inferred by mapping partially buried chains and observing how many links were buried or exposed after a flood (Matthaei et al. 1999b). A more costly method, but perhaps more realistic, to access both movement and burying of particles, is the mapping of labeled stones and several subsequent visits to search for those ones missing.

In this study we tested the importance of contrasting stream sites inside a same catchment on the frequency of stone movement and burying. Specifically, we tested if frequency of movements is related to stream size and time of the year. The assessment of movement and burying of particles was done directly, using painted stones.

Study sites

The study was carried out in the Carmo River catchment, at Parque Estadual Intervales (24°18'S, 48°25'W), São Paulo State, Brazil. The vegetation is tropical ombrophilous submontane-montane forest, commonly known as tropical rain forest (Mueller-Dombois and Ellenberg 1974). The mean annual precipitation in the area is 2040 mm (rain gauge located 20 km from the nearest stream site studied and subjected to the same precipitation regime of the studied catchment; 25 y record). Rainfall is unevenly distributed across two seasons: one wet (150-400 mm/mo) and warm (15-30 °C) from September through March and another dry (60-150 mm/mo) and cold (0-25 °C) from April to August. During the first year of the study, September 1999 through August 2000, 1928 mm of precipitation were recorded. The months with highest precipitation were February and March with 313 and 318 mm respectively. In the second year, 1867 mm were recorded and the two rainiest months were September and December with 260 and 259 mm respectively.

Experiments were performed in five stream sites. Site 1 was a first order stream ranging 0.5-1 m in width. The streambed was composed mainly of sand and stones (10-40 cm long). The studied stream reach had several pools and small waterfalls formed by fallen trees and accumulated twigs, leaves, and sand. Stream sites 2 and 3 were of second and third order, and in the studied reach width ranges were 2.5-3.5 and 3-4 m respectively. In both sites, the stream bottoms were predominantly composed of stones and boulders. Pools and waterfalls were sparse. Sites 4 and 5 are fourth order streams and were very similar to each other. For both sites the width range is 9-11 m and like sites 2 and 3, the streambeds were composed mainly of stones and boulders. No pools and waterfalls were present in the studied reaches of sites 4 and 5. Studied stream sites 1-5 represent sites 1, 4, 6, 8, and 9 respectively in the Fig. 1 of Melo and Froehlich (2001), where additional information on physical characteristics of streams and invertebrate assemblages are provided.

Materials and methods

Fieldwork

In each stream site, two sets of 15 stones each were painted using automobile spray cans. The first set was composed by stones around 10 cm long and here is called small stones. The second set comprised stones around 18 cm long and are hereafter called large stones. Each stone was identified using a small plastic label attached to the stone using angling nylon line. As density of stones are likely to affect movement frequency, we standardized stone type by selecting sedimentary stones (shales, siltstones, and sandstones), frequent in the five sites studied.

The painted stones were placed in riffles in rows perpendicular to the main flow. In sites 1 and 2, each row comprised one small and one large stone, totaling 15 rows. In sites 3, 4, and 5, each row was composed of two stones of each size, except by the most downstream row which contained three stones of each size, totaling seven rows. Plastic strips tied to the surrounding vegetation were used to indicate the position of each row in the study reach.

The experiment was started in September 1999 and followed through one year. During the period, we visited each stream site every two months and recorded the movements and burying status of each stone and replaced the dislodged and buried stones to their original position. Stones that remained in the original row were removed and then placed back in the same position to ensure that all stones were in the same condition of embedding for the next period. When necessary, stones were repainted.

Missing stones were searched during 1-2 hours by two people. In cases in which a stone was not found, a new stone of the same size class was painted and replaced, but with a different label code. In subsequent visits, several previously lost stones were found. For these cases, we considered that the stone was completely buried and moved once during the original two-month period.

In the last bimonthly visit in August 2000, we also returned all stones to the original positions in order to assess how many stones moved from the original location in the following unit time frame of one year.

Statistical analysis

We tested the importance of stream site on the proportion of movements of stones using logistic regression. Despite our main interest being the importance of stream site, we also included in the model the variables stone size and period of year as explanatory variables, instead of including them as control variables. This allowed us to assess possible significant interaction between these two variables, what was considered relevant. For example, small stones could move more frequently than large stones in periods of low to medium floods, while both stone sizes could move with the same high frequency during periods of severe floods. All three explanatory variables were treated as dummy variables. Model selection was done using a hierarchical backward elimination approach (Kleinbaum 1994). Low movement frequency during the dry periods and the large number of combinations of the explanatory variables (5 sites x 2 stone sizes x 6 periods = 60) resulted in zero-frequency (no stone movement) data in some combinations of the variables in study. The presence of zero values in logistic analysis causes the non-stabilization of the Log Likelihood statistic in the iteration process, and the production of extremely high, misleading parameters estimates. Thus, we performed the analysis using the original data as well as adding a 0.1 constant to 0-values and subtracting 0.1 from the correspondent 15-values representing the frequency of non-movements (Agresti 1996, p. 192). Log Likelihood statistics were similar between the two analyses and the same final model was attained.

Frequency of burying was too low in most of the sites studied, precluding a reliable use of logistic regression. Data regarding small and large stones were pooled and are presented graphically.

Results

The simplest final model that still fitted the data for stone movements was composed by an interaction term between the factors stream site and time of year, and the main factor stone size. Small stones tended to move more than large stones, and although the final model did not contain an interaction term with time of year, such differences were more conspicuous during periods of high frequency of events (Fig. 1). Overall, the highest frequency of movements were observed in January-February, followed by the September-October period. However, as indicated by the significant interaction term in the selected model, periods of highest movements were not constant through all stream sites. Stones moved with a lower frequency in site 5 during September-October, but at higher frequency in March-April than in the other four sites (Fig. 1). For most periods, site 4 presented the highest frequency of movements, while movements in site 2 occurred at the lowest frequency.

Burying of stones occurred most frequently during January-February (Fig. 2). Burying was rare in sites 2-5, but occurred at high frequency in site 1 during January-February. Sediments covering stones in sites 2-5 were mainly gravel and pebbles. Covering material in site 1 was predominantly sand, derived from debris dams broken during high flow events.

During the second year of the study, most of the stones moved and/ or were buried (range 80-100 and 67-93%, respectively for small and large stones) (Fig. 3). In sites 1, 2, and 4 no small stone remained in place. Frequency of disturbed stones was lowest in site 5, but still high (80 and 67%, respectively for small and large stones).

