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Tese de Doutorado

Efeitos de processos regionais e locais sobre comunidades, populações e interações em peixes de riachos

[Effects of regional and local processes on communities, populations and interactions in stream fishes]

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Porto Alegre, maio de 2016

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Tese de Doutorado apresentada ao Programa de Pós-Graduação em Ecologia, do Instituto de Biociências da Universidade Federal do Rio Grande do Sul, como parte dos requisitos para obtenção do título de Doutor em Ecologia.

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Resumo

Os ecossistemas aquáticos são afetados por processos que ocorrem em escalas finas (locais) e amplas (regionais). Os processos locais incluem, por exemplo, filtros ambientais e interações interespecíficas, enquanto que os regionais abrangem principalmente questões relacionadas à dispersão de indivíduos. O entendimento de como esses processos atuam sobre comunidades, populações e interações em peixes de riachos é fundamental para a conservação dos ecossistemas aquáticos, pois permite predizer as consequências de alterações antrópicas e fornece subsídios para ações de manejo e políticas de conservação. Na presente tese, eu desenvolvi cinco estudos em distintas escalas espaciais. Cada um é apresentado em capítulos distintos. Nos capítulos 1 e 2, eu abordei questões relacionadas à compreensão de como alterações antrópicas feitas em distintas escalas espaciais influenciam as diversidades alfa e beta de comunidades de peixes de riachos. No capítulo 3, eu procurei entender como processos previstos na teoria de metacomunidades influenciam mudanças temporais na composição e abundâncias de espécies em comunidades locais. No capítulo 4, eu estudei como os impactos antrópicos levam a alterações no papel trófico e no intestino de populações de uma espécie generalista e persistente. Por fim, no capítulo 5, eu usei uma abordagem de aninhamento, desenvolvida inicialmente na Ecologia de Comunidades, para avaliar a ocupação de larvas de uma espécie de quironomídeo (Diptera) sobre o corpo de seu hospedeiro (uma espécie de peixe da família Loricaridae).

Palavras-chave: agricultura, vegetação ripária, impactos antrópicos, habitat, qualidade d’água, filtros ambientais, sistemas dendríticos, dispersão, metacomunidades, interação interespecífica, aninhamento.

Abstract

Aquatic ecosystems are influenced by processes that occur at fine (local) and broad (regional) scales. Local processes include, for example, environmental filters and interspecific interactions, whereas regional processes encompass mainly questions regarding individual dispersion. Knowledge on how these processes affect communities, populations and interactions in stream fish is essential for conservation of aquatic ecosystems, as it allows predicting consequences of human-alterations and provides subsidy for management actions and conservation policies. In this dissertation, I developed five studies using distinct spatial scales. I presented each one in a separate chapter. In the 1st and 2nd chapters, I addressed questions concerned with the understanding of how human alterations at different spatial scales influence alpha and beta diversity of stream fish communities. In the 3rd chapter I looked for understanding how processes predicted in metacommunity theory influence mid- to long-term changes in composition and species abundances of local communities. In the 4th chapter, I studied how anthropic impact drives modification in the trophic role and intestine of a generalist and persistent fish species. Lastly, in the 5th chapter, I employed nestedness approach previously developed for Community Ecology to evaluate occupation of chironomid species larvae (Diptera) on the body of its host (an armored catfish species of the family Loricariidae).

Keywords: agriculture, riparian vegetation, anthropic impacts, habitat, water quality, environmental filters, dendritic systems, dispersion, metacommunity, interspecific interaction, nestedness.

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Introdução geral

Assim como em ambientes terrestres, os ecossistemas aquáticos são governados por processos que atuam localmente, em escala mais fina e, regionalmente, por processos que ocorrem em escalas amplas e afetam as comunidades, populações e interações (Ricklefs, 1987). Um olhar mais distante, procurando avaliar processos em escalas regionais, pode revelar muito mais de um ecossistema e fornecer uma fotografia mais completa para entender por que os sistemas naturais apresentam o estado e as condições em que são encontrados atualmente. Localmente, as comunidades, populações e interações são afetadas, por exemplo, pela temperatura da água, que modula o metabolismo e, indiretamente, a densidade de indivíduos e de espécies. Este fator pode determinar a força da competição entre espécies distintas e entre indivíduos da mesma espécie ou, ainda, pode afetar a forma de interação entre duas espécies onde uma depende intimamente da outra para completar seu ciclo de vida. Em escala regional, a imigração de indivíduos oriundos de outras manchas de habitat pode ter grande influência sobre a biota local. Entretanto, tal influência dependerá de várias questões, incluindo o tamanho e qualidade das manchas de habitat, as suas distâncias, e a facilidade com que os indivíduos conseguem se dispersar, seja este determinado pela estrutura do sistema em si, ou pela habilidade individual.

Drenagens hídricas são sistemas dendríticos que apresentam uma conformação e organização espacial peculiar se comparado aos ambientes terrestres (Benda et al., 2004; Altermatt, 2013). Nesses ambientes, a fauna aquática, principalmente os peixes, é condicionada a se movimentar por dentro dos braços e das ramificações das redes de drenagens. Apesar dessa restrição a movimentação, os ambientes aquáticos são extremamente favoráveis a dispersão de organismos, devido a características próprias da água e do sistema (e.g. transporte pelo fluxo corrente) (Altermatt, 2013). Isso é particularmente relevante para peixes, uma vez que suas formas e estruturas corporais permitem movimentações ativas para regiões à montante e à jusante, ou ainda lateralmente e verticalmente na coluna da água. Essas duas últimas formas de movimentação são mais relacionadas a processos que ocorrem localmente, uma vez que as posições horizontal (margem ou centro dos riachos) e vertical (superfície, meia água, ou bentônica), são mais determinantes de preferências por microhabitats específicos (Casatti & Castro, 2006). Já as dispersões no sentido longitudinal, como montante-jusante, estão mais relacionadas a processos regionais, incluindo a troca de indivíduos e espécies com distintas machas de habitat espalhados na rede de drenagem.

Os processos locais e regionais que afetam comunidades podem muitas vezes ser análogos àqueles que afetam populações. Por exemplo, limitações à dispersão podem ser observadas em níveis de comunidade e de população. Limitação a dispersão pode acarretar na ausência de uma ou mais populações mesmo em locais que apresentam as condições e recursos necessários para a sua ocorrência e, consequentemente, pode reduzir a riqueza de espécies na comunidade. Embora características como rochas grandes e velocidade da correnteza moderada possam proporcionar maior *fitness* para uma espécie hipotética de peixe bentônico, ainda assim será possível encontrar indivíduos desta espécie em ambientes próximos menos preferenciais. Assim, espécies com maiores abundâncias e grandes capacidades de dispersão podem ocorrer em locais distantes e menos favoráveis e alterar a composição de comunidades em distintos segmentos das redes de drenagem.

Da mesma forma que uma certa espécie de peixe pode depender de um microhabitat específico, em interações interespecíficas onde uma depende intimamente da outra para sobreviver, alguns locais específicos no corpo do hospedeiro podem reunir as melhores condições e recursos para aumentar o *fitness* individual do hóspede (seja este comensal, parasita ou mutualista). Nesses casos, processos análogos – filtros ambientais e dispersão –

podem levar a ocupação de hóspedes em partes do corpo de seus hospedeiros a apresentar um padrão semelhante ao encontrado na distribuição espacial dos organismos em seu habitat. Esses processos podem levar, por exemplo, a um padrão aninhado de oupação se os locais ou regiões pouco favoráveis só são ocupadas quando as preferências já estão preenchidas.

Muita ênfase foi dada no passado para desvendar se processos os regionais ou os locais são mais determinantes das características encontradas em diferentes ecossistemas. Atualmente, se reconhece que ambos têm papel fundamental para determinar estrutura e dinâmica de comunidades, populações e interações entre espécies (Thompson & Townsend, 2006). Revelar quais processos causam os padrões observados na natureza, e se os mesmos processos levam aos mesmos ou a distintos padrões, são provavelmente os grandes desafios dos ecólogos atualmente. Isso é principalmente relevante se considerarmos que esse conhecimento pode ajudar a entender e predizer as consequências das alterações antrópicas e servir como base para a preservação da biodiversidade global e para a manutenção ou restauração do funcionamento dos ecossistemas.

A abordagem de múltiplas escalas tem sido empregada com o objetivo de encontrar padrões em comunidades de peixes de riachos e associá-los a processos locais e regionais que determinam a montagem de comunidades (Morley & Karr, 2002; Allan, 2004). Em escala local, vários estudos têm abordado relações de características do habitat e da qualidade d'água com a biota local, e também têm avaliado como estes aspectos bióticos e abióticos de dentro dos riachos são afetados pelo estado de conservação da vegetação ripária imediatamente adjacente aos sítios amostrais (Casatti *et al.*, 2009). Em escala um pouco maior, ao longo de segmentos extensos de riachos na rede de drenagem (e.g. 1 km a montante), há evidência de que tanto as características da vegetação ripária quanto a estrutura morfológica do canal (condicionada pela geomorfologia) influenciam a ocorrência de espécies e as dinâmicas populacionais (Jones *et al*., 1999). Na escala de sub-bacia hidrográfica, considerando a área de drenagem a montante, o uso do solo por atividades antrópicas (Wang *et al*., 1997), assim como características como tamanho da área de drenagem (Hughes *et al*., 2011), e formato e relevo das bacias (Montgomery, 1999) tem se demonstrado influentes sobre comunidades avaliadas em nível local. Além disso, características a jusante dos trechos amostrais também são relevantes. Por exemplo, estudos têm encontrado que o tamanho da conexão na confluência imediatamente a jusante, ou seja, o tamanho do curso d'água a jusante no qual um tributário avaliado deságua, exerce grande influência sobre a fauna local, invocando processos relacionados à dispersão de organismos (Thornbrugh & Gido, 2010).

Na presente tese, eu desenvolvi cinco estudos observacionais buscando fazer associações entre padrões encontrados e processos locais e regionais que podem estar causando estes padrões. Eu espero que estes estudos contribuam, pelo menos em parte, para a compreensão desses processos.

Objetivo (s)

Nesta tese eu desenvolvi cinco estudos, cada um apresentado como um capítulo separado, que buscam entender como processos locais e regionais afetam peixes de riachos. Nos dois primeiros capítulos (capítulos 1 e 2) eu abordo questões relacionadas à compreensão de como alterações antrópicas agrícolas feitas em distintas escalas espaciais influenciam as diversidades alfa e beta de comunidades de peixes de riachos. No capítulo 3, eu avalio como os impactos antrópicos alteram o papel trófico de populações de uma espécie de peixe generalista e persistente. No capítulo 4, eu procuro entender como processos previstos na teoria de metacomunidades influenciam mudanças temporais na composição e abundâncias em comunidades locais em médio-longo prazo. No capítulo 5, eu uso uma abordagem de aninhamento, comum na área de Ecologia de Comunidades, para avaliar a ocupação de locais no corpo de uma espécie de peixe (Loricariidae) por larvas de uma espécie de quironomídeo (Diptera).

CAPÍTULO 1 - Revealing the pathways by which agricultural land-use affects stream fish communities in South Brazilian grasslands*

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SUMMARY

1. Understanding mechanisms by which agricultural practices affect freshwater ecosystems help inform land-use policies and management strategies aimed at mitigating effects of agriculture on biodiversity.

2. Land-use activities in the catchment, riparian and local scales likely influence stream fish communities via multiple pathways, for instance, by modifying the instream habitat.

3. We investigated the mechanisms driving local stream fish taxonomic richness and functional diversity in South Brazilian grasslands by testing a theoretical path model in which we specified *a priori* relationships based on decades of scientific literature, predicting how land-use at multiple scales affects instream habitat and fish communities.

4. We found agricultural activities adjacent to streams (i.e. local impact on the streambank) and catchment scale cropland area positively related to macrophyte cover and negatively to coarse particulate organic matter (CPOM, i.e. woody debris and leaf litter). Local impact also increased substrate siltation and homogenization. Riparian vegetation in the upstream buffer ameliorated instream habitat condition by dampening macrophyte proliferation and providing CPOM.

5. Fish species richness increased with macrophyte cover and CPOM, whereas functional diversity decreased with substrate siltation. Thus, agricultural activities at the streambanks decreased functional diversity via substrate siltation, whereas species richness responded to multiple pathways linked to multiple scales. Agricultural streams showed a replacement of benthic and lithophilic species by a larger number of morphologically similar and macrophyte-associated nektonic fishes.

6. Our study indicates land-use adjacent to streams and upstream riparian zones are critical for maintaining taxonomically and functional diverse fish communities due to their strong effects

on instream habitat. Protection and recovery of riparian zones from agricultural conversion can mitigate the effects of agriculture on fish communities in South Brazilian grasslands.

Introduction

Rapid expansion of agriculture over the past century has left an indelible mark on the world as croplands and livestock pastures now cover an area larger than many of Earth's natural biomes (Foley *et al*., 2005). Ongoing agricultural expansion is expected to have significant consequences for global biodiversity because it is concentrated in the species-rich tropics (Gibbs *et al*., 2010; Ray *et al*., 2012). However, our understanding of how agriculture affects tropical biodiversity is centred primarily on forest ecosystems and the terrestrial taxa therein (e.g. Naughton-Treves *et al*., 2003; Wilcove *et al*., 2013), whereas the potential effects on freshwater ecosystems remain much less understood (Castello *et al*., 2013).

The mechanisms by which agricultural conversion affect freshwater communities are complex; they are often scale-dependent and involve numerous interacting processes and pathways (Fausch *et al*., 2002; Allan, 2004). Agriculture at the catchment scale increases sediment and nutrient load to streams (Burdon, McIntosh & Harding, 2013), causing reduced habitat quality for benthic and substrate-spawning species (Rabeni & Smale, 1995). The interaction between fine sediment and nutrient inputs can alter primary productivity (e.g. increasing macrophyte cover), which in turn influence the trophic structure of communities (Bunn, Davies & Mosisch, 1999). At a finer scale, the loss of forest cover in the riparian zone allows increased input of solar radiation and consequent increase of primary production (Burrell *et al*., 2014), reduction of woody debris and leaf litter input (Hyatt & Naiman, 2001), and reduction of terrestrial food subsidies for fishes (Evangelista *et al*., 2014).

At both catchment and riparian scales, the replacement of native vegetation with agriculture affects taxonomic and functional composition of fish communities (Walser & Bart, 1999; Wang *et al*., 2003; Casatti *et al*., 2015; Giam *et al*., 2015). Increased siltation and concomitant changes in habitat quality caused by agriculture, measured at the catchment scale, has been shown to reduce fish species richness (Roth, Allan & Erickson, 1996; Walser & Bart, 1999; Sutherland, Meyer & Gardiner, 2002) as well as fish biotic integrity (Wang *et al*., 1997; Rowe, Pierce & Wilton, 2009). At the riparian scale, vegetation removal decreases species richness and functional diversity as well as alters the composition of fish communities through reductions in woody debris and leaf litter inputs (Stauffer, Goldstein & Newman, 2000; Giam *et al*., 2015). Removal of woody riparian vegetation is also related to instream habitat homogenization due to high input of terrestrial sediment that in turn causes modifications in fish species composition (Casatti, Ferreira & Carvalho, 2009) and in the abundance of trophic guilds (Zeni & Casatti, 2014). Conversely, there is evidence that riparian deforestation may promote higher fish species richness by elevating primary production (Lorion & Kennedy, 2009; Teixeira-de Mello *et al*., 2015) owing to increased nutrient loading and greater light input (Burrell *et al*., 2014). Moreover, agricultural land-use at catchment may also be positively linked to stream fish species richness (e.g. Harding *et al*., 1998). Species gains, however, comprise mostly tolerant, water column species and macrophyte specialists (Harding *et al*., 1998; Wang, Robertson & Garrison, 2007; Casatti *et al*., 2012). These differential responses of fishes to human disturbance highlight the importance of considering simultaneously the multiple pathways by which human land-use affect stream biodiversity both in terms of taxonomy and functional trait composition (Olden *et al*., 2010).

A common strategy to mitigate agricultural effects on stream biota is the retention of natural vegetation in the riparian zones (i.e. riparian buffers or reserves; Allan, 2004).

Riparian buffers can reduce nutrient and sediment inputs by filtering upland groundwater and surface runoff (Lowrance *et al*., 1997) and limit stream eutrophication via shading (Burrell *et al*., 2014). Whereas riparian buffers are generally effective in maintaining instream habitat quality, they were not always effective in protecting fish communities (e.g. see Giam *et al*., 2015 vs. Wang, Lyons & Kanehl, 2002; 2006). This suggests that fishes may respond to factors other than local habitat structure when catchments are converted to agricultural activities.

The South Brazilian grasslands (*Campos*) are an example of a understudied region that is under severe pressure from agricultural conversion to croplands of maize, soy, and rice and forest plantations, despite supporting a large number of endemic plant and animal taxa (MMA - Ministério do Meio Ambiente, 2007; Pillar *et al*., 2009). There is limited information on how agriculture may threaten terrestrial taxa [reduced avian species richness (Silva, Dotta & Fontana, 2015) and snake abundance (Winck, Santos & Cechin, 2007)] and even less is known regarding freshwater taxa such as fishes. This is in spite of the fact that the *Campos* encompasses freshwater ecoregions with a large number of native and range-restricted fish species (Abell *et al*., 2008; Albert, Petry & Reis, 2011; FEOW, 2015), and that understanding the mechanisms by which agricultural conversion affect these communities will help inform their conservation and management.

Whereas previous research has found contrasting results and indicated that fish communities are influenced by agricultural activities developed at multiple spatial scales, such as catchment, riparian and local (e.g. Roth *et al*., 1996; Lammert & Allan, 1999; Sály *et al*., 2011; Feld, 2013), there is less understanding on how the mechanisms involved vary when multiple spatial scales are evaluated simultaneously. Partially, this occurs because of the correlative nature of multiple scales of agricultural development (King *et al*., 2005). For instance, one may expect pristine catchments to present preserved riparian zones, whereas it is much likely that agriculture-dominated catchments also have altered riparian zones. The spatially correlated nature of agricultural development points to the importance of building models that incorporate multiple spatial scales simultaneously. This is particularly relevant if the goal is to better understand the drivers of agricultural effects on fish communities, as agricultural land-use can be a proxy for multiple environmental factors (e.g. water quality, pesticides, discharge dynamics and habitat modifications). In our study, we focused on three key instream habitat characteristics to understand how agriculture affects stream fish diversity (substrate, woody debris/leaf litter and macrophyte).

We constructed a global model to test the hypothesis that part of the influence of agricultural land-use on fish communities arises from its effect on instream habitat characteristics. We explicitly tested whether agricultural activities at different scales influence fish communities through the same or different pathways of habitat modifications, as well as how these pathways behave when multiple scales are evaluated simultaneously. We aimed to answer the following two specific questions: (1) how does agriculture at catchment, riparian and local spatial scales affect taxonomic and functional diversity of stream fish communities? (2) Can riparian vegetation mitigate effects of agriculture on stream habitat and on fish diversity in agriculture-dominated catchments? Results from this study broadly contribute to essential knowledge regarding how agriculture affects freshwater biodiversity, specifically helping to inform stream conservation in Neotropical grasslands and similar non-forested ecosystems globally.

Methods

Study area

The South Brazilian *Campos* comprises the Pampa grasslands and the high-altitude grasslands of the Atlantic forest biome (Fig. 1). Mosaics of shrubland and forest patches are spread across natural grassland, especially along riparian zones. Horses, cattle and sheep were introduced to the *Campos* in the seventeenth century and since then have become widely used for beef production (Overbeck *et al*., 2007). In the last three decades, a large part of the native vegetation of *Campos* has been converted, mainly due to a rapid expansion of soybean, rice, maize and wheat croplands (Overbeck *et al*., 2007; Overbeck *et al*., 2015; IBGE, 2015).

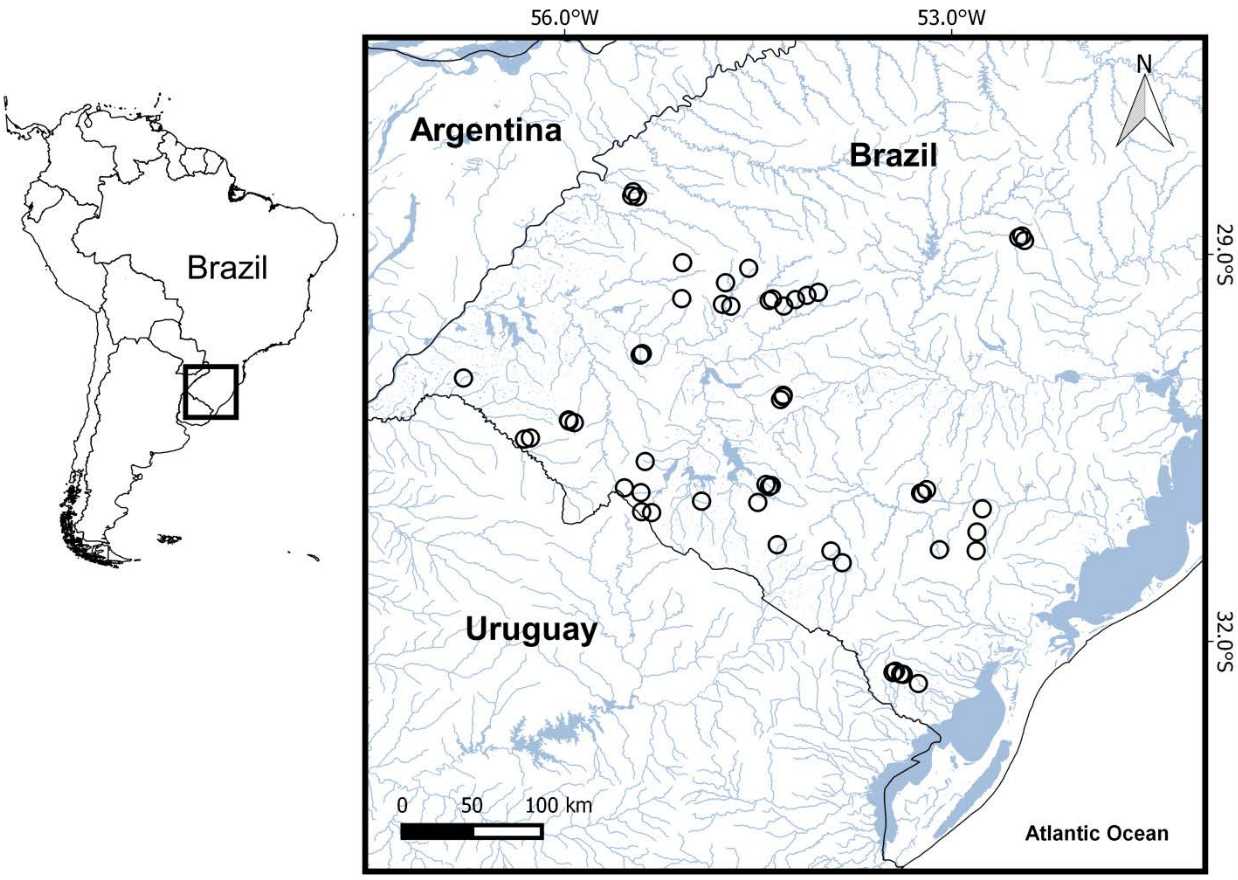

Fig. 1 Location of the 54 wadeable streams sampled across South Brazilian grasslands (*Campos*).

Stream sites and fish sampling

We surveyed 54 wadeable stream sites across South Brazilian grasslands (Fig. 1; Table 1). Each site was sampled once at an independent catchment (independent upstream area). Sampled streams comprised second (16), third (29) and fourth (nine) Strahler order. The sampled streams belong to two large river basins: the Uruguay river (36 streams) and the Patos lagoon (18 streams) basins. Whereas the species pool may present some differences between these two river basins, the occurrence patterns of fish groups are very similar (such as the predominance of Characidae and Loricariidae fish species). Average pairwise linear overland distance between all the sampling sites was 196 km (SD 101 km), ranging from 1.1 to 472 km.

We sampled fishes from 150-m stream reaches using single-pass electrofishing (EFKO GmbH model FEG 1500). Single-pass electrofishing has been shown to be adequate to detect trends in fish abundance and species richness given standardized effort (Bertrand, Gido & Guy, 2006; Sály *et al.*, 2009; Teixeira-de Mello *et al.*, 2014), and is cost-effective for large-geographic-scale studies (Meador, McIntyre & Pollock, 2003). We sampled standardized 150-m long reaches because previous evaluations indicated that representative samples of fish species for small streams can be obtained from reaches 30-40 times the mean wetted width or at least 150-m (Angermeier & Smogor, 1995; Reynolds *et al.*, 2003; Sály et al., 2009; David *et al.*, 2012). We restricted sampling to wadeable streams (mean wetted width = 4.7 m, SD 1.8 m) to ensure comparability of sampling reaches.

Prior to fish sampling, we blocked reach extremities with 1.2-mm gill nets. The same team sampled all sites by performing a controlled sampling effort (~three hours electrofishing). We sampled all habitats encountered along the 150-m stream reaches. Sampling was conducted during spring and summer, between October 2013 and April 2014 and between October 2014 and March 2015. Specimens were anesthetized with clove oil and preserved in 10% formalin for identification in the lab.

Table 1. Variables describing 54 stream sites sampled across South Brazilian grasslands. Values in parentheses are standard deviation. Min = minimum; Max = maximum.

Variables	Mean (SD)	Min	Max
Catchment size - upstream area from sample site (km^2)	23.1 (21.7)	1.6	148.1
Catchment urban land-use (%)	<0.1 (<0.1)	0	0.3
Catchment cropland area (%)	22.4 (20.1)	0.3	73.4
Catchment livestock density (animal/km^2)	87.1 (25.1)	40.4	155.5
Riparian 50-m wide 1-km long woody vegetation (%)	44.1 (22.3)	0	80.7
Local impact (crops and livestock at streambank) (%)	12.0 (17.3)	0	100
Above sea level elevation (m)	232 (131)	75	677
Macrophyte cover 150-m stream segment (%)	3.8 (8.5)	0	43.4
Silt cover (grain size < 0.06 mm) (%)	4.9 (15.0)	0	91.9
Substrate homogeneity (inverse Shannon-Wiener, 0-1)	0.39 (0.22)	0	1
Woody debris 150-m stream segment (%)	15.4 (11.6)	0	45.7
Leaf litter covering streambed (%)	6.6 (7.7)	0	44.3
Rarefied species richness (site-level)	13.9 (4.2)	5.4	23.6
Functional diversity (Rao's Q)	12.8 (1.9)	8.3	17.1

Instream habitat variables

We characterized the instream habitat at the 150-m fish sampling reach by quantifying (1) % macrophyte cover, (2) % of fine sediment on the stream bottom (silt, grain size < 0.06 mm), (3) substrate homogeneity (i.e., the inverse of the Shannon-Wiener index of substrate grain size composition standardized to 0–1), (4) % leaf litter cover, and (5) % woody debris cover. See Appendix S1 and Fig. S1 in Supporting Information for a detailed description of how we quantified these variables.

Local-, riparian- and catchment-scale

We assessed the local streambank condition by evaluating the extent and effect of agricultural activities developed along 10-m wide margins of the sampling reaches. We visually estimated % cropland area and livestock use intensity at both stream sides in 11 cross sections along the sampled reaches. We averaged these scores to obtain values per sampling site (See Appendix S1 and Fig. S1 for details).

We characterized land-use and land-cover at the catchment scale (i.e. the entire basin area upstream from a given sampling reach) and the riparian scale (i.e. 50-m wide riparian zones on both sides of the stream extending 1-km upstream from a given sampling reach; Morley & Karr, 2002) (Fig. 2) by performing supervised classification of 5-m resolution RapidEye satellite imagery (Geo Catálogo, 2015). Agricultural effects at the catchment scale were quantified as % cropland cover. Because natural riparian zones of larger streams (≥ second-order) in the *Campos* are usually dominated by shrubland and forest, we calculated % woody vegetation at the riparian scale to examine whether preservation of the riparian zone may ameliorate agricultural effects at the catchment scale. In addition, we estimated the density of livestock (i.e. cattle, horse and sheep) at the catchment scale using municipal livestock density data (IBGE, 2006).

Functional diversity

Functional diversity was based on 14 morphological traits (Table S1; Fig. S2) that reflect fish trophic guilds (e.g. Albouy *et al*., 2011), spatial occupation in the water column, and habitat use (e.g. Leal, Junqueira & Pompeu, 2011). For each trait of each species, an average value was calculated based on measurements of individuals from five size classes whenever possible (Table S1).

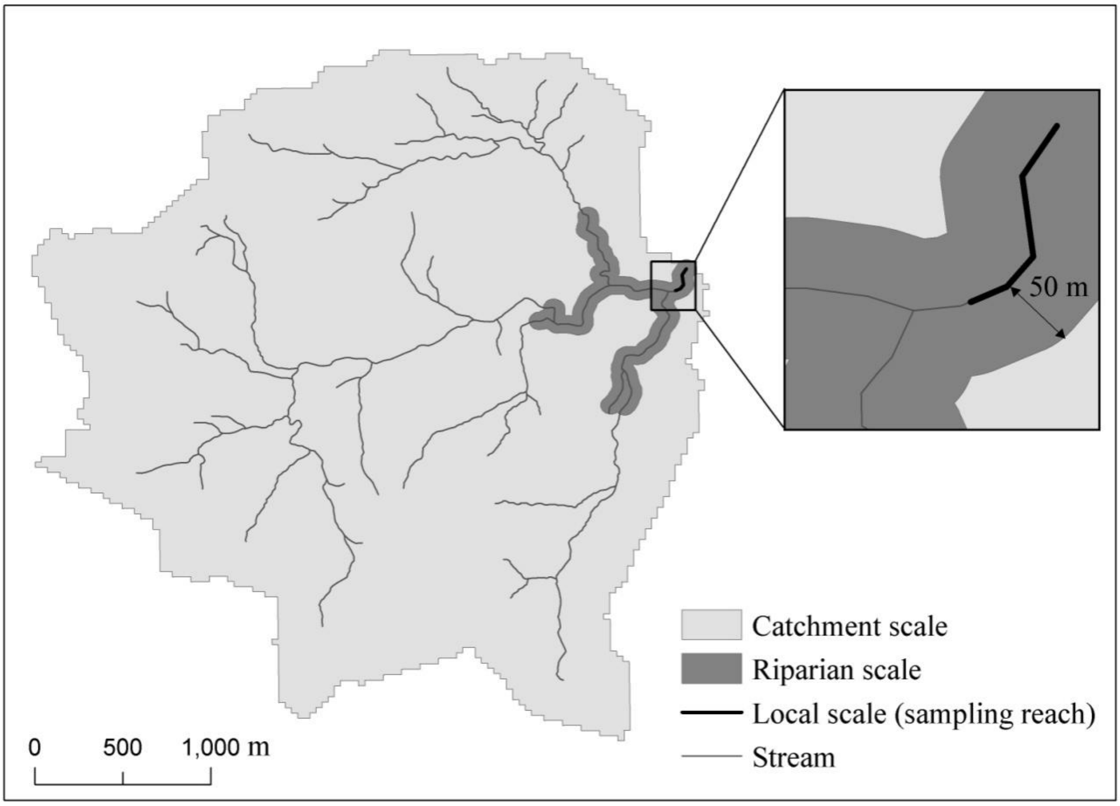

Fig. 2 Spatial scales (catchment, riparian and local) at which agricultural land-use was assessed. The catchment scale comprised the entire area upstream from the sample site. The riparian scale comprised a 1-km long upstream buffer from the sample site (50-m width at both stream sides). The local scale comprised a 10-m width streambank from both stream sides along the 150-m sampling reach. Catchment and riparian scale data were obtained with GIS, while local scale was visually assessed *in situ*.

We used Rao's quadratic entropy (Rao's Q) to quantify fish functional diversity in each sampling site based on species presence-absence data. Rao's Q measures the mean pairwise distance between species in trait space. Therefore, communities with species that have very different traits will have larger Rao's Q values than those comprising higher number of species with very similar traits. Rao's Q does not increase monotonically with species richness (Botta-Dukát, 2005), hence ensuring that a high functional diversity value is not merely an artifact of a high species richness value. We used *FD* package (Laliberté, Legendre & Shipley, 2014) in R Statistical Environment (R Core Team, 2015) to calculate Rao's Q values.

Path models

Based on the literature, we developed a global theoretical model of how agriculture at different spatial scales may affect fish communities by altering instream habitat (Fig. 3). We performed Structural Equation Modelling (SEM; Fox, 2010) to examine the support for two sub-models that differed in the catchment scale land-use (cropland or livestock). We used the *lavaan* package (Rosseel, 2012) in R (R Core Team, 2015) to perform SEM. We fitted different models for cropland and livestock because whereas these variables were partially correlated (r = -0.41), they may affect fishes in different ways. The two path models had the following structure linking terrestrial modifications, instream habitat characteristics and fish diversity (species richness and functional diversity) (Table 1):

(1) Terrestrial agricultural effects were specified at the local (streambank), riparian and catchment scales (Figs 2 and 3). We predicted that riparian native vegetation coverage and agricultural activities at these scales would affect fish communities by modifying different aspects of instream habitat.

(2) Instream habitat was represented by (i) % macrophyte cover (which was measured directly) and two other latent variables: (ii) substrate siltation and the amount of (iii) coarse particulate organic matter (CPOM). We assumed substrate siltation could be assessed from % silt cover on stream bottom and substrate homogeneity (i.e. high values of both observed variables indicate poor substrate condition). We also assumed that % leaf litter cover and %

woody debris cover are representative of both the amount of CPOM available in the stream reach and the structural complexity and microhabitat for fishes.

(3) Two alpha diversity measures of fish community diversity, which we predicted to be affected differently by instream habitat variables, were used: (i) rarefied species richness and (ii) functional diversity as measured by Rao's Q. Individual-based rarefaction allowed us to account for the variation in the number of individuals sampled among sites to estimate species richness. It is well known that differences in species number can be an artifact of different individual numbers sampled in communities, rather than actual differences in species richness (Gotelli & Colwell, 2001). Hence, the rarefaction procedure allows for meaningful standardization and comparison of communities with different numbers of individuals (Gotelli & Colwell, 2001). We performed rarefaction in our study because the number of sampled individuals per stream reach was quite variable (ranging from 105 to 1212 individuals), even though sampled area were similar.

We assessed the goodness-of-fit of each model using the chi-square test (χ^2): models with low value of χ^2 and nonsignificant p-values ($p > 0.05$) were considered as good models because they indicate consistency between observed data and the hypothesized model (Grace, 2006). In addition to calculating the direct standardized effect sizes between pairs of variables, we interpreted the results of our path models by calculating the indirect effects (*IE*) of agricultural land-use on fish diversity. Thus, within each model, we calculated the *IE* of local-, riparian-, and catchment-scale agriculture on fish taxonomic richness/functional diversity via instream habitat variables by tracing (i.e. multiplying) significant ($p \leq 0.05$) and marginally significant ($0.05 < p < 0.1$) standardized path coefficients from each of the agricultural effect variables to the community response variable (Legendre & Legendre, 2012).