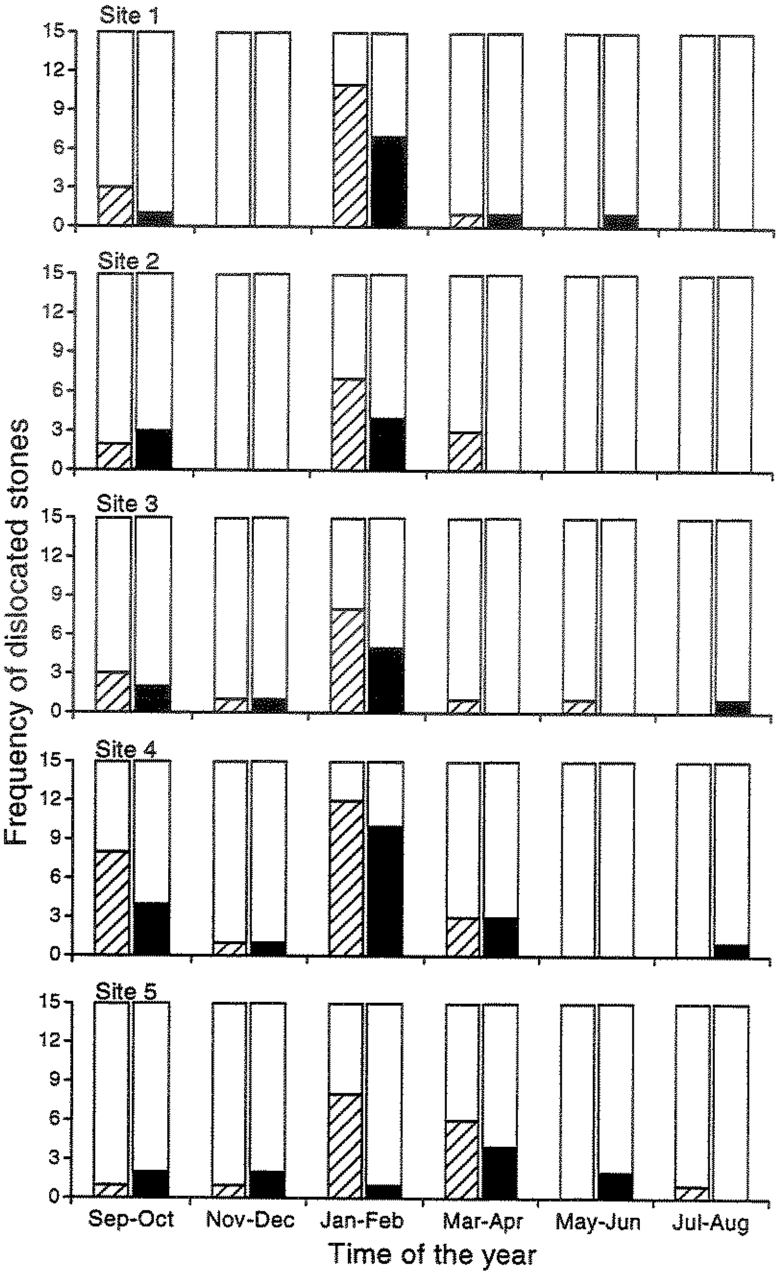

Fig. 1. Frequency of stone movements in five stream sites during one year. Hatched bars indicate movements of small stones (around 10 cm) and black bars movements of large stones (around 18 cm).

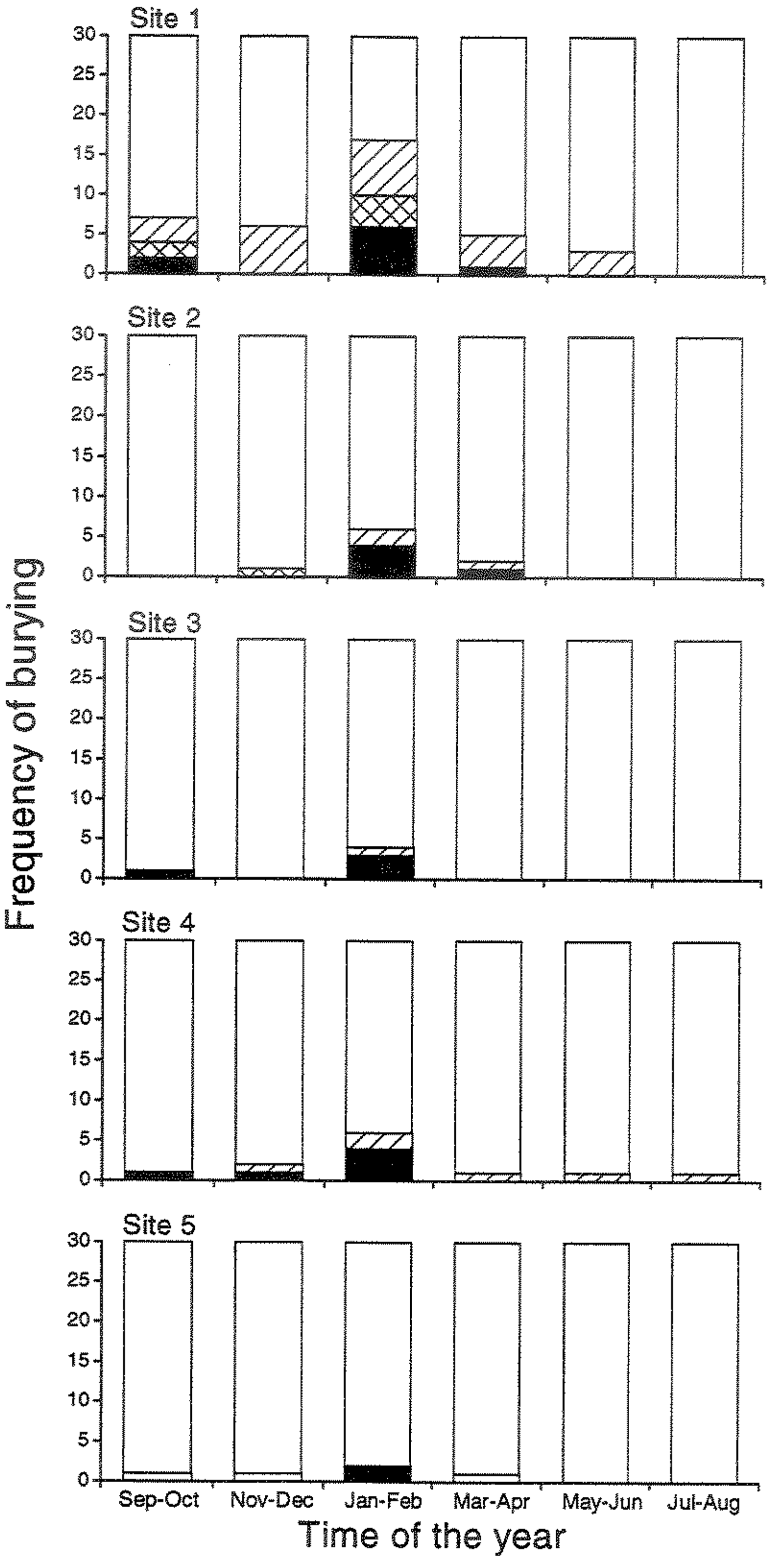

Fig. 2. Frequency of stone burying in five stream sites during one year. Small and large stones pooled. Hatched, cross-hatched, and black portions of bars indicate respectively 25-50, 50-75, and 75-100% of burying.

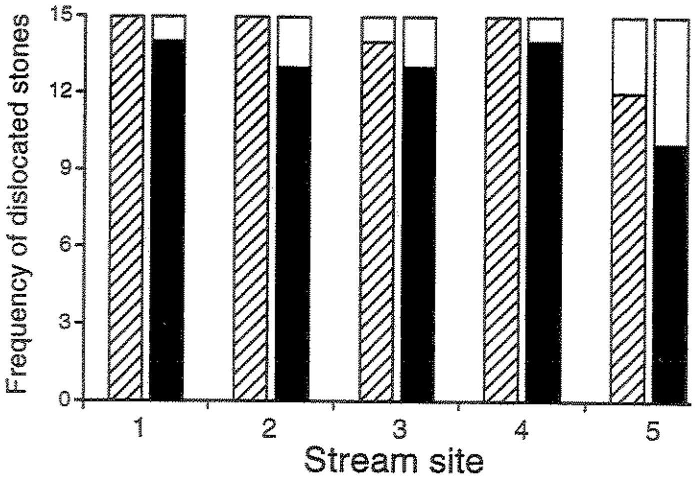

Fig. 3. Frequency of stone movements during a one-year period. Hatched bars indicate movements of small stones and black bars movements of large stones.

Discussion

Small stones moved more frequently than large stones, agreeing with previous results (Downes et al. 1998; Matthaei et al. 1999a). Movement occurred with highest frequency during January-February in all stream sites, followed by the periods September-October and March-April. As would be expected, the period of highest frequency of movements coincides with the peak of the rainy season, while the two others represent the start and the end of the rainy season in the studied area.

Downes et al. (1998) found that disturbance levels were similar in third- and fourth-order streams during the dry, summer season. During winter, disturbance levels in upstream sites remained unchanged, but doubled in downstream sites. Based on results of Downes et al. (1998) we expected that frequency of movements would increase downstream, at least during rainy periods. However, we did not find a clear relation of frequency of stone movements and stream size (Fig. 1). Moreover, frequency of burying was even much higher in the smallest site than in the other four sites (Fig. 2).

Differently from the other streams studied, the first-order stream contained several small debris dams formed by fallen trees and subsequent accumulation of twigs, leaves, and sand. We observed that the process of formation and the posterior partial or total destruction of dams in this stream was very dynamic. After rupture of debris dams, stones located within 5-10 m upstream are moved downstream as a consequence of sand and gravel movements. In downstream areas ranging from 5 to 15 m long, stones are covered by sand and gravel derived from patches located in the eroded areas upstream of the ruptured dam. Hax and Golladay (1998) observed that debris dams increased the retention of wood debris, the main substrate used by invertebrates in a sandy stream. They argued that wood trapped by debris dams might be an important refuge, slowing the downstream transport of invertebrates during floods. While this might be true in some cases (Palmer et al. 1996), our data suggest that this statement is far from being a rule. Debris dams that acted as refuges for a particular flood, might act as a very unstable site during a posterior event when decaying key logs in the dam are no longer strong enough to withstand high flow.