Fig. 3 Global theoretical model developed to predict agricultural effects at three spatial scales on α-diversity of fish communities (species richness or functional diversity) via modifications of three key instream habitat characteristics for fishes. CPOM = coarse particulate organic matter.

Spatial autocorrelation

Because spatial autocorrelation could influence our data since some streams were relatively close to each other, we carried out a correction of sample sizes and standard errors of the endogenous variables (response variables) in the path models fitted with SEM. This procedure was performed with the function *spatialCorrect* available in the package *semTools* (Pornprasertmanit, 2016) for R (R Core Team, 2015), using geographical coordinates of sampled sites. This function employs Moran's *I* statistics to calculate the degree of spatial autocorrelation and the effective sample sizes for all endogeneous variables, as well as returns adjusted standard errors and test statistics for each parameter estimate for each endogeneous variables. Only space-corrected results are presented.

RLQ analysis

To understand potential changes in functional composition caused by agricultural effects mediated by instream habitat modifications, we performed a RLQ analysis using species presence-absence data (Dray *et al*., 2014). RLQ analysis involves ordinations of the environmental (R), species occurrence (L) and traits (Q) matrices to assess trait associations along environmental gradients. We used the multivariate fourth-corner method permutation test (type I error fixed at 0.05, adjusting the significance level from $\sqrt{\alpha}$ to α, ter Braak, Cormont & Dray, 2012) to evaluate the relationships between the R, L and Q matrices (Dray *et al*., 2014). Subsequently, we performed a trait-based clustering of species to identify species clusters associated with changes in instream habitat (Kleyer *et al*., 2012; Table S2). These analyses were performed using the *ade4* package (Dray, Dufour & Thioulouse, 2015) in R (R Core Team, 2015).

Results

Fish communities

Overall, the sampled stream sites had a total of 116 fish species, with a mean number species per site of 18.5 (SD 6.7). All species were native to the studied region. A total of 16 families were represented, with the richest families, Characidae, Loricariidae and Cichlidae, having 39, 23 and 14 species respectively, and accounting for 65.5% of the total number of species. The most frequently caught species were the catfish *Heptapterus mustelinus* (90.1%) and the characid *Bryconamericus iheringii* (88.8%), followed by *Characidium pterostictum*

(74.1%), *Rineloricaria stellata* (61.1%), *Astyanax laticeps* (55.5%) and *Rhamdia quelen* (53.7%).

Pathways of agricultural influence on fish diversity

We found support for the two tested path models (Table 2; $p > 0.05$), what indicates that agricultural land-use at different spatial scales shapes patterns in fish species richness and functional diversity via different pathways of instream habitat modifications. Species richness was indirectly affected by land-use at three spatial scales (local, riparian and catchment) via macrophyte and CPOM. On the other hand, functional diversity was only and indirectly related to land-use at the local scale (local impact) via substrate siltation (Fig. 4).

Agricultural activity along stream margins, expressed as local impact, had a positive effect on species richness by increasing macrophyte cover [*IE* = 0.09 (Fig. 4a), and *IE* = 0.08 (Fig. 4b)], but also had a negative effect on species richness by decreasing CPOM (*IE* = -0.10; Fig. 4b). Also, our models suggest that local impact decreased functional diversity via increase of substrate siltation [*IE* = -0.18 (when cropland area was the catchment-scale covariate) and *IE* = -0.19 (when livestock density was the catchment-scale covariate)] (Fig. 4a, b).

Compared to agricultural activities at local scale, catchment cropland had a similar relationship with species richness via macrophyte and CPOM (Fig. 4). An increased cropland area in the upland catchment area had a positive indirect effect on species richness by stimulating macrophyte cover (*IE* = 0.12), but also a negative indirect effect on species richness by decreasing CPOM (*IE* = -0.13) (Fig. 4a). Conversely, livestock density at catchment scale was positively associated to CPOM and thus to species richness (*IE* = 0.12; Fig. 4b).

The amount of riparian vegetation cover in 1-km long and 50-m wide buffer upstream from sampled site was also linked to species richness via its influence on macrophyte cover and CPOM, but exerting an opposite effect compared to local impact and catchment cropland (Fig. 4). Thus, riparian vegetation attenuated the influence of local impact and catchment cropland on species richness by controlling macrophyte cover [*IE* = -0.13 (Fig. 4a); *IE* = -0.13 (Fig. 4b)], and by promoting the input of CPOM [*IE* = 0.13 (catchment-scale covariate = livestock density; Fig. 4b)]. However, riparian vegetation had no effect on CPOM in the path model using cropland area as the catchment-scale covariate (Fig. 4a). Notably, neither catchment agriculture nor riparian vegetation cover were linked to functional diversity, as they did not have a significant relationship with substrate siltation, which was the only proximate instream habitat driver of functional diversity.

Table 2. Structural Equation Modelling (SEM) results for the 2 models tested to predict agricultural effects on fish communities via instream habitat modifications (endogenous variables). Low value of χ^2 and non-significant p-value ($p > 0.05$) indicate low difference between observed data and the hypothesized model. N = number of sample sites; χ^2 = chi-square; df = degrees of freedom; *p* = global probability.

Models	Endogenous variables	N	χ^2	df	*p*	R^2 endogenous variables
a) Cropland		54	30.7	22	0.103	
	Macrophyte					0.39
	Substrate siltation					0.49
	CPOM					0.40
	Species richness					0.25
	Functional diversity					0.27
b) Livestock		54	31.5	22	0.087	
	Macrophyte					0.29
	Substrate siltation					0.43
	CPOM					0.33
	Species richness					0.32
	Functional diversity					0.24

Fig. 4 Structural equation models showing the pathways by which agricultural activities developed at multiple scales affect species richness and functional diversity. CPOM = coarse particulate organic matter. Arrow thickness is proportional to the estimated standardized effect size (single headed) or correlation to other variables (double headed), and their values are indicated over the arrows. Squared multiple correlations (R^2) indicate the variance of instream (endogenous) factors explained by exogenous factors (catchment, riparian and local).

Functional composition

Species traits were significantly associated with environmental variables (RLQ analysis; Fig. 5; *p* = 0.005). The first RLQ axis had the strongest positive correlation with riparian vegetation (r = 0.60) and negative correlation with local impact and macrophyte (r = -0.71 and -0.64). The second RLQ axis was more positively correlated with woody debris and leaf litter (r = 0.77 and 0.62) and more negatively correlated with cropland at the catchment (r = -0.78) (Table S2). Clustering species according to their traits resulted in four distinct groups, and their locations in the RLQ ordination reflect their occurrences in relation to environmental variables (Fig. 5b). Groups A and B increased in agricultural streams, while groups C and D were more common in streams with preserved riparian zones (Fig. 5b).

Traits associated with environmental conditions typical of agriculturally developed streams (i.e. high macrophyte cover and substrate siltation) included compressed and deep body shapes, compressed caudal peduncle, large eyes, terminal to superior mouth position and large head. Species with these traits comprised mainly nektonic omnivorous characins, detritivorous Curimatidae and piscivorous Erythrinidae and Characidae species (Cluster A), as well as several cichlids (Cluster B). Most of these species are water-column dwellers and feeders, and more typical of slow-water habitats. By contrast, fishes with long peduncles, dorsal and small eyes, inferior mouth and depressed-body, such as the bottom-dwellers periphyton-grazing armored catfishes (Cluster C), and also nektobenthic elongated-body species of varied groups (Cluster D), were associated to streams with preserved riparian vegetation and with higher CPOM and scarce fine sediment, which typifies streams that experienced low agricultural activity (Table S3, Fig. S3).

Fig. 5 RLQ analysis of the multiple relationships between species traits and environmental variables related to agricultural activities (a) and the 4 groups of species clustered according to their set of functional traits (b). First and second axes summarized 60.3% and 32.4% of variation, respectively. The lower-left position of the ordination represents sites with more agricultural effects and upper-right position represents sites with integer riparian vegetation (a). Each point in the ordination plot within clusters (b) represents the species position modelled according to its traits on RLQ axes 1 and 2, and each colour or letter represents a different group. Groups A and B were associated to agricultural streams with increased siltation and macrophyte coverage, while groups C and D were more common in streams with preserved riparian zones and higher density of woody debris and leaf litter.

Discussion

We found evidence that the effects of agriculture on stream fish communities are scale-dependent; the spatial grain of the agricultural footprint determines the magnitude of the effect on fish communities as well as the pathways through which these effects are likely manifested. Effects of agriculture on fish functional diversity appear to be greater at the local scale due to stream bottom siltation, whereas species richness was linked to terrestrial modifications at multiple scales. The conversion of upland catchment natural grassland vegetation and also of riparian zone woody vegetation (shrubland and forests) to agriculture was associated with elevated species richness in adjacent stream reaches. The increased availability of light owing to riparian vegetation removal together with increased nutrient input from upland cropland and from adjacent cropland and farming likely allowed macrophytes to proliferate (Burrell *et al*., 2014). These modifications on ecosystem conditions may favour the invasion of native species typically found in larger streams, such as detritivorous species (Lorion & Kennedy, 2009), and species tolerant to water quality/temperature and habitat structure modifications (Scott & Helfman, 2001). Hence, the increased species richness owing to riparian vegetation removal occurs along with changes in species composition (Teixeira-de Mello *et al*., 2015). Particularly, we found an increase in those species belonging to richer fish groups and associated to macrophyte habitat (see Fig. 5, groups A and B). Increasing catchment cropland area and local impact was correlated with reduced species richness via decreased CPOM (i.e. leaf litter and woody debris), whereas riparian vegetation attenuated this effect by maintaining CPOM input to the stream. Higher CPOM may help support a species-rich fish community by spatially concentrating resources such as invertebrates and biofilm (Pringle *et al*., 1988; Wallace *et al*., 1997) and acting as favourable microhabitats (Sazima *et al*. 2006).

Functional diversity was more strongly linked to agricultural activity on adjacent streambanks (local scale). But unlike species richness, the negative association between functional diversity and local agriculture was mediated by a decline in substrate condition by increasing siltation. Increased soil erosion resulting from open cropland and livestock trampling likely contributed to stream bottom siltation and to increase overall substrate homogeneity, which then resulted in the loss of benthic-dwelling and lithophilic fish species. The set of species associated with a better substrate condition (Cluster C; RLQ analysis; Fig. 5) was more diffuse in trait space despite having fewer members than the species sets associated with locally impacted, macrophyte-rich stream reaches (Cluster A). Therefore, as streambanks become more impacted by agriculture, our results suggest that species belonging to the functionally diverse benthic and lithophilic species guilds will be replaced by members of the more species-rich but functionally redundant nektonic guild typified by traits such as a deep and compressed body, superior mouth position and large head and eyes. This also explains why functional diversity declined despite an increase in species richness as streams become more affected by agriculture at the local scale.

The retention of woody vegetation in the riparian zone (1-km long 50-m wide buffer) appeared to be important for attenuating the effect of catchment-scale agriculture on species richness by increasing CPOM in streams. Also, by dampening the increase in macrophyte cover, our results suggest that the conservation of riparian vegetation can reduce the effect of agriculture on species richness. Importantly, in addition to the effects of local and riparian scales on CPOM, we found that cropland at catchment scale reduced CPOM. This suggests catchment cropland may have additional effects on riparian vegetation and that these were not captured at the riparian scale as defined by us. Thus, our results suggest that retaining long continuous stretches (≥ 1 km) of riparian vegetation is necessary for effective mitigation of catchment-scale agricultural effects on instream habitat and fish species communities. These findings are consistent with previous studies showing that effects at riparian zone can have more pronounced effects on instream habitat, affecting fish communities and ecosystem functioning (Jones *et al*., 1999; Lorion & Kennedy, 2009; Giam *et al*., 2015).

In contrast to our expectation, no evidence was found for catchment agriculture effects on substrate siltation. A possible reason is that the study area covered a large region (*ca*. 110.000 km^2), including several soil types with different sensibility to erosion. Moreover, the measure of agricultural land-use in the studied catchments did not distinguish different types of cropland, what may have increased the uncertainty regarding how cropland influenced stream siltation. It is plausible, however, that agriculture will have a pronounced effect on stream siltation when it affects streambank vegetation. Also, the footprint of cropland at catchment scale of our sampling sites is low-to-moderate (only seven of the 54 sites with > 50% cropland area and one > 70%), and there is some previous evidence of stronger effects at higher levels of catchment agricultural cover (Wang *et al*., 2003; 2006). Thus, we were not able to assess whether riparian vegetation can be effective in preventing agricultural impacts when larger catchment proportions become converted to agricultural use.

Whereas our findings are consistent with previous studies demonstrating the effects of agricultural activities measured at multiple spatial scales on instream habitat and fish communities (e.g. Wang *et al*., 1997; Iwata, Nakano & Inoue, 2003; Lorion & Kennedy, 2009, Giam *et al*., 2015), we provide additional evidence on the different pathways by which agricultural activities at each scale likely affect fish communities. Our results suggest the mechanisms affecting aquatic communities may depend on the spatial scale used to evaluate human impacts. Also, inconsistency in the literature regarding positive or negative responses of species richness to agricultural land-use may in part be a consequence of underlying mechanisms associated with different spatial scales (such as macrophyte production versus CPOM input). This result highlights the importance of taking into account the effects of multiple spatial scales (and the different mechanisms operating at each scale) in assessing anthropic impacts on catchments and their stream networks.

As unique non-forest ecosystems, such as the *Campos* in Brazil, are increasingly being converted to cropland or used for cattle ranching (Overbeck *et al*., 2015), an efficient conservation policy is required to protect aquatic biodiversity from the effects of agricultural activities. In 2012, Brazilian legislation on native vegetation protection was modified (Federal Law 12651/2012 or "*Native Vegetation Law*", see Appendix S1 in Overbeck *et al*., 2015), allowing landowners to use larger portions of riparian areas (Sparovek *et al*., 2012; Garcia *et al*., 2013). Ecologists have warned that the reduction in riparian buffer width could result in irreversible environmental degradation including the loss of valuable biodiversity (Metzger *et al*., 2010; Garcia *et al*., 2013). Our results provide strong empirical support to these concerns. In the absence of any changes to the current legislation and of policies encouraging the protection riparian zones, we suggest that landowners should voluntarily preserve continuous riparian buffers to effectively reduce negative effects of catchment- and local-scale agriculture. Particularly important is the notion that upstream riparian reserves should be considered as targets for conservation actions.

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Revealing the pathways by which agricultural land-use affects stream fish communities in South Brazilian grasslands

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Supporting Information

Appendix S1. Description of how instream habitat variables were measured in each sampling site, using a modification of the protocol detailed in Kaufmann et al. (1999).

For measuring instream habitat variables and streambank condition we separate each sampled 150-m stream segment into 11 cross sections (see Appendix S2) where we performed repeated measures according to Kaufmann et al. (1999). We used in our study the instream variables related to (1) substrate composition, (2) macrophyte cover and (3) density of leaf litter and woody debris. These variables were recorded at each cross section by a visual estimative of the cover percentage according to five size classes, namely 0) absent; 1) <10%; 2) 10-40%; 3) 40-75%; 5) >75%. Macrophyte cover included the cover percentage of both submerged and emergent aquatic plants. Leaf litter included its cover percentage in relation to the stream bottom. Woody debris included the relative cover percentage of submerged or partially submerged wooded vegetation inside the wetted channel. Substrate composition comprised the cover at the stream bottom percentage of different grain size, including: 1) silt: <0.06 mm; 2) sand: 0.06-2 mm; 3) small pebbles: 0.2-1.6 cm; 4) large pebbles: 1.6-6.4 cm; 5) cobbles: 6.4-25 cm; 6) small boulders: 25-50 cm; medium boulder: 50-100 cm; large boulders; 100-400 cm; bedrock: >400 cm.

Streambank conditions for each sampled site were assessed at each one of the 11 cross sections per 150-m stream segment at both right and left stream margins. This assessment comprised an evaluation of the percentage cover of agriculture up to 10-m from the stream channel and also an estimative of livestock use by direct (animal visualization) and indirect signals (trampling marks, damage on vegetation and cattle manure). The quantification of these crop and animal land-use at each cross section was visually estimated into five classes: 0) absent; 1) <10%; 2) 10-40%; 3) 40-75%; 5) >75%. The mean percentages standardized by range (0 to 100%) was used to infer local impact for each sample site. We also measured canopy openness with a densiometer at each cross section on streambanks, but we did not include in our analysis because it was correlated with local impact (r = 0.65).

Table S1. Fourteen functional traits indicative of habitat use/occupation and feeding behaviour used to calculate Rao's Q functional diversity for stream fish communities. See Fig. S2 for abbreviations.

Trait	Equation	Function
Body compression		Related to manoeuvrability. Compressed bodies are usually found in lentic waters (Watson & Balon 1984).
Body depth		Inversely related to flow velocity and it determines the ability to perform vertical movement in the water column (Gatz 1979).
Head size		Related to prey size (Gatz 1979).
Eye position		Indicates vertical habitat preference (Gatz 1979). Varies from 0 to 1. High values mean superior eyes.
Eye size		Indicates the importance of vision for feeding (Gatz 1979). Values relative to head size.
Mouth position		Indicates vertical position at which fish forages (Albouy et al. 2011). Varies from 0 to 1. High values mean superior mouth.
Peduncle length		Longer caudal peduncles indicate good swim ability (Gatz 1979).
Peduncle compression		Compressed causal peduncles indicate poor swim activity (Gatz 1979).
Pectoral position		Related to manoeuvrability (Dumay et al. 2004). High values mean dorsal located pectoral fins in relation to the most ventral body part.
Pectoral fin area		High values are indicative of benthic habit species, where pectoral fins are used as anchors to stand in fast water (Watson & Balon 1984).
Ventral fin area		Large ventral fins are used as support for benthic species (Casatti & Castro 2006).
Caudal fin area		Large caudal fins are associated to high propulsion (Gatz, 1979).
Dorsal fin area		Fish that prefer fast flow has usually small dorsal fins (Casatti & Castro 2006).
Biomass		Indicates contribution to the system via metabolism (Albouy et al. 2011).

Table S2. Correlations between functional traits, environmental variables and the 2 first RLQ axes.

Category	Variable	Axis 1	Axis 2
Functional traits	Body compression	-0.91	-0.32
	Body depth	-0.77	-0.59
	Superior eye position	0.71	0.42
	Eye size	-0.78	-0.35
	Superior mouth position	-0.78	0.02
	Head size	-0.17	-0.78
	Peduncle length	0.76	0.50
	Peduncle compression	-0.39	-0.22
	Superior pectoral position	0.09	-0.06
	Pectoral area	0.49	-0.62
	Ventral area	0.57	-0.64
	Dorsal area	0.26	-0.79
	Caudal area	-0.18	-0.89
	Biomass	0.38	-0.57
Environmental variables	Catchment cropland	-0.03	-0.78
	Livestock density	-0.55	0.29
	Riparian vegetation	0.61	0.40
	Local impact	-0.71	-0.21
	Macrophyte	-0.64	-0.47
	Woody debris	0.13	0.77
	Leaf litter	0.13	0.62
	Fine sediment	-0.51	-0.17
	Substrate homogeneity	-0.48	0.49

Table S3. List of fish species surveyed in 54 wadeable streams across South Brazilian grasslands, with indication of their family and respective group assigned according to functional traits. All the species are native.

Group	Species	Family
A	*Aphyocharax anisitsi*	Characidae
A	*Apistogramma commbrae*	Cichlidae
A	*Astyanax dissensus*	Characidae
A	*Astyanax eigenmanniorum*	Characidae
A	*Bryconamericus iheringii*	Characidae
A	*Characidium occidentale*	Characidae
A	*Characidium orientale*	Crenichidae
A	*Characidium zebra*	Crenichidae
A	*Charax stenopterus*	Characidae
A	*Cheirodon ibicuhiensis*	Characidae
A	*Cheirodon interruptus*	Characidae
A	*Crenicichla punctata*	Cichlidae
A	*Diapoma alegretensis*	Characidae
A	*Diapoma tipiaia*	Characidae
A	*Diapoma uruguayensis*	Characidae
A	*Diapoma speculiferum*	Characidae
A	*Diapoma terofali*	Characidae
A	*Heterocheirodon yatai*	Characidae
A	*Hyphessobrycon anisitsi*	Characidae
A	*Hyphessobrycon luetkenii*	Characidae
A	*Hyphessobrycon meridionalis*	Characidae
A	*Hypobrycon* sp.	Characidae
A	*Mimagoniates inequalis*	Characidae
A	*Moenkhausia dichroura*	Characidae
A	*Odontostilbe pequira*	Characidae
A	*Pseudocorydopoma doriae*	Characidae
A	*Serrapinnus calliurus*	Characidae
A	*Steindachnerina brevipinna*	Curimatidae
B	*Astyanax fasciatus*	Characidae
B	*Astyanax henseli*	Characidae
B	*Astyanax jacuhiensis*	Characidae
B	*Astyanax laticeps*	Characidae
B	*Astyanax procerus*	Characidae
B	*Astyanax saguazu*	Characidae
B	*Astyanax* sp. "a"	Characidae
B	*Astyanax* sp. "b"	Characidae
B	*Astyanax stenohalinus*	Characidae

B	*Astyanax xiru*	Characidae
B	*Australoheros facetus*	Cichlidae
B	*Australoheros minuano*	Cichlidae
B	*Australoheros scitulus*	Cichlidae
B	*Callichthys callichthys*	Callichthiydae
B	*Cichlasoma dimerus*	Cichlidae
B	*Corydoras paleatus*	Callichthiydae
B	*Corydoras* sp.	Callichthiydae
B	*Corydoras undulatus*	Callichthiydae
B	*Crenicichla lepidota*	Cichlidae
B	*Crenicichla scotti*	Cichlidae
B	*Cyphocharax saladensis*	Curimatidae
B	*Cyphocharax spilotus*	Curimatidae
B	*Cyphocharax voga*	Curimatidae
B	*Gymnogeophagus gymnogenys*	Cichlidae
B	*Gymnogeophagus labiatus*	Cichlidae
B	*Gymnogeophagus mekinos*	Cichlidae
B	*Gymnogeophagus meridionalis*	Cichlidae
B	*Gymnogeophagus pseudolabiatus*	Cichlidae
B	*Gymnogeophagus rhabdotus*	Cichlidae
B	*Hoplias malabaricus*	Erythrinidae
B	*Hyphessobrycon togoi*	Characidae
B	*Oligosarcus jacuiensis*	Characidae
B	*Oligosarcus jenynsii*	Characidae
B	*Oligosarcus oligolepis*	Characidae
B	*Oligosarcus robustus*	Characidae
B	*Oligosarcus* sp.	Characidae
B	*Steindachnerina biornata*	Curimatidae
C	*Ancistrus brevipinnis*	Loricariidae
C	*Ancistrus taunay*	Loricariidae
C	*Bunocephalus doriae*	Aspredinidae
C	*Hemiancistrus fuliginosus*	Loricariidae
C	*Hemiancistrus punctulatus*	Loricariidae
C	*Hypostomus aspilogaster*	Loricariidae
C	*Pseudobunocephalus iheringii*	Aspredinidae
C	*Pseudohemiodon laticeps*	Loricariidae
C	*Rhamdella longiuscula*	Heptapteridae
C	*Rhamdia quelen*	Heptapteridae
C	*Rineloricaria baliola*	Loricariidae
C	*Rineloricaria cadeae*	Loricariidae
C	*Rineloricaria longicauda*	Loricariidae
C	*Rineloricaria microlepidogaster*	Loricariidae
C	*Rineloricaria* sp.	Loricariidae

C	*Rineloricaria stellata*	Loricariidae
C	*Rineloricaria strigilata*	Loricariidae
D	*Brachyhypopomus bombilla*	Hypopomidae
D	*Characidium pterostictum*	Crenichidae
D	*Characidium tenue*	Crenichidae
D	*Cnesterodon decemmaculatus*	Poeciliidae
D	*Eigenmannia trilineata*	Sternopygidae
D	*Gymnotus carapo*	Gynmotidae
D	*Heptapterus mustelinus*	Heptapteridae
D	*Heptapterus* sp.	Heptapteridae
D	*Heptapterus sympterygium*	Heptapteridae
D	*Hisonotus armatus*	Loricariidae
D	*Hisonotus charrua*	Loricariidae
D	*Hisonotus laevior*	Loricariidae
D	*Hisonotus notopagos*	Loricariidae
D	*Hisonotus ringueleti*	Loricariidae
D	*Homodiaetos anisitsi*	Loricariidae
D	*Hypostomus commersonii*	Loricariidae
D	*Hypostomus uruguaiensis*	Loricariidae
D	*Imparfinis mishky*	Heptapteridae
D	*Ituglanis australis*	Trichomycteridae
D	*Ituglanis* sp.	Trichomycteridae
D	*Microglanis cottoides*	Pseudopimelodidae
D	*Otocinclus arnoldi*	Loricariidae
D	*Otocinclus flexilis*	Loricariidae
D	*Phalloceros caudimaculatus*	Poeciliidae
D	*Pimelodella australis*	Heptapteridae
D	*Pseudoheptapterus* sp.	Heptapteridae
D	*Rhamdella eriarcha*	Heptapteridae
D	*Scleronema* aff. *operculatum*	Trichomycteridae
D	*Scleronema minutum*	Trichomycteridae
D	*Scleronema operculatum*	Trichomycteridae
D	*Scleronema* sp. "ibicui"	Trichomycteridae
D	*Scleronema* sp. "quarai"	Trichomycteridae
D	*Synbranchus marmoratus*	Synbranchidae
D	*Trichomycterus poikilos*	Trichomycteridae

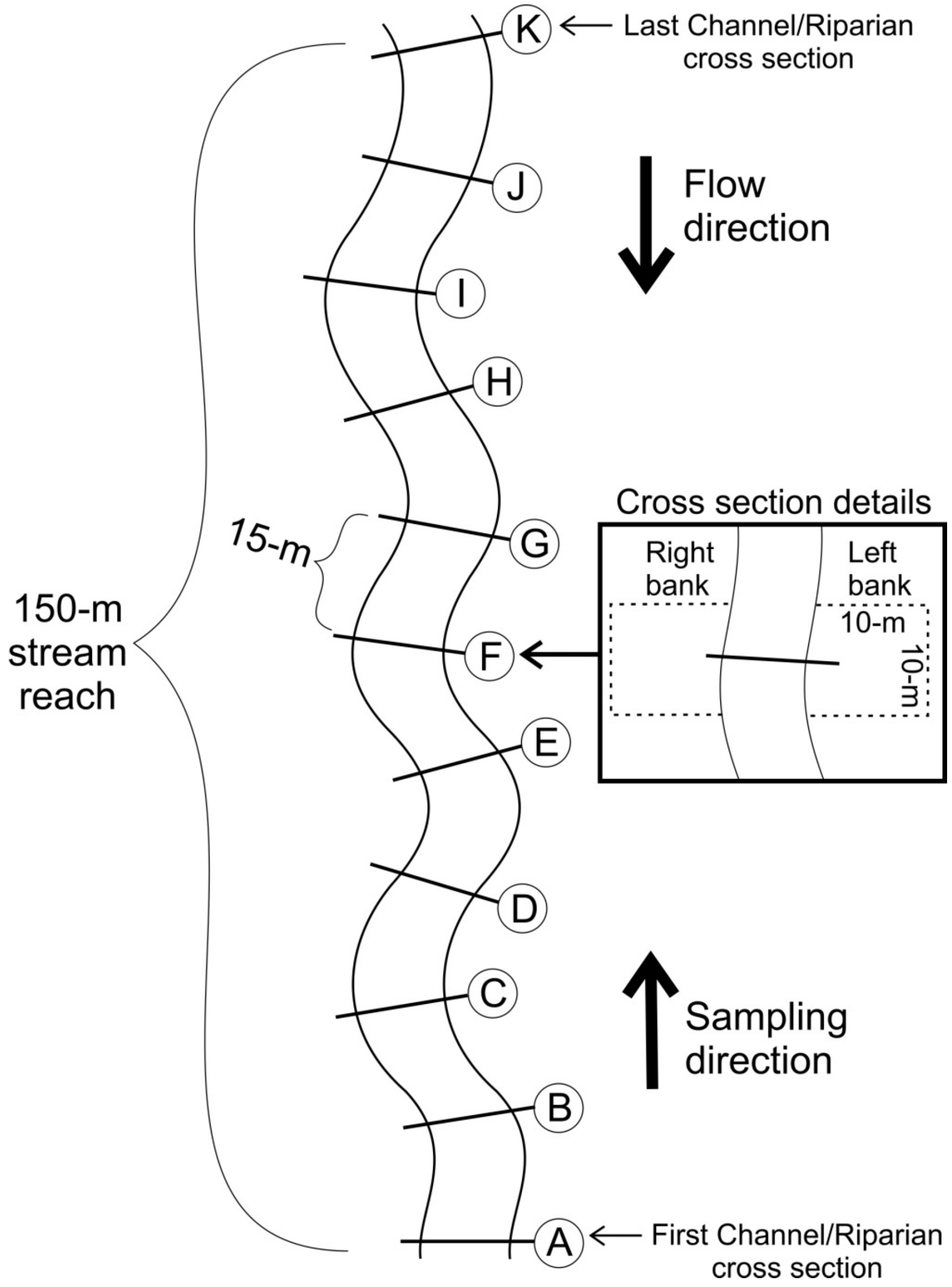

Figure S1. Illustration of how instream habitat variables and streambank conditions were measured in each sampling site, as a modification of the protocol detailed in Kaufmann *et al*. (1999). Fish communities were sampled in the same stream reaches.

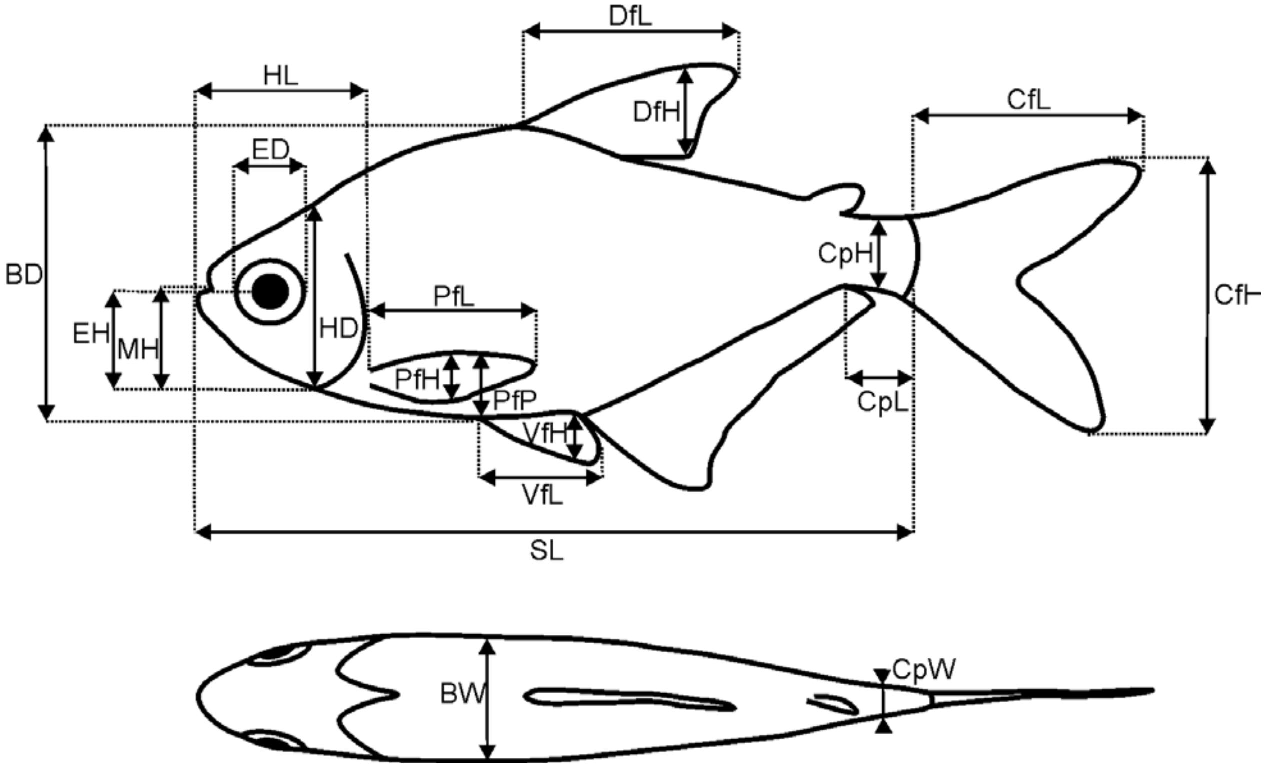

Figure S2. Illustration of the 20 morphometric measures used to calculate functional traits related to habitat use and feeding behaviour. SL = Standard length; BD = Body depth; BW = Body width; HL = Head length; HD = Head depth; ED = Eye diameter; EH = Eye height; MH = Mouth height; PfL = Pectoral fin length; PfH = Pectoral fin height; PfP = Pectoral fin position; VfL = Ventral fin length; VfH = Ventral fin height; DfL = Dorsal fin length; DfH = Dorsal fin height; CpL = Caudal peduncle length; CpH = Caudal peduncle height; CpW = Caudal peduncle width; CfL = Caudal fin length; and CfH = Caudal fin height.

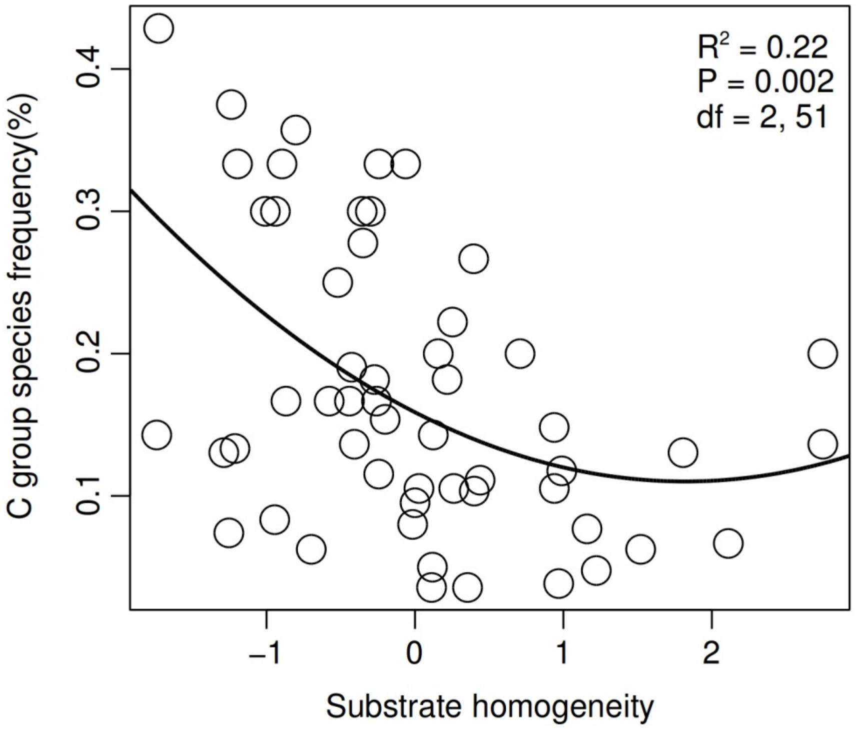

Figure S3. Decrease in the relative frequency of occurrence of benthic species (group C) in relation to substrate homogeneity (inverse of Shannon-Wiener diversity index of substrate grain size composition).