Frequency of burying was low in stream sites 2-5 and in most of these cases stones were actually wedged by other stones. This is in agreement with previous findings that floods cause higher frequency of movements than of burying (Gintz et al. 1996). Nevertheless, burying was frequent in the first-order site, indicating that effects of flood disturbance might be site-dependent. Most recent studies of disturbance in streams have only taken in account stone movements as consequences of high flow events (Matthaei et al. 1997). Our data reinforce the suggestion of Matthaei et al. (1999b) that more attention should be paid by stream ecologists to burying of stones, which in some cases might be as important as stone movements.

In the second year of study, we were not able to locate most of the disturbed stones because most of the ink coat had been scoured away. However, the plastic label remained attached to the rocks, allowing us to assess whether stones remained in the original positions or were dislocated. For this entire one-year period, 95% of the small and 85% of the large stones were dislodged

Our study indicates that disturbance of stones in streams is frequent, regardless of stream size. However, the causes of theses movements might differ between sites differing in physical structure. In the small stream studied, perhaps most of the stone movements were caused by rupture of debris dams, while in the four large streams the main cause was likely the direct force of water during high flow. Also, physical differences among stream sites (i.e. the presence of debris dams) were a cause of frequent events of burying.

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Capítulo 7

Community structure and persistence of stream macroinvertebrates in tropical streams[1]

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[1] Melo, A. S. and C. G. Froehlich. Community structure and persistence of stream macroinvertebrates in tropical streams. Unpublished manuscript.

Abstract

We used data of stream macroinvertebrates in five streams sampled twice a year during five years to assess four topics related to persistence of communities: (1) Are there distinct and recurrent summer and winter communities? (2) Are communities in seasons of low environmental variability (dry, winter season) more constant and similar to each other over years than communities in seasons of high environmental variability (rainy, summer season)? (3) Is community variability related to environmental variability? (4) Does community variability increase with time? Communities in each stream site were distinct from each other as revealed by cluster analysis. For the five streams, all 10 samples collected in each stream over five years were classified in a single group. Winter samples tended to cluster together at high similarity levels and were nested within summer samples. We used Kendall's coefficient of concordance (*W*) to assess persistence of summer and winter communities over the five years period studied. For the five streams, persistence was higher in winter than in summer communities. Persistence was not related to environmental stability of the five streams studied. There was not an overall significance of the correlations between persistence of communities (measured by the coefficients of concordance *W*), and three measures of site stability (frequency of dislocated and buried stones and the scores of the Pfankuch index of channel stability). There was a decrease in community concordance with time, although the decrease was nearly four times larger in summer than in winter. Results support the view that stream communities vary along the year from a non-equilibrium state during the rainy season when disturbance by high flow is frequent, to an equilibrium state during the dry season when flow is stable.

Introduction

Communities are expected to change with time. In a time scale of one year, short-lived species usually experience changes in abundance as a response to seasonal changes in resources, biotic interactions and environmental constraints (Wolda 1988). Although a number of works has addressed in detail these within-year, seasonal changes, few studies have addressed whether these changes are repeated in successive years, particularly at the community level (e.g. Lawton and Gaston 1989). Differences detected in community structure (i.e. species composition and relative abundances) between two seasons in a one or two years study may reflect a particular event, and not a recurrent characteristic of the assemblage in study. Although results obtained in such a study may add evidence to previous results, and in this sense be relevant, the study by itself is inconclusive (Underwood 1991).

In a longer time-scale, it is of great importance to know to which extent community structure persists over time. If species composition and relative abundance change randomly between years, our prediction ability likely would be very low. We would not be able to assess for example whether an assemblage was impacted by some human-related perturbation, as we would not have a reliable reference condition (Karr and Chu 1999 p. 141).

Despite the importance of works encompassing several years using uniform methods, there are few studies in the literature, most of them dealing with temperate faunas (Wolda 1978; McElravy et al. 1989; Jackson and Sweeney 1995). In this study we use an extensive dataset of benthic macroinvertebrates from five streams collected twice a year in summer and winter, during five years, to assess four issues regarding persistence of communities. Following Rahel (1990), we use the term persistence to describe the constancy in absolute abundance or abundance ranking of species over time.

Floods are indicated by stream ecologists as one of the main forces structuring benthic macroinvertebrate assemblages (Resh et al. 1988; Townsend 1989). Following a flood event, abundances of species are often decreased (Flecker and Feifarek 1994). After small to medium floods, recolonization of depleted patches is fast and original abundance levels can be reached within 30-120 days depending on flood intensity (Mackay 1992). First colonizers generally are small, short-lived species with high dispersal power. In places where precipitation is concentrated in one period of the year, it is often possible to distinguish a rainy season when stream flow is variable, with occasional floods, from a dry season when flow is constant. Minshall et al. (1985) predicted that in such cases, community structure varies from non-equilibrium to equilibrium conditions along the year. During periods of high flow variability, stream assemblages would be governed mainly by abiotic forces such as floods, resulting in a non-interactive community, but shifts toward a more equilibrium state during stable flows, when biotic interactions became preponderant (Minshall and Petersen 1985). Following this reasoning we would expect the presence of two distinct community states during the year, and greater similarity among stable-flow (dry season) communities than among variable-flow (rainy season) communities over years. In other words, we asked: (1) Are there distinct and recurrent summer and winter communities? (2) Do communities in seasons of low environmental variability (dry season) are more constant and similar to each other over years than are communities in seasons of high environmental variability (rainy season)?

Similarly to the second question above, we extend the prediction about the extent of recurrent community structure over years to the spatial scale. It would be expected that stream sites experiencing high environmental variability would present lower concordance in community structure over years than sites subjected to low environmental variability

(Oberdorff et al. 2001). So, (3) Does community variability is related to environmental variability?

Finally we investigate persistence of communities over time. Bengtsson et al. (1997) using a large dataset of bird occurrence in Great Britain over 21 years observed that community variability increased over time, as measured by changes in rank-abundance of species. We comment the way Bengtsson et al. (1997) computed the rank-abundance statistic and re-assess the question: (4) Does community variability increases with time? Our data included a much shorter time period than that used by Bengtsson et al. and this could potentially increase autocorrelation between adjacent years. High persistence of communities in this sense would be a result of high survival of individuals between years. However, although macroinvertebrate species likely varied greatly in time generation, it is probable than for most of them time generations were less than one year. Thus, the five years included in the study should have been enough for at least five generations for most species. Bengtsson et al. mention that the 21-year period was enough for 4-5 generations in most of the bird species included in their study.

Materials and methods

Study area

The study was carried out in the Carmo River catchment, at Parque Estadual Intervales (24°18'S, 48°25'W), São Paulo State, Brazil. The vegetation is tropical ombrophilous submontane-montane forest, commonly known as tropical rain forest. The mean annual precipitation in the area is 2040 mm (25 y record). Rainfall is unevenly distributed across two seasons: one rainy (150-400 mm/mo) and warm (15-30 °C) from September through March (austral summer) and another dry (60-150 mm/mo) and cold (0-25 °C) from April to August (winter).

Five stream sites were studied. Site 1 was a first order stream ranging 0.5-1 m in width. The streambed was composed mainly of sand and stones (10-40 cm long). The studied stream reach had several pools and small waterfalls formed by fallen trees and accumulated twigs, leaves, and sand. Stream sites 2 and 3 were second and third order, and in the studied reach width ranges were 2.5-3.5 and 3-4 m respectively. In both sites, the stream bottoms were predominantly composed of stones and boulders. Pools and waterfalls were sparse. Sites 4 and 5 are fourth order streams and were very similar to each other. For both sites the width range is 9-11 m and like sites 2 and 3, the streambeds were composed mainly of stones and boulders. No pools and waterfalls were present in the studied reaches of sites 4 and 5. Positions of studied sites in the catchment basin is shown in Fig. 1 and additional information on physical characteristics in Table 1.