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CAPÍTULO 2 - Agricultural land-use drives subsidy-stress beta-diversity response of stream fish communities*

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Abstract

Agricultural land-use is often associated to multiple stressors that modify physical habitat, water quality and biota of aquatic ecosystems. These modifications can lead to faunal homogenization, which occurs when compositional dissimilarity (β-diversity) among stream fish communities decreases. In opposite, agricultural development can also be hypothesized to increase fish composition dissimilarity by inducing a phenomenon called native invasion, when enviromental modification facilitates the occurrence of species usually found only in downstream areas. If these two opposite trends manifest one can expect a subsidy-stress response of β-diversity to agricultural land-use, a modal curve that describes both positive and negative effects. We investigated this question using fish communities sampled in 54 streams across South Brazilian grassland region and three indicators of agricultural disturbance (catchment cropland percentage, local impact and canopy openness). We found taxonomic and functional β-diversities showed a subsidy-stress response to, respectively, percentage of agriculture at catchment scale and local impact. This suggests that agricultural activities modify the balance between stochastic and deterministic process affecting fish communities assembly, by altering the strength of niche filtering. A negative effect was observed when agriculture exceeded 40% of catchment area, and when local impact exceeded 30%, suggesting a transition point to manage catchments in order to prevent faunal homogenization. The results have implications for understanding the effects of agriculture on biodiversity at regional scale and to conservation and management planning of watersheds.

Introduction

Agriculture expansion over natural vegetation is a worldwide phenomenon, and imposes a challenge to the scientists trying to understand and predict its environmental consequences (Foley et al., 2005; 2011). Riverine ecosystems are particularly sensitive to agricultural land-use as drainage networks receive nonpoint pollutants through surface runoff, including fine sediment, nutrients and pesticides (Lowrance et al., 1984; Allan, 2004). Conversion of native vegetation to cropland and pastures may also increase stream temperature (Macedo et al., 2013) and cause modifications on stream flow (Davis et al., 2015). These multiple stressors can be exacerbated if agricultural activities reach and impair riparian zones, and lead to complex effects on ecosystem functioning and stream communities (Allan, 2004; Giam et al., 2015; Dala-Corte et al., submitted).

Diversity of stream fish communities have been demonstrated to be affected by the replacement of native vegetation for agricultural land-use (Wang et al., 1997; 2003; Casatti et al., 2015; Giam et al., 2015). For instance, agriculture is expected to reduce benthic specialists due to stream bottom siltation (Walser & Bart, 1999; Dala-Corte et al., submitted). Also, shading reduction owing to riparian vegetation removal coupled with increased nutrient from agriculture may cause proliferation of algae and macrophytes (Lowrance et al., 1997; Burrell et al., 2014), and modify the proportion of autochthonous and allochthonous organic matter into the food webs (Minshall, 1978; Nakano et al., 1999; Majdi et al., 2015). These modifications can benefit the occurrence of a great number of nektonic and tolerant fish species (Casatti et al., 2015; Teresa et al., 2015; Dala-Corte et al., submitted).

Studies report that species richness of stream fish communities may decrease in agriculture-perturbed watersheds (Roth et al., 1996; Walser & Bart, 1999). On the other hand, there is also evidence that the environmental consequences of agriculture can trigger native invasion of fish species typically found in downstream parts of drainage networks and augment local species richness (Lorion & Kennedy, 2009; Teixeira-de Mello et al., 2015; Dala-Corte et al., submitted). Whereas local diversity (α-diversity) of stream fish communities can be either negatively or positively influenced by agriculture, concomitant to shifts in species composition, there is less knowledge on whether agricultural land-use increases or decreases community differentiation among streams.

Beta diversity (β-diversity) is a measure of among-community variability and distinctiveness that reflects the rate of changes in species composition across space and time (Melo et al., 2012; Heino et al., 2015). Beta diversity has been largely used for comparing communities between two or multiple sites that differ in environmental conditions (e.g. Condit et al., 2002; Costa & Melo, 2008; Melo et al., 2009; Leprieur et al., 2011). The term was first introduced by Whittaker (1960) and several different measures were posteriorly developed to quantify the level of heterogeneity among communities (e.g. Anderson et al., 2006; Tuomisto & Ruokolainen, 2006; Baselga, 2010; Legendre & De Cáceres 2013). Beta-diversity will be high if communities present a high spatial (or temporal) turnover of species composition or distinct species richness, and will be low if communities harbor a great proportion of shared species and are similar in species richness.

Human-induced disturbances are often linked to faunal homogenization, i.e. when local community distinctiveness decreases compared to other sites or regions (Walters et al., 2003; Smart et al., 2006; Pool & Olden, 2012). Different mechanisms are involved with faunal homogenization (Rahel, 2002), such as invasion or introduction of nonnative species (Olden & Poff, 2004; Hermoso et al., 2012). Additionally, it is hypothesized that fish community homogenization can result from environmental modifications that benefit occurrence of cosmopolitan fish species at the expense of the endemic ones (Walter et al., 2003). For agricultural stressors, for example, increased siltation, temperature and nutrients can favor the occurrence of tolerant and widespread fish species, usually encountered in warmer, more turbid, sediment- and nutrient-rich streams of lower portions of watersheds (Scott & Helfman, 2001). In this case, agricultural land-use could decreases β-diversity among stream fish communities.

Environmental disturbance sometimes enhances biological response at low-levels of impact and degrades it at higher levels, rather than cause a linear negative effect (Odum et al., 1979). This phenomenon generates a subsidy-stress curve, in which the response variable increases up to a certain disturbance level or threshold at which it starts to decreases (Odum et al., 1979). Subsidy-stress responses to agriculture have been found, for example, for several invertebrate stream health metrics, such as Ephemeroptera–Plecoptera–Trichoptera density and richness (Niyogi et al., 2007). Also, fish biomass has been found to response as subsidy-stress shape to phosphorous gradient (King & Richardson, 2007). One potential mechanism behind this relationship is that agricultural development at low-levels lead to nutrient enrichment of naturally nutrient-poor streams, and increases primary production to a certain level that subsidies macroinvertebrates and fishes production (Niyogi et al., 2007; King & Richardson, 2007). On the other hand, catchments dominated by agricultural land-use might lead to increases in nutrient, sediment and temperature of streams to such a level that become stressful for many taxa (Niyogi et al., 2007; Wagenhoff et al., 2011).

Studies have suggested that more restrictive environmental conditions drive increased similarity of community composition (Chase, 2007; 2010). For instance, β-diversity is hypothesized to augment with primary productivity, because low productivity limits community assembly to deterministic processes, whereas high productivity allows stochasticity to play a more important role on community assembly (Chase, 2010). In this case, instead to decrease β-diversity, agricultural disturbance could drive fish communities to become more distinct across the landscape. If agricultural land-use lead streams to harbor a random subset of the region pool of species, community β-diversity will increase, but if environment modifications caused by agricultural development allows only the occurrence of those tolerant and cosmopolitan fish species, commonly found in downstream areas of watersheds, β-diversity will decreases. Hence, if these opposite trends manifest for agricultural disturbance, one can expect a subsidy-stress response of fish community β-diversity to agricultural land-use.

The threshold at which a negative response is observed may be dependent on the disturbance type and on the response variable sensibility. Taxonomic and functional composition have been shown to differ in their response to agricultural stressors. For instance, Dala-Corte et al. (submitted) found that whereas species richness can increase with environment modifications linked to cropland and farming activities, functional diversity decreases by the addition of species with similar function in the fish communities, concomitant with reduction of benthic specialists. Therefore, if a large number of different species with similar functions are incorporated in impacted streams, one can expect different responses of β-diversity when evaluating taxonomic versus functional compositions. Identifying the response type and determining environmental threshold for agricultural land-use effects on stream fish β-diversity are particularly relevant if the goal is to manage watersheds in order to maximize the conservation of aquatic ecosystem.

We investigated here how β-diversity of stream fish communities responds to agricultural land-use. Specifically, we asked (1) How taxonomic and functional β-diversities of stream fish communities respond to agricultural land-use? (2) Is there a threshold for agricultural land-use at which we observe an abrupt loss of β-diversity? (3) Do taxonomic and functional diversities present different responses to agricultural land-use? By responding these questions we can contribute to understand the impacts of agriculture on freshwater fish diversity at regional scales and to subsidy conservation strategies.

Methods

Study area and fish sampling

We sampled 54 wadeable streams distributed across South Brazilian grasslands (Fig. 1). This area comprises the Pampa lowlands region and the high altitude grasslands of Atlantic Forest biome in the southernmost Brazilian State - Rio Grande do Sul. Regional climate is classified as humid temperate with hot summer (Cfa type) according to Köppen-Geiger classification (Peel et al., 2007). Although grassland is the dominant vegetation across the landscape, riparian zones commonly develop woody vegetation composed of shrubland and forest.

Agricultural land-use has been expanded in South Brazilian grasslands in the last three decades, with an increased portion of native vegetation being converted mainly to soybean, rice, maize and wheat plantations (Overbeck et al., 2007; IBGE, 2015). In addition, cattle and sheep farming are traditional activities since the 17th century, widely used for beef production (Overbeck et al., 2007). Therefore, regional streams are susceptible to different levels of environmental disturbances coming from agricultural land-use. To avoid potential confounding factors, all the sampled streams had less than 1% of urban land-use at upland catchment area and were dominated by a gradient from natural land-cover (grassland, shrubland or forest) to cropland land-use at catchment scale.

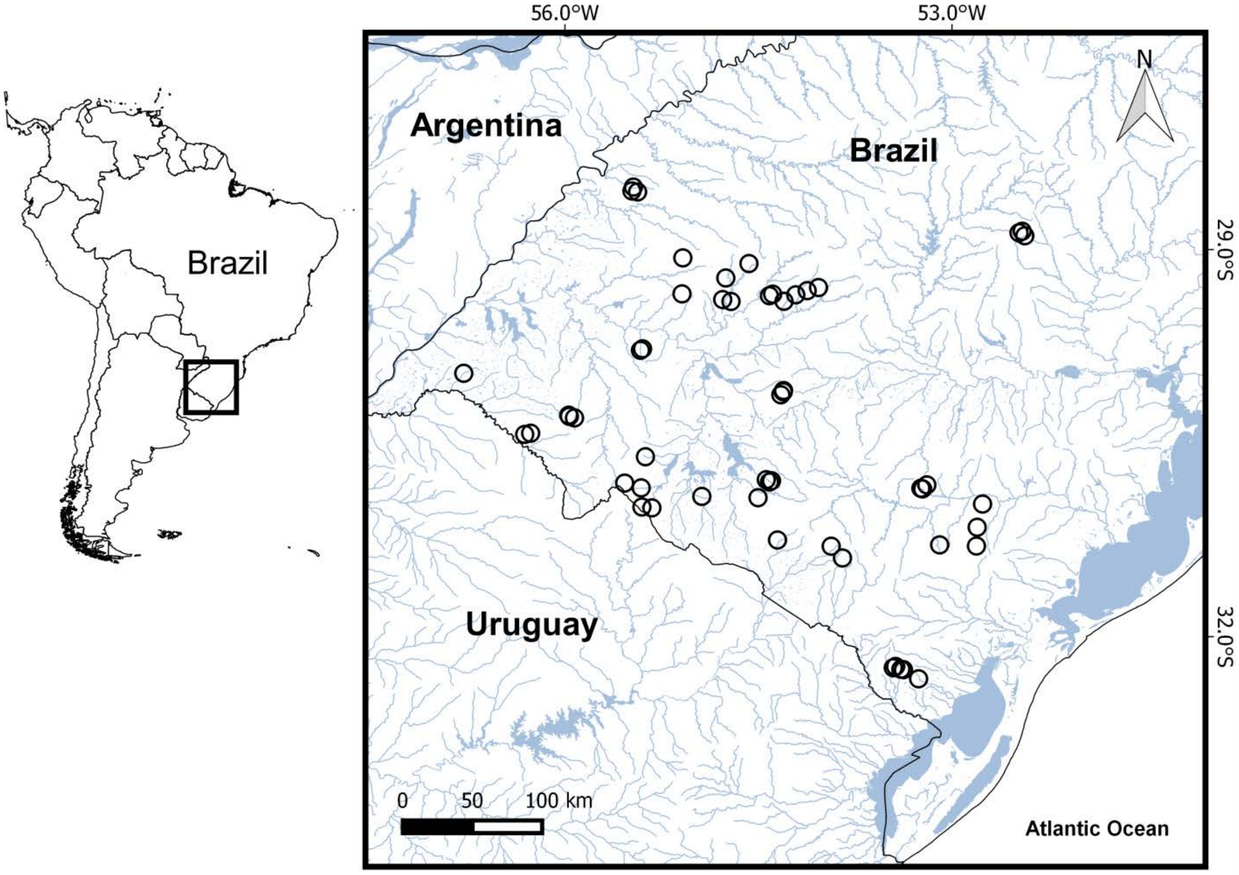

Fig. 1 – Location of the 54 sampled sites in different streams of South Brazilian grasslands region.

Fishes were sampled by performing electrofishing in 150-m long reaches. Sampled reaches always comprised different types of habitats, such as riffles, runs, pools and glides. A single pass electrofishing was carried out in each stream reach with an averaged time of three hours sampling effort. Fishes were anesthetized and subsequently preserved in 4% formaldehyde in accordance with national ethical guidelines (CEUA-UFRGS #24433) and with pre-approved permits (SISBIO #39672-1). Species were identified in laboratory with assistance of taxonomists.

Functional traits

We quantified functional traits of each fish species by measuring morphological traits related to habitat preference and to feeding behavior (e.g. Albouy et al., 2011; Leal et al., 2011). These traits have been useful to study response of fish communities to human-induced disturbances (Dala-Corte et al., submitted). We measured 21 morphometric variables of ten individuals of each fish species whenever possible, or measured all individuals in cases of low abundance species. Then we used these morphological measures to calculate 14 functional traits, which consisted in percentage values relative to body or head sizes and also biomass (see Table S1 and Fig. S1 for more details).

Instream habitat

We followed Kaufmann et al. (1990) and quantified instream physical habitat variables at 11 cross sections along the same 150-m long reaches where we sampled fishes. The 11 measures of each variable were used to calculate mean values of habitat characteristics per sampled reach. Detailed description of the method used can be found in Dala-Corte et al. (submitted). Instream habitat characteristics used included (1) averaged depth; (2) percentage cover of the substrate classes bedrock, boulder, cobble, large pebble, small pebble, sand, silt and hardpan (compacted clay); and (3) percentage cover of microhabitat important for fishes, macrophytes, woody debris, submerged tree roots, leaf litter, hanging vegetation and undercut bank.

Agricultural disturbance indicators

We used three indicators for agricultural disturbances measured at three spatial scales: (1) cropland percentage at catchment scale, (2) local impact, and (3) canopy openness. The last two were obtained from the assessment of the stream margins made during habitat quantification, based on Kaufmann et al. (1999). Local impact was the sum of agricultural influence visually estimated as the percentage cover of agricultural land-use at both stream margins along the 150-m sampled reaches. It included cropland cover percentage and density of signals left by livestock use (trampling, manure and animal visualization) up to 10-m from stream bank at each cross section of the sampled reaches. Canopy openness was the averaged value of 11 repeated measures made with a densiometer along each sampled stream reach. Catchment cropland percentage was estimated based on supervised classifications of land-cover and land-use made on 5-m resolution RapidEye satellite images from year 2012 (Geo Catálogo, 2015). Catchment scale comprised the entire upland area from each sampled site.

Data analyses

Continuous β-diversity

To test taxonomic and functional β-diversity responses to agricultural impact we obtained individual β-diversity values for each one of the 54 sampled sites. We accomplish that by coding a script that arranges sites from the lowest to the highest values of a given environmental gradient, and calculate different measures of β-diversity by comparing each site to its neighbor sites in the environmental gradient. In other words, each site β-diversity value was calculated by comparing community composition among sites having similar values in a (moving) window of the environmental gradient under study. By doing this we can ask whether sites impaired by agriculture have higher or lower β-diversity values than sites with less human alterations. Mainly, this method allowed us to build models using β-diversity as a continuous variable to test against a single or multiple explanatory variables. The function we have coded in the statistical environment R (R Core Team, 2016) is available in supplementary material (Appendix S1). Prior to conduct our analysis, we tested this function using a simulated community with a known sites-by-species distribution in order to guarantee that our results were reliable (Appendix S2; Figure S2).

To obtain beta-diversity values for each sampled site we carried out the following steps: (i) sorted sites according to an environmental gradient vector from lowest to highest values; (ii) sorted sites in the sites by species matrix (or sites by traits matrix when calculating functional diversity) to match the same set of sites ordered accordingly to the environmental gradient ordination; (iii) selected window size, which was the number of neighbor sites to the focal site in the environmental gradient used to compute β-diversity metrics. For example, for a window size of four sites, the selected sites were always the two sites below and the two sites above the focal site with the nearest values in the environmental gradient; (iv) calculated six β-diversity metrics for the selected window (see below); (v) repeated steps iii and iv for the sampled sites to obtain a matrix with individual β-diversity values for each site. Beta diversities were only obtained for focal sites with complete sets of neighborhood sites in the window (i.e. not for focal sites in the extremities of the gradient). To increase site independence, our window size was two. Accordingly, we obtained 54-2=52 beta diversity values.

The following six metrics were used as measures of taxonomic and functional β-diversity: (i) mean.diss.pairs – mean pairwise dissimilarity between sites in the window; (ii) mean.diss.focal – mean dissimilarity between focal site and the other sites in the window; (iii) mean.dist.cent – mean distance of window sites from their centroid group in a principal coordinate space (betadisper, Anderson et al., 2006). Ordination was done once for all the 54 sites and group centroids calculated multiple times using sites in a window; (iv) multi.diss – multiple-site dissimilarities for the window, measured as Jaccard dissimilarity, only calculated for taxonomic presence-absence data (Baselga, 2010); (v) SS.group – total sum of squares (SS) of the window sites (Legendre & De Cáceres, 2013); (vi) SS.focal – Local contributions to beta diversity (LCBD), which represents the degree of uniqueness of the sites in terms of their species compositions or how much a contributed to the total window SS (Legendre & De Cáceres, 2013). We multiplied percentage LCBD values by total window SS to obtain each focal site SS values (SS.focal) (Oksanen et al., 2013). Bray-Curtis dissimilarity was chosen for sites by species matrix and Euclidean was chosen for sites by traits matrix.

Beta-diversity models

We calculated the above described continuous β-diversity metrics for (i) taxonomic composition with log-transformed species abundances; (ii) functional composition with traits weighted by log-transformed species abundances. We selected the Bray-Curtis dissimilarity index to calculate β-diversity for taxonomic composition and Euclidean distance for the functional composition.

First, taxonomic and functional β-diversity responses to agricultural disturbance variables (catchment cropland, local impact and canopy openness) were tested using single non-linear models. We fitted non-linear models (cubic) using each one of the agricultural disturbance variables separately to better visualize the shape of β-diversity responses. This resulted in three models for each one of the six β-diversity metrics for taxonomic β-diversity (in a total of 18 models) and three models for five β-diversity metrics for functional β-diversity (in a total of 15 models). Taxonomic and functional β-diversity resulted in different number of models because it was not possible to use multi.diss metric for calculating functional β-diversity, as multi.diss is only applicable to presence-absence data (such as species by traits matrix). Non-linear cubic model fitting was carried out using the package *splines* (Bates & Venables, 2014) in R environment (R Core Team, 2016).

Later, we constructed multiple linear models including geographic distance and habitat dissimilarity as covariates of agricultural disturbance effects on fish community β-diversity. We included these two variables as controlling variables because they are expected to have a great influence on spatial variation in species composition and thus may hinder the understanding of agricultural land-use effects on β-diversity. For example, sites very distant to each other will share less species than sites near to each other owing to dispersion limitation, whereas habitat characteristics may restrict species occurrences by niche-filtering. Geographical distance variable was calculated with mean.diss.focal metric, using decimal degree coordinates data and Euclidean distance in the same way we calculated β-diversity values. In other words, it was the mean distance of the focal site from the others in a given window of the environmental gradient. Habitat dissimilarity was calculated with SS.group metric using instream habitat variables and Euclidean distance. Model fitting was carried out in R (R Core Team, 2016).

Results

Fish community composition

We found a total of 106 fish species in 54 stream sites across South Brazilian grasslands. Mean species number per site was 18.5 (SD 6.7), ranging from 6 to 32 species. Eight species were highly frequent and occurred in more than 50% of the total sampled sites, including *Heptapterus mustelinus*, *Bryconamericus iheringii*, *Characidium pterostictum*, *Rineloricaria stellata*, *Astyanax laticeps*, *Rhamdia quelen*, *Pseudocorynopoma doriae* and *Crenicichla lepidota*. On the other hand, 67 species had their occurrence restricted to less than 10% of the sampled sites. All the caught fish species are indigenous from the studied region.

Catchment cropland

Univariate models revealed a non-linear subsidy-stress response of taxonomic β-diversity to catchment cropland percentage (Fig. 2). Models with taxonomic β-diversity calculated with metrics that addressed total or mean group/window dissimilarities among sites (mean.diss.pairs, mean.dist.cent, multi.diss, SS.group) yielded more similar responses to catchment cropland percentage, but the same pattern was detected in the models evaluating focal site distance from the other group sites metrics (mean.diss.focal; SS.focal) (Fig. 2). Best model fitting was β-diversity calculated with multi.diss metric ($r^2 = 0.47$; df = 4, 47; $p < 0.001$; Fig. 2d). This pattern was not observed, however, for functional β-diversity (Fig. 3).

In the multiple linear models, taxonomic β-diversity was affected by catchment cropland percentage and by habitat dissimilarity but not by geographical distance: highest explanation model included mean.diss.pairs β-diversity metric (adjusted-$R^2 = 0.43$; df = 3, 48; $P < 0.001$; Table 1). Functional β-diversity was only influenced by habitat dissimilarity.

Local impact

In the single variable models, taxonomic β-diversity was not affected by local impact ($p > 0.05$; Fig. 4). In contrast, functional β-diversity showed a subsidy-stress response shape; although a great variation was observed up to 20% of local impact (Fig. 5). The model with best fit was functional β-diversity calculated with mean.diss.focal metric ($r^2 = 0.37$; df = 4, 47; $p < 0.001$; Fig. 5b).

Multiple linear models showed an influence of geographical distance and local impact (for mean.diss.pairs and mean.dist.cent metrics only), but not of habitat dissimilarity, on taxonomic β-diversity (Table 2): the best fit was with multi.diss metric (adjusted-$R^2 = 0.37$; df = 3, 48; $P < 0.003$). Functional β-diversity was influenced by habitat dissimilarity and local impact (best fit mean.diss.pairs; adjusted-$R^2 = 0.25$; df = 3, 48; $P < 0.001$; Table 2).

Canopy openness

We did not observe influence of canopy openness on taxonomic and functional β-diversities in the univariate models ($p > 0.05$; Figs 6 and 7). Also, canopy openness was not significant in the multiple linear models including habitat dissimilarity and geographical distance to explain functional and taxonomic β-diversity (Table 3). Geographical distance was significant in all the β-diversity models, whereas habitat dissimilarity was significant only for functional traits ($P < 0.05$; Table 3).

Table 1 – Multiple linear models with catchment cropland percentage (cropland), habitat dissimilarity (habitat) and geographical distance (dist.geo) as predictors of taxonomic and functional β-diversities of stream fish communities calculated with different metrics. Significant variables in the models are indicated with an asterisk (*). Adjusted R^2 = adj-R^2; degrees of freedom = df; Overall model probability with F-test = P. Note that multi.diss was calculated only for taxonomic data.

β-diversity	Response variables	Explanatory variables	adj-R^2	df	P
Taxonomic	mean.diss.pairs	(+)cropland*, (+)habitat*, dist.geo	0.43	3, 48	<0.001
	mean.diss.focal	(+)cropland*, (+)habitat*, dist.geo	0.24	3, 48	<0.001
	mean.dist.cent	(+)cropland*, (+)habitat*, dist.geo	0.44	3, 48	<0.001
	multi.diss	(+)cropland*, (+)habitat*, dist.geo	0.31	3, 48	<0.001
	SS.group	(+)cropland*, (+)habitat*, dist.geo	0.40	3, 48	<0.001
	SS.focal	(+)cropland*, habitat, dist.geo	0.11	3, 48	0.032
Functional	mean.diss.pairs	cropland, (+)habitat*, dist.geo	0.10	3, 48	0.042
	mean.diss.focal	cropland, (+)habitat*, dist.geo	0.08	3, 48	0.072
	mean.dist.cent	cropland, (+)habitat*, dist.geo	0.11	3, 48	0.031
	SS.group	cropland, (+)habitat*, dist.geo	0.09	3, 48	0.053
	SS.focal	cropland, (+)habitat*, dist.geo	0.05	3, 48	0.138

Table 2 – Multiple linear models with local impact (local.impact), habitat dissimilarity (habitat) and geographical distance (dist.geo) as predictors of taxonomic and functional stream fish community β-diversity calculated with different metrics. Significant variables in the models are indicated with an asterisk (*). Adjusted R^2 = adj-R^2; degrees of freedom = df; Overall model probability with F-test = P. Notice that multi.diss was calculated only for taxonomic data.

β-diversity	Response variables	Explanatory variables	adj-R^2	df	P
Taxonomic	mean.diss.pairs	(+)local.impact*, habitat, (+)dist.geo*	0.31	3, 48	<0.001
	mean.diss.focal	local.impact, habitat, (+)dist.geo*	0.22	3, 48	0.002
	mean.dist.cent	(+)local.impact*, habitat, (+)dist.geo*	0.32	3, 48	<0.001
	multi.diss	local.impact, habitat, (+)dist.geo*	0.37	3, 48	<0.001
	SS.group	local.impact, habitat, (+)dist.geo*	0.30	3, 48	<0.001
	SS.focal	local.impact, habitat, (+)dist.geo*	0.09	3, 48	0.052
Functional	mean.diss.pairs	(+)local.impact*, (+)habitat*, dist.geo	0.25	3, 48	<0.001
	mean.diss.focal	(+)local.impact*, (+)habitat*, dist.geo	0.23	3, 48	0.001
	mean.dist.cent	local.impact, (+)habitat*, dist.geo	0.25	3, 48	<0.001
	SS.group	local.impact, (+)habitat*, dist.geo	0.23	3, 48	0.001
	SS.focal	local.impact, (+)habitat*, dist.geo	0.10	3, 48	0.049

Table 3 – Multiple linear models with canopy openness (canopy), habitat dissimilarity (habitat) and geographical distance (dist.geo) as predictors of taxonomic and functional stream fish community β-diversity calculated with different metrics. Significant variables in the models are indicated with an asterisk (*). Adjusted R^2 = adj-R^2; degrees of freedom = df; Overall model probability with F-test = P. Notice that multi.diss was calculated only for taxonomic data.

β-diversity	Response variables	Explanatory variables	adj-R^2	df	P
Taxonomic	mean.diss.pairs	canopy, habitat, (+)dist.geo*	0.29	3, 48	<0.001
	mean.diss.focal	canopy, habitat, (+)dist.geo*	0.22	3, 48	0.002
	mean.dist.cent	canopy, habitat, (+)dist.geo*	0.30	3, 48	<0.001
	multi.diss	canopy, habitat, (+)dist.geo*	0.27	3, 48	<0.001
	SS.group	canopy, habitat, (+)dist.geo*	0.31	3, 48	<0.001
	SS.focal	canopy, habitat, (+)dist.geo*	0.12	3, 48	0.030
Functional	mean.diss.pairs	canopy, (+)habitat*, (+)dist.geo*	0.25	3, 48	<0.001
	mean.diss.focal	canopy, (+)habitat*, (+)dist.geo*	0.23	3, 48	0.002
	mean.dist.cent	canopy, (+)habitat*, (+)dist.geo*	0.26	3, 48	<0.001
	SS.group	canopy, (+)habitat*, (+)dist.geo*	0.22	3, 48	0.002
	SS.focal	canopy, (+)habitat*, dist.geo	0.13	3, 48	0.022

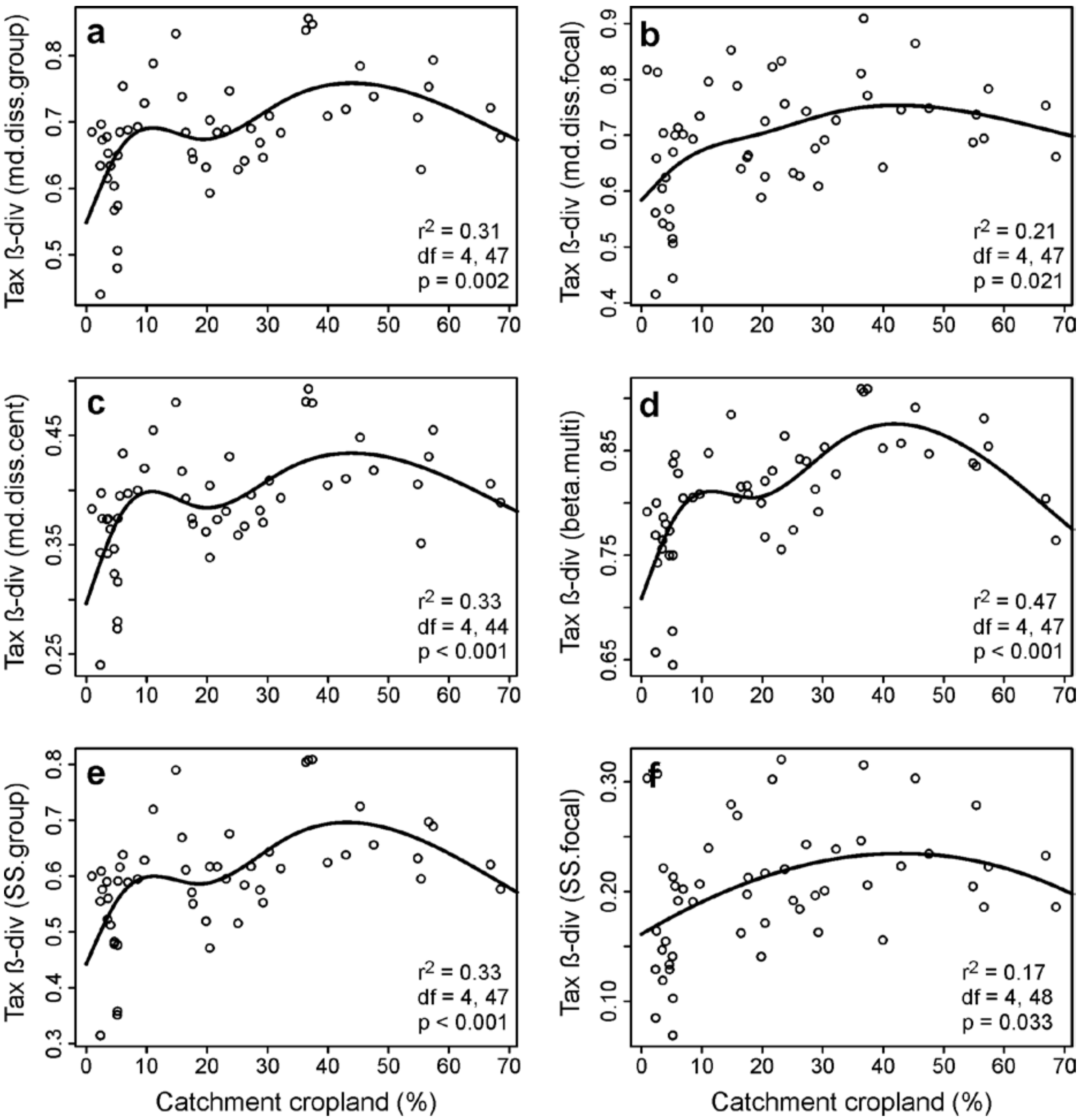

Fig. 2 – Subsidy-stress response of different taxonomic β-diversity (Tax β-div) metrics to cropland percentage measured at catchment scale for 54 sampled streams in South Brazilian grassland region.

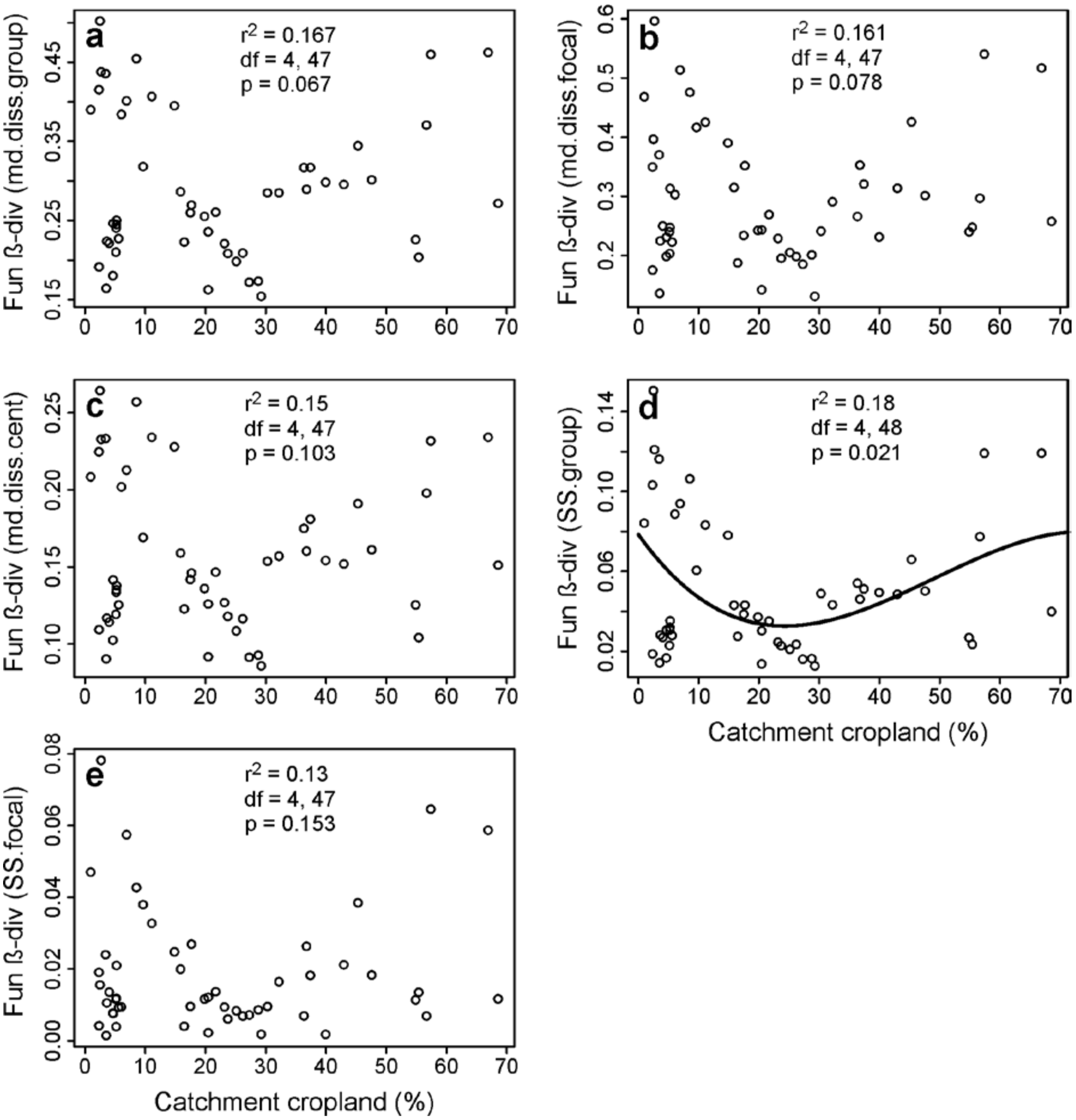

Fig. 3 – Relationship between functional β-diversity (Fun β-div) metrics and cropland percentage measured at catchment scale for 54 sampled streams in South Brazilian grassland region.