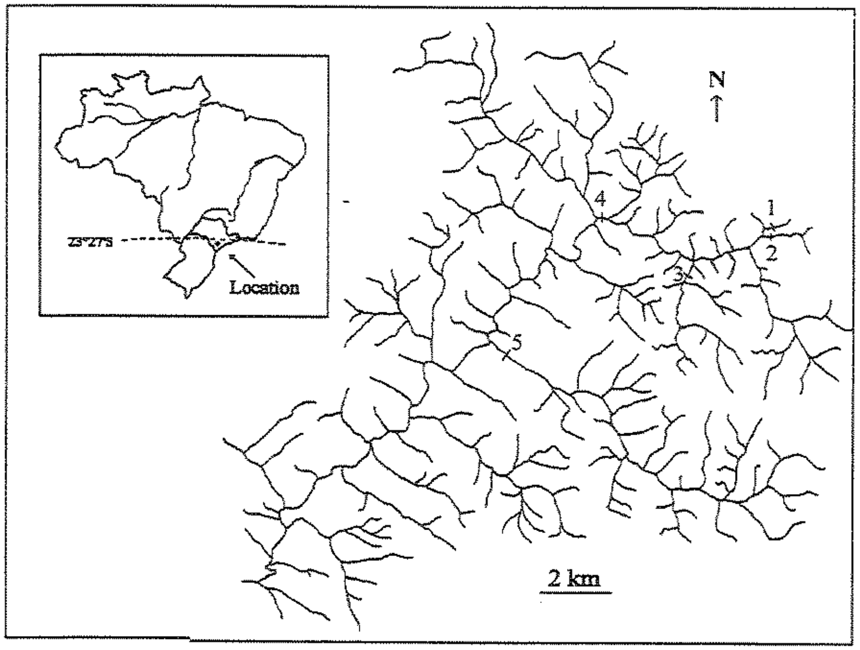

Fig. 1. Location of studied stream sites in the Rio do Carmo catchment basin, Parque Estadual Intervales, São Paulo, Brazil.

Table 1. Physical characteristics of the five sampling sites in Parque Estadual Intervales, São Paulo, Brazil.

Parameters	Stream sites				
	1	2	3	4	5
Stream order	1	2	3	4	4
Link magnitude[a]	1	2	6	43	36
Width (m)	1	3.5	4	10	10
Summer discharge (m^3/s)	0.010	0.091	0.339	1.158	1.281
Winter discharge (m^3/s)	0.005	0.059	0.092	0.657	0.750
Canopy cover (%)	100	100	98	94	84
Stream gradient (%)	0.07	0.02	0.03	0.03	0.02
Altitude (m)	680	680	700	520	400
Conductivity (winter, µs/cm)	51	48	45	30	36
Sediments over rocks[b]	2	1.6	1.6	1.6	2.3
Diel max-min temp. (winter, °C)	15-15	15-13	16-13	17-14	16-15

[a] Number of 1st-order streams included in the sub-catchment

[b] Scored qualitatively as the amount of sediment collected by washing a defined area of stones and filtered through laboratory filter paper. The presented values are mean of 3 stones and the range used was 1 to 5 where 1 is no visible residue and 5 is the largest quantity sampled over the 24 filter papers

Sampling and processing of material

Sampling occurred from 1997 through 2001. Stream sites were sampled in February-March and July-August, during the peak of rainy (summer) and dry (winter) seasons respectively, except for the summer collection of 2001 that was in December 2000.

We used individual stones of ~18 cm maximum diameter as sample units. In each stream and date, one sample containing 25 sample units (individual stones) was collected from a reach of at least 100 m in length. The stones were taken from the streambed of riffles using a U-net sampler with a 250-µm mesh. The material collected in this device was transferred to a white tray and all visible invertebrates removed and fixed in 80% ethanol. Stones were also examined for attached individuals.

Because of the poor knowledge of the stream macroinvertebrate fauna in Brazil (Hurlbert et al. 1981), particularly for immature insects, it was difficult to identify specimens to the species level. Hence, individuals were identified to the family level using available literature and then separated as morphospecies. When separation of organisms into one or two morphospecies was doubtful, we used a conservative approach and left them in a single class, a procedure also used by Townsend et al. (1987). Additional information on sampling and identification procedures can be found elsewhere (Melo and Froehlich 2001a; 2001b).

Analysis

We assessed whether samples collected in summer and winter represent distinct communities using agglomerative cluster analysis. Total abundance for each species occurring on the 25-stone sample collected at each stream and date were transformed using $\log_{10}(x + 1)$. Cluster analysis on samples (i.e. among each 25-stone set collected on each site and date) was done using Sørensen distance and the unweighted pair-group method using arithmetic averages (UPGMA).

Our second hypothesis, that winter communities should be more persistent over years than summer communities, was assessed using Kendall's coefficient of concordance (*W*). The statistic is a non-parametric multisample correlation based on the rank-abundance of species. It ranges from 0 (no concordance) to 1 (total concordance). It is analogous to the two-sample Spearman rank correlation (Zar 1999, p. 446). Summer and winter *W* values were computed separately for each stream site over five years. As presence and abundance of rare species in samples are subjected to higher sample errors than are common species, only the latter were used. For each stream site and season, we used the 15 most common species obtained from the summed abundances over the five years. We expected higher values of *W* for winter than for summer communities.

In order to assess whether community persistence is related to environmental variability, we calculated correlations of summer and winter *W*-values from the five streams over five years with three measures of bed stability. Overall statistical significance of the hypothesis that persistence is related to streambed stability was assessed using the consensus combined *p*-value test (Rice 1990). The test evaluated the significance of the general hypothesis using the 6 *p*-values obtained from the correlations (2 seasons X 3 bed stability metrics). Used metrics were the frequency data of moving and burying of stones in the five streams studied (Capítulo 6) and the Pfankuch index of channel stability. Surface movements and burying of stones are pointed in the literature as mechanisms by which floods disturb benthic invertebrates and have been used recently to assess streambed stability (Death and Winterbourn 1995; Townsend et al. 1997; Matthaei et al. 1999). Data of moving and burying of stones were obtained from 30 labeled stones placed in the streambed of each stream. Six bi-monthly visits were done from September 1999 through August 2001 to check movement and burying of stones and replace dislocated stones to original positions. Half of the stones were of size around 10 cm long and half composed by stones around 18 cm. We used the mean percentage of stone movement of the two stone size classes as a measure of bed movement. Burying was quantified by the mean percentage of stones buried at least 50%. Data from the two stone size classes were pooled. Analyses were done separately for summer and winter. For summer, we used pooled data from the three bi-month records for the period of September through February and for winter data from March through August. The Pfankuch channel stability index is a subjective, composite index developed by Pfankuch (1975). It is calculated by summing the scores of 15 variables relating to the resistance of mountain stream channels to the detachment of bed and bank materials caused by high flow (Collier 1992; Death and Winterbourn 1995; Townsend et al. 1997).

To test the hypothesis of increase of community variability with time, Bengtsson et al. computed *W*-values for different time intervals: the complete 18-22 year period and non-overlapping sequences of lengths 2, 4, 6, and 8 years (see Fig. 1 in Bengtsson et al. 1997). However, a potential problem in computing *W*-values in this way is that for each time interval, there are different numbers of samples (or years) on which the statistic is calculated. For two years apart, there are three samples (or years), while for four years apart there are five samples. We empirically assessed whether this difference in number of samples affect the *W*-values computed. We randomly chose six samples of 25-sampling units (stones) each from a data set of stream macroinvertebrates associated to 150 stones collected in a same stream reach and time (Melo and Froehlich 2001a). We then used these six samples to calculate *W*-values in the same way described above, but using overlapping sequences because of the reduced number of samples available. Samples were given numbers one up to six and all combinations of "time sequences" from one up to five "years" apart calculated. The procedure was repeated 10 times. If number of samples ("years") do not affect *W*-values, we would expect similar *W*-values for all different "time sequences". We compared results obtained using the procedure above with results obtained using the same 10 random sets of six samples but with pairs of "years" instead of sequences of "years". For example, for four "years" apart, instead of using the sequence 1-2-3-4-5, we used only the pair 1-5. Note that for 1 "year" apart data were the same.

We found a clear trend of decrease in *W*-values as more samples are included (Fig. 2), invalidating thus the procedure used by Bengtsson et al. (1997). On other hand, the procedure using pairs of samples did not produce a clear trend with samples further apart, as expected.

We thus assessed whether the hypothesis that community variability increase with time computing *W*-values for each site and season using pairs of years with different intervals apart. For one year apart, *W*-values were obtained from pairs: 97-98, 98-99, 99-00 and 00-01; for two years apart: 97-99, 98-00, 99-01; for three years: 97-00, 98-01; and for four years: 97-01. Bengtsson et al. also calculated *W*-values in this way, but to test differences among woodland types and to correlate with variables associated with study plots or community composition (Table 2 and Fig. 2 in Bengtsson et al. 1997). Data used to calculate *W*-values were clearly not independent from each other and some years are used more times than others. Further, for different time intervals there are different numbers of *W*-values. These three problems prevented the use of parametric statistical inference to reveal trends in *W*-values over time. We thus assessed correlation between W-values with time intervals using a Mantel test. The first matrix contained distance in years and the second one distance in non-concordance (1-W). The matrix of non-concordance values was obtained by averaging data of the five stream sites for each combination of pairs of years. Calculations were done using winter and summer data separately. As an indicative of magnitude of trend over time, we further calculated slopes of *W*-values regressed with time for both seasons. As noted above, used data were not independent to each other and thus we did not evaluate the statistical significance of the difference between slopes obtained from winter and summer data.

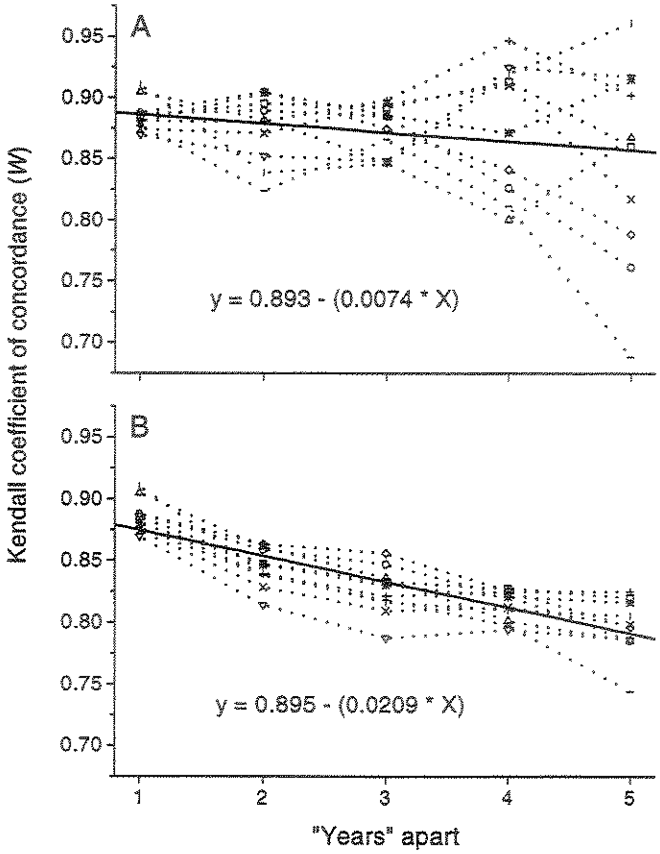

Fig. 2. Kendall rank concordance *W*-values among pairs (A) and sequences (B) of randomly obtained samples at increasingly numbers of "years" apart (dotted lines). Used data are not independent from each other and thus regressed lines (straight lines) are only indicatives of trends. Note that the slope for sequences (B) is nearly three times larger than for pairs (A). Each value represents the average of all possible combinations of pairs or sequence of years. For the distance of one "year" there are five combinations (1-2; 2-3; 3-4; 4-5; 5-6) and for the distance of five years only one combination (1-6 using pairs of years and 1-2-3-4-5-6 using sequences of years). Notice that values for one "year" apart are the same for calculations using pairs or sequences. The increased spread of values in the calculations using pairs is due to two factors: (1) the average values are based on decreasing number of combinations of "years" and for the five "years" apart there is only one combination, (2) for each run using pairs of years only a fixed random fraction of sampling units are used (50 of 150 = 0.33), whether an increasing proportion of sampling units are used in the calculations using sequences (0.33, 0.5, 0.66, 0.83, and 1 respectively for sequences 1, 2, 3, 4, and 5 "years" apart).

Results

Macroinvertebrate assemblages at each site were distinct from each other and were classified in five groups corresponding exactly to the five stream sites studied (Fig. 3). The intermediatesized stream 3 was classified farthest from the four other streams, reflecting the presence of several species restricted to this site. The two smallest streams (sites 1 and 2) formed a distinct group as well as the two largest streams (sites 4 and 5). Assemblages in summer and winter were in general distinct from each other. Winter assemblages sampled in sites 2 and 3 were classified as perfect groups within their respective streams. For sites 1 and 4, four of the five winter samples were classified in the same group. In all streams, winter communities tended to classify at high similarity levels, forming a compact nested group in relation to summer samples. Within stream and season groups, there was not a tendency for samples collected in adjacent years to be classified in nearby positions.

For all stream sites, Kendall rank concordance statistic (*W*) was higher for winter than for summer communities (Table 2). Rank-abundances for the 15 most common species in winter were more persistent over the five years period than for the 15 most common species in summer (Wilcoxon signed rank test, n = 5, T = 0, $p = 0.043$). This further support the result obtained with the cluster analysis, that winter communities are more similar to each other than are communities in summer.

Table 2. Kendall coefficient of concordance (*W*) among five years for five streams and two seasons.

Stream	Winter	Summer
1	0.726	0.698
2	0.594	0.486
3	0.582	0.521
4	0.640	0.609
5	0.676	0.519

The hypothesis that persistence of communities is related to environmental stability was not supported. Using the six *p*-values together (Table 3) to assess the hypothesis, the consensus combined *p*-value test produced a non-significant value of $p = 0.430$. *W*-values and streambed stability metrics were weakly correlated, and only in one case the *p*-value was lower than 0.05 (Table 3). Despite the lack of statistical significance, in all cases correlation coefficients were positive, contradicting the original expectation that persistence would be negatively related to environmental stability.

Table 3. Correlations among three measures of stream bed stability and Kendall coefficient of concordance (*W*) over five years for summer and winter samples. In all cases n = 5 stream sites.

	Summer		Winter	
Metric	r	p	r	p
Stone movement	0.514	0.375	0.297	0.627
Stone burying	0.899	0.038	0.252	0.682
Pfankuch index	0.358	0.554	0.182	0.769

There was a decrease in *W*-values as pairs of years were further apart, as assessed by the Mantel test (p = 0.007 for winter and p = 0.016 for summer). For both seasons community variability increased with time. The slope of the regressed W-values over time for summer data was nearly four times larger than for winter data (Fig. 4). Although community variability increased with time during both seasons, the increase was much more prominent for summer communities.