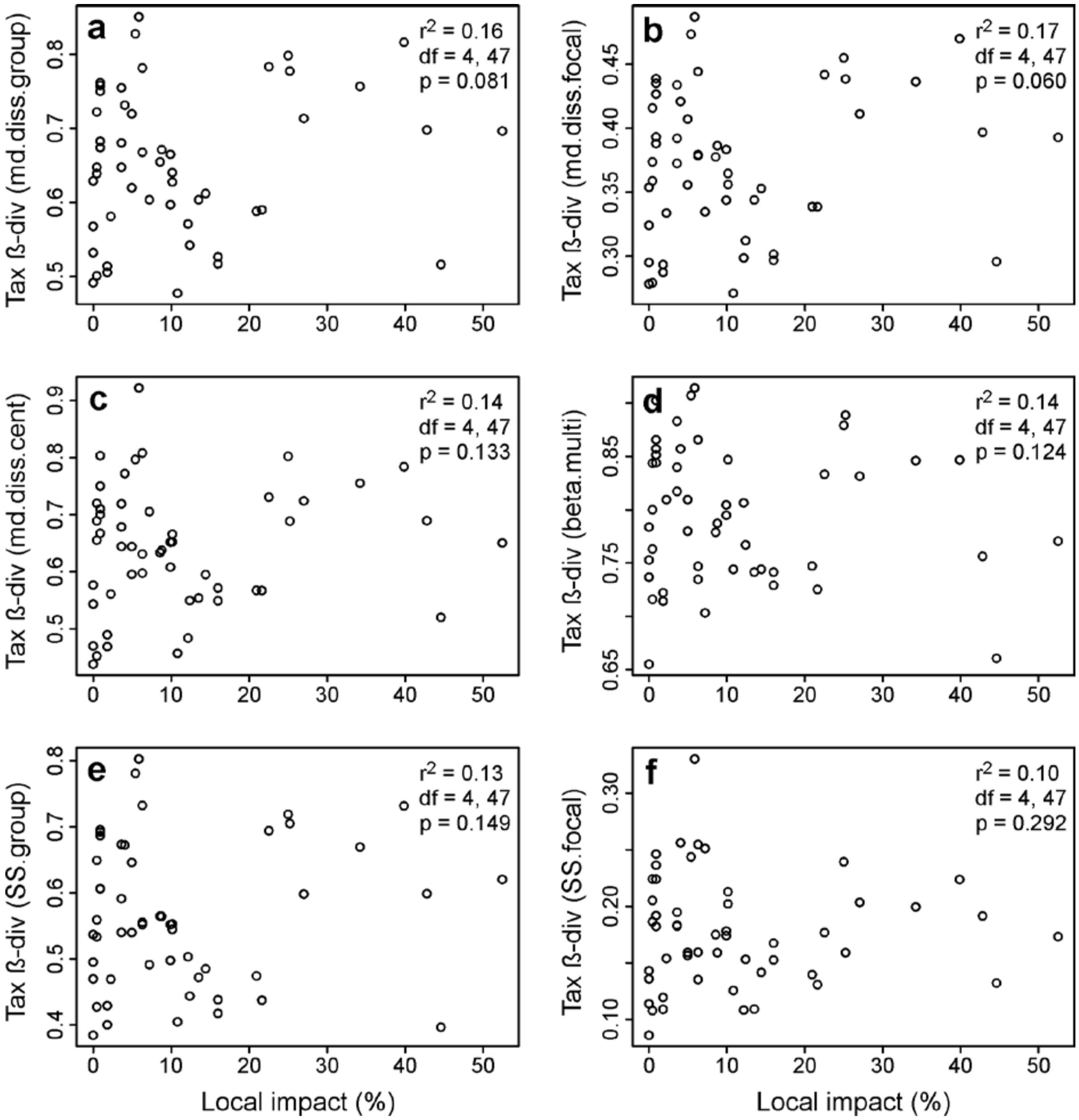

Fig. 4 – Relationship between different taxonomic β-diversity (Tax β-div) metrics and local impact (streamside crops and livestock signals) for 54 sampled streams in South Brazilian grassland region.

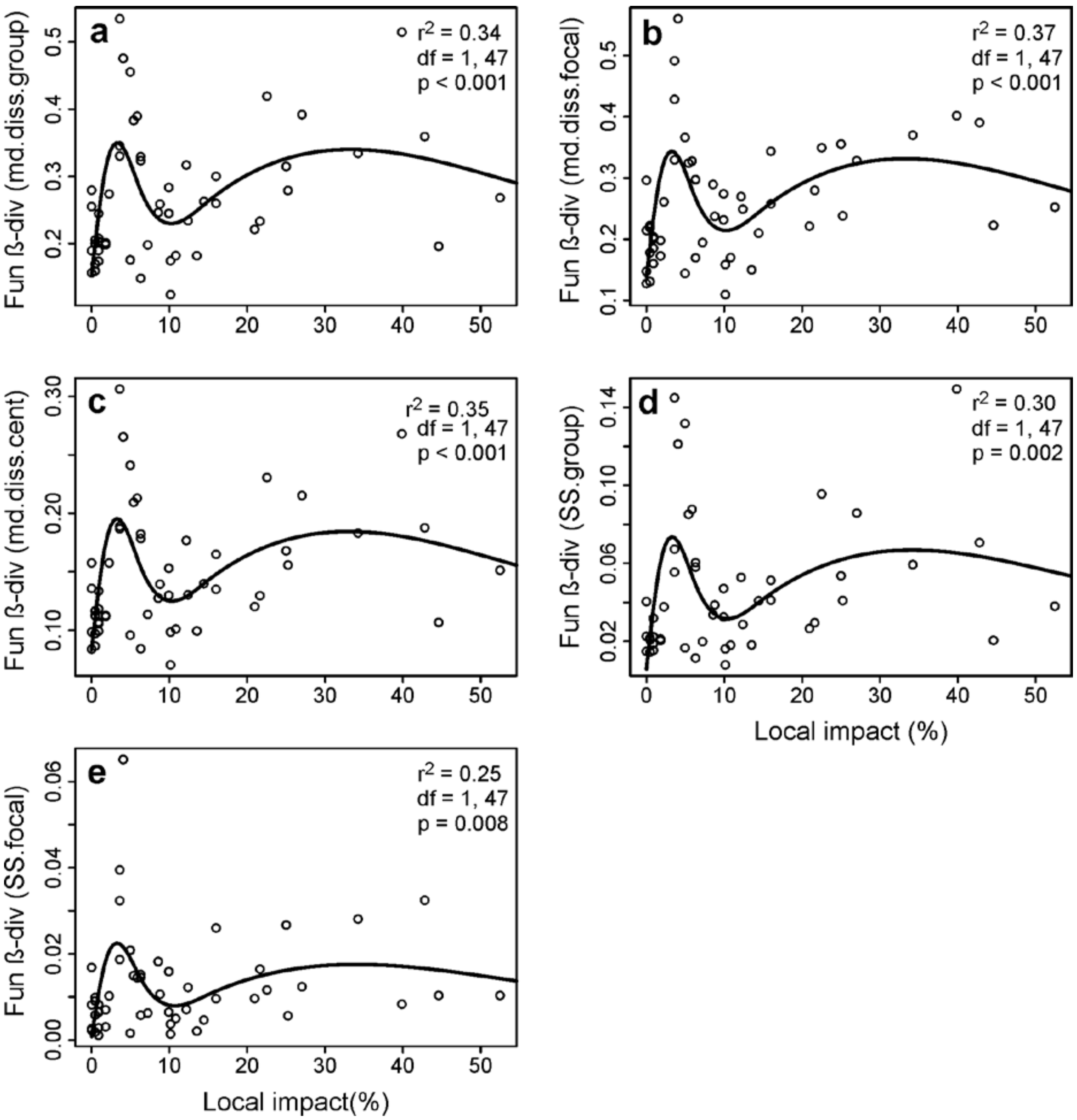

Fig. 5 – Relationship between functional β-diversity (Fun β-div) metrics and local impact (streamside crops and livestock signals) for 54 sampled streams in South Brazilian grassland region.

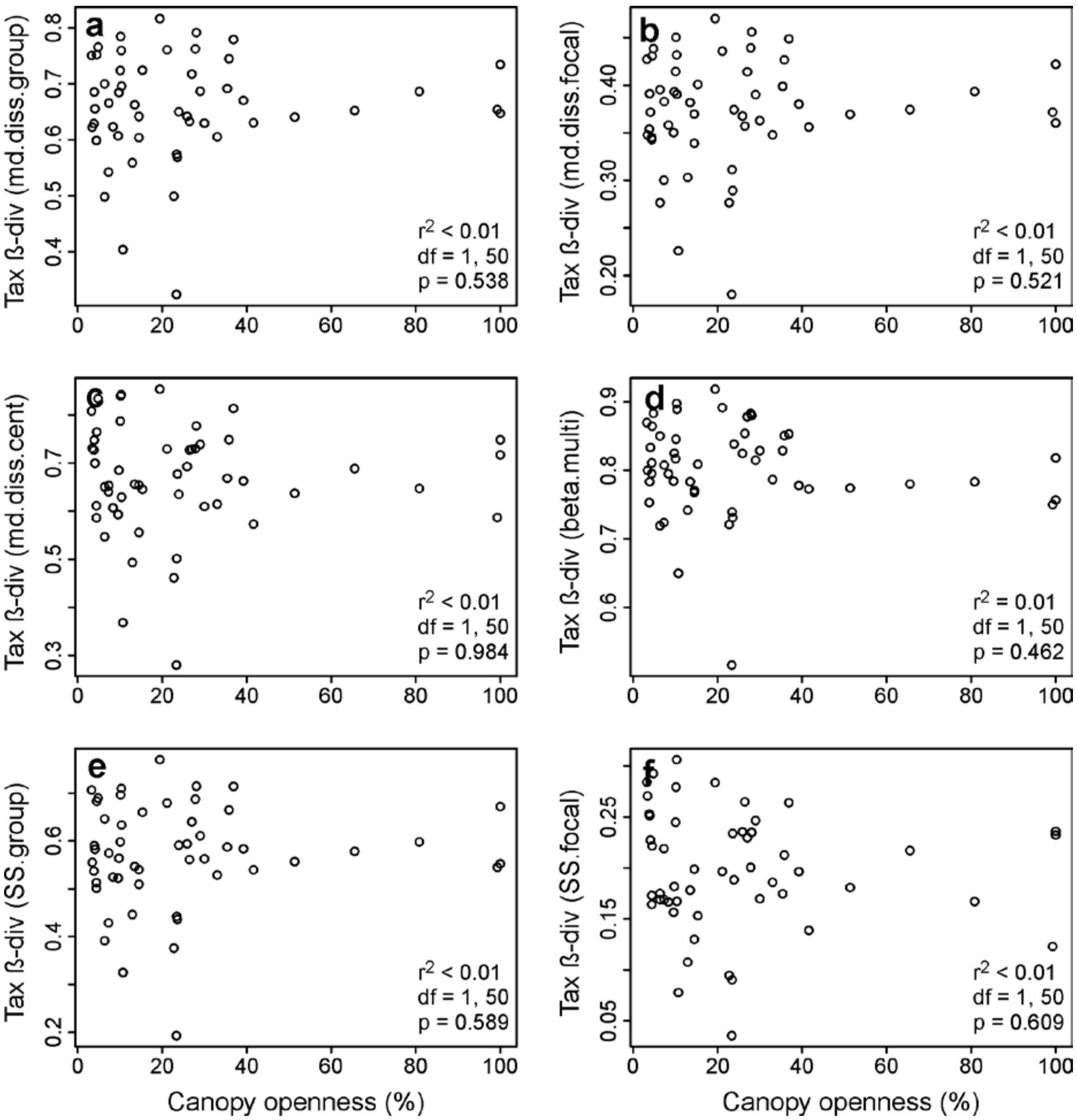

Fig. 6 – Relationship between different taxonomic β-diversity (Tax β-div) metrics and canopy openness for 54 sampled streams in South Brazilian grassland region.

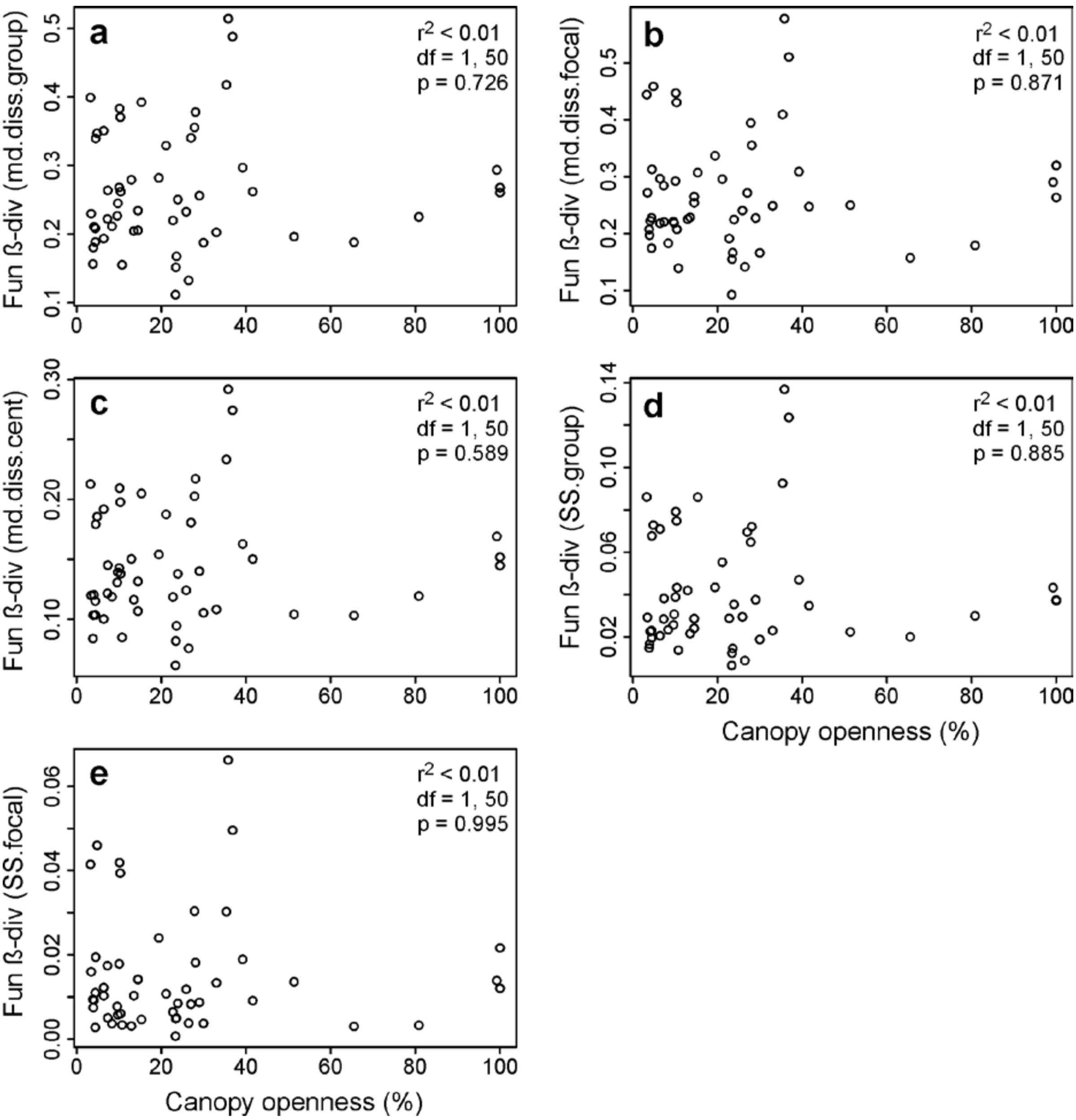

Fig. 7 – Relationship between functional β-diversity (Fun β-div) metrics and canopy openness for 54 streams sampled in South Brazilian grassland region.

Discussion

We found evidence that both taxonomic and functional differentiation among stream fish communities were influenced by agricultural land-use. Our results indicated that agricultural percentage at catchment scale and local impact drive subsidy-stress response of taxonomic and functional β-diversities, respectively. Low percentages of agricultural development gradually increased taxonomic β-diversity up to a plateau, from which we observed a gradual decline of β-diversity values as agriculture percentage increased. This indicates a threshold around 40% at which agricultural development starts to have negative effects on stream fish taxonomic β-diversity. For functional β-diversity response to local impact, the threshold was around 30%.

Subsidy-stress response of fish community β-diversity found here may result from different processes affected by agricultural development. Agriculture percentage at catchment scale, measured as the entire drainage area upstream from sampled site, is an indicator of several disturbance factors to streams (Morley & Karr, 2002). For instance, agriculture land-use has been associated to nutrient enrichment, fine sediment deposition and increased temperature (Allan, 2004; Niyogi et al., 2007; Macedo et al., 2013; Burrell et al., 2014). Although these variables can be stressful for some fish species, especially for those found in clear and cold waters and benthic specialists of rocky substrates (Jones et al., 1999; Walser & Bart 1999; Dala-Corte et al., submitted), various other slow-water species or those typically found in larger downstream segments may be favored by these modifications (Casatti et al., 2015; Teresa et al., 2015; Dala-Corte et al., submitted). In fact, studies have found that agricultural disturbance may trigger a phenomenon called native invasion, which consisted of an increase in local species number from the regional pool species (Scott & Helfman, 2001;

Lorion & Kennedy, 2009; Teixeira-de Mello et al., 2015). To our knowledge, the effects of native invasion on fish beta diversity is yet to be studied.

The subsidy-stress response found here for stream fish β-diversity to agricultural land-use may be explained by alterations in the balance between stochastic and deterministic processes driving community assembly. It is hypothesized that community β-diversity is influenced by the strength of ecological filters affecting the relative importance of stochastic (such as colonization and extinction) versus deterministic factors (niche filtering) on community assembly (Chase, 2007; 2010). For example, experimental ponds with low productivity or submitted to frequent drought have shown reduced β-diversity, whereas ponds with high productivity or with permanent water had increased community dissimilarity (Chase, 2007; 2010). The possible underlying mechanism behind this pattern may be something similar to the priority effect, which is the effect that a given species can have on community due to its prior arrival or colonization (Young et al., 2001). Many fish species can be favored by agricultural environmental modifications if they are not stressful, such as low-levels of nutrient and sediment enrichment, and increased temperature. This may allow a higher number of species to occur, those of native invasion phenomenon (Dala-Corte et al., submitted). If these environmental modifications lead to a more relaxed environmental filtering, then stochasticity will play a more important role on community assembly, increasing among-stream β-diversity (the subsidy part of the curve). On the other hand, when agriculture reaches a level at which environmental modifications become stressful for most of the fish species, at which only a reduced number of fish species can live (same taxa and function), it imposes a stronger environmental filter to species occurrence. Thus among-stream fish β-diversity will decreases due to deterministic processes (the stress part of the curve). Whereas modification in the strength of stochastic versus deterministic processes driving community assembly is a plausible explanation for our results, further studies are needed to test this hypothesis.

Taxonomic β-diversities had a positive response to agriculture development up to 40% of the catchment area and a negative response when agriculture exceeded this threshold. On the other hand, functional β-diversity started to decline when local impact exceeded 30%. The proximity of streams may be important to determine the threshold point at which human alterations cause negative effect on β-diversity. For example, the impacts on stream ecosystems may be exacerbated if agriculture reaches riparian zone (Lowrance et al., 1984; Giam et al., 2015; Dala-Corte et al., submitted). The different response of taxonomic and functional β-diversities highlights the importance of addressing different community aspects when focusing on understanding human effects on biodiversity.

At low values of catchment agriculture and local impact (up to 20%), we found a great variability in β-diversity values (highlighted by the first mode in the subsidy-stress curve) indicating minor effects on faunal differentiation. In this case, other factors we also found relevant to explain regional variation in community composition, such as geographical distance and habitat dissimilarity, may be playing a more important role on fish β-diversity. As agricultural development raises (>20%), it influences on fish community β-diversity started to increase, and at high percentages (>40%) a negative effect was observed. However, these effects may be dependent on whether farmers adopt best management practices to reduce agricultural impacts (e.g. riparian fencing) or traditional techniques (Wang et al., 2002; 2006). For instance, in another study, we found stronger effect of local impact (alterations on streamside vegetation) on habitat and fish community α-diversity (Dala-Corte et al., submitted); and herein we found evidence that threshold of negative effects of local impact on fish β-diversity occurs at lower percentage. Testing whether best management practices alter threshold at which agriculture causes negative effects on stream fish β-diversity will greatly contribute with information to subsidy aquatic conservation policies.

Rather than evaluate whether faunal composition change or not along agricultural gradients, we were interested here in uncover the shape of β-diversity response to agricultural disturbance. Even if agricultural development at low levels (<40%) has a positive effect on fish β-diversity, as our evidences suggest, this increased compositional dissimilarity may still result from changes in the natural fish species composition expected to be found in pristine streams. Therefore, other community characteristics important for ecosystem functioning and for conservation not evaluated by us, such as unique functions or endemic species, which are often found in pristine streams, can still be negatively affected by low amounts of agricultural disturbances.

Our findings indicate β-diversity of stream fish community responds as subsidy-stress to agricultural land-use. Our study adds new evidences on how agriculture-induced disturbances affect diversity patterns of stream ecosystem at regional (among-streams) scale, which have implications on the understanding of how much change agriculture have already left on diversity of fish communities and for planning conservation strategies. Taxonomic β-diversity decreased only when cropland cover exceeds about 40% of the total catchment area, and functional β-diversity decreased when local impact exceeded 30%. This suggests that avoiding agricultural land-use to exceed 40% of the catchment areas and protecting riparian zone could be useful to prevent faunal homogenization of stream fish communities, at least for small catchments (<50 km^2).

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Supplementary material

Appendix S1. Function coded in R to calculate six metrics of continuous β-diversity:

```
#Function betaRegDisp (continuous β-diversity)
#Y must be a matrix where rows are sites and species columns.
#X must be a vector of environmental gradient under study.
#n must be the window size, i.e. the number of sites to compute distances.
#method is the dissimilarity used to β-diversity metrics (see vegdist() options).

#Warning: multi.diss is calculated only for presence/absence data. taxonomic.data=T must be specified.

betaRegDisp <- function(Y, X, n=2, method="euclidean", taxonomic.data=F){
   require(vegan)
   require(betapart)
   Y <- Y[order(X, decreasing = F), ]
   X <- X[order(X, decreasing = F)]
   size <- length(X)
   result <- matrix(0,size,7)
   colnames(result) <- c('grad','mean.diss.pairs','mean.diss.focal','mean.dist.cent','multi.diss',
                                     'SS.group','SS.focal')
   result[,'grad'] <- X
   rownames(result) <- names(X)
   disT <- vegdist(Y, method=method)
   N <- n/2
   x11()
   for(i in (N+1):(size-N)){
      group <- rep('B',times = size)
      sites <- (i-N):(i+N)
      sites <- c(i, sites[sites!=i])
      plot(1:size, X)
      points(sites, X[sites], cex=2, col='blue')
      points(i, X[i], cex=3, col='red', pch=0)
      group[sites] <- 'A'
      mat <- Y[sites,]

      #Mean dissimilarity
      dis <- vegdist(mat, method=method)
      result[i,'mean.diss.pairs'] <- mean(dis) #Mean for all sites
      result[i,'mean.diss.focal'] <- mean(dis[1:n]) #Mean for only focal site

      #beta disper of Anderson et al (2006)
      #Distance from group centroid
      mod <- betadisper(disT, group = group)
      d <- mod$distances
      result[i,'mean.dist.cent'] <- mean(d[group=="A"])

      #beta multi of Baselga (2010)
      #Overall beta diversity measured as Jaccard dissimilarity
      #It's only computed if taxonomic data=T
      #Only usefull for species/site matrix
      if(taxonomic.data==T){
         beta.b <- beta.multi(ifelse(mat>0 , 1, 0), index.family="jaccard")
         result[i,'multi.diss']<-beta.b$beta.JAC
      }
```

```
    #Sum of Squares (SS) of Legendre and De Caceres 2013 for the window
    source ('beta.div.R') #Load function downloaded from
    #http://adn.biol.umontreal.ca/~numericalecology/Rcode/
    #Also available in Legendre and De Caceres (2013)
    #Total Group SS
    res.SS <- beta.div(mat, ifelse(method=="euclidean","euclidean","hellinger"), nperm=9)
    result[i,'SS.group'] <- res.SS$SStotal_BDtotal[2]

    #Site contribution to total group SS
    result[i,'SS.focal'] <- res.SS$LCBD[1]*res.SS$SStotal_BDtotal[2]

  }

  result <- result[(N+1):(size-N),]
  return(result)
}
```

Appendix S2. We tested the reliability of our continuous β-diversity function and calculated metrics by coding a function that simulates communities with the same sample size, total species richness and a fixed species richness values per site, which was the mean species richness found in our study. The function randomly sample the occurrence of species at each site, from the first to the last sampled site. At each round, possibility of occurrence of species not shared with other sites increases. For instance, in the first site, species occurrence was restricted to only the first 18 species, whereas in the last site species occurrence could occur at random in any one of the 116 species. Coded function is the following:

```
simComu <- function(su=54, ric=18, Stot=116){
  #su = sampling size
  #ric = richness in each su
  #Stot = total richness
  quais <- seq (ric,Stot,length.out = su)
  teste <- matrix(0,ua,Stot)
  for(i in 1:su){
    teste[i, sample(1:quais[i],ric)] <- 1

  }
  rownames(teste) <- paste("a", 1:su, sep="")
  return(teste)
}
```

Table S1. Functional traits indicative of habitat use/occupation and feeding behaviour of fish species used to calculate functional β-diversity for stream fish communities. See Fig. S1.

Trait	**Equation**	**Function**
Body compression	$\frac{\mathrm{BW}}{\mathrm{BD}}$	Related to manoeuvrability. Compressed bodies are usually found in lentic waters (Watson & Balon 1984).
Body depth	$\frac{\mathrm{BD}}{\mathrm{SL}}$	Inversely related to flow velocity and it determines the ability to perform vertical movement in the water column (Gatz 1979).
Head size	$\frac{\mathrm{HL}}{\mathrm{SL}}$	Related to prey size (Gatz 1979).
Eye position	$\frac{\mathrm{EH}}{\mathrm{HD}}$	Indicates vertical habitat preference (Gatz 1979). Varies from 0 to 1. High values mean superior eyes.
Eye size	$\frac{\mathrm{ED}}{\mathrm{HL}}$	Indicates the importance of vision for feeding (Gatz 1979). Values relative to head size.
Mouth position	$\frac{\mathrm{MH}}{\mathrm{HD}}$	Indicates vertical position at which fish forages (Albouy *et al*. 2011). Varies from 0 to 1. High values mean superior mouth.
Peduncle length	$\frac{\mathrm{CpL}}{\mathrm{SL}}$	Longer caudal peduncles indicate good swim ability (Gatz 1979).
Peduncle compression	$\frac{\mathrm{CpH}}{\mathrm{CpW}}$	Compressed causal peduncles indicate poor swim activity (Gatz 1979).
Pectoral position	$\frac{\mathrm{PfP}}{\mathrm{BD}}$	Related to manoeuvrability (Dumay *et al*. 2004). High values mean dorsal located pectoral fins in relation to the most ventral body part.
Pectoral fin area	$\frac{\mathrm{PfL} \times \mathrm{PfH}}{\mathrm{SL}}$	High values are indicative of benthic habit species, where pectoral fins are used as anchors to stand in fast water (Watson & Balon 1984).
Ventral fin area	$\frac{\mathrm{VfL} \times \mathrm{VfH}}{\mathrm{SL}}$	Large ventral fins are used as support for benthic species (Casatti & Castro 2006).
Caudal fin area	$\frac{\mathrm{VfL} \times \mathrm{VfH}}{\mathrm{SL}}$	Large caudal fins are associated to high propulsion (Gatz, 1979).
Dorsal fin area	$\frac{\mathrm{DfL} \times \mathrm{DfH}}{\mathrm{SL}}$	Fish that prefer fast flow has usually small dorsal fins (Casatti & Castro 2006).
Biomass	$\mathrm{Weight}(g)$	Indicates contribution to the system via metabolism (Albouy *et al*. 2011).

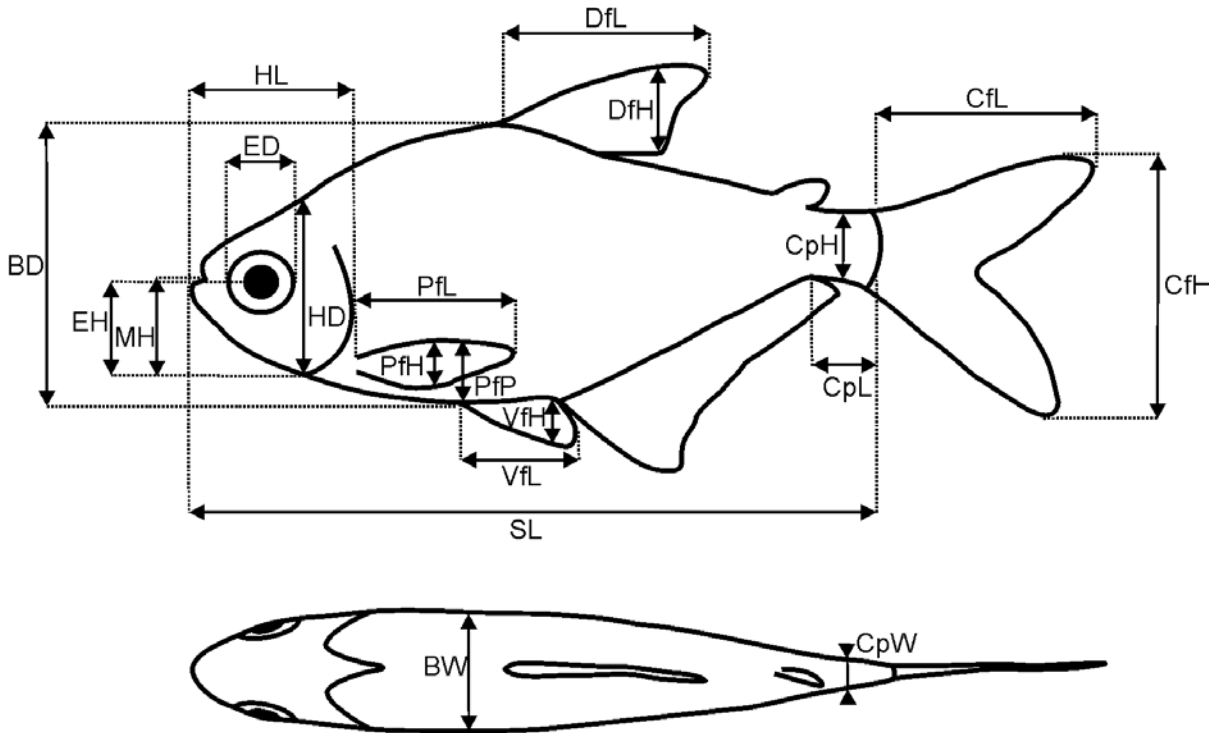

Figure S1. Illustration of the morphometric measures used to calculate 14 functional traits related to habitat use and feeding behaviour. SL = Standard length; BD = Body depth; BW = Body width; HL = Head length; HD = Head depth; ED = Eye diameter; EH = Eye height; MH = Mouth height; PfL = Pectoral fin length; PfH = Pectoral fin height; PfP = Pectoral fin position; VfL = Ventral fin length; VfH = Ventral fin height; DfL = Dorsal fin length; DfH = Dorsal fin height; CpL = Caudal peduncle length; CpH = Caudal peduncle height; CpW = Caudal peduncle width; CfL = Caudal fin length; and CfH = Caudal fin height.

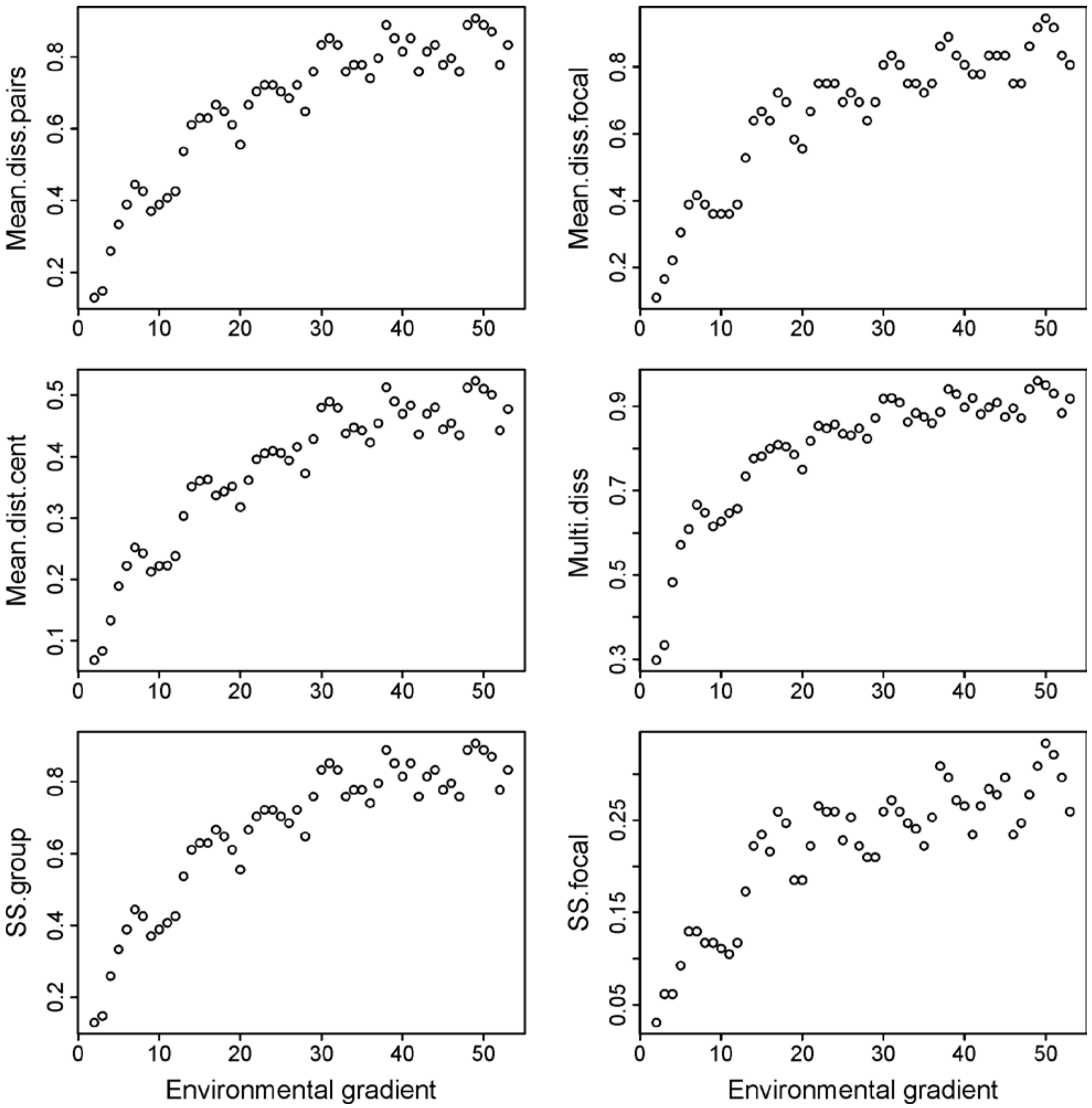

Figure S2. Plots of the six β-diversity metrics calculated with a simulated community with a known decrease in the number of shared species along a continuous environmental gradient.

CAPÍTULO 3 - The importance of metacommunity processes for long-term turnover of riffle-dwelling fish assemblages depends on spatial position within dendritic network*

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Abstract: Spatial position within dendritic network may determine environmental filters and connectivity with source of immigrants, influencing species composition and variation in metacommunities. We investigated how long-term turnover of riffle-dwelling fish assemblages is affected by niche- and dispersal-related processes. Multiple linear model selection resulted in four variables important to explain assemblage turnover: (i) habitat change, (ii) channel slope, (iii) dlink - a proxy for connectivity - and (iv) catchment area. These four variables were related to turnover in species abundance, while turnover in species occurrence was only related to habitat change. Models generated for each species indicated they were differently affected by niche- and dispersal-related processes. Our findings provide support for the hypothesis that metacommunity processes affecting temporal turnover of stream fish communities are dependent on the spatial position within dendritic network, but also vary among species. Our study has implications for sampling design in monitoring programs, as the degree of dissimilarities in fish communities depends not only on local habitat change, but on spatial position within the drainage network and on species particularities.

Introduction

Long-term variation in species occurrence and population density is a central theme in ecology and allows understanding and predicting how communities will behave facing particular environmental conditions (Magurran et al. 2010). Changes in species occurrence and abundance between years have been widely studied in stream ecosystems and linked mainly to variations in environmental conditions (e.g., Mykrä et al. 2011; Matthews et al.1988, 2013). This seems to be of particular importance in stream ecosystems, as environmental conditions may be highly variable in time due to slow and continuous processes (erosion, deposition) and to episodic disturbances caused by drought and spates that lead to channel reconfiguration (e.g., Matthews et al. 1988; Grossman et al. 1998; Matthews and Marsh-Matthews 2006). However, less attention has been given to the fact that flow regime and the severity of hydrological disturbances may depend on the spatial position within a drainage network (the “Process Domain Concept”, Montgomery 1999) and that its spatial structure affects the dispersal of aquatic organisms (Hitt and Angermeier 2008; Altermatt 2013). According to the Process Domain Concept, “spatial variability in geomorphic processes governs temporal patterns of disturbances that influence ecosystem structure and dynamics”.

The importance of metacommunity theory to explain patterns and processes of freshwater assemblages in dendritic networks has received increasing attention (Altermatt 2013; Heino 2013; Heino et al. 2015). Metacommunity theory posits that local assemblages are determined by i) environmental and biotic (e.g. competition) filtering, operating at different spatial scales, ii) dispersal limitation dependent on the spatial connectivity and on dispersal abilities of species, and iii) neutral processes (Leibold et al. 2004; Heino et al. 2015). In this sense, local assemblages are recognized as open systems that are dependent on other local communities. For instance, dispersal may influence population size and preclude local species extinctions in sub-optimal or sink patches (Leibold et al. 2004).

Metacommunity theory has been more broadly used to explain spatial patterns in species occurrence and in population abundance of local assemblages, in both terrestrial (Schmidt et al. 2008; Driscoll and Lindenmayer 2009; Myers et al. 2013) and dendritic aquatic networks (e.g. Thompson and Townsend 2006; Perkin and Gido 2012; Altermatt 2013). However, it can also address processes affecting the temporal dynamics of local assemblages (Leibold et al. 2004). For instance, metacommunity theory can be evoked to predict which communities will be more or less stable between years and over mid- to long-term periods (i.e., more than 10 years). Communities well connected in a network should receive many migrants that buffer against demographic stochasticity and allow fast recovery from disturbance events.

The hydrological regime of riverine ecosystems, including flood and drought disturbances, is probably the most important factor driving temporal variation in stream habitats and faunal composition (Poff and Ward 1989; Grossman et al. 1990; Matthews et al. 2013). Variation in flow regime can affect immigration and extinction rates of stream fishes (Taylor and Warren 2001). Due to the spatially structured geomorphologic characteristics of river basins, upstream segments of mountainous streams are expected to have steep slopes, entrenched and straight channels, to retain large substrates and to experience high stream flow energy (Rosgen 1994; Montgomery 1999; Rice et al. 2001). Also, steep channels will respond more quickly to rainfall and will produce higher and shorter peak discharges compared to streams with gentle slopes in the lower section of watersheds (Resh et al. 1988; Gordon et al. 2004; Nippgen et al. 2011). Therefore, fish assemblages in stream segments with steep channels will be subjected to strong environmental filtering owing to continuous high flow energy and to severe hydrological disturbances caused by spates, which may influence temporal turnover of fish assemblages (changes in species occurrence and population abundance).

A second important aspect associated with the dendritic structure of stream networks is that the faunal movement is highly dependent on the connectivity between branches of the network and on how the network structure is spatially organized (Benda et al. 2004; Altermatt 2013). In contrast to dispersal in terrestrial landscapes, fish and other aquatic species are constrained to disperse through branches and confluences (or edges and vertices) of the stream network (Fagan 2002; Altermatt 2013; Altermatt et al. 2013). Two particular features of such dendritic structure are that small streams can be connected to rivers of different sizes depending on their location within the basin (central or peripheral), and within a given stream there is a longitudinal distance gradient from the connection to the mainstem channel (Fausch et al. 2002; Campbell-Grant et al. 2007). The influence of the dendritic structure of drainage networks on spatial variation in occurrence and abundance of fish species has been demonstrated (Osborne and Wiley 1992; Grenouillet et al. 2004; Thornbrugh and Gido 2010). A likely mechanism for such influence is that large rivers with increased habitat capacity act as sources of migrants, altering local richness and community structure of tributaries (Osborne and Wiley 1992). Accordingly, the distance of a stream segment from its downstream confluence with a large stream has been shown to be important for local composition of stream communities (Thornbrugh and Gido 2010). Also, centrally located stream segments are more connected to other habitat patches and to other fish populations distributed across the drainage network. Thus, isolated stream habitats in the periphery of the basin are expected to receive fewer migrants compared to more connected stream habitats in the center of the basin (Taylor 1997). Even if the extinction rates are similar between peripheral and central sites, the populations in the latter are more likely to be ‘rescued’ from local extinction by high immigration rates. Additionally, immigration rates have been demonstrated to increase with stream size (Taylor and Warren 2001). This effect may be dependent on the combined effect of patch size and dendritic connectivity of drainage networks, which increase simultaneously in more centrally located sites (Carrara et al. 2014). Therefore, we should expect that distance from sources of colonists may lead to differences in temporal turnover of assemblages regarding species occurrence and abundance.

Considering the influences that niche- and dispersal-related processes may have on fish assemblages in dendritic networks, we investigated how long-term turnover of riffle-dwelling fish assemblages is affected by habitat change, channel slope, and spatial position within drainage network. We used habitat and slope variables as proxies for niche-related processes and spatial position variables as proxies for dispersal-related processes. We hypothesized that (1) large changes in instream physical habitat structure (i.e. changes in environmental conditions) should lead to high temporal turnover, (2) high-gradient channels should lead to high temporal turnover due to severe stochastic flood events, and (3) proximity to a large stream should lead to low temporal turnover of riffle-dwelling fish assemblages due to a high influx of downstream immigrants that dampens disturbance effects. We addressed these hypotheses using 22 stream fish assemblages sampled 13 years apart in a subtropical catchment.

Methods

Study area

This study was conducted in the Maquiné river basin (total area ca. 550 km^2), an Atlantic coastal drainage located in southern Brazil between longitudes 50°05’W and

51°21’W and latitudes 29°20’S and 29°50’S (Fig. 1). Headwaters of the river Maquiné are located on a basalt plateau (ca. 1000 m above sea level) and its final segment flows into lake Quadros (ca. 10 m above sea level). Regional climate is humid subtropical (Moreno 1961) with mean temperatures higher than 22°C in the warmest month and between 13°C and 15°C in the coldest month (Hasenack and Ferraro 1989). There is no typical dry season and annual rainfall is high (1400 mm to 1800 mm) (Hasenack and Ferraro 1989). Rain storms are common over the year (mainly in spring and summer) resulting in high-energy flash floods, which physically affect stream habitats (Becker et al. 2008). The pronounced elevation profile of the Maquiné basin and the steep and entrenched stream channels (mainly in its upper and intermediate segments), contribute to episodes of harsh hydrological conditions for the fish fauna.

Sampling sites

Fish sampling was conducted in 1999 and 2012. There were at least three catastrophic flood events in the period caused by concentrated rainfall that physically affected stream habitats: 206 mm in three days (2001), 172 mm in two days (2007) and 306 mm in three days (2008). There was more accumulated rainfall in one day in the 2008 event than in any other day between 1999 and 2012 period, exceeding the mean rainfall expected for an entire month (Appendix, Fig. A1). In fact, two of the authors (ASM, FGB) were able to visit the area immediately after the 2008 intense rains and observed a severe flood episode that caused profound channel reconfiguration in some of the stream segments, particularly those in low valley areas (< 250 m). Average monthly rainfall of the two sampling years were similar to each other (131 mm for 1999 and 123 mm for 2012) and to the average monthly rainfall of the entire 13 years sampling interval (1999 to 2012; mean = 145 mm; Appendix, Fig. A1).

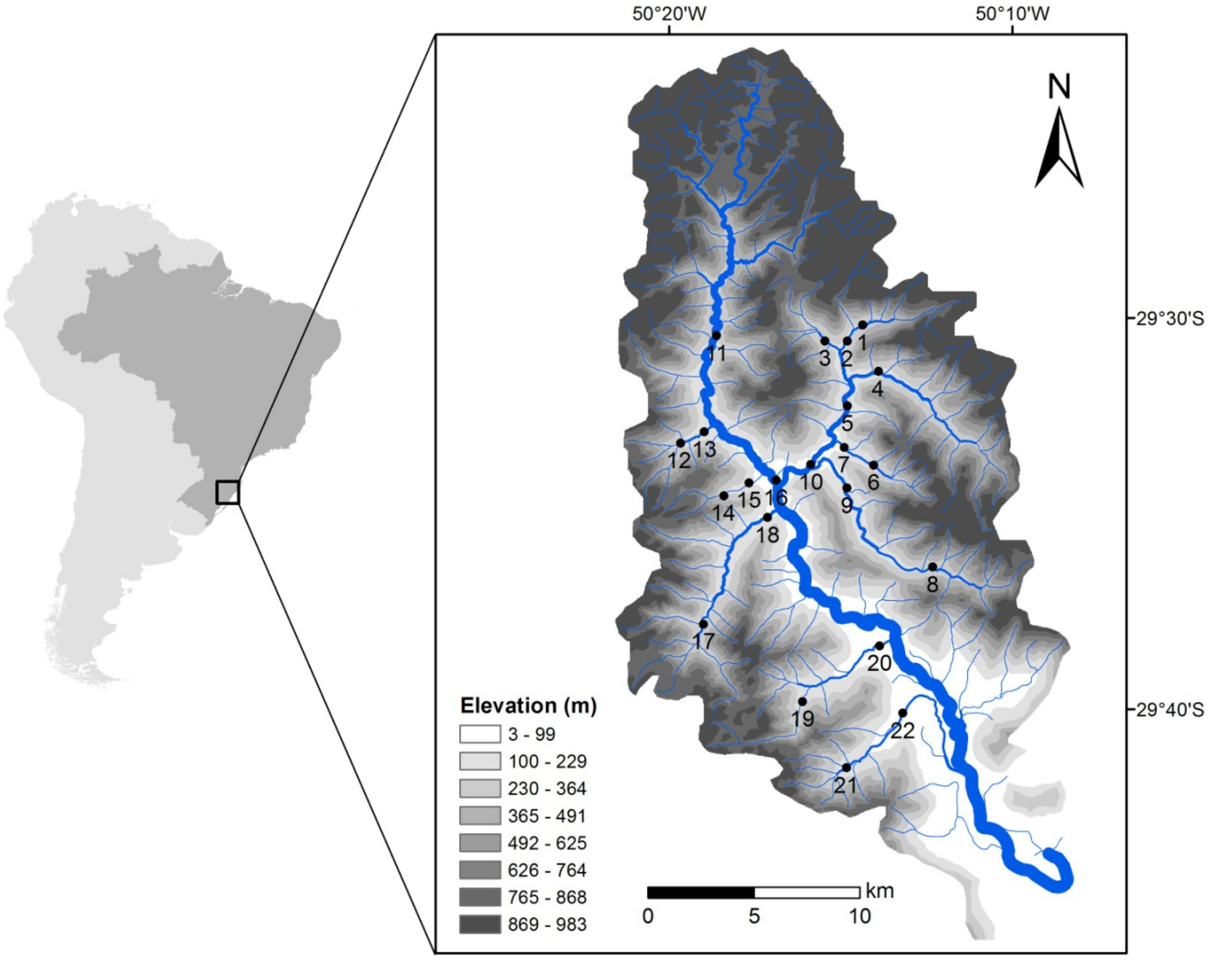

Fig. 1. Sampling site location in the Maquiné river basin, southern Brazil. The hydrographic line thickness is proportional to stream size. Note the abrupt change in steep elevation within a short distance from headwater to lower valleys. Sampling was restricted to lower valley sections (< 250 m of elevation).

Streams of the Maquiné basin are composed of riffle–pool sequences with clear and fast-flowing water and substrate composed mainly of pebbles, cobbles and boulders (Becker et al. 2008). We sampled riffle habitats with an average depth of 22 cm and average wetted width of 8.8 m (ranging from 4.0 m to 19.5 m). Catchment area of the sample sites ranged from 6.4 km^2 to 165.9 km^2, average 38.1 km^2 (Appendix, Tables A1, A2). Sampling sites were restricted to lower valley sections (< 250 m of elevation) and to the tributaries of the main river (Maquiné). In each stream, we selected riffle sites according to accessibility (roads) and to distance between sites (a minimum of 1 km network distance between sites).

Fish sampling

We sampled 22 riffle sites (Fig. 1) that differed in channel steepness and distance from the mainstem channel. At each site, we employed kick-sampling to capture fish along a 40-m stream riffle segment using a rectangular dip-net (80 x 40 x 40 cm with 4 mm mesh), sampling all the flowing-water micro-habitats by disturbing stream bottom. A benthic-sampler (Fisher 1987) similar to the kick-sampling procedure we used has been shown to be reliable for obtaining quantitative samples of small benthic fishes in riffle habitats. In addition, we observed that kick-sampling is efficient (compared to trawl-net, gill-net and electrofishing) for capturing fish species in riffle habitats of the study area, particularly when the focus is on riffle-dwelling specialists such as benthic species (e.g., most of loricariids; R. B. Dala-Corte and F. G. Becker, personal observation). For instance, we noted that several shocked armored catfish (loricariids) individuals get stuck among bottom rocks because of their benthic, cryptic habit and body morphology, reducing their catchability in comparison to kick-sampling (R.B. Dala-Corte, personal observation). Indeed, Champeau et al. (2009) reported that armored catfish species were less vulnerable to electrofishing gear and may be underrepresented in electrofishing samples. Thus, although electrofishing may be more efficient than kick-sampling to sample the whole fish community, kick-sampling should be as good as electrofishing for the benthic riffle fishes in the study region. Most importantly, however, is that any potential sampling bias introduced by the chosen sampling method was standardized between the two sampling periods, as we used precisely the same sampling protocol and sampling effort.

We sampled 40-m long riffle segments because a previous assessment has demonstrated that the total number of riffle species does not increase substantially when more than 40 m are sampled (F. G. Becker, unpublished data). We were able to catch most of the species that one can find in riffles of the Maquiné river basin (25 out of 27 species) according to a previous extensive sampling effort performed throughout the whole basin (Becker 2002). We sampled each site once in the spring–summer of 1999 and once in the spring–summer of 2012. In both years, we captured fishes during low-flow periods and at least one week after rainfall events. Thus, fish catchability was unlikely to be affected by discharge variability between samples. In each site, we employed the same sampling effort in the two sampling years. Sampling performed in 2012 was supervised by the author who sampled the sites in 1999 (FGB). Most South American freshwater fishes usually live up to 5–6 years (Dei Tos et al. 2010) and, therefore, a 13-year period should be enough for at least 1 to 2 full replacements of all individuals in the population.

The fish species pool of the Maquiné basin is well known, although a few species are still not formally described (Malabarba et al. 2013), allowing us to identify individuals *in situ* during field work. Only two of the caught species have not been formally described to date (*Heptapterus* sp. and *Trichomycterus* sp.), but they were readily identifiable during field work and thus included in the analyses. We returned all the sampled fishes to the stream site immediately after each sampling in 2012.

In order to reduce noise in statistical analyses, we removed from the data matrix those species for which kick-sampling is not an efficient method and also those species typical of other habitats (e.g. pools or stream shore aquatic vegetation), or which were only accidentally caught. After these criteria, we kept 14 species in the data matrix out of the total of 25 species captured by kick-sampling. Therefore, this study focused on benthic species that are typically found in riffle habitats. Removal of these species was based on previous studies in streams of the same basin, including electrofishing, seining and gill-nets (Becker 2002; Vogel 2012) and underwater observation (FGB and RBD, personal observation). Out of the 11 low-abundant species caught with kick-sampling and that were removed from analyses, five were among the most abundant species sampled using electrofishing (*Rhamdella zelimai*, *Crenicichla maculata*, *Astyanax laticeps*, *Astyanax douradilho*, *Rhamdia quelen*) and three were among the most abundant species caught with seine nets (*Odontostoechus lethostigmus, Hyphessobrycon luetkenii*, *Deuterodon stigmaturus*). These are all nektonic (*A. laticeps*, *A. douradilho*, *O. lethostigmus, H. luetkenii*, *D. stigmaturus*) or nektobenthic (*C. maculata, R. zelimai*, *R. quelen*) species. The other three species removed (*Mimagoniates rheocharis*; *Microglanis cibelae*; *Phalloceros caudimaculatus*) are typical of other habitats, such as lateral backwater channels and vegetated slow water pools, and are not usually caught in riffles (Menezes and Weitzman 2009; Malabarba et al. 2013).

Local habitat variables and habitat change

In 1999 and 2012 we measured local habitat variables at five cross sections perpendicular to stream flow, and placed every 10 m along each 40-m stream segment where fish were sampled. We measured bankfull and wetted width, and depth at three equidistant points in each transect. Substrate size composition and variation were quantified by visually estimating the percent cover of five size classes of substrate at the same locations where we measured depths: 1) < 2 cm; 2) 2–7 cm, 3) 7–25 cm, 4) 25–50 cm, 5) > 50 cm. Percent cover corresponding to each substrate size were visually estimated in the following classes: 1) 1–

25%; 2) 25–50%; 3) 50–75%; 4) > 75%. We used these measures to generate 13 habitat variables per site: 1–4) mean and coefficient of variation of bankfull and wetted width; 5) wetted:bankfull width ratio; 6–7) mean and coefficient of variation of water depth; 8–12) averages of percent cover for each of the five substrate classes and 13) Shannon–Wiener diversity index based on substrate classes. Differences in habitat variables between 1999 and 2012 were evaluated with paired *t*-tests, using sampled sites as blocking factor.

Based on the 13 aforementioned local habitat variables, we calculated habitat change between 1999 and 2012 for each sampling site using Euclidean distances on variables standardized by their ranges with the vegan package (Oksanen et al. 2013). Thus, habitat change was measured as the Euclidean distance based on how much a site differed in time regarding its habitat characteristics. All the statistical analyses were carried out using R Statistical Environment (R Development Core Team 2015).

Stream size, channel slope and network position

Eight variables regarding stream size, channel slope and spatial position within the drainage network were used to investigate temporal turnover of fish assemblage using species occurrence and species abundance (Table A2). They were obtained from a digital elevation model (DEM) generated for the Maquiné basin using ArcGIS 10.2. The DEM was generated from topographic data and stream network lines available from a 1:50.000 scale digital spatial database (Hasenack and Weber 2010). These eight explanatory variables included one metric of stream size, four metrics of network position and three metrics of stream channel slope.

Stream size. To represent stream size we used square root transformed upstream catchment area, which is the cumulative catchment area (km^2) for each sampling site. Squared-transformed upstream catchment area is an indicator of discharge volume, distance from river source and channel width, and thus can be used as a stream size descriptor (Leopold & Maddock, 1953). Notice, however, that small streams can be located either in the periphery or in the center of the basin, so that catchment area does not express differences in linkage to large downstream streams (i.e., streams or rivers with greater water volume; Osborne and Wiley 1992); therefore we also calculated network position metrics for each site (see below).

Network position metrics. For each sampled stream segment we calculated the following network position metrics: downstream link magnitude, downstream order, betweenness centrality, and closeness centrality. Downstream link magnitude and downstream order of a given site are metrics that represent size of the stream after the next downstream confluence, as proposed by Osborne and Wiley (1992). We used downstream Shreve order to measure downstream link magnitude and downstream Strahler order to measure downstream order. Strahler order increases only after the confluence of two streams of same order (Strahler 1957), while Shreve order of a given network site is the sum of the number of all upstream tributaries (Shreve 1966). Consequently, stream sites with the same upstream catchment area can differ both in terms of downstream link and downstream order, depending on their spatial location within a drainage network. For computing stream order, we follow Osborne and Wiley (1992) in not considering the most immediate downstream confluence when they were small (first order) tributaries, because of the potential influence of the larger downstream watercourse. Betweenness centrality was measured as the number of shortest paths among all nodes that pass through a given node of interest (Newman 2010). Thus, central nodes in the drainage network will supposedly receive a high number of paths. Closeness centrality of a node was the inverse of the average length of the shortest paths from the focal node to all other nodes in the graph (Newman 2010). Betweenness centrality and closeness centrality were calculated with the igraph package (Csardi and Nepusz 2006). We used packages shp2graph (Lu 2014) and maptools (Bivand and Lewin-Koh 2014) to convert shapefiles of the drainage network and sites into igraph-class objects. Sample sites and confluences were defined as nodes. See Fig. A2 (Appendix) for a schematic representation of how network position metrics were calculated.

Stream channel slope (stream gradient). We assessed the three following stream slope metrics: average slope between upstream and downstream confluences of the sampled site, average slope in a 1-km segment upstream from the sampled site, and average local slope. The first two metrics were obtained in ArcGIS 10.2 using the slope tool in the Spatial Analyst toolbox. Average local slope was obtained from field measurements of channel steepness between the five cross sections along each sampling site (see sub-section Local habitat variables and habitat change). We included stream channel slope measured at three different spatial scales because there is still little information in the literature on which scale slope should be measured to represent the influence of hydrological process on local assemblages.

Assemblage turnover and model selection

We used multiple linear model selection to evaluate three hypotheses about the influence of habitat change, stream channel slope and within-network spatial position on temporal turnover of riffle-dwelling fish assemblages. The response variable used in the models was within-site dissimilarity of assemblages between 1999 and 2012 (temporal beta diversity). We tested models using two different dissimilarity measures: 1) Sorensen dissimilarity based on species presence and absence data, to assess turnover based on species occurrence data, and 2) Bray–Curtis dissimilarity, as an indicator of assemblage change based on log-transformed species abundance data.

We generated two linear regression models, one for Sorensen dissimilarity (species occurrence) and the other for Bray–Curtis dissimilarity (species abundance) as response variables, including eight variables regarding spatial position, plus 1999-2012 changes in instream physical habitat (Table A2). We then used Variance Inflation Factor (VIF) analysis (Fox and Monette 1992) to evaluate multicollinearity in the models. Variables with higher values of VIF were removed one-by-one from the models until all remaining variables presented VIF values lower than two (Fox and Monette 1992). From the nine initial explanatory variables, two presented VIF values higher than two (betweenness centrality and downstream order) and were removed from the models (Appendix, Table A3). The remaining seven explanatory variables were kept for model selection using the second-order Akaike Information Criterion (AICc).

Reduced models with all possible combinations of the seven explanatory variables and their respective AICc values were generated with the dredge function from the MuMIn package (Barton 2014). Reduced models were ranked according to AICc differences (delta) and Akaike weights. Models with higher Akaike weights may be interpreted as being more probable given the data (Burnham and Anderson 2002). We also estimated the relative importance of each explanatory variable by summing Akaike weights (SW) of all models in which a given predictor was included (Burnham and Anderson 2002). The validity of using SW to quantify the relative importance of explanatory variables in models has been recently demonstrated (Giam & Olden 2015). By using SW, even if a variable was not included in the best models it can be detected as important if it appeared several times in the other models. Furthermore, coefficients of the explanatory variables were standardized (beta argument in the dredge function) to make them comparable (MuMIn package; Barton 2014). Only models with delta AICc values less than two were retained. Subsequently, we included interaction terms between the most important variables (based on their Akaike weights). For example, channel slope could interact with habitat change to drive fish assemblage turnover.

Ordination and overall species variation

To best interpret the turnover of fish assemblages, we performed a Principal Coordinate Analysis (PCoA) with Sorensen (species occurrence) and Bray–Curtis (species abundance) dissimilarities using the package vegan (Oksanen et al. 2013). Species spatial position in the ordinations was proportional to their correlation with PCoA axes. We performed PCoA with 1999 and 2012 assemblage composition data.

We calculated the overall relative frequency of occurrence in the sample sites (*fr%*) and the relative abundance of individuals (*ab*%) as mean values for each species caught in 1999 and 2012. Overall differences in *fr%* and *ab%* between years were calculated as the absolute differences in these values for each species.

Individual species models

In order to assess whether fish species differed in their response to environmental variables regarding turnover from 1999 to 2012, we fitted individual models for each species. These models were performed for each one of the species that occurred at least in 10 sampling sites in 1999 or 2012 samples (9 out of the 14 studied species), using as explanatory variables only the most important variables according to SW in the overall model selection performed for assemblage turnover (see details in the sub-section above “Assemblage turnover and model selection”). The response variable in each species model was the difference in species abundance regarding an expected relationship, which indicated how much a species changed from 1999 to 2012. This was performed by fitting a linear model between 1999 and 2012 abundances for each species and using its residuals as response variables, i.e. how much the species abundance differed from expected for all sites.

Correlation between habitat variables and assemblage turnover

Posterior to model selection, we carried out an exploratory analysis to find out which instream habitat variables used to quantify habitat change were the most important variables contributing to explain the relationship between habitat change and assemblage turnover. We generated all possible combinations of the 13 instream habitat variables (from one to 13 variables) to calculate site-level habitat change (Euclidean distance). Subsequently, we tested the Pearson’s correlation between habitat dissimilarities, calculated with different combinations of habitat variables with assemblage turnover, in order to find those combinations of variables that maximized the correlation between habitat change and assemblage turnover.

Results

Instream habitat and assemblage composition

Sampling sites changed from 1999 and 2012 regarding instream habitat characteristics (Table 1). Significant differences between years were observed in the coefficient of variation of depth, in the substrate diversity and in most of the categories of substrate size. Depth and was higher in 1999 samples, while stream width and cover percentage of larger categories of substrate size increased in 2012 (Table 1).

Fourteen species of riffle-dwelling fishes were sampled at the 22 sites in the Maquiné river basin. Mean species richness per site including 1999 and 2012 data was 8.5, ranging from 5 to 11 species. One species (*Hemiancistrus punctulatus*) was sampled only in 1999, but no species occurred exclusively in 2012. Siluriformes was the richest order, with 11 species, followed by Characiformes and Cyprinodontiformes, with two and one species respectively.

The most frequent and abundant species caught for both 1999 and 2012 samples were *Rineloricaria aequalicuspis*, *Pareiorhaphis nudulus*, *Pareiorhaphis hypselurus*, *Epactionotus bilineatus* and *Characidium pterostictum* (Table 2). Overall, species that most varied in frequency of occurrence from 1999 to 2012 were *Ancistrus multispinis*, *Diapoma itaimbe* and *P. nudulus*; whereas the species that most varied in their relative abundance from 1999 to 2012 were *E. bilineatus, D. itaimbe, R. aequalicuspis* and *C. pterostictum* (Table 2). All sampled species are resident and thus turnover of the studied assemblages was not influenced by diadromous species.

Turnover of fish assemblages

We found that habitat change, i.e., local habitat dissimilarity between years, was the single important variable in the models explaining turnover in species occurrence ($R^2 = 0.23$; standardized coefficient = 0.47; Tables 3 and 4). Habitat modifications over time increased turnover of species occurrence, indicating that streams that presented more changes in local environmental characteristics also showed more variation in fish species composition (Figs 2 and 3). Interaction terms of explanatory variables were not important to explain assemblage turnover (Table 4).

Nine best models (delta AICc < 2) were selected to explain temporal turnover of assemblage based on species abundance and they included different combinations of seven explanatory variables (Table 3). Four of these seven variables were the most important to explain turnover of species abundance, since they had relative importance values greater than 0.5 and the greatest standardized coefficients (Table 4). These variables were habitat change, slope between confluences, downstream link magnitude and catchment area (Figs 4 and 5). Thus, turnover in species abundance differed from turnover in species occurrence because in addition to local habitat change, turnover in assemblages using species abundance was also influenced by channel slope, downstream link magnitude and catchment area (Table 4). Turnover of species abundance was negatively related to channel slope and downstream link magnitude and positively related to catchment area and local habitat change (Table 4).

Differences were observed in the individual response of species to the four most important variables selected to overall assemblage turnover in abundance, namely: habitat change, slope between confluences, downstream link magnitude and catchment area (Table 5). Models generated separately for each species showed some species changed more from 1999 to 2012 due to the influence of habitat change (*E. bilinelatus*), downstream link magnitude (*R. aequalicuspis*, *P. nudulus* and *E. bilinelatus*), slope between confluences (*P. nudulus*) or catchment area (*D. itaimbe*) (Table 5).

Instream habitat variables that most contributed to maximize the correlation between habitat change and assemblage turnover were the combination of coefficient of variation of depth and mean bankfull width for turnover in species occurrence (r = 0.74; Appendix, Table A4); and the combination of mean depth and cover percentage of substrate size class 25 to 50 cm for turnover in species abundance (r = 0.52; Appendix, Table A5).

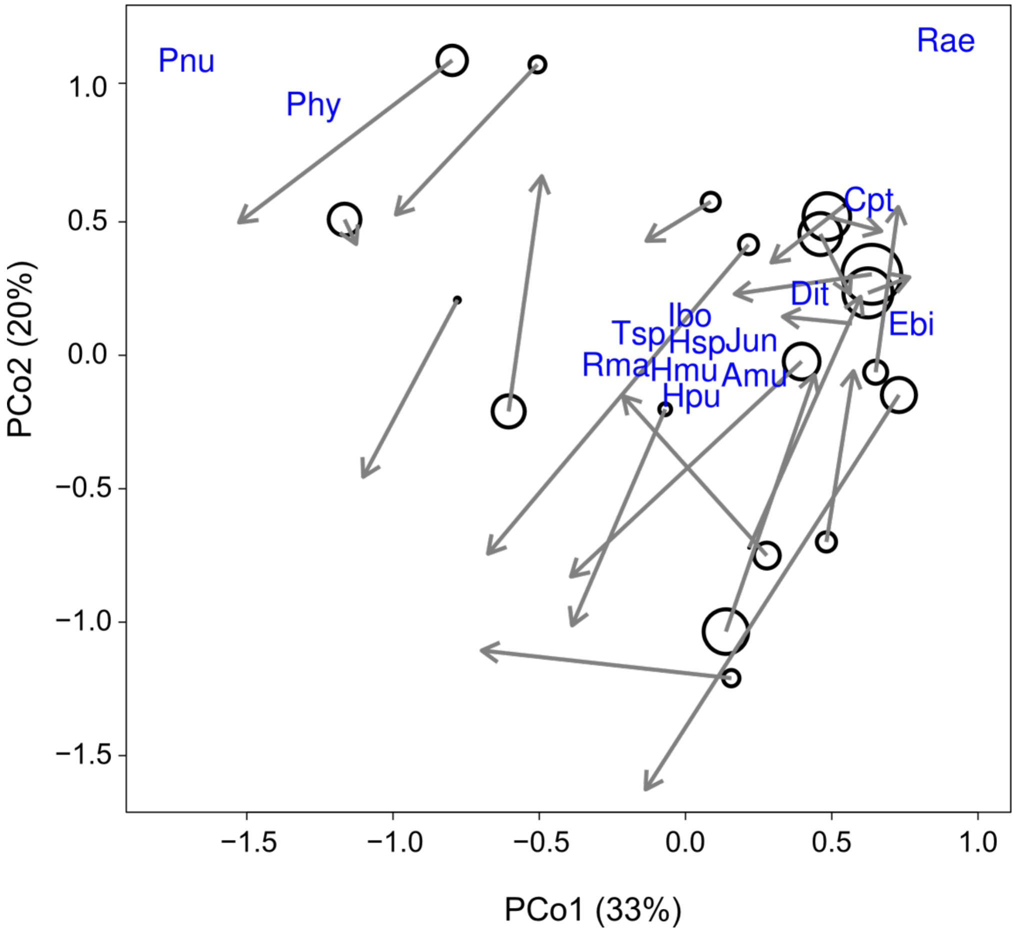

Fig. 2. Principal coordinates analysis (PCoA) based on species occurrence dissimilarities (Sorensen) showing turnover in fish assemblages from 1999 (circles) to 2012 (arrowhead). Arrow length is proportional to assemblage turnover and circle size is proportional to habitat change, which were the only important variables explaining assemblage turnover (AICc model selection). See Table 2 for species name abbreviations.