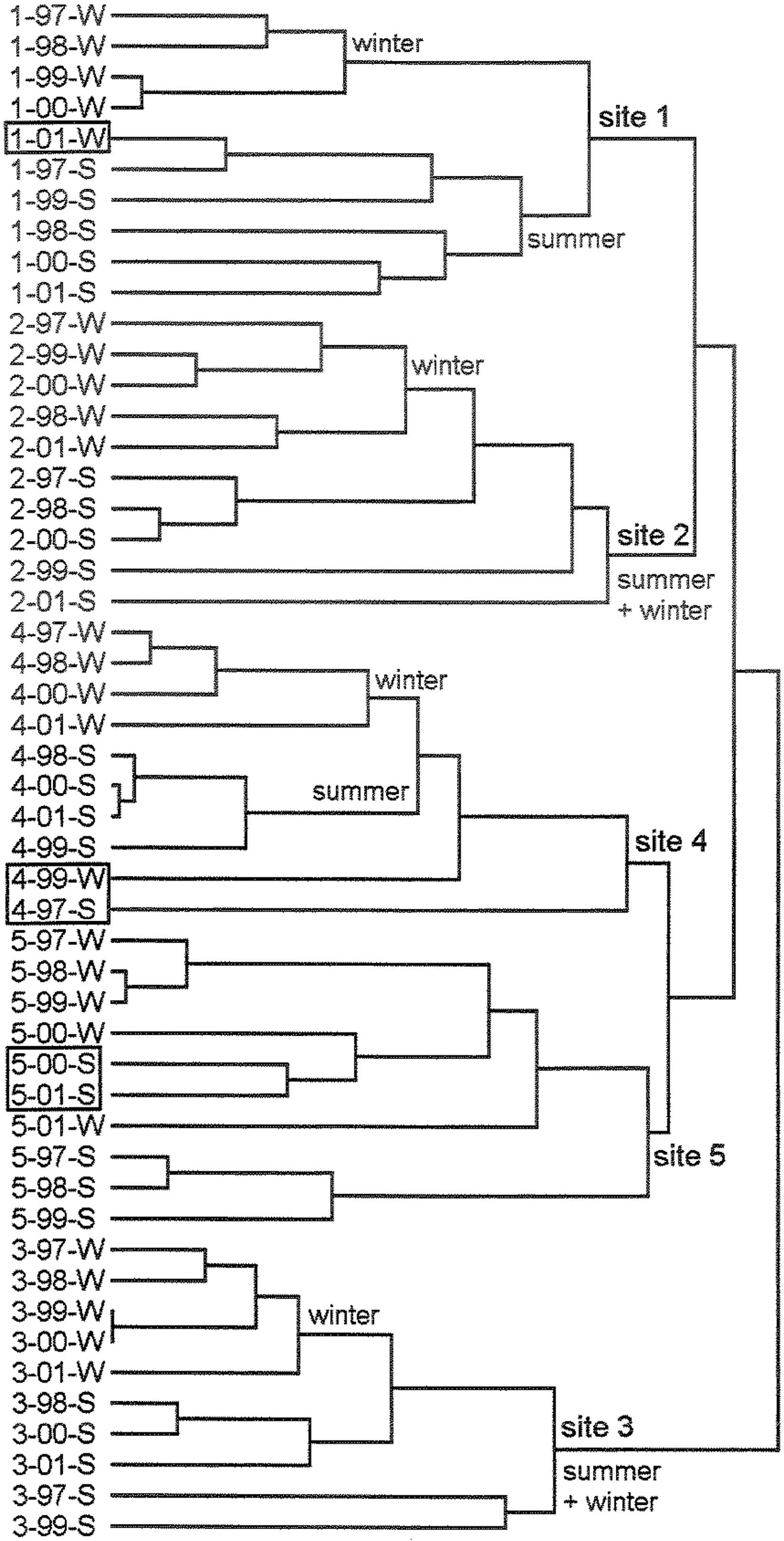

Fig. 3. Classification of stream sites and seasons by unweighted pair-group method using arithmetic averages (UPGMA) and Sørensen distance. 1, 2, 3, 4 and 5 = stream sites. 97, 98, 99, 00 and 01 = years. S = summer, W = winter. Terms indicate consistent groups and boxes "misclassified" samples.

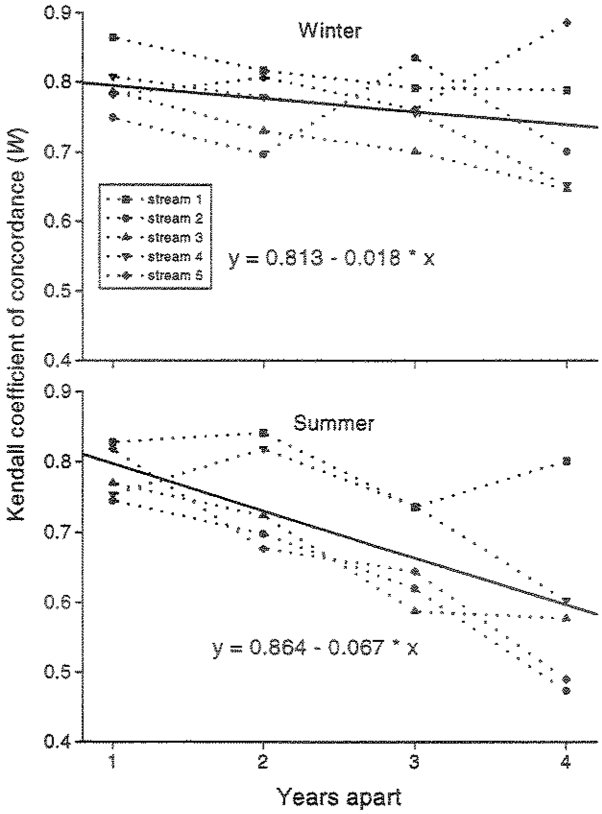

Fig. 4. Kendall rank concordance *W*-values among pairs of samples at increasingly numbers of years apart for each stream site and season (dotted lines). Used data are not independent from each other and thus regressed lines (straight lines) are only indicatives of trends. Note that slope in summer (B) is nearly four times larger than in winter (A).

Discussion

Differences in species composition and relative abundance among streams were distinct enough to classify samples collected over the years in each stream site into distinct groups. For each stream group, winter samples tended to cluster together, generally at high similarity levels, and nested within summer samples. High similarity of winter samples detected in the cluster analysis was further supported by high concordance of rank-abundance values over years using the most common species. In other hand, summer samples tended to differ more from each other, and these differences were highest as sampled years were farthest apart.

Minshall et al. (1985) predicted that stream communities vary along the year from a non-equilibrium state during the season in which environmental variability is highest, to an equilibrium state when environmental variability tends to decrease. The authors supported their predictions using a composite statistics and data collected at several sites and two seasons in a single year. Using the same approach, we did not find such a result in a previous work (Melo and Froehlich 2001b). However, if we define equilibrium state as a recurrent and predictable state in terms of community parameters (species richness, species composition, relative abundance), then our results obtained over five years support the view that stream communities varies annually from a non-equilibrium state during the rainy season, to a equilibrium state during the dry season, when environmental variability tend to decrease.

Connell and Souza (1983) argued that in order to consider a community as persistent, two fundamental criteria should be satisfied. The community should be subjected to disturbance factors and present a distinct equilibrium state. A persistent community should remain in the same state after a disturbance (i.e. high resistance) and/ or return to the original equilibrium state after the disturbance factor ceases. Connell and Souza (1983, p. 806) admit that these criteria are "...quite restrictive, and few of the studies reviewed were rigorous enough to establish unambiguously the relative stability and/or persistence of the populations or community being studied." It is thus interesting to notice that our results fits not only the largely untested theoretical predictions of alternate non-equilibrium and equilibrium states made by Minshall et al. (1985) for stream communities, but also fulfill the two criteria of Connell and Souza (1983) required to regard a community as persistent.

Two specific characteristics of stream invertebrate communities are worthy to notice in order to a better understanding of the recurrent community states and persistence over years: (1) the quite predictable occurrence of disturbance by high flow during the rainy season and (2) the existence of efficient mechanisms of recovery following a disturbance event. Seasonal disturbance by high flow events and the consequent reduction in abundance for most species is an almost universal characteristic in streams (Resh et al. 1988; Townsend 1989; Lake 2000), and in fact are regarded by stream ecologists as one of the most, if not the most, important factor structuring stream invertebrate communities. In this sense, stream invertebrates are regarded to have low resistance to disturbance events. In other hand, they are regarded to have high resilience, as pre-disturbance abundance levels are attained shortly after the disturbance event. The quick return to original levels is the result of a number of recovery processes, including drift from upstream areas, dispersion from nearby unaffected patches (i.e. refuges), and ovipositing aerial adults (Mackay 1992). The importance of the two first mechanisms are likely to be most important in cases of low to medium disturbance events, when the streambed is affected at the level of stones or microhabitats (Lancaster and Hildrew 1993; Matthaei et al. 2000). In cases of catastrophic flood events, it is likely that both upstream areas and local refuges are greatly affected, and in theses cases aerial oviposition should be the most important recovery process (Scrimgeour et al. 1988; Mackay 1992). During our five years study, we did not observe any evidence of catastrophic flood and it is thus reasonable to assume that drift from upstream areas and dispersion from refuges were important. The period when high flow events occur in the studied area, the warm and rainy summer, is also the period of emergence of most of the stream insect species (Ferreira and Froehlich 1992; Marinoni and Almeida 2000). The three recovery mechanisms cited summed to the relative large period of stable flow (from the end of the rainy season around March until the dry sampling period in July-August) very likely provided enough conditions to the disturbed communities in the rainy season to return to an equilibrium state during the dry season. It would be interesting to see if the recurrent equilibrium state during the dry season would occur after a rainy season with a catastrophic flood and a consequent strong reduction in total abundance over all parts of the streambed.