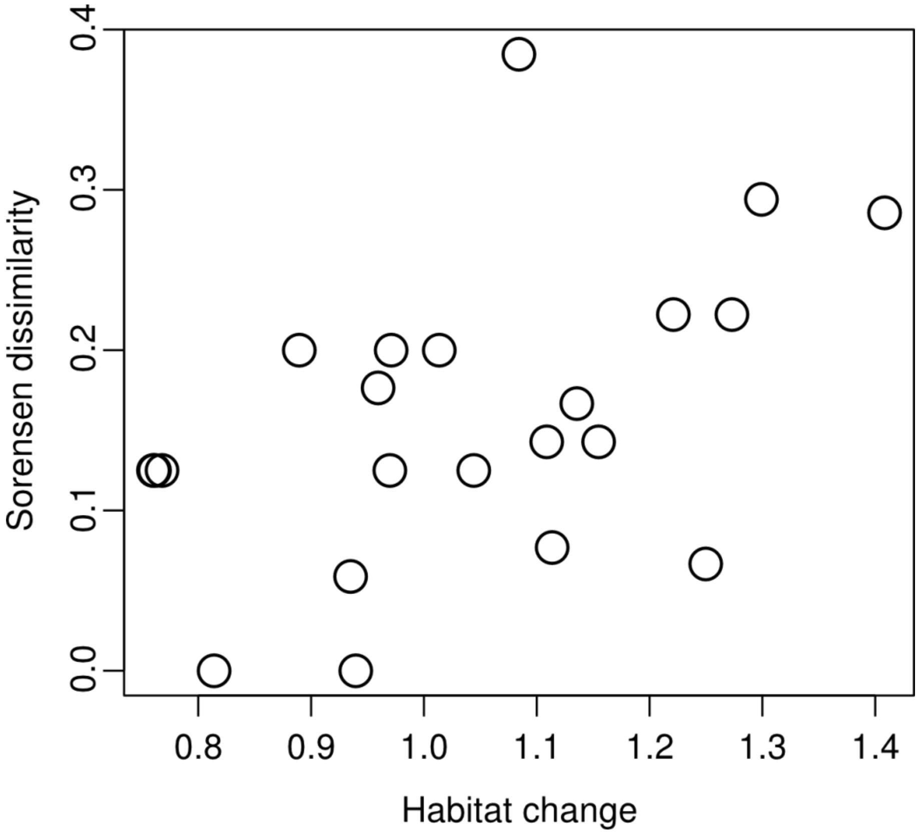

Fig. 3. Long-term turnover in species occurrence of riffle-dwelling fish assemblages calculated as pairwise Sorensen dissimilarity in relation to instream habitat change. Samples were obtained in 1999 and 2012, in 22 sites at the Maquiné river watershed, South Brazil.

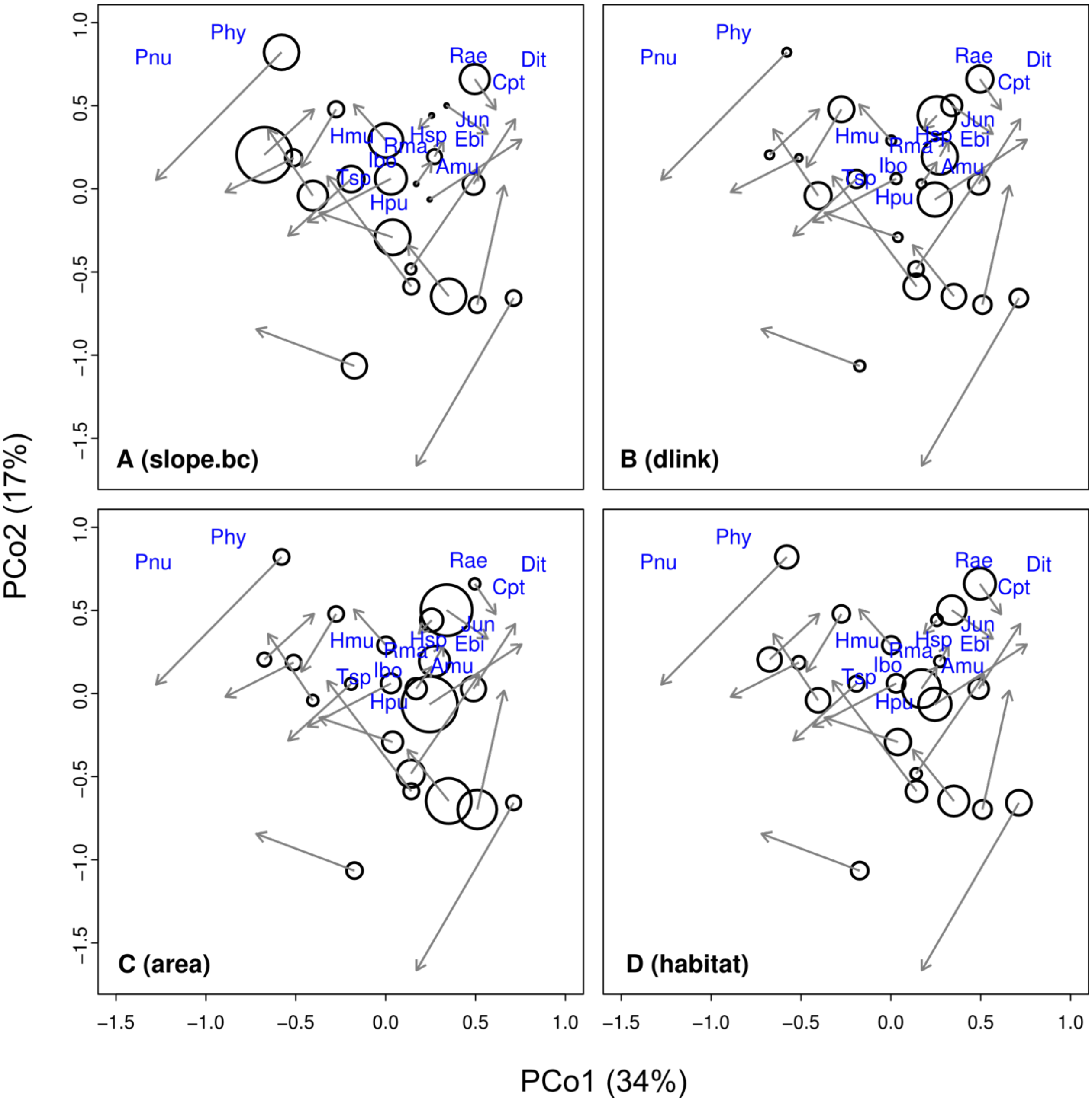

Fig. 4. Principal coordinates analysis (PCoA) based on species abundance dissimilarities (Bray-Curtis) showing turnover in fish assemblages from 1999 (circles) to 2012 (arrowhead). Arrow length is proportional to assemblage turnover and circle size is proportional to the four most important variables explaining assemblage turnover (AICc model selection). These variables were (A) slope between confluences, (B) downstream link magnitude; (C) catchment area, and (D) habitat change. See Table 2 for species name abbreviations.

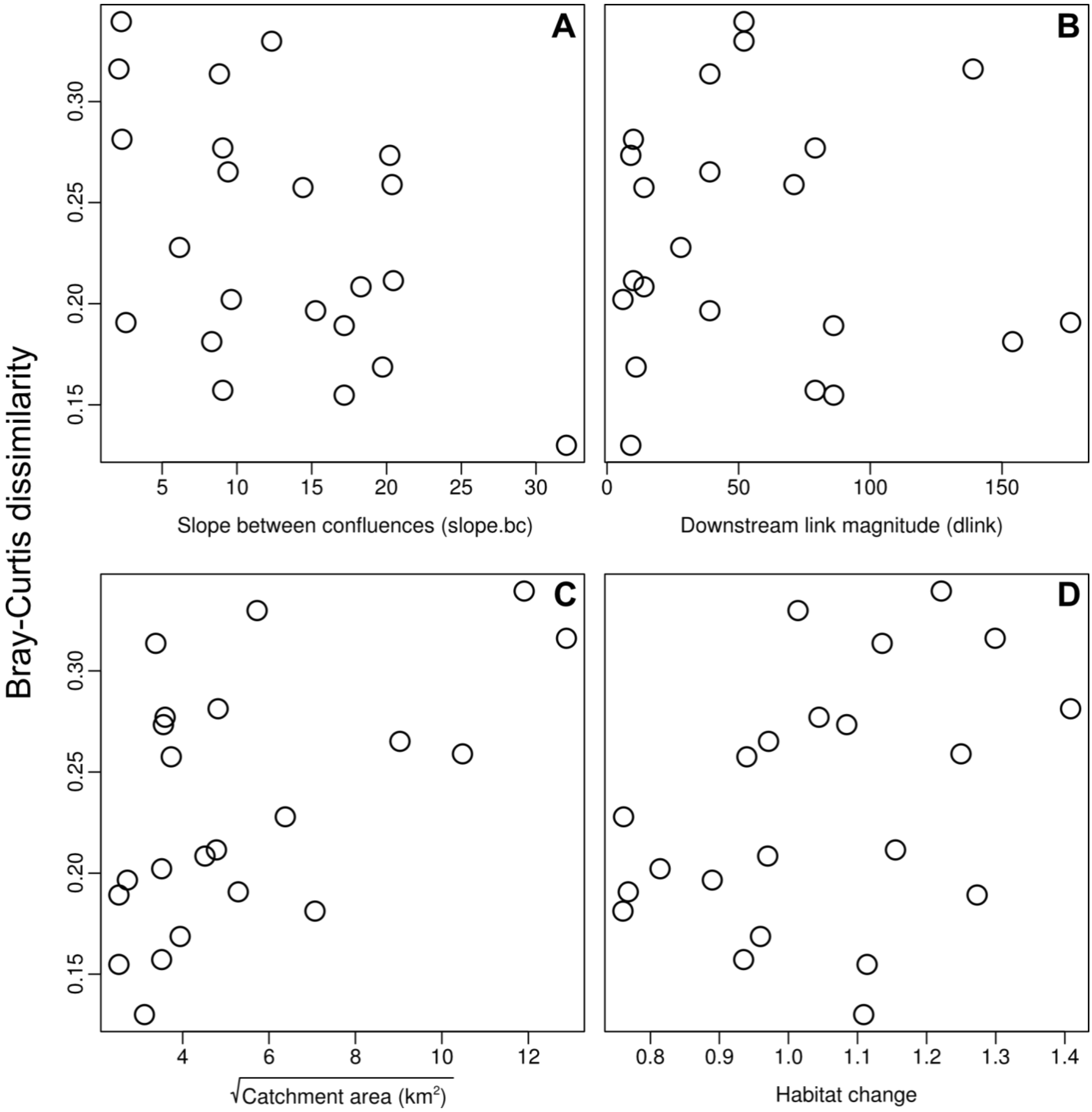

Fig. 5. Long-term turnover in species abundance of riffle-dwelling fish assemblages calculated as pairwise Bray-Curtis dissimilarity in relation to the four most important variables, according to multiple model selection. These variables were (A) slope between confluences, (B) downstream link magnitude; (C) square root transformed catchment area, and (D) habitat change. Samples were obtained in 1999 and 2012, in 22 sites at the Maquiné river watershed, South Brazil.

Discussion

The hypothesis that high habitat change would lead to high turnover of communities was supported. Turnover in species occurrence of riffle-dwelling fish assemblages in the 13-years interval was explained only by temporal changes of habitat characteristics, with higher turnover occurring at sites where physical habitat structure changed more. As well as species occurrence, turnover in species abundance was explained by habitat change. Additionally, turnover in species abundance was also explained by other factors that do not change with time in the studied temporal scale, such as channel slope, downstream link magnitude (a proxy for connectivity), and catchment area. Differences between 1999 and 2012 in channel size, depth and substrate composition were the most important habitat changes explaining turnover in fish assemblages. Processes of erosion and deposition, affected by regular flow and also by episodic severe disturbances such as flash floods, may cause channel reconfiguration by moving substrate and altering local habitat structure. Habitat change over the years may thus affect the set of species capable of inhabiting the new prevailing conditions or resources, affecting population abundance and species occurrence; a view consistent with species sorting of metacommunities (Leibold et al. 2004).

The reduced turnover of species abundance found in high-gradient sites is opposite to what we expected from our second hypothesis, which predicted that steep channels should lead to high temporal turnover due to severe stochastic flood events. Steeper channels tend to have higher flow energy and rapid response to rainfall, which will produce short and intense spates (Gordon et al. 2004; Nippgen et al. 2011). The implications for stream biota are high flow variability and harsh hydrological conditions (Resh et al. 1988), which may increase temporal variation in abundance of stream fishes (Ross et al. 1985). Moreover, flow variability has been demonstrated to be positively associated with extinction rates and negatively associated with immigration rates of stream fishes (Taylor and Warren 2001). Thus, it would be plausible to expect that stream segments with steep channels should show high assemblage changes. Despite this predicted positive relation between assemblage turnover and stream slope (as a proxy for habitat harshness), previous studies in aquatic ecosystems suggested that harsh environmental conditions may instead reduce community turnover (Chase 2007; 2010). For example, Chase (2007) found that communities in experimental ponds that experienced drought had much lower dissimilarity to each other than permanent ponds. Moreover, Chase (2010) found that community dissimilarity of producers and animals was much lower in ponds that experienced low productivity than ponds with medium or high productivities. Chase argues that niche selection may be the mechanism whereby species that could not tolerate such harsh environmental conditions were filtered out from the regional pool (Chase 2007; 2010). In contrast, in less restrictive environmental conditions, communities would be open to a large pool of colonists and thus would vary in species occurrence and abundance (Chase 2007; 2010). Indeed, the dominant species we sampled at sites with steep channels, such as the suckermouth armored catfishes *Pareiorhaphis nudulus* and *P. hypselusus*, are able to resist or avoid extremely high flows by seeking refuge among larger rocks and crevices at the stream bottom. Accordingly, we suggest that the negative relation between channel slope and turnover of species abundance was due to species sorting (Leibold et al. 2004), in which a restricted pool of species is able to maintain constant populations in hydrologically harsher (steeper slopes) environments. We highlight, however, that we measured slope in three different ways, but relationship with abundance turnover was important only when using slope between confluences, a large scale measure. Thus, the way that slope is measured may affect the detection of influence on assemblage turnover. Also we did not evaluate faunal composition immediately after hydrological disturbances, such as floods and droughts. Therefore, our results are valid for a time span of a decade and should not be extended to short periods in which assemblage turnover may be affected by episodic disturbance events (Rahel 1990).

We found that stream segments flowing directly into larger streams (i.e., sites with high downstream link magnitude) showed reduced temporal turnover in species abundance. As previously demonstrated by Osborne and Wiley (1992), downstream link magnitude was a useful proxy for size of downstream connection and connectivity within drainage network. This finding corroborates our third hypothesis, that proximity to larger streams should lead to low temporal turnover of riffle-dwelling fish assemblages, and also other studies reporting that communities living in stream segments close to confluences with main channels are more influenced by the pool of downstream immigrants (Osborne and Wiley 1992; Grenouillet et al. 2004; Thornbrugh and Gido 2010; Hitt and Angermeier 2011). The importance of spatial distance for dispersal limitation has been well demonstrated for stream ecosystems, even for highly mobile organisms (e.g., Thompson and Townsend 2006). The influx of immigrants from downstream pools tends to be lower at isolated and peripheral sites within the drainage network (Ai et al. 2013; Miyazono and Taylor 2013). As a consequence, colonization is expected to decrease and extinction to increase at peripheral segments (Gotelli and Taylor 1999). However, our findings indicate that the effects of the potential increase in the influx of immigrants in more centrally located sites, measured as downstream stream size, was important to population abundance, but not for species occurrence. This is consistent with the experimental study of Altermatt et al. (2011), which demonstrated that local population densities of protozoa and rotifers had an increased rate of post-disturbance recovery in connected patches compared with isolated patches. In this sense, our results indicate that tributaries centrally located in the stream network (i.e. higher connectivity), and which are directly connected to the mainstem, had relatively stable abundance of riffle species over time, whereas occurrence of species was mostly determined by habitat change.

We observed that sites with large upstream catchment area presented high turnover in species abundance. We had no *a priori* expectation regarding the effects of catchment size on the turnover of stream fish assemblages. However, we included catchment size in the models because it is expected to be partially correlated with variables we hypothesized to affect assemblage turnover (Appendix, Table A3). For instance, although small streams may flow into either small or large streams, large streams necessarily flow into large streams. Nonetheless, the finding that sites with larger upstream catchment area had higher turnover are not entirely surprising, given that previous studies have observed a positive relationship between stream size and immigration rates (e.g. Taylor and Warren 2001; Miyazono and Taylor 2013). The relationship between square root of catchment area and mean discharge has been well-known to hydrologists for decades (Leopold and Maddock 1953), and ecologists have recently used it as an indicator of stream size and volume (Gordon et al. 2004, Hughes et al. 2011). We thus suggest that large streams may function as corridors along which a temporally variable set of dispersing individuals from different species are constantly tracking suitable habitats. Large streams are not only more centrally located in the drainage network, but may present larger species pool and populations (habitat capacity), providing immigrants for restructuring local communities. Thus, the observed positive relationship between assemblage turnover and catchment area may not only reflect the influence of habitat size by itself, but the combined effects of stream size and dendritic connectivity of drainage networks (Carrara et al. 2014).

Our study was limited to riffle-specialist species; thus pool-specialists were not considered, as well as transient species that use the interface between riffle and pool habitats, most of which are nektonic species. One important implication is that the benthic riffle-specialist species sampled in our study (mostly loricariids) are commonly sedentary and/or short distance swimmers (Casatti and Castro 2006), a characteristic that may affect the immigration rates and thus the temporal turnover of fish assemblages. For example, Hitt and Angermeier (2008, 2011) observed that the most important factor influencing differences in fish species composition of centrally located tributaries (compared to isolated tributaries in the network periphery) was the influx of cyprinids with high dispersal ability from mainstem segments. Therefore, riverine species with high dispersal abilities may exert stronger influence on assemblages of tributaries near to mainstem river confluences through mass effects (Hitt and Angermeier 2011). Although our study focused on benthic riffle species, we found lower turnover in assemblages at stream sites connected to larger confluences. The studied basin is a small drainage (550 km^2) in a mountainous region with large proportion of riffle habitats in tributaries and mainstem. Thus, mainstem segments can act as potential sources of immigrants for riffle communities inhabiting adjacent tributaries. Also, mainstem segments are centrally located and can act as corridors connecting their flowing tributaries (Altermatt 2013). Distant peripheral stream segments, on the other hand, are more spatially isolated and more difficult to reach by dispersing individuals, which could influence local population dynamics (e.g. after a catastrophic event causing extinction of a local population). Hence, our study indicates that even benthic (and perhaps low-dispersing) riffle communities of tributaries near to mainstem may be more easily and frequently accessed by dispersing individuals of different species than isolated and peripheral riffle communities.

Differences in species responses to habitat change, stream channel slope, connectivity (dlink) and catchment area indicate that populations are differently affected by niche- and dispersal-related processes. For instance, connectivity may be more important to occurrence of some species for which local habitat characteristics are not very suitable. In this case, species with high dispersal abilities may have an advantage to maintain their presence in unsuitable but connected sites via immigration. Therefore, fluctuations in population abundance in mainstem or tributaries will influence species occurrence in other connected tributaries. However, our results indicate that this influence will be conditioned by how connected or how close a tributary is to a larger connection within a drainage network.

A caveat of our study is that it was limited to two sampling events with 13-years interval and does not account for seasonal and annual variation in species occurrence and abundance, and in physical habitat. Consequently, we were not able to assess multiple temporal replicates which would allow documenting the direction of assemblage changes (e.g. Matthews et al. 2013). Nevertheless, by using a long-term interval we were able to study a temporally independent replicate (i.e. turnover of all individuals). A second issue is that a relevant proportion of the total variation in assemblage turnover was not explained by the variables used in our models. Additional likely important sources of variation are intra-annual variation in species occurrence (movement patterns), inter-annual oscillation in population abundance due to suitable breeding events, stochastic environmental variation, and also sampling error. Also, habitat change was based only on physically-based variables, e.g. substrate composition and channel size, so we did not consider other potentially important factors to assemblage turnover, such as water chemistry and streambed stability. Although not taking into account all these other potential sources of variation, our models explained a relevant amount of variation in species occurrence (r^2 = 0.23) and abundance (r^2 = 0.39 to 0.53).

We found that the dynamic of stream fish communities was affected by multiple factors, including environmental filters (slope and habitat change) and the potential for communities to receive dispersing individuals (connection to larger streams). As these factors can be expected to vary throughout the stream network, our study shows the spatial position within dendritic network can affect metacommunity processes that drive turnover in stream fish assemblages. In addition, our study indicates that species differ in their response to metacommunity processes, such as habitat filtering and dispersal limitation (connectivity). These results have implications for sampling design in monitoring programs, since the degree of temporal dissimilarities in fish assemblages depends not only on local habitat change, but on spatial position of the sampled reaches within the drainage network and species particularities. Sampling design should then incorporate spatial position (e.g. downstream link) and take into account different species responses.

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by Instituto Chico Mendes de Conservação da Biodiversidade - ICMBio (sampling permit SISBIO #10650).

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Table 1. Instream habitat variables used to quantify habitat change between 1999 and 2012 for 22 sampling sites in the Maquiné river basin, Southern Brazil. CV = coefficient of variation; SD = standard deviation; *p* = probability of difference between years according to paired *t*-test.

Variables	Abbreviation	1999 Mean (SD)	2012 Mean (SD)	*p*
Mean wetted width	MeanWettWid	8.6 (4.7)	9.1 (4.8)	<0.001
CV wetted width	CVWettW	22.0 (10.8)	24.5 (14.1)	ns
Mean bankfull width	MeanBankWid	11.9 (6.5)	14.7 (7.2)	<0.001
CV bankfull width	CVBankW	16.6 (6.2)	15.4 (13.7)	ns
Ratio wetted/bankfull width	RatioWB	70.5 (13.9)	62.8 (15.2)	ns
Mean depth	MeanDepth	20.5 (7.0)	23.9 (5.0)	<0.001
CV depth	CVDepth	41.1 (13.0)	31.9 (7.4)	ns
%Substrate 2 cm	Subs2cm	5.8 (3.99)	1.8 (4.7)	0.014
%Substrate 2-7 cm	Subs2to7	13.5 (5.5)	10.5 (7.4)	ns
%Substrate 7-25 cm	Subs7to25	47.9 (10.9)	39.5 (11.9)	0.016
%Substrate 25-50 cm	Subs25to50	26.3 (11.1)	34.2 (11.5)	0.044
%Substrate >50 cm	Subs50cm	6.5 (6.2)	14.1 (12.3)	0.013
Shannon diversity substrate	DiverSubs	0.9 (0.1)	0.8 (0.2)	ns

ns = nonsignificant difference (p>0.05)

Table 2. Variation in fish species sampled in 22 riffle habitats between 1999 and 2012 samples. Abbrev = name abbreviations; *fr%* = mean relative frequency of occurrence; *fr% diff* = absolute differences in relative frequency of occurrence between 1999 and 2012; *ab%* = mean relative abundance; *ab% diff* = absolute difference in relative abundance between 1999 and 2012.

Species	Abbrev	1999 (*fr%*)	2012 (*fr%*)	*fr% diff*	1999 (*ab%*)	2012 (*ab%*)	*ab% diff*
Rineloricaria aequalicuspis	Rae	100	100	0	42.6	33.9	8.7
Pareiorhaphis nudulus	Pnu	86.4	72.7	13.7	20.6	18.8	1.8
Pareiorhaphis hypselurus	Phy	90.9	95.5	4.6	20.9	18.6	2.3
Epactionotus bilineatus	Ebil	100	100	0	26.5	12.6	13.9
Characidium pterostictum	Cpt	100	95.6	4.4	17.9	9.9	8
Diapoma itaimbe	Dit	31.8	54.6	22.8	1.3	10.9	9.6
Jenynsia unitaenia	Jun	63.6	63.6	0	2.5	2.8	0.3
Heptapterus sp.	Hsp	72.7	77.3	4.6	1.8	3.3	1.5
Ancistrus multispinis	Amu	68.2	40.9	27.3	2.6	0.6	2
Rineloricaria maquinensis	Rma	9.1	13.6	4.5	0.4	0.4	0
Heptapterus mustelinus	Hmu	22.7	13.6	9.1	0.6	0.3	0.3
Ituglanis boitata	Ibo	0	9.1	9.1	0	0.3	0.3
Hemiancistrus punctulatus	Hpu	4.6	0	4.6	0.1	0	0.1
Trichomycterus sp.	Tsp	4.6	4.6	0	0.1	0.1	0

Table 3. Best linear regression models for fish assemblage turnover selected using AICc statistics (delta AICc < 2). Response variables were turnover of species occurrence (Sorensen dissimilarity) and species abundance (Bray-Curtis dissimilarity) of riffle-dwelling fish assemblage between years 1999 and 2012. Explanatory variables were channel slope, position in the network, local habitat change and stream size. Variables representing channel slope, site position within stream network, local habitat change and stream size are: slope between confluences (slope.bc); downstream link magnitude (dlink); upstream catchment area (area); habitat change (habitat); closeness centrality (centr.cl); slope 1 km upstream from the sampling site (slope.1k) and local slope (slope.loc). AICc = Akaike information criterion; df = degrees of freedom. Delta = AICc difference from the best model; Weight = Akaike weights; adj. R^2 = adjusted coefficient of determination. Only models with delta AICc < 2 are shown.

Response variables	Explanatory variables	AICc	df	Delta	Weight	adj. R2
Occurrence	habitat	-41.6	3	0.0	0.34	0.23
Abundance	slope.bc+dlink+habitat+area	-64.1	6	0.0	0.14	0.51
	slope.bc+habitat	-64.0	4	0.1	0.14	0.39
	slope.bc+dlink+area	-63.9	5	0.2	0.13	0.44
	slope.bc+dlink+centr.cl+area	-63.7	6	0.4	0.12	0.50
	slope.bc+dlink+habitat	-63.5	5	0.7	0.10	0.43
	slope.bc+habitat+slope.1k	-63.2	5	0.9	0.09	0.42
	slope.bc+habitat+area	-63.0	5	1.1	0.08	0.42
	dlink+slope.1k+area	-62.7	5	1.4	0.07	0.41
	slope.bc+dlink+habitat+centr.cl	-62.6	6	1.5	0.07	0.47
	slope.bc+dlink+habitat+centr.cl+area	-62.2	7	1.9	0.06	0.53

Table 4. Relative importance of explanatory variables based on summed Akaike weights (SW) and their average standardized (beta) coefficients resulting from all combinations of AICc models of assemblage turnover (see Table 3 for the best models). Slope between confluences (slope.bc); downstream link magnitude (dlink); upstream catchment area (area); habitat change (habitat); closeness centrality (centr.cl); slope 1 km upstream from the sampling site (slope.1k) and local slope (slope.loc). Interaction between two explanatory variables is indicated with a colon.

Response variables	Explanatory variables	Standardized coefficients	Relative importance
Species occurrence	**habitat**	**0.47**	**0.78**
	slope.bc	-0.12	0.26
	area	0.12	0.25
	dlink	-0.06	0.20
	slope.loc	-0.17	0.20
	slope.1k	-0.04	0.17
	centr.cl	-0.01	0.16
	slope.bc:habitat	-0.28	0.04
	area:habitat	-0.35	0.03
	dlink:habitat	-0.15	0.02
	area:slope.bc	-0.47	0.03
	slope.bc:dlink	-0.42	0.02
	area:dlink	0.26	<0.01
Species abundance	**slope.bc**	**-0.54**	**0.75**
	dlink	**-0.49**	**0.66**
	area	**0.42**	**0.61**
	habitat	**0.37**	**0.57**
	slope.loc	-0.34	0.25
	centr.cl	0.31	0.30
	slope.1k	-0.07	0.23
	slope.bc:dlink	-0.20	0.08
	dlink:habitat	0.06	0.03
	slope.bc:habitat	-0.04	0.06
	area:habitat	0.03	0.03
	area:slope.bc	0.13	0.05
	area:dlink	0.09	0.05

Boldface highlights the most important variables (scores > 0.5).

Table 5. Relative importance based on summed Akaike weights (SW) and average standardized (beta) coefficients resulting from all combinations of AICc models of species abundance change. Only the four most important variables explaining overall assemblage turnover (see Table 3) were used for each species models. See Table 2 for species names abbreviations and Table 3 for variable name abbreviations.

Species	Explanatory variable	Standardized coefficients	Relative importance (SW)
Rae	**dlink**	**0.50**	**0.83**
	area	0.31	0.40
	habitat	0.20	0.27
	slope.bc	-0.08	0.19
Pnu	**slope.bc**	**0.60**	**0.93**
	dlink	**0.32**	**0.46**
	area	-0.30	0.39
	habitat	0.13	0.20
Phy	area	-0.28	0.35
	habitat	-0.16	0.23
	dlink	-0.14	0.22
	slope.bc	0.05	0.20
Ebi	**dlink**	**0.42**	**0.64**
	habitat	**0.38**	**0.59**
	area	0.35	0.44
	slope.bc	0.10	0.20
Cpt	habitat	-0.15	0.23
	area	0.16	0.22
	slope.bc	-0.10	0.21
	dlink	0.03	0.19
Dit	**area**	**0.66**	**0.98**
	habitat	-0.17	0.25
	slope.bc	-0.18	0.25
	dlink	-0.05	0.18
Hsp	habitat	0.27	0.35
	dlink	0.23	0.30
	area	0.09	0.20
	slope.bc	-0.10	0.20
Jun	habitat	0.25	0.32

	area	0.26	0.32
	dlink	0.19	0.25
	slope.bc	-0.14	0.22
Amu	area	-0.04	0.19
	dlink	0.07	0.20
	habitat	-0.05	0.20
	slope.bc	-0.03	0.19

Boldface highlights the most important variables according to SW (values ≥ 0.45).

APPENDIX

Table A1. Overall description of the 22 sampled sites in Maquiné river basin, Brazil. Slope between confluences (slope.bc); upstream catchment area (area); downstream link value (dlink). Large boulder = l. boulder.

Site	Mean wetted width (m)	Mean depth (cm)	Catchment area (km^2)	Slope.bc (%)	Dlink	Dominant substrates (> 20%)
1	6.0	20.4	12.6	20.2	9	cobble
2	7.9	17.0	20.4	18.3	14	cobble and pebble
3	4.8	21.0	14.0	14.4	14	boulder and cobble
4	8.1	33.9	40.6	6.2	28	boulder, cobble and l. boulder
5	19.5	22.1	81.5	9.4	39	cobble and boulder
6	4.6	20.2	7.4	15.2	39	cobble and boulder
7	4.6	15.4	11.4	8.8	39	cobble and boulder
8	12.0	23.2	15.6	19.7	11	cobble, boulder and l. boulder
9	12.8	18.0	32.8	12.3	52	cobble and boulder
10	14.5	30.5	141.6	2.3	52	cobble and boulder
11	15.0	36.8	109.7	20.4	71	cobble and boulder
12	6.9	18.8	12.4	9.0	79	cobble and boulder
13	5.8	20.5	12.9	9.0	79	cobble and boulder
14	4.0	17.7	6.4	17.2	86	cobble and boulder
15	5.5	13.4	6.5	17.2	86	Cobble
16	15.2	22.8	165.9	2.1	139	cobble, pebble and boulder
17	6.2	24.4	22.9	20.5	10	cobble and boulder
18	12.9	21.6	49.9	8.3	154	cobble and boulder
19	6.5	19.6	9.7	32.0	9	cobble, boulder and l. boulder
20	10.4	20.0	28.0	2.6	176	Cobble
21	4.9	23.3	12.4	9.6	6	cobble and boulder
22	6.7	27.3	23.2	2.3	10	cobble and boulder

Table A2. Descriptive summary of explanatory variables used in multiple regression models of turnover in species occurrences and abundances. Dorder and centr.bt were highly correlated with other variables (see Table 3) and were not used in the model selection procedure. Slope between confluences (slope.bc); slope one kilometre upstream of site (slope.1k); local slope (slope.loc); upstream catchment area (area); downstream link magnitude (dlink); downstream order (dorder); closeness centrality (centr.cl); betweenness centrality (centr.bt) and habitat change (habitat). Values in parentheses are standard deviation.

	Mean (SD)	Minimum	Maximum
slope.bc	12.59 (7.67)	2.09	32.03
slope.1k	11.42 (5.38)	1.08	19.08
slope.local	3.03 (1.32)	1.75	6.99
area	38.07 (45.40)	6.37	165.86
dlink	54.64 (49.91)	6.00	176.00
dorder	3.55 (0.91)	2.00	5.00
centr.cl	5.02×10^{-05} (1.08×10^{-05})	2.66×10^{-05}	6.48×10^{-05}
centr.bt	10459 (11850)	1263	43788
habitat	44.02 (9.83)	27.52	66.27

Table A3. Pairwise Pearson's correlation matrix of nine explanatory variables. Slope between confluences (slope.bc); slope one kilometre upstream of site (slope.1k); local slope (slope.loc); upstream catchment area (area); downstream link magnitude (dlink); downstream order (dorder); closeness centrality (centr.cl); betweenness centrality (centr.bt) and habitat change (habitat).

	slope.1k	slope.bc	area	dlink	dorder	centr.cl	centr.bt	habitat
slope.loc	0.50	0.70	-0.51	-0.50	-0.49	-0.36	-0.51	-0.09
slope.1k		0.68	-0.29	-0.55	-0.46	-0.30	-0.19	-0.26
slope.bc			-0.41	-0.45	-0.32	-0.20	-0.34	0.08
area				0.34	0.46	0.40	0.97	0.33
dlink					0.85	0.60	0.34	-0.13
dorder						0.74	0.45	0.01
centr.cl							0.39	0.17
centr.bt								0.35

Table A4. Combinations of instream habitat variables to calculate habitat change (dissimilarity) based on Euclidean distances that maximize the correlation with fish assemblage turnover of species occurrences (Sorensen dissimilarity). See Table 1 for variable names abbreviations.

Variable combinations	Correlation (Pearson's *r*)
CVDepth	0.590
CVDepth + MeanBankWid	0.741
CVDepth + MeanBankWid + Subs2cm	0.786
CVDepth + MeanBankWid + Subs2cm + Subs2to7	0.784
CVDepth + MeanBankWid + Subs2cm + Subs2to7 + Subs50cm	0.802
CVDepth + MeanBankWid + Subs2cm + Subs2to7 + Subs50cm + MeanDepth	0.833
CVDepth + MeanBankWid + Subs2cm + Subs50cm + MeanDepth + MeanWettWid + DiverSubs	0.820
CVDepth + MeanBankWid + Subs2cm + Subs25to50 + Subs50cm + MeanDepth + MeanWettWid + DiverSubs	0.812
CVDepth + MeanBankWid + Subs2cm + Subs25to50 + Subs50cm + MeanDepth + MeanWettWid + DiverSubs + RatioWB	0.738
CVDepth + MeanBankWid + Subs2cm + Subs2to7 + Subs25to50 + Subs50cm + MeanDepth + MeanWettWid + DiverSubs + RatioWB	0.711
CVDepth + MeanBankWid + Subs2cm + Subs2to7 + Subs25to50 + Subs50cm + MeanDepth + MeanWettWid + RatioWB + CVWettW + DiverSubs	0.675
CVDepth + MeanBankWid + Subs2cm + Subs2to7 + Subs25to50 + Subs50cm + MeanDepth + MeanWettWid + RatioWB + CVWettW + DiverSubs + Subs7to25	0.640
CVDepth + MeanBankWid + Subs2cm + Subs2to7 + Subs25to50 + Subs50cm + MeanDepth + MeanWettWid + RatioWB + CVWettW + DiverSubs + Subs7to25 + CVBankW	0.479

Table A5. Combinations of instream habitat variables to calculate habitat change (dissimilarity) based on Euclidean distances that maximize the correlation with fish assemblage turnover of species abundances (Bray-Curtis dissimilarity). See Table 1 for variable abbreviations.