Equilibrium and non-equilibrium models of population and community dynamics have been a greatly debated topic in ecology, particularly during the 1970s and 1980s (DeAngelis and Waterhouse 1987). Theoretical models suggest that a transient non-equilibrium state community state should occur at small spatial scales due in part to destabilizing biotic interactions, but tends to a more equilibrium state as one moves to larger spatial scales. The fundamental idea is that extinction in patches or "cells" are counterbalanced by immigration from other patches. Community composition would vary greatly at the patch level, but would be persistent if one considers the summation of all patches together. In this sense, reports of equilibrium states are trivial, as they are likely to be found in large spatial scales. However, as for most other systems, it is hard to define for stream systems the real size of patches or cells and the respective landscape or grid in which they are nested. Some species of insects live in single rocks during nearly their entire aquatic stage, as for example species of caddisfly that build houses attached to stones (e.g. *Leucotrichia* and *Zumatrichia*). On the other hand, small-bodied mayflies (e.g. Baetidae) and blackflies (Simuliidae) are efficient drifters, entering the water column frequently either to search for good feeding places or to escape predation. Further, most stream invertebrates are insects with aerial adults, and no data exist for most species regarding how much females disperse before laying eggs. These characteristics of stream invertebrate communities make difficulty the placement of our study in the continuum from small (patch) to large (landscape) scales.

From a more practical point of view, however, the spatial scale of our study is similar to those employed in most stream ecology studies, allowing thus comparative works, and to those used in biological monitoring of water quality. For the last type of study, our results support the view that stream communities are not too variable to monitor, allowing thus reliable assessment of stream integrity (Karr and Chu 1999, p. 154).

The hypothesis that community persistence is related to environmental stability was not supported. This is in disagreement with previous studies of stream fishes, where community persistence was negatively related to environmental variability (Matthews et al. 1988; Oberdorff et al. 2001). Two characteristics of our study may concur to explain this lack of relationship. Our work was restricted to five stream sites and they were of different sizes. It is possible that some confounding factor related to stream size may have overridden the effects of streambed stability on community persistence.

Our results support the hypothesis that community persistence decrease with time, although the magnitude of persistence is contingent on the season considered. This result conforms well to the alternate equilibrium and non-equilibrium communities states discussed above. The picture forming from the results of alternate equilibrium states and the higher persistence of communities in winter than in summer is that disturbance during summer dislocate community states into an unpredictable direction. As the stream environment becomes more stable, community state converges to a recurrent, equilibrium state during winter. It would be interesting to see if such model of community dynamics holds for other systems in which disturbance occur seasonally.

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Conclusões gerais de cada capítulo

A seguir apresento as principais conclusões obtidas em cada capítulo da tese. Após estas conclusões individuais, segue uma conclusão geral sobre estimadores de riqueza e perturbações em riachos. No final apresento um anexo contendo a relação de morfoespécies encontradas no Parque Estadual Intervales durante os trabalhos componentes desta tese.

Capítulo 1 – Evaluation of methods for estimating macroinvertebrate species richness using individual stones in tropical streams

1. Todos estimadores avaliados foram dependentes do tamanho amostral, produzindo estimativas maiores em tamanhos amostrais maiores.

2. O método SV estimou a riqueza na amostra total usando o menor tamanho subamostral. No entanto, o método mostrou comportamento errático com tamanhos subamostrais pequenos e as curvas estimadas para os seis conjuntos de dados foram muito diferentes entre si.

3. O método Bootstrap foi o melhor em termos de constância do tamanho subamostral necessário para estimar a riqueza na amostra total. No entanto, o tamanho da subamostra necessária para estimar a riqueza total foi excessivamente grande.

4. O método Jackknife 2 foi o segundo melhor tanto em termos do tamanho necessário para estimar a riqueza total como na constância deste tamanho nos seis conjuntos de dados usados na avaliação.

5. Visto a dependência de todos estimadores em relação ao tamanho amostral, são discutidos alguns aspectos da validade no uso de tais métodos na estimativa de riqueza em comunidades biológicas.

Capítulo 2 –On the use of jackknife and related non-parametric techniques to estimate species richness in an area

1. Eu mostro que estimativas de riqueza de espécies na comunidade produzidas por métodos não-paramétricos são tão dependentes do tamanho amostral, que tal dependência pode até ser expressa como uma relação linear entre os tamanhos amostrais necessários para coletar e para estimar um determinado valor de riqueza.

2. Métodos não-paramétricos de estimativa de riqueza pressupõem que a proporção de espécies raras (i.e. com 1 ou 2 indivíduos ou com ocorrência em 1 ou 2 unidades amostrais) deve diminuir com tamanhos amostrais sucessivamente maiores. Usando diversos conjuntos de dados provenientes de comunidades biológicas distintas, eu mostro que a proporção de espécies raras não diminui. Ao contrário, as curvas de espécies raras aumentam até um certo patamar e depois estabilizam.

3. Eu concluo que métodos não-paramétricos não são confiáveis para estimar a riqueza de espécies em comunidades biológicas.

Capítulo 3 – Comparing species richness among assemblages using sampling units: why not use extrapolation methods to standardize different sample sizes?

1. O estimador Log-série foi o melhor avaliado, seguido pelos métodos Binomial Negativo, SO-J1, Logarítmico, SV e Weibull.

2. As estimativas do método Log-série foram acuradas e precisas mesmo usando conjuntos diversos de dados e diferentes magnitudes de extrapolação.

3. A variabilidade das estimativas produzidas pelo estimador Log-série foi similar àquela obtida a partir de valores de riqueza observada em amostras independentes de conjuntos reais de dados.

4. Os métodos avaliados em geral produziram melhores estimativas em conjuntos ricos em espécies.

5. Boas estimativas foram obtidas com extrapolações de até 1.8-2.0 vezes o tamanho da amostra disponível.

6. Os bons resultados obtidos com os estimadores acima citados permitem a recomendação de seu uso em situações reais.

Capítulo 4 –Resistance, resilience and patchiness of invertebrate assemblages in native tussock and pasture streams after a hydrological disturbance

1. A abundância de invertebrados diminuiu após a perturbação experimental, mas a densidade de espécies e a riqueza de espécies (rarefeita) ficaram estáveis.

2. Após oito dias da perturbação, a abundância nos riachos foi semelhante àquela observada na amostra controle.

3. Não houve diferenças em resistência e resiliência entre riachos em tussock e aqueles em pastagens.

4. Após a perturbação, a agregação da comunidade aumentou significativamente, mas voltou a valores semelhantes ao controle em oito dias.

Capítulo 5 – Colonization by macroinvertebrates of experimentally disturbed stones in three streams differing in size

1. Os padrões de colonização foram semelhantes entre si, e portanto a hipótese original de que a colonização seria mais rápida no riacho de tamanho intermediário foi rejeitada.

2. A colonização das pedras foi rápida, atingindo valores similares aos controles em 8-16 dias.

3. A similaridade entre a fauna das amostras nos diferentes dias de colonização e nos controles tendeu a aumentar ao longo do processo de colonização, como o esperado. No entanto, os valores finais (dia 64) foram menores do que aqueles obtidos entre as duas amostras controle. Apesar da recuperação das densidades e riquezas nas pedras individualmente, a abundância relativa e a composição de espécies como um todo (i.e. na somatória das 10 unidades amostrais) não atingiram níveis similares aos dos controles.

Capítulo 6 – Disturbance in streams by flood-caused moving and burying of stones: variation among stream sites along a catchment

1. Como o esperado, maiores frequências de rolagem e enterramento foram obtidas na época chuvosa. No entanto, houve diferença na época de maior rolagem entre os riachos.

2. Não houve relação entre frequência de rolagem ou enterramento com o tamanho do riacho.

3. Rolagens foram muito mais frequentes do que enterramentos em quatro dos cinco riachos estudados. No menor riacho, a proporção de pedras enterradas foi considerável e atingiu níveis similares aos de rolagem durante a época chuvosa.

4. Os eventos de enterramento no menor riacho foram em grande parte devido a ruptura parcial ou total de pequenas cachoeiras formadas por troncos caídos e acúmulo de folhas e gravetos.

Capítulo 7 – Assemblage structure and persistence of stream macroinvertebrates in tropical streams

1. As comunidades de macroinvertebrados em cada riacho foram relativamente distintas entre si, sendo posicionadas em grupos compactos na análise de classificação.

2. Para os cinco riachos, houve maior concordância das comunidades ao longo dos anos durante o inverno.

3. Não houve relação entre medidas de estabilidade dos riachos e concordância da comunidade ao longo dos cinco anos.

4. A variabilidade das comunidades no inverno e verão aumentaram com o tempo, embora o aumento no verão tenha sido muito maior do que no inverno. Amostras coletadas em anos próximos foram mais semelhantes entre si do que aquelas coletadas distantes no tempo.