Variable combinations	Correlation (Pearson's *r*)
Subs25to50	0.392
MeanDepth + Subs25to50	0.522
MeanDepth + Subs25to50 + MeanBankWid	0.544
MeanDepth + Subs25to50 + Subs2to7 + CVBankW	0.585
MeanDepth + Subs25to50 + Subs2to7 + CVBankW + Subs2cm	0.603
MeanDepth + Subs25to50 + Subs2to7 + CVBankW + Subs2cm + MeanBankWid	0.605
MeanDepth + Subs25to50 + CVBankW + Subs2cm + MeanWettWid + DiverSubs + Subs7to25	0.604
MeanDepth + Subs25to50 + CVBankW + Subs2cm + MeanWettWid + DiverSubs + Subs7to25 + CVDepth	0.605
MeanDepth + Subs25to50 + CVBankW + Subs2cm + MeanWettWid + DiverSubs + Subs7to25 + CVDepth + MeanBankWid	0.607
MeanDepth + Subs25to50 + CVBankW + Subs2cm + MeanWettWid + DiverSubs + Subs7to25 + CVDepth + MeanBankWid + Subs2to7	0.596
MeanDepth + Subs25to50 + CVBankW + Subs2cm + MeanWettWid + DiverSubs + Subs7to25 + CVDepth + MeanBankWid + Subs2to7 + Subs50cm	0.566
MeanDepth + Subs25to50 + CVBankW + Subs2cm + MeanWettWid + DiverSubs + Subs7to25 + CVDepth + MeanBankWid + Subs2to7 + Subs50cm + RatioWB	0.514
MeanDepth + Subs25to50 + CVBankW + Subs2cm + MeanWettWid + DiverSubs + Subs7to25 + CVDepth + MeanBankWid + Subs2to7 + Subs50cm + RatioWB + CVWettW	0.405

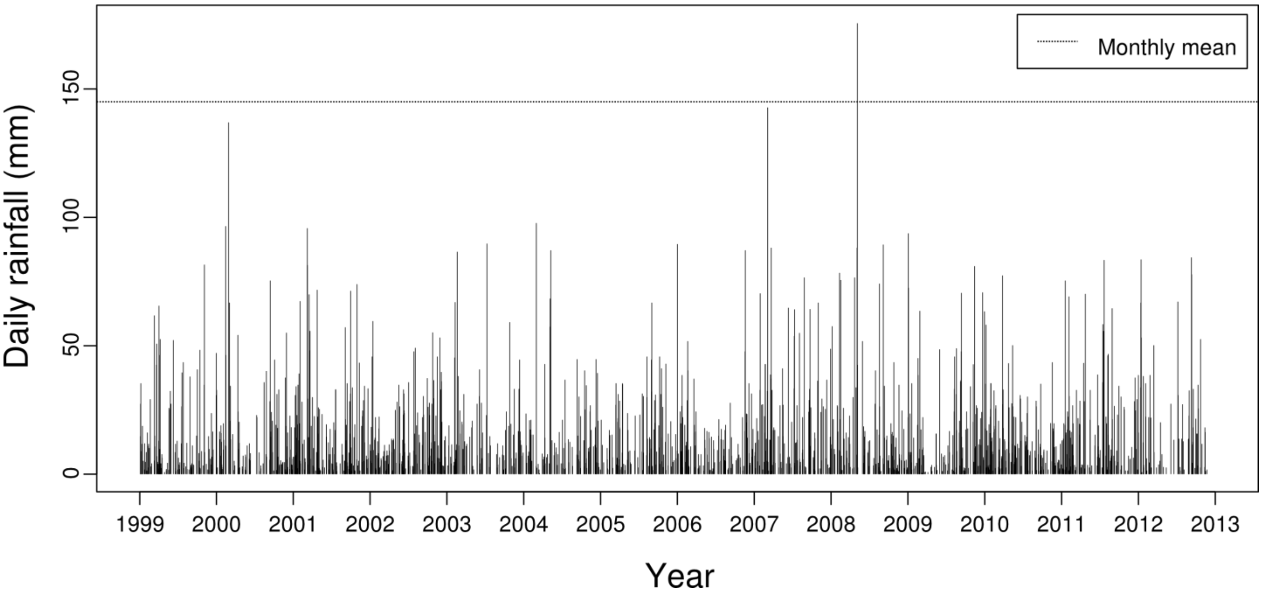
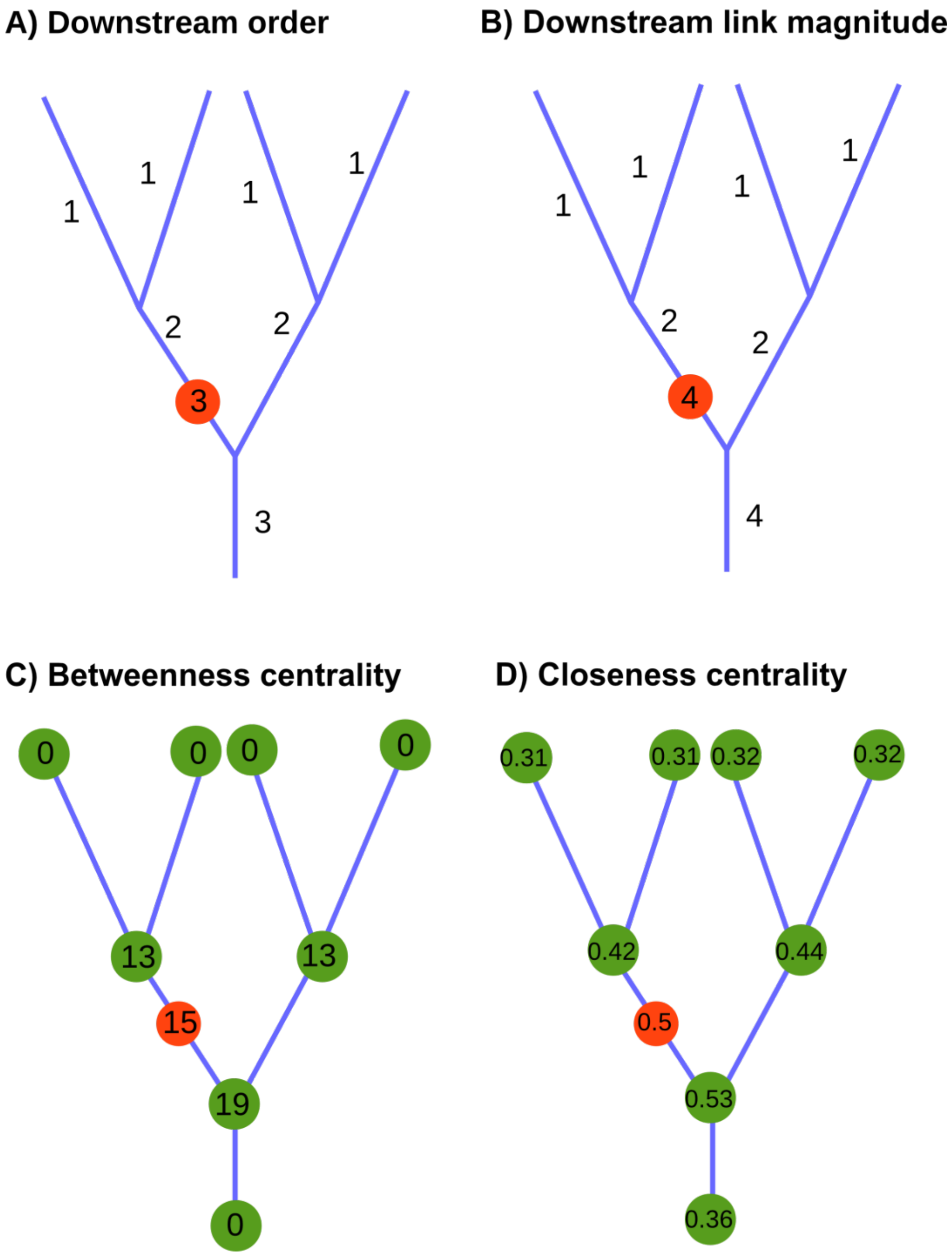


Fig. A1. Daily rainfall from 1999 to 2012 for the studied region, southern Brazil. Data from Centro Estadual de Meteorologia do Rio Grande do Sul – CemetRS of the Fundação Estadual de Pesquisa Agropecuária – Fepagro.



A) Downstream order

B) Downstream link magnitude

C) Betweenness centrality

D) Closeness centrality

Fig. A2. Schematic representation of a hypothetical network showing how network position metrics were calculated for each sampling site. Downstream order (A - Dorder) and Downstream link magnitude (B - Dlink) are based on values of downstream segment after confluences, whereas betweenness centrality and (C) and closeness centrality (D) are calculated based on the nodes. Red circle represents the network position value for a hypothetical sampling site. Values for C and D were calculated using the package igraph.

CAPÍTULO 4 - Anthropic modification of riparian zone affects trophic function and intestine length of a generalist fish species*

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Abstract. Human activities in the riparian zone can affect feeding of stream fish as they alter autochthonous production (periphyton, macrophytes and aquatic insects) and allochthonous inputs (terrestrial insects, leaves, seeds and fruits). We investigated how the diet and the intestine length of a persistent and generalist fish species (*Bryconamericus iheringii)* responds to anthropic impacts in the riparian zone in 31 subtropical streams. We hypothesised that intestinal length would be longer in populations inhabiting streams with highly converted riparian vegetation as consequence of a greater consumption of indigestible and low-energy/low-protein diet. Populations of *B. iheringii* from streams with an altered riparian zone decreased the consumption of terrestrial plants and invertebrates, and increased the ingestion of filamentous algae, macrophytes and detritus. Also, mean intestinal length was positively related to canopy openness. These results indicate that human alterations of riparian zones and increased canopy openness triggered a shift in the trophic position and function of *B. iheringii* populations. Our findings suggest that plasticity in the intestine length is an important characteristic to determine whether fish populations can persist in a variety of habitat conditions, and cope with the digestion of a greater proportion of low-quality and low-protein food items in human-altered environments.

Introduction

Riparian zones are linked to freshwater ecosystems by several pathways and affect energetic subsidy for aquatic communities (Nakano *et al*. 1999; Pusey and Arthington 2003). Riparian vegetation provides coarse particulate organic matter (CPOM) to the streams through the input of leaf litter and woody debris, which in turn support invertebrate prey for fish (England and Rosemond 2004). Large pieces of wood material from the riparian vegetation serve not only as food subsidies for aquatic invertebrates but also influence invertebrate density by providing shelter and retention of CPOM (Pringle *et al*. 1988; Wallace *et al*. 1997). Also, riparian zones provide fruit and terrestrial invertebrates, which are widely recognised as essential food resources for fish (Kawaguchi and Nakano 2001; Allan *et al*. 2003). Moreover, some generalist and omnivorous fish, as well as highly specialist species like the wood-grazers, forage directly on CPOM (Agostinho and Zalewski 1995; Lujan *et al*. 2011; Manna *et al*. 2012). Accordingly, terrestrial food items may constitute a high percentage of fish diets (ca. 40% or more), as recorded for different fish groups worldwide (Bojsen 2005; Chan *et al*. 2008; Sullivan *et al*. 2012; Leite *et al*. 2015).

Riparian shading mediates the amount of light that reaches the stream water and controls algal accrual and macrophyte production (Wootton 2012; Burrell *et al*. 2014; Majdi *et al*. 2015). Thus, removal of woody vegetation adjacent to streams contributes to a shift in the trophic state of stream ecosystems from heterotrophic to autotrophic (Minshall 1978; Dodds 2007). Riparian vegetation also promotes stream bank stability, reducing bank erosion and stream bottom siltation (Casatti *et al*. 2006; Casatti *et al*. 2009). Moreover, marginal vegetation can act as filter for non-point pollution sources in human altered landscapes, impeding nutrients and fine sediment coming from upland surface runoff entering streams (Lowrance *et al*. 1997; Ferreira *et al*. 2012a). Accordingly, modifications in the riparian zone should cause changes in the food availability and influence occurrence and abundance of trophic guilds in fish communities (Zeni and Casatti 2014).

Many freshwater fish species can tolerate human-induced environmental modifications to a certain degree (Karr *et al*. 1986; Teresa *et al*. 2015). These are usually widely distributed and are generalist fish species that occur along heterogeneous environmental conditions and feed on a wide range of food types. These species are usually omnivorous and opportunistic; which means that an individual is able to shift its diet according to food availability (Karr *et al*. 1986; Karr 1991; Schmitter-Soto *et al*. 2011). For example, fish species can vary their diets according to the spatial availability of food items at upstream and downstream sites (Manna *et al*. 2012).

Anthropogenic alteration of riparian vegetation can induce fish species to shift their diets from terrestrial to aquatic arthropods (Nakano *et al*. 1999; Baxter *et al*. 2004). Also, there is evidence that fish species may ingest a greater proportion of detritus because of the increase of suspended and deposited fine sediment in streams with impacted riparian zones (Ferreira *et al*. 2012b). Moreover, algae and macrophytes often represent a large proportion of fish diet in impacted streams due to reduced shading that benefits autochthonous production (Bojsen 2005; Wootton 2012). Hence, diet plasticity may be important for generalist fish species to cope with modifications in the environmental conditions and resources and to allow population maintenance in streams with impacted riparian vegetation.

Fish species exhibit a well-known relationship between feeding habit and intestinal length, which relates to the capacity of digestion of different food items and to nutrient absorption (Kramer and Bryant 1995*a*; Kramer and Bryant 1995*b*). Longer intestines are related to items that are more difficult to digest, because they can retain food items for a longer period and extract more nutrients (Kramer and Bryant 1995*b*). In fact, there is evidence that the intestines of detritivorous and herbivorous fish species are longer than those of omnivorous ones, which in turn are longer than those of insectivorous and carnivorous fish (Kramer and Bryant 1995*b*; Davis *et al*. 2013). There is also some evidence that the relationship between intestinal length and consumed food items exists not only for different species, but also for populations of a single species (Wagner *et al*. 2009; Scharnweber *et al*. 2011). Accordingly, intestinal length of plastic species may be related to the consumption of food items which are harder to digest and to obtain nutrients from, as well as less proteic, such as algae and detritus.

We investigated whether human modifications of the riparian zone cause shifts in the trophic role and in the intestinal length of a generalist stream fish species capable of maintaining populations in streams with varied environmental conditions and resources. We hypothesised that riparian-impacted streams drive increases in the intestinal length of the characid fish *Bryconamericus iheringii*, owing to shifts in the diet composition and to reduced quality of the consumed food items. Specifically, we expected an increase in the consumption of food items which are difficult to digest and have low nutritional value (low-protein) in riparian-impacted streams, such as detritus and vegetable matter, which would be positively related to the intestinal length. Our study contributes to a better understanding of: (1) how trophic structure and ecosystem functioning of streams respond to human modifications; (2) how anthropogenic modification in the riparian zone propagates through the trophic web of streams; and (3) how generalist fish species maintain populations in riparian impacted streams.

Methods

Studied species

Bryconamericus iheringii is a small-sized characid fish with maximum standard length of 7.3 cm that occurs in the Laguna dos Patos and Uruguay River basins, South America, encompassing Argentina, Uruguay and Southern Brazil (Lima *et al*. 2003). This species is commonly found in different types of environment, from rocky clear water streams to sandy lakes with vegetated margins. Frequently, *B. iheringii* is reported among the most abundant and widely distributed species across sampling sites in distinct fish community studies (Fernández *et al*. 2008; Volcan *et al*. 2012; Bertaco and Azevedo 2013). This species is referred as benthopelagic with omnivorous opportunistic feeding habits. Its stomach contents can include different plant items (algae and vascular plants), detritus (mineral sediment plus particulate organic matter) and small aquatic or terrestrial invertebrates coming from riparian zones (Oliveira and Bennemann 2005; Oricolli and Bennemann 2006). The reproduction of *B. iheringii* occurs during spring and summer of the Southern Hemisphere (Lampert *et al*. 2004); mean standard length (SL) at sexual maturity is around 40 mm for both males and females (Lampert 2003). Overall, *B. iheringii* can be classified as a generalist species, as it is able to persist in a wide range of habitat conditions, has a wide geographical distribution and usually is among the most abundant species in the communities where it occurs.

Studied sites and sampling procedures

Sampling sites were distributed across Southern Brazilian grasslands (Fig. 1). This unique biome is under threat due to the extensive landscape conversion for agricultural practices in the last three decades (Overbeck *et al*. 2007). We sampled *B. iheringii* at 31 wadeable streams, with different levels of riparian integrity and environmental conditions including: variation in riparian coverage, fine sediment at stream bottom, and local impact on stream bank by agricultural practices (*i.e.* cattle trampling and crops). Each sampling site comprised a 150 m long stream reach where fish were sampled using a single-pass electrofishing (EFKO GmbH model FEG 1500). All specimens were anesthetised with clove oil, preserved in formalin and subsequently transferred to ethanol for analyses of stomach content and measurements of morphological traits in the laboratory. The sampling procedures were conducted in accordance with ethical protocols and were previously authorised (Comissão de Ética no Uso de Animais of the Universidade Federal do Rio Grande do Sul, CEUA-UFRGS #24433).

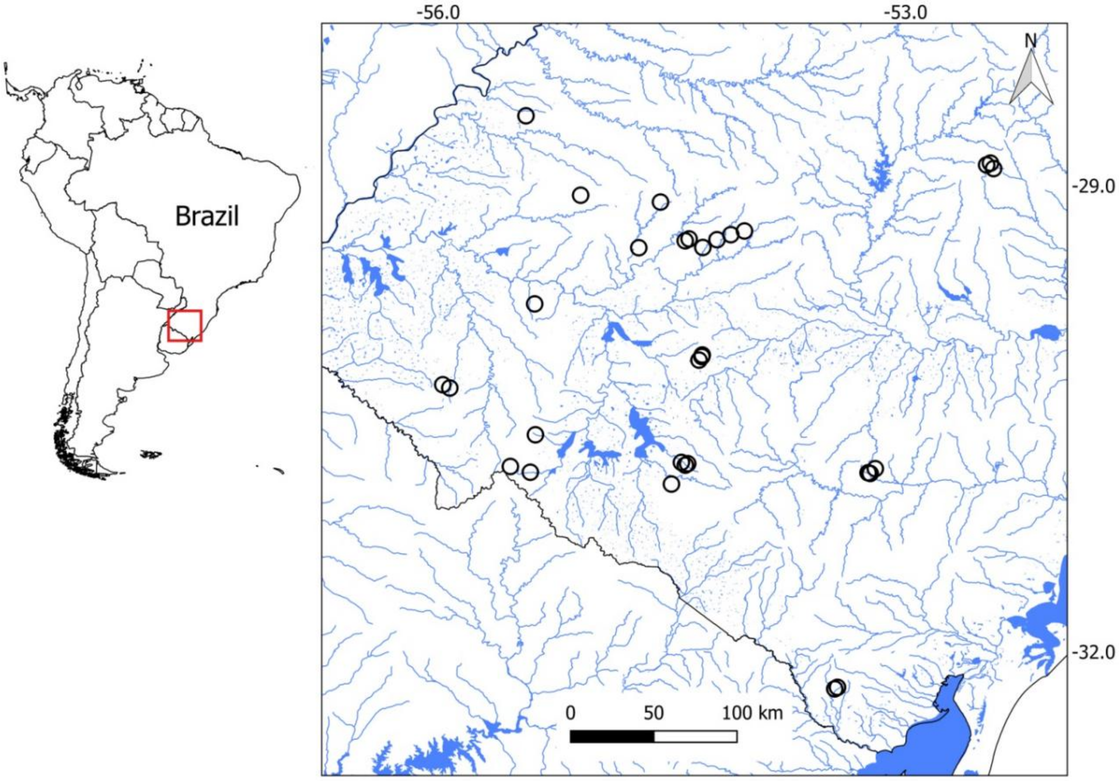

Fig. 1. Location of the 31 sampling sites of *Bryconamericus iheringii* populations in Southern Brazil.

Environmental variables

The following five variables were used to explain modifications in diet composition and intestinal length of *B. iheringii*:

1) Fine sediment. It was defined as the percentage cover of fine sediment deposited at the stream bottom (silt; grain size < 0.02 mm), and was visually estimated (Kaufmann *et al*. 1999). Fine sediment assessment was made at the same 150 m long stream reach where fish sampling was carried out. Each stream reach was segmented into 11 cross sections where repeated estimates (n=11) of fine sediment cover percentage were made. The mean cover percentage per sampling site was calculated and used as an explanatory variable.

2) Riparian vegetation coverage (riparian 1 km). It consisted of the percentage of riparian woody vegetation cover in a 1 km long 50 m wide buffer upstream from each sampling site along the drainage network. This variable was obtained by performing a supervised classification of 5 m RapidEye satellite images (Geo Catálogo 2015).

3) Canopy openness. This was the mean value of repeated estimates (three per cross section) of local canopy openness made with spherical densiometer in the same reaches where the fish were sampled (Kaufmann *et al*. 1999).

4) Local impact. The anthropic effects on stream banks at the 150 m long stream reaches were obtained from visual estimates of percentage cover of cropland on both stream margins and also estimates of livestock use density by direct (animal visualisation) and indirect signals (trampling marks, damage on vegetation and cattle manure). The quantification of local cropland cover and livestock use density was carried out at 11 cross sections along each sampled site by visually estimating their percentage into five classes: 0) absent; 1) <10%; 2) 10–40%; 3) 40–75%; 5) >75%. Mean percentage per sampling site was used to infer local impact (adapted from Kaufmann *et al*. 1999).

5) Elevation. Elevation above sea level for each sampling site was included to account for differences between mountain and lowland streams.

Morphometry and stomach contents

Stomach content analyses were performed along with measures of intestinal length and body size. Standard length (SL, in mm) was used as a body size measure and was obtained from the tip of the snout to the distal portion of caudal peduncle, *i.e.* excluding caudal fin. Intestinal length (IL, in mm) was measured from the intestinal joint with stomach to the most distal part of the intestine (ending at urogenital opening), with the intestine fully extended. All the measures were made with a digital caliper with 0.01 mm precision.

Stomach contents of 374 adults of *B. Iheringii* from the 31 streams were analysed. As dietary composition is dependent on body size and changes during ontogenetic development for several characid species (Winemiller 1989; Ward-Campbell and Beamish 2005; Vitule *et al*. 2008), juvenile individuals (smaller than 40 mm SL) were not included in our analysis (based on mean length at sexual maturity, Lampert 2003). The number of individuals per site ranged from 8 to 20, 12.1 ± 2.7 (mean ± SD). The size of individuals (SL) ranged from 40 to 71.2 mm, 52.3 ± 7.1 mm. Intestinal length (IL) ranged from 22.3 to 87.1 mm, 46.8 ± 11.3 mm.

Food items of each stomach were identified and quantified by estimating the occupied volume (mm^3) using an adaptation of the points method (Hynes 1950; Hyslop 1980). Food items found in the stomachs were accommodated to 1 mm height in a graph paper with 1 mm^2 cells in order to allow estimation of the number of cells occupied by each food items (mm^3), by using a stereomicroscope. This method allowed the relative volume of each food item in the stomach to be calculated.

Food items were grouped into categories prior to statistical analyses. These categories were: filamentous algae (Chlorophyta and Cyanophyta); aquatic plants (fragments of aquatic plants, *e.g. Podostemaceae*); terrestrial plants (seeds, fruits, leaves, flowers and other allochthonous plant fragments); aquatic invertebrates (immatures and adults); terrestrial invertebrates; detritus (inorganic and organic particulate items); and organic matter (unidentified/digested food items).

Statistical analyses

Initially the correlation between IL and SL was investigated in order to assess the association of intestinal length and body size. As this correlation was high (r = 0.71), we regressed IL against SL to obtain the component of the intestinal length not explained by body size (residuals of IL). These residuals represent an estimation of how much the IL of each individual differs from the value expected according to SL (Fig. 2). This variable will be referred hereafter as IL-SL-residuals.

To test whether riparian alteration by human use affects intestinal length we fitted linear regression models using IL-SL-residuals as response variable to the five environmental variables above described. We carried out backward model selection using F-statistic and probability (P) values as criteria to remove explanatory variables one-by-one from the full model. Only significant variables remained in the final model. Model slopes (beta) were standardised to obtain comparable effect sizes (Std-beta).

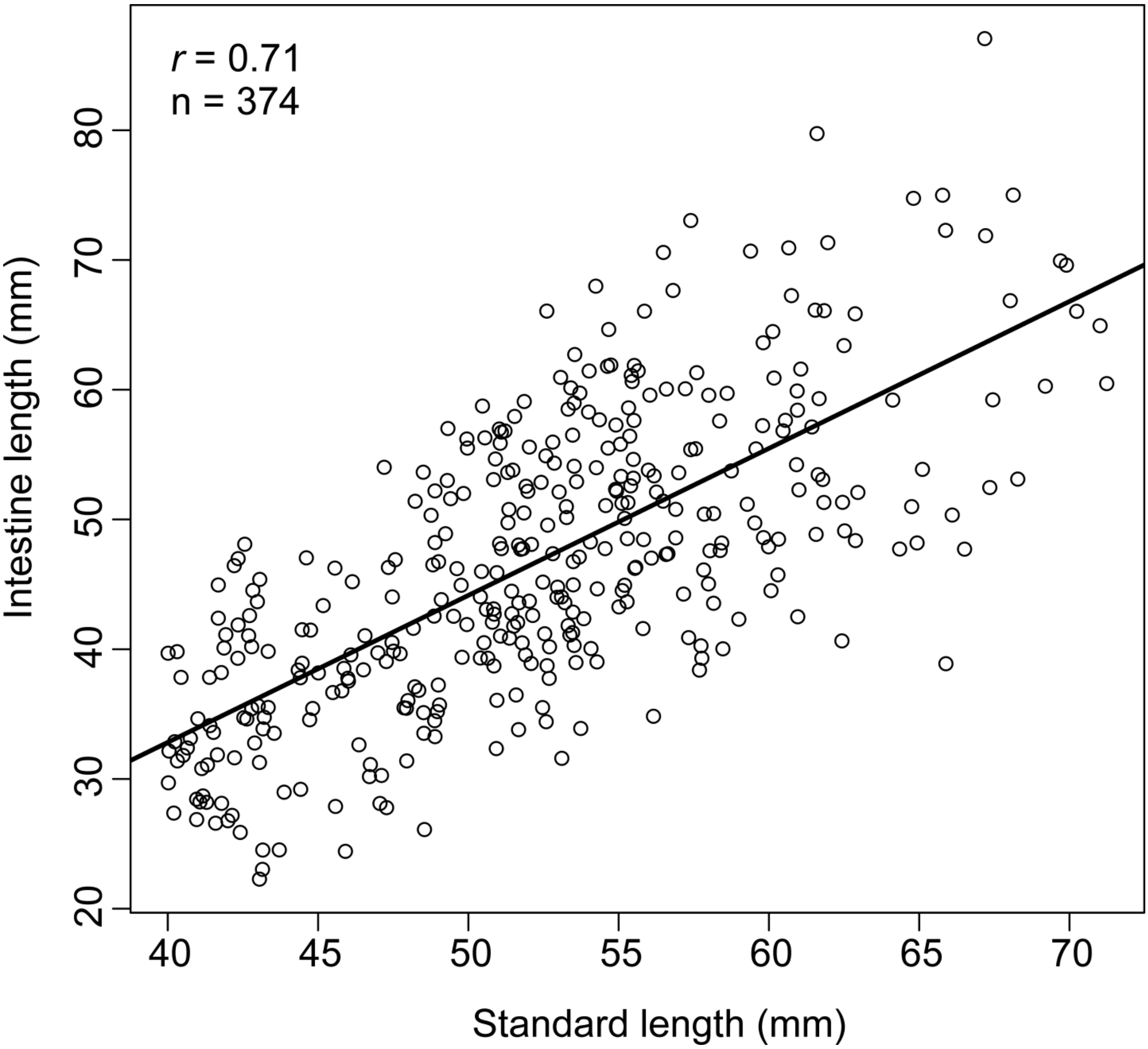

Fig. 2. Correlation between standard length and intestine length of *Bryconamericus iheringii* individuals. Line represents a fitted linear regression used to extract residuals between the two variables and to obtain values of intestine length that differ from expected, i.e. values that are independent of body size.

The relationship between diet composition of *B. iheringii* and the five environmental variables described above plus IL-SL-residuals and body size (SL) was assessed by performing a Constrained Analysis of Principal Coordinates (CAP) (Table 1). IL-SL-residuals and SL were included as predictors because both intestine and body sizes can constrain diet composition of fish individuals. We used a Bray-Curtis dissimilarity matrix obtained from Hellinger-transformed volume of food items to perform the CAP. The Hellinger transformation has been shown to provide best solutions to summarise variance and to increase explanations (Legendre and Gallagher 2001). The diet matrix included the averaged values of food categories consumed by all individuals per sampling site. We first checked for collinearity of explanatory variables used in CAP models with the Variance Inflation Factor (VIF). Usually, VIF values over 10 indicate redundant constraints, which should be removed from constrained models (Oksanen 2012). As all the variables had low VIF values (< 2), all were retained in the CAP models. Two CAP models were fitted: 1) a full model containing all the environmental variables and 2) a reduced model containing only the important variables selected by backward model selection. Significant variables according to permutation tests and with lowest AIC values were excluded one by one to generate the reduced model. The overall significance of the models, as well as the significance of the ordination axes and constrained variables predicting diet variation, were assessed with 9999 permutations. These analyses were performed with the package *vegan* (Oksanen *et al*. 2013) for the R environment (R Core Team 2015).

Results

Food items that occupied the highest overall volume in *B. iheringii* stomachs were terrestrial plant fragments (mean 38.5%), aquatic invertebrates (mean 21%) and detritus (mean 14.1%). Each of the remaining food items occupied less than 10% of the stomach volume, namely filamentous algae, unidentified organic matter, terrestrial invertebrates and aquatic plant fragments (Table 2). Among the aquatic invertebrates, unidentified aquatic insect fragments occupied the highest stomach volume (13.7%), followed by Ephemeroptera nymphs (2.2%) and Coleoptera larvae (1.8%). Among the terrestrial invertebrates, Hymenoptera adults (1.9%) and Lepidoptera larvae (1.5%) occupied the highest volume (Table S1, Supporting information).

Table 1. Explanatory variables used in Constrained Analysis of Principal Coordinates to explain diet variation in 31 populations of *Bryconamericus iheringii*.

Variables	Abbreviation	Mean	SD	Minimum	Maximum
Canopy openness (%)	Cano_open	19.7	18.4	2.7	80.8
Fine sediment covering (%)	Fine_sedim	3.4	7.0	0.0	25.9
Local impact (0–1)	Local_imp	0.2	0.2	0.0	1.0
Riparian covering (%)	Riparian	47.8	21.9	3.1	80.7
Above sea level elevation (m)	Elevation	264.7	152.4	115.4	677.4
Intestine length (residual)	IL-SL-residuals	0.5	5.9	-8.0	14.4
Standard length (mm)	Body_length	52.1	4.5	42.9	60.8

Linear model selection showed that the single most important variable to explain mean IL values independent of SL (IL-SL-residuals) of *B. iheringii* individuals was local canopy openness ($F_{1, 29} = 16.5$; Std-beta = 0.60; $P < 0.001$). After taking into account body size, longer intestines were observed in fish from stream sites with open canopies (Fig. 3).

There was an overall relationship between ingested food items and environmental variables in the full CAP model (explained variation = 37.4%; constrained axes = 7, Df = 7, 23; $P = 0.002$), as well as in the reduced CAP model (constrained variation = 27%; axes = 3, Df = 3, 27; $P < 0.001$). The first and second CAP axes constrained 27.3% and 24.7% of the total diet variance for the full and reduced CAP models, respectively ($P < 0.001$). The full model showed that ingestion of detritus and filamentous algae increased at sites with higher canopy openness, local impact and fine sediment cover on stream bottom, and populations with this diet had longer intestines (Table 3; Fig. 4). The reduced model also showed that detritus and filamentous algae increased in specimens with longer intestines from sites with high local impact at stream margins (Table 4; Fig. 5). In contrast, fish in stream sites with low local impact and closed canopies tended to ingest more aquatic and terrestrial invertebrates as well as terrestrial plant fragments. Also, stream elevation was associated with populations that ingested less terrestrial plants and more aquatic plants.

Table 2. Overall percentage values of food categories used in Constrained Analysis of Principal Coordinates to summarize diet variation of 374 *Bryconamericus iheringii* individuals.

Food categories	Abbreviation	Mean	SD	Minimum	Maximum
Terrestrial plants	TerrPlants	38.5	42.2	0	100
Aquatic invertebrates	AquaInv	21.0	33.1	0	100
Detritus (particulate matter)	Detritus	14.1	30.7	0	100
Organic matter not identified	OrgMatnId	8.2	23.1	0	100
Filamentous algae	FilamAlgae	7.8	23.4	0	100
Terrestrial invertebrates	TerrInv	7.3	19.4	0	100
Aquatic plants	AquaPlants	3.2	13.4	0	91.1

Table 3. Significance test (9999 permutations) and variation explained by the variables predicting diet variation of *Bryconamericus iheringii* in the full model fitted with Constrained Analysis of Principal Coordinates (CAP). See Table 1 for name abbreviations.

	Df	Variance	Proportional variance (%)	F	*P*
Cano_open	1	0.31	7.9	2.91	0.009
Fine_sedim	1	0.11	2.7	1.01	0.413
Local_imp	1	0.15	4.0	1.47	0.189
Riparian	1	0.09	2.4	0.87	0.529
Elevation	1	0.34	8.8	3.24	0.006
IL-SL-residuals	1	0.29	7.5	2.74	0.013
Body_length	1	0.16	4.1	1.48	0.175
Residual	23	2.41	62.6		

Table 4. Significance test (9999 permutations) and variation explained by the variables predicting diet variation of *Bryconamericus iheringii* in the reduced model selected with AIC and fitted with Constrained Analysis of Principal Coordinates (CAP). See Table 1 for name abbreviations.