5. Os resultados dão suporte a idéia de que as comunidades de invertebrados em riachos variam ao longo do ano de um estado de não-equilíbrio, durante a época chuvosa, para um estado de equilíbrio durante a época seca.

Conclusões gerais da tese

Estimadores de riqueza na comunidade deveriam produzir valores próximos aos valores encontrados na área utilizando um esforço amostral sensivelmente menor do que aquele necessário para observar todas as espécies durante um inventário. No entanto, os resultados obtidos no Capítulo 1 mostram que nenhum dos estimadores avaliados atingiu este objetivo. Apesar da indicação de uso do estimador Jackknife 2 sobre os demais, tal indicação é feita baseando-se em quatro métricas específicas. Tais métricas foram usadas em parte para avaliar a possibilidade de uso de tais estimadores em situações comparativas, onde mesmo uma estimativa viciada da riqueza pode potencialmente ser melhor do que os valores de riqueza observada.

A dependência das estimativas com a riqueza observada é em grande parte devido ao não decréscimo da frequência de espécies raras conforme aumenta-se o tamanho amostral. Este fato aconteceu não só com dados provenientes de invertebrados em riachos, mas também com conjuntos de aranhas, árvores, *Drosophila* spp., opiliões e anuros (Cap. 2).

Apesar dos problemas para se estimar a riqueza em comunidades biológicas, pode-se estimar a riqueza em tamanhos amostrais maiores, pré-definidos, com grande segurança (Cap. 3) . Entre os diversos estimadores avaliados para esta segunda questão, seis métodos tiveram desempenho bom, em especial o método Série Logarítmica. Tais métodos foram em geral robustos frente a dados provenientes de diversas comunidades biológicas. Uma única exceção ocorreu em comunidades muito pobres em espécies, onde a inclusão ou não de uma espécie rara pode produzir estimativas bastante distintas.

Na literatura, perturbações por enchentes em riachos são apontadas, entre outras coisas, como causas de rolagem e enterramento de substrato e redução da densidade de macroinvertebrados. De fato, pude constatar que o leito dos riachos estudados é muito dinâmico. Durante o período de 1 ano em torno 90% das pedras entre 10 e 18 cm de diâmetro presentes em cinco riachos foram deslocadas (Cap. 6). No entanto, tais movimentos parecem não afetar a comunidade em termos de persistência a longo prazo (Cap. 7). Usando experimentos, pude observar que a colonização de substrato perturbados é muito rápida, atingindo níveis de densidade semelhantes à regiões perturbadas em apenas oito dias (Caps 4 e 5). Após uma enchente, é provável que, pelo menos para perturbações de baixa intensidade, além da redução de densidade de organismos exista também uma redistribuição de indivíduos no leito de riachos. De fato, apesar de em média haver uma redução no leito de riachos, alguns pontos acabam recebendo indivíduos de outras regiões, atuando portanto como refúgios. Embora não tenha estudado especificamente rotas de colonização, encontrei evidências de que a dispersão sobre o substrato (movimentos ambulatórios) pode ser tão importante quanto a colonização por indivíduos provenientes da coluna d'água (deriva) (Cap. 5).

Numa escala temporal maior, pude observar que a estrutura da comunidade (composição e abundância relativa) de invertebrados em riachos varia de um estado de menor equilíbrio na época chuvosa (verão) para um estado de maior equilíbrio na época seca, quando o fluxo de água é menos variável (Cap. 7). Por equilíbrio entenda-se aqui a repetição da mesma configuração da comunidade no tempo. Durante o verão, a comunidade possui um estado menos previsível do que o inverno. Um modelo simples seria a ocorrência de (1) um estado de equilíbrio no inverno, (2) subsequente mudança para um estado menos previsível no verão seguinte, e (3) retorno no inverno seguinte para uma configuração semelhante à do inverno anterior.

Apêndice 1. Grupos de macroinvertebrados encontrados no Parque Estadual Intervales. Números em parênteses indicam o número de morfoespécies encontradas no grupo. No total foram encontradas 217 morfoespécies.

Ordem ou outra categoria taxonômica	Família ou outra categoria taxonômica	Gênero	Observações
Ephemeroptera (24)			
	Baetidae (7)		
		Baetodes sp. (1)	
		Camelobaetidius spp. (2)	
	Eutyphlociidae (1)		
		Campylocia sp. (1)	
	Leptohyphidae (7)		
	Leptophlebiidae (8)		
		Perissophlebiodes sp. (1)	
		Askola sp. (1)	
		Farrodes sp. (1)	Provavelmente mais de uma espécie.
		Massartella sp. (1)	
		Thraulodes spp. (2)	
		Hagenulopsis sp. (1)	
	Caenidae (1)		
Odonata - Anisoptera (3)			
	Gomphidae (1)		
Odonata - Zygoptera (7)			
Plecoptera (22)			
	Gripopterygidae (9)		
		Paragripopterix spp. (3)	
		Gripopterix spp. (4)	
		Tupiperla sp. (1)	
		Guaranyperla sp. (1)	
	Perlidae (13)		
		Anacroneuria spp. (5)	Talvez uma morfoespécie não seja válida.
		Kempnyia spp. (6)	Talvez uma morfoespécie não seja válida.
		Macrogynoplax spp. (2)	Talvez uma morfoespécie não seja válida.

Ordem ou outra categoria taxonômica	Família ou outra categoria taxonômica	Gênero	Observações
Hemiptera (2)			
	Naucoridae (2)		
		Cryphocricos sp. (1)	
		Limnocoris sp. (1)	
Megaloptera (1)			
	Corydalidae (1)		Representa várias espécies de *Corydalus* e uma de *Chloronia*.
Trichoptera (63)			
	Anomalopsychidae (1)		
		Contulma sp. (1)	
	Calamoceratidae (3)		
		Phylloicus spp. (3)	
	Ecnomidae (2)		
		Austrotinodes spp. (2)	Talvez uma morfoespécie não seja válida.
	Glossosomatidae (2)		
	Helicopsychidae (7)		
	Hydrobiosidae (2)		
		Atopsyche spp. (2)	
	Hydropsychidae (8)		
		Smicridea spp. (5)	
		Leptonema sp. (1)	
	Hydroptilidae (16)		
		Alisotrichia sp. (1)	
		Leucotrichia sp. (1)	
		Zumatrichia sp. (1)	
	Leptoceridae (10)		
		Grumichella spp. (2)	
		Nectopsyche sp. (1)	
		Triplectides sp. (1)	
	Odontoceridae (4)		
		Marilia spp. (3)	
		Barypenthus sp. (1)	
	Philopotamidae (3)		
		Chimarra spp. (2)	Talvez uma morfoespécie não seja válida.
	Polycentropodidae (3)		
	Sericostomatidae (1)		
		Grumicha sp. (1)	
	Xiphocentronidae (1)		

Ordem ou outra categoria taxonômica	Família ou outra categoria taxonômica	Gênero	Observações
Lepidoptera (2)			
	Pyralidae (2)		
		Petrophila sp. (1)	
Coleoptera (36)			
	Dryopidae (1)		
	Elmidae (18)		
	Gyrinidae (2)		
	Hydraenidae (3)		
	Hydrophilidae (5)		
	Psephenidae (5)		
	Scirtidae (2)		
Diptera (50)			
	Blephariceridae (1)		
	Ceratopogonidae (17)		Talvez algumas morfoespécies não sejam válidas.
	Dixidae (2)		
	Empididae (2)		
	Muscoidea (11)		
	Psychodidae (5)		Quatro morfoespécies foram raram e talvez sejam terrestres ou semi-aquáticas.
	Simuliidae (2)		Uma morfoespécie talvez corresponda a uma espécie. A outra certamente engloba várias espécies.
	Tipulidae (10)		
Platyhelminthes (1)			
	Turbellaria (1)		
Annelida (2)			
	Oligochaeta (1)		Certamente engloba mais de uma espécie.
	Hirudinea (1)		

Ordem ou outra categoria taxonômica	Família ou outra categoria taxonômica	Gênero	Observações
Mollusca (2)			
	Gastropoda (2)		Uma espécie é comum no trecho Alecrim (riacho 3 do Capítulo 5), a outra talvez seja terrestre.
Crustacea (2)			
	Aeglidae (1)		
		Aegla sp. (1)	Provavelmente inclui mais de uma espécie.
	Hyalellidae (1)		