	Df	Variance	Proportional variance (%)	F	*P*
IL-SL-residuals	1	0.37	9.6	3.52	0.002
Elevation	1	0.41	10.6	3.89	0.001
Local_imp	1	0.25	6.6	2.42	0.027
Residual	27	2.82	73.2		

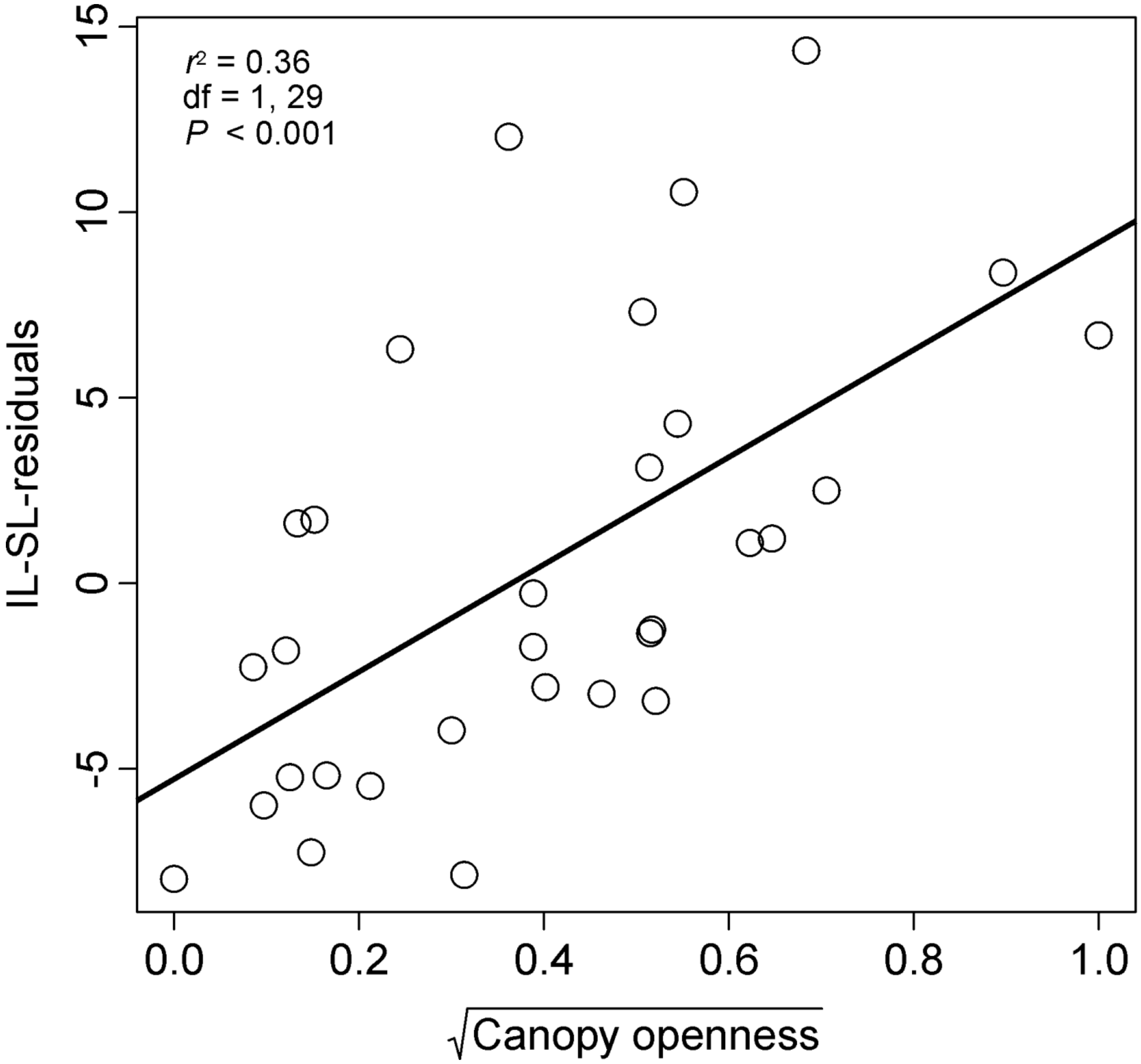

Fig 3. Relationship between intestine length independent of standard length (IL-SL-residuals) of *Bryconamericus iheringii* individuals and canopy openness (square root transformed) for 31 streams sampled in South Brazil. Each point represents the mean value for a sampling site.

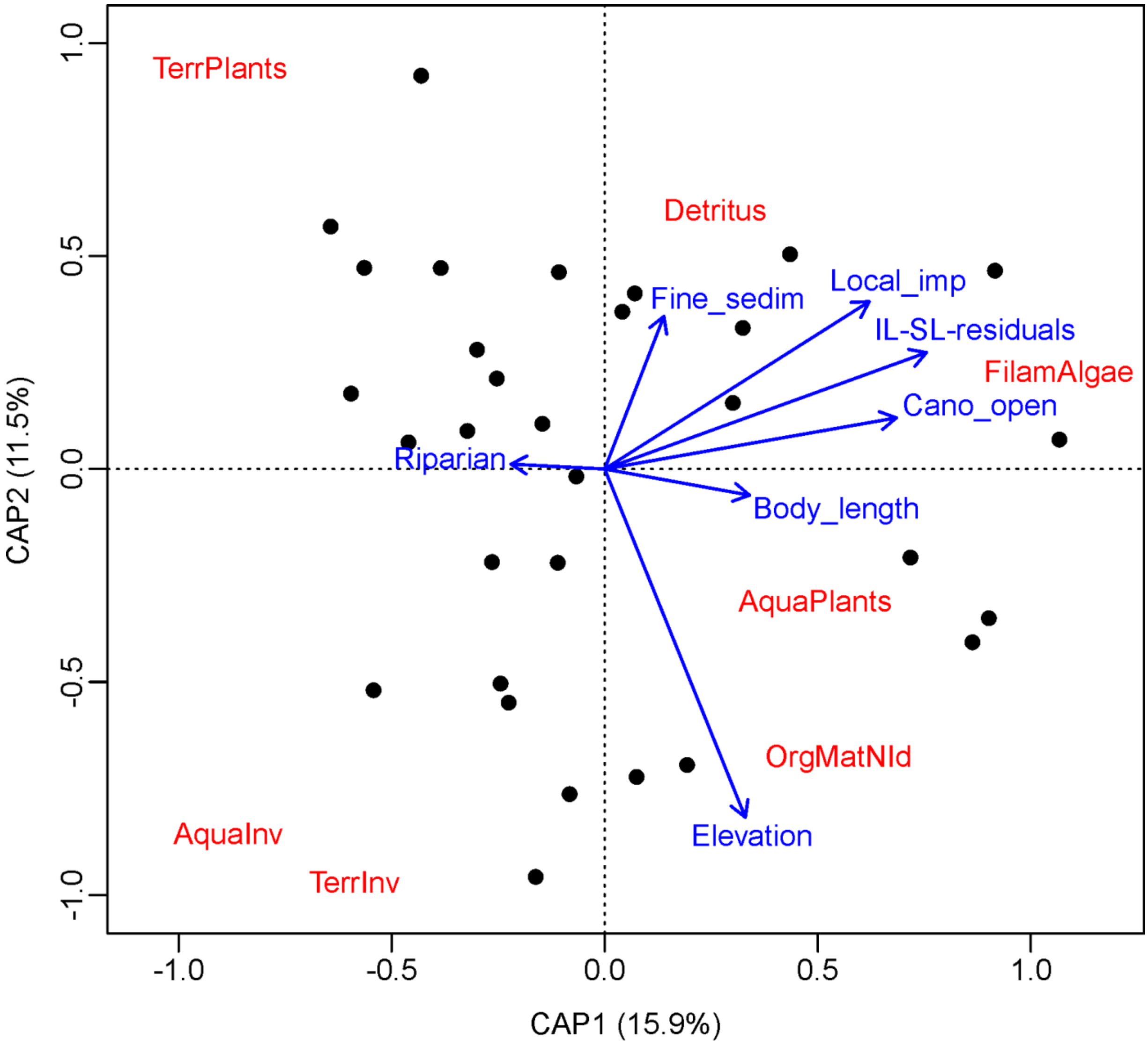

Fig. 4. Constrained Analysis of Principal Coordinates (CAP) showing the relationship between exploratory variables of the full model (blue arrows) and diet variation of *Bryconamericus iheringii* in 31 stream sites of Southern Brazil (black dots). See Tables 1 and 2 for name abbreviations.

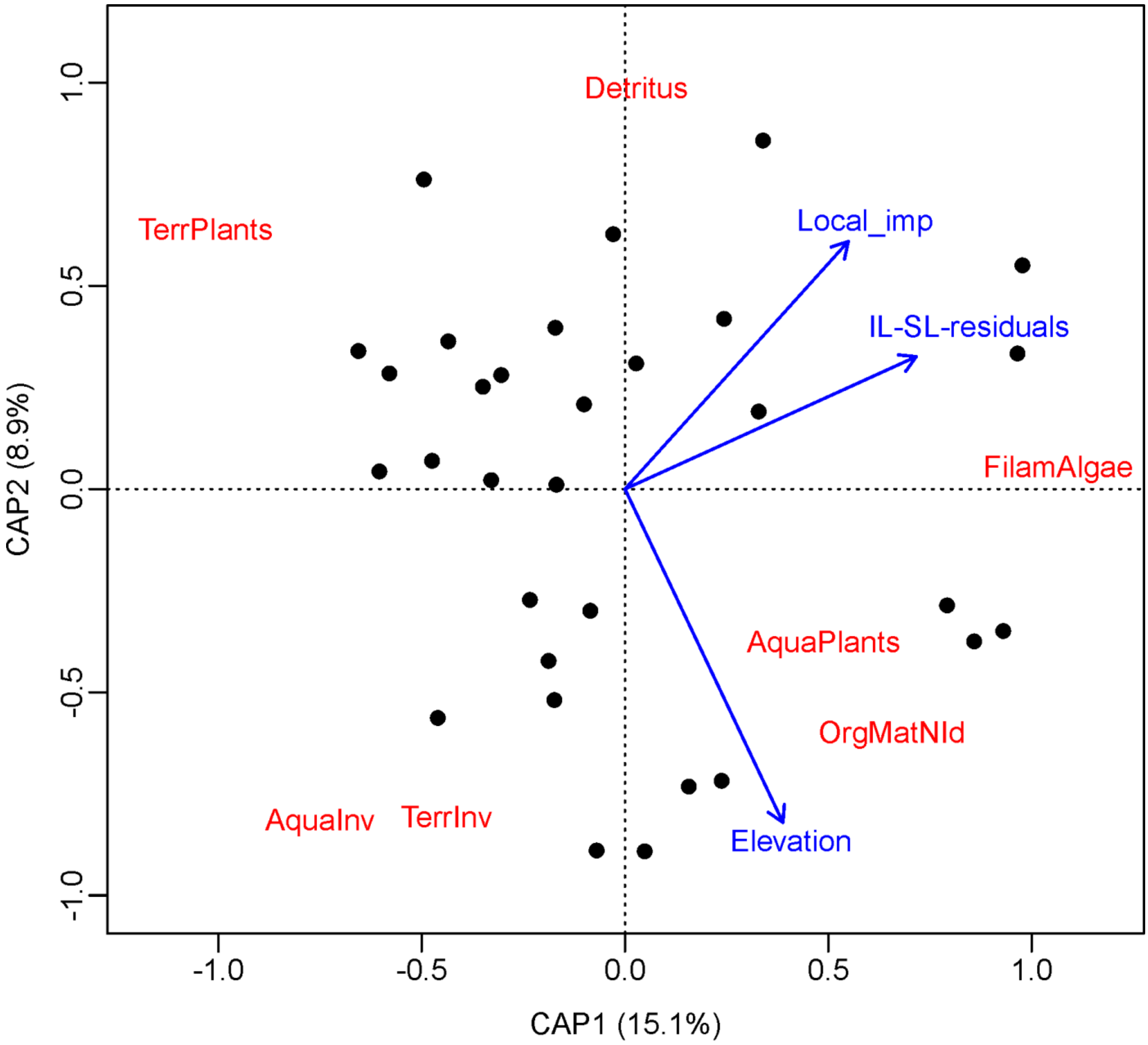

Fig. 5. Constrained Analysis of Principal Coordinates (CAP) showing the relationship between exploratory variables of the reduced model (blue arrows) and diet variation of *Bryconamericus iheringii* in 31 stream sites of Southern Brazil (black dots). See Tables 1 and 2 for name of abbreviations.

Discussion

We found support for the hypothesis that reduced riparian vegetation coverage and increased agricultural impacts along stream margins drive shifts in the trophic role of fish species capable to persist in varied environmental conditions. Populations of *B. iheringii* in riparian impacted streams increased the proportional consumption of detritus, filamentous algae and aquatic plants, and reduced the ingestion of aquatic and terrestrial invertebrates and of terrestrial plant fragments. Also, we found evidence for the hypothesis that increased ingestion of low-protein and indigestible food items in riparian-impacted streams are associated to the selection of longer intestines.

The influence of human modification of riparian zones on the diet composition of *B. ihenrigii* may be associated to changes in the available food resources caused by removal of riparian vegetation. One of the consequences expected from the reduction of woody vegetation at riparian buffers is the increment of solar radiation combined with reduction of particulate organic inputs from terrestrial ecosystems (Pusey and Arthington 2003). These modifications can alter the trophic state of streams and diminish the contribution of allochthonous material and increase the contribution of autochthonous material to the food webs (Minshall 1978; Wootton 2012; Burrell *et al.* 2014). Our study indicated that generalist and persistent fish species can respond to these environmental alterations caused by riparian vegetation removal and concomitant local impacts by consuming a greater proportion of autochthonous material, including filamentous algae and vascular plants (macrophytes), instead of allochthonous material, such as terrestrial plant fragments and terrestrial invertebrates.

We also found that *B. iheringii* reduced consumption of aquatic invertebrates (autochthonous) in streams with impacted riparian zones but increased the ingestion of detritus. In addition to the greater canopy openness, the impacted streams in this study had greater cropland area and cattle trampling along the riparian buffers, *i.e.* had increased local impact. Agricultural activities are known to increase the input of fine sediment to streams (Allan 2004). In another study in the same streams, we found that local (riparian) agricultural impacts had a positive relationship with fine sediment deposited on streambed, and reduced the occurrence of benthic fish species (Dala-Corte *et al*. unpublished data). Several studies have demonstrated the pervasive effects of fine sediments on benthic macroinvertebrates (Matthaei *et al*. 2006; Burdon *et al*. 2013). These modifications of the stream bottom may explain why *B. iheringii* consumed less aquatic invertebrates and more detritus (which included fine sediment and particulate organic matter) in streams with greater local impact. This result helps to understand the mechanisms by which some species distribute in extremely heterogeneous habitat conditions, *i.e.* not only environmental tolerance is important, but plasticity in resource utilisation is also imperative.

The longer intestines found in *B. iheringii* individuals inhabiting streams with impacted riparian zones may enhance the digestion efficiency of low-quality (low-protein) and indigestible food items. Algae, macrophytes and detritus ingested in greater proportion by *B. iheringii* in streams with impacted riparian zone are food items commonly found in diets of fish species with longer digestive tracts (Kramer and Bryant 1995*b*). Low-protein food items are demonstrated to have a positive relationship with intestinal length, even for individuals of the same fish species (German and Horn 2006; Zandonà *et al*. 2015). The functional significance of longer intestines is that they have a greater absorptive surface that maximises the digestive efficiency of low-quality and poorly digestible food items. Species with longer intestines usually occupy lower trophic position in the food web of freshwater ecosystems as their diets are primarily based on producers (Kramer and Bryant 1995*b*; Wagner *et al.* 2009). Our study provides evidence that the increased low-quality food items for fish in riparian-impacted streams may also trigger shifts in the trophic function and position of generalist fish species. Furthermore, we speculate that intestinal plasticity may be an important trait to determine whether fish populations will be able or not to persist in streams with impacted riparian vegetation. Experimental studies will provide valuable information to evaluate this hypothesis.

A limitation of our study is that we did not evaluate food availability in the sampling sites. Therefore, we cannot state whether diet differences between *B. iheringii* populations were caused by differences in food availability or by the influence of other environmental factors that lead individuals to shift their foraging behaviour, such as intra or interspecific competition or food items detectability. However, there is a strong support in the literature towards the hypothesis that riparian impacts lead to modifications in the food availability (Nakano *et al*. 1999; Wootton 2012; Burrell *et al*. 2014; Majdi *et al*. 2015). In addition, our study was focused on understanding how generalist fish species that can be found in a wide range of stream sites with different degree of human impacts respond to environmental alterations. Hence, our results should not be extrapolated to all species in a stream fish community but to generalist and persistent species.

Whereas the majority of the studies focus on the relationship between species distribution and a variety of environmental gradients, and are centred on species rarity or abundance, we show here that ubiquitous and abundant species can provide valuable information to understand how environmental alterations caused by human activities affect organisms and ecosystems. In addition, we observed that a species function can vary in the ecosystem as response to environmental variation. This result reinforces the importance of taking into account intraspecific variation by studies focusing on understanding functional role of species in aquatic ecosystem or for other trait-based studies. For instance, using unique trait values for species, or classifying them into specific group/functions, such as trophic groups, will ignore species function variation, mainly for the generalists. Furthermore, intestinal length should be better explored as a proxy variable for intraspecific feeding variation.

Our study indicated that human modifications of riparian zone triggered a shift in the trophic role of generalist fish species that can persist in streams with varied environmental conditions. Removal of riparian vegetation and human activities impacting riparian zone may lead to increased intestinal length of generalist fish species, which is associated to the ingestion of a greater volume of indigestible and low-protein food items. Hence, persistence of some fish species in streams with impacted riparian zones may be dependent on shifts in the function within the trophic web, and also on shifts in the way of obtaining nutrients and energy from the food items with different digestibility and nutritional quality. Our study sheds new light on the mechanisms underlying fish responses to environmental modifications and adds evidence on the effects that impacts of riparian zones have on the trophic structure and functioning of stream ecosystems.

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Supporting information

Table S1. Overall percentage values of the food items found in stomach content analyses of 374 *Bryconamericus iheringii* adult individuals.

Food items	Mean	SD	Minimum	Maximum
Chironomidae aquatic larvae	0.9	4.6	0.0	50.0
Simuliidae aquatic larvae	0.3	2.2	0.0	34.9
Ceratopogonidae aquatic larvae	0.0	<0.01	0.0	0.1
Empididae aquatic larvae	0.1	0.7	0.0	11.7
Tabanidae aquatic larvae	0.2	3.8	0.0	70.0
Diptera aquatic pupae	0.1	0.8	0.0	8.7
Trichoptera aquatic nymph	0.4	2.6	0.0	38.9
Ephemeroptera aquatic nymph	2.2	11.0	0.0	100
Plecoptera aquatic nymph	0.1	0.6	0.0	10.8
Odonata aquatic nymph	0.4	3.6	0.0	44.4
Lepidoptera aquatic larvae	0.3	4.8	0.0	91.6
Hemiptera aquatic adult	0.3	2.4	0.0	34.0
Megaloptera aquatic larvae	<0.01	0.6	0.0	11.4
Coleoptera aquatic larvae	1.8	9.0	0.0	98.5
Unidentified aquatic insect fragments	13.7	27.6	0.0	100
Aegla (Decapoda) aquatic adult	0.3	4.7	0.0	84.9
Bivalvia (Mollusca)	<0.1	0.1	0.0	1.8
Gastropoda (Mollusca)	<0.1	0.3	0.0	3.2
Ostracoda (Crustacea)	<0.1	0.1	0.0	1.1
Diptera terrestrial adult	1.1	9.5	0.0	100
Stratiomyidae terrestrial adult	<0.1	0.4	0.0	6.7
Lepidoptera terrestrial larvae	1.5	9.5	0.0	96.7
Hemiptera terrestrial adult	0.3	5.1	0.0	97.8
Hymenoptera terrestrial adult	1.9	8.5	0.0	100
Coleoptera terrestrial adult	1.1	7.6	0.0	100
Diplopoda terrestrial	0.1	1.0	0.0	19.4
Unidentified terrestrial insect fragments	0.9	6.6	0.0	83.3
Araneae terrestrial	0.3	2.5	0.0	28.0
Aphidoidea terrestrial adult	<0.1	<0.1	0.0	0.8
Formicidae terrestrial adult	<0.1	0.2	0.0	3.5
Aquatic plant fragments	3.2	13.4	0.0	91.1
Terrestrial plant fragments	38.5	42.2	0.0	100
Filamentous algae	7.8	23.4	0.0	100
Sediment (particulate inorganic matter)	2.4	9.4	0.0	94.1
Unidentified organic matter	8.2	23.1	0.0	100
Detritus (particulate matter)	11.7	27.9	0.0	100

CAPÍTULO 5 - Living on a stream-dwelling armored catfish: nested occupation of chironomid larvae on host's body surface*

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Abstract

Interaction where one species lives on other species is a worldwide phenomenon. In most cases, the symbiont species has preferential attachment sites on host’s body and thus some sites are only occupied when the supposed preferential sites are already occupied. This spatial distribution might be expected to generate a nested pattern. Moreover, the host size can interfere on the site quality for symbionts given aspects such as available area and host behavior. We used the NODF nestedness metric to explain the spatial distribution of chironomid larvae (*Ichthyocladius* sp.) on their host armored catfish (*Pareiorhaphis hypselurus*) of streams from Southern Brazil. As predicted, we found that (1) suboptimal sites on hosts were usually occupied by larvae only when optimal sites were already occupied and (2) sites occupied by larvae on small hosts were a subset of the sites occupied on large hosts. Our study suggests that nestedness is expected to occur in cases where symbionts have preferential sites for attachment on host’s body and thus a sequential occupation from the optimal to the least favorable sites. We also demonstrated that host size can interfere on the suitability of sites to symbionts, although the mechanisms whereby this process occurs still lack studies.

Introduction

Interspecific interactions where a species lives on specific sites of the body of its host is a common phenomenon found in a wide range of ecosystems (*e.g.* Waage, 1979; Ter Hofstede, Fenton & Whitaker, 2004; Wool, 2004). Symbionts, hereafter defined as any species living on or with other species, may occupy different sites of their hosts, although it can be expected they be more frequent on those which best fulfill their specific requirements (Tokeshi, 1993; Cook, Chubb & Veltkamp, 1998; Bittencourt & Rocha, 2002; Pennuto 2003).

Accordingly, the differential benefits for symbionts regarding the attached position on the host's body surface may generate a spatial distribution on hosts in which suboptimal sites are only occupied if optimal sites are already occupied. Also, host size may affect resource quality and quantity used by symbionts and thus it can be expected that large individuals be occupied not only by more symbionts but also on low-quality sites.

Chironomid midges comprise a highly diversified group of dipterans that lives in a wide range of aquatic and humid habitats (Pinder, 1986). Most larvae are free-living, although some species may build cases that are attached to the substrate, live inside the mesophyll of submersed leaves or attached to the surface of several host groups (Tokeshi 1993; Roque *et al*., 2004). Regarding the later habit, previous studies have documented chironomid midges living on several insect groups such as Plecoptera (Dosdall, Mason & Lehmkuhl, 1986; Giberson, Macinnis & Blanchard, 1996; Doucett, Giberson & Power, 1999), Ephemeroptera (Svensson 1980; Tokeshi, 1986; Jacobsen, 1998), Megaloptera (de la Rosa, 1992; Pennuto, 2000; Pennuto, Wooster-Brown & Belisle, 2002) and Odonata (Dosdall & Parker, 1998; Rosa *et al*., 2009). Although less frequently, Chironomidae larvae have also been recorded living on fishes (Freihofer & Neil, 1967; Mendes, Andersen & Saether, 2004), including catfish species of the South American families Astroblepidae and Loricariidae (*e.g*. Freihofer & Neil, 1967; Mendes *et al*., 2004; Roque *et al*., 2004; Sydow *et al*., 2008). The symbiont species of chironomids that live on fishes belongs to the genus *Ichthyocladius* Fittkau 1974, which has been suggested to have a commensal relationship with species of fishes (Freihofer & Neil, 1967; Sydow *et al*., 2008). Benthic habits, relatively large body size and intermediate mobility, rather than sedentary or highly mobile swimming species, appear to be the chosen host's characteristics for occurrence of symbiont chironomid larvae (Tokeshi, 1993). Members of both Astroblepidae and Loricariidae fish families move over rock and plant surfaces scraping the periphytic layer ingesting algae and other organisms or feed on the bottom detritus, and usually stay stationary in contact with the bottom or other surfaces for many hours during inactivity periods (Freihofer & Neil, 1967). Various chironomid species are also bottom-dwellers of rock and plant surfaces in lotic ecosystems and feed mostly by grazing the periphyton layer and gathering or filtering the suspended algae and detritus (Pinder, 1986; Gresens & Lowe, 1994). Thus, one probable explanation for the evolution of the association of chironomids with fishes is the common habitat and the advantage that the chironomid larvae may obtain by feeding on the suspended algae during scraping activities of the fishes (Sydow *et al*., 2008).

Ichthyocladius larvae were initially reported as occurring mainly on the interopercular tuft and near the base of the fins in Loricariidae and also on the leading rays of pelvic fins in Astroblepidae (Freihofer & Neil, 1967). Furthermore, the occurrence of *Ichthyocladius* on three species of armored catfishes (loricariids) was studied by Sydow *et al*. (2008), which found that larvae were more common on the pectoral and anal fins. Selection for specific sites of fish's body surface is suggested to benefit larvae regarding anchorage suitability and food acquisition (Freihofer & Neil, 1967; Sydow *et al*. 2008). The suspended material resulting of the foraging behavior of fishes is pointed out as the food source for *Ichthyocladius* and the site in which larvae is attached on fish's body is suggested to determine the quality and quantity of food that is received by the individuals (Freihofer & Neil, 1967; Sydow *et al*. 2008). The selection of preferential sites for attachment by symbiont chironomids is also supported by studies analyzing others hosts groups (*e.g*. Giberson *et al*., 1996; Pennuto, 2003). For instance, Pennuto (2003) found that chironomid larvae tend to attach on the ventral thoracic region of insect megalopteran larvae. However, when more than one larva occurred on a host, the smaller larvae were dislodged to the posterior portion of the host's body.

The number of chironomid larvae per host has been demonstrated to be positively associated to the host size (*e.g.* Tokeshi, 1986; Giberson *et al.*, 1996; Sydow *et al.* 2008). Possible causes for such relationship are the larger available area for colonization, the longer exposure time and also the concentration of chemical cues (Svensson, 1980; Pennuto, 2002). In addition, behavior of large fishes is suggested to increase the number of attached chironomids, once large individuals tend to dislocate over large areas and suspend more particulate food that benefit larvae (Sydow et al. 2008). Furthermore, the number of occupied sites on hosts may be expected to increase with the number of larvae per host given the territorialism behavior documented for symbiont chironomid larvae (Pennuto, 2003). Thus, we should expect that body sites occupied by larvae in small host individuals are a subset of those body parts occupied by larvae in larger host individuals, resulting in a nestedness pattern.

In ecology, nestedness is a concept originally developed for the spatial distribution of species in which species of poor communities are a proper subset of those species found in rich communities (Ulrich & Almeida-Neto, 2012). Although nestedness metrics are usually used in ecological studies aimed to understand patterns of metacommunities (*e.g.* Hylander *et al.*, 2005; Bloch, Higgins & Willig, 2007; Heino, Mykrä & Muotka, 2009) and of networks of interacting species (*e.g.* Bascompte *et al.*, 2003; Ollerton *et al.*, 2007; Guimarães *et al.*, 2007; Piazzon, Larrinaga & Santamaría, 2011), its use can be extended to any phenomenon that hypothetically generates nestedness among objects described by a set of variables. For instance, preferential occupation of symbionts for specific sites on the host's body surface has been suggested by several studies (*e.g.* Laihonen & Furman, 1986; Giberson *et al.*, 1996; Cook *et al.*, 1998; Whittington & Ernst, 2002) and if these preferences follows a ordered sequence of occupation, it can be hypothesized that spatial distribution of symbionts on their hosts should conform to a nested pattern.

We investigated the occupation pattern of the chironomid larvae *Ichthyocladius* sp. on the body surface of the armored catfish *Pareiorhaphis hypselurus* (Pereira & Reis, 2002). We predicted a nestedness spatial distribution of larvae on the host body and tested two hypotheses: 1) potential sites on the fish body differ in suitability for chironomid larvae and, thus, suboptimal sites will be occupied only when optimal sites are already occupied; 2) among individuals, small fish specimens support a reduced number of chironomid larvae and, thus, sites occupied in small host individuals are a subset of the body sites occupied by chironomids in large individuals.

Methods

Study area

We recorded associations between the chironomid larvae *Ichthyocladius* sp. and the armored catfish *Pareiorhaphis hypselurus* in streams of the Maquiné river basin, state of Rio Grande do Sul, southern Brazil. The Maquiné river is an Atlantic coastal drainage comprising a total area of 550 km^2 which is located between the longitudes 50°05’W and 51°21’W and the latitudes 29°20’S and 29°50’S. The regional climate type is humid subtropical (Moreno, 1961), with regular high rainfall throughout the year (1400 mm to 1800 mm) and mean temperatures greater than 22 °C in the warmest month and between 13-15 °C in the coldest month (Hasenack & Ferraro, 1989). Streams of the Maquiné basin are characterized by riffle–pool sequences with clear and fast flowing water and the substrate composed mainly of pebbles, cobbles and boulders (Becker, Carvalho & Hartz, 2008). Our sampled sites comprised riffle habitats with average depth of 22 cm and average wetted width of 8.8 m (ranging from 4.0 m to 19.5 m).

Fish and chironomid larvae sampling

We employed the kick-sampling method to capture fishes along 40 m stream riffle segments using a rectangular dip-net (80 x 40 x 40 cm with 4 mm mesh) in 22 sites distributed throughout the Maquiné basin. Fishes were kept alive after each sampling round and were immediately examined for number and location of chironomid larvae on their bodies. We recorded chironomid larvae at 16 body sites of the fish host, including pectoral and ventral fins (left and right), dorsal, anal, adipose and caudal fins, odontodes (left and right), operculum bony, lip, flank, back, abdomen and caudal peduncle (Fig. 1). We used incidence data and, thus, for the few cases in which more than one larva was present in a fish body site (e.g. ventral or dorsal face of the fins) we recorded it simply as presence. We also measured the total length (mm) of all captured fishes. Subsequently, we returned all fishes to their respective stream sites.

Nestedness analyses

We used the NODF metric for nestedness analyses (Almeida-Neto *et al.*, 2008) to test our two hypotheses about the occupation pattern of chironomid larvae on the body surface of fishes. The NODF requires a previous ordering of rows and/or columns of the incidence matrix. This can be done simply by ordering rows and/or columns by decreasing incidences. However, an informed way to compute NODF is to order rows and/or columns according to specific hypotheses (Almeida-Neto *et al.*, 2008; Melo, Cianciaruso & Almeida-Neto, 2014).

The NODF metric is based on paired comparisons of rows and/or columns of a given matrix. For each pair, the first step is to evaluate if the supposedly incidence-rich row (or column) is in fact richer than the supposedly incidence-poor row (or column). If this condition is not met, N_{paired} is zero. If the condition is met, N_{paired} is the percentage of shared incidences in relation to the total of incidences of the supposedly incidence-poor row (or column) (Almeida-Neto *et al*., 2008). The degree of nestedness for rows of a matrix is the average of all N_{paired} comparisons ($NODF_{rows}$). The same process is applied for columns depending on the question of interest ($NODF_{columns}$). Finally, the degree of nestedness for the whole matrix (NODF) can by calculated as the average of all row and column N_{paired} comparisons (Almeida-Neto *et al*., 2008). The significance of the observed statistics is obtained by contrast to a distribution generated by a proper null model or a permutation test, that is, by evaluating whether the observed nestedness value differs from a null expectation (Ulrich, Almeida-Neto & Gotelli, 2009). We raised hypotheses for both columns (site occupation on body surface) and rows (fish size).

NODF for columns – chironomid larvae distribution on fish body surface

We hypothesized that there are optimal places on fish's body surface for the occurrence of chironomid larvae and thus the remaining body sites of a given fish will be occupied only when the optimal sites are already occupied. That is, sites less often used by chironomids tend to be occupied only in the fish individuals for which better sites are already occupied. Columns of our matrix were sites of the fish's body potentially occupied by chironomid larvae and rows of the matrix were individuals of the armored catfish *P. hypselurus*. Despite of previous findings that some sites of the fish body are more often used than others, we lacked a clear and independent hypothesis regarding preference of occupation for all potential sites. Accordingly, columns were sorted simply from the most (left) to the least (right) occupied sites of the fish bodies. Our expectation was that columns on the right side of the matrix (least occupied sites) were a subset of columns located in the left side of the matrix. As this hypothesis refers only to columns, order of rows of the matrix did not affect results (Fig. 2).

We performed paired comparisons between all matrix columns to calculate an average observed $\mathrm{NODF}_{\mathrm{columns}}$ (Fig. 2). We had no *a priori* expectation of nestedness regarding comparisons of symmetrical body parts (e.g. pectoral fins; Fig. 1), and thus did not use these N_{paired} to compute $\mathrm{NODF}_{\mathrm{columns}}$. We computed observed and expected N_{paired} values in a blocked way as fish individuals were sampled at several stream sites, allowing us to control expected variation occurring between sampling sites (Fig. 2). Accordingly, we first obtained N_{paired} values involving pairs of columns for each stream site matrix separately. These matrices only included columns with incidences. Then, N_{paired} values were combined across sites and their average obtained ($\mathrm{NODF}_{\mathrm{columns}}$).

To test whether our observed $\mathrm{NODF}_{\mathrm{columns}}$ differed from a null expectation, we used a null model where only the row marginal totals were preserved equal to the original matrix, using the method r0 of the *commsimulator* function available in the vegan package (Oksanen *et al*., 2013) for the R environment (The R Development Core Team, 2014). Method r0, or fixed-equiprobable according to the nomenclature of Gotelli (2000), fills presences anywhere on the row independent of column. Therefore, this method maintained the number of chironomid larvae per fish individuals. This is important because number of larvae per fish may be limited by some particular characteristics of the individuals (e.g. body size). We repeated the procedure 999 times to generate an expected distribution of $\mathrm{NODF}_{\mathrm{columns}}$ values.

NODF for rows – chironomid larvae occupation according to fish size

We hypothesised that chironomid larvae occupies body sites in smaller size individuals of the armored catfish *P. hypselurus* that are a subset of the body sites occupied in largersized individuals of the fish. We tested this hypothesis by sorting the rows (fish individuals) of the incidence matrix from the largest to the smallest fish individuals (Fig. 3). Because our hypothesis regards only the rows of the matrix, column order did not affect results.

The nestedness degree of matrix rows ($\text{NODF}_{\text{rows}}$) was calculated as the average of observed N_{paired} values of all pairs of rows (Fig. 3). Because we sampled fishes at multiple sites in the Maquiné river basin, $\text{NODF}_{\text{rows}}$ were calculated separately for each stream site. Our expectation was that nestedness would be high between individuals with greater body size differences and low between individuals with small body size differences. Therefore, we weighed N_{paired} values with the respective differences between total lengths of the individuals being compared. We did that by multiplying each N_{paired} value by the difference between total lengths of the pair being compared relative to the maximum difference in total lengths among all pairs of individuals in a stream site.

Because we are testing a specific hypothesis, represented in a specific order of the rows of the matrix, we did not need to use traditional null models to assess the significance of the observed NODF. Instead, we employed a permutation test in which the expected distribution of random $\text{NODF}_{\text{rows}}$ values was generated by permuting positions of the matrix rows (*i.e.* fish individuals), keeping the entire matrix intact (Fig. 3). Randomized N_{paired} were obtained in a blocked way, as done for the observed values. That is, random weighted N_{paired} were obtained for each stream site separately and them combined to obtain an average ($\text{NODF}_{\text{rows}}$). We performed 999 within-site permutations of the original matrix and for each permuted matrix we calculated the expected $\text{NODF}_{\text{rows}}$.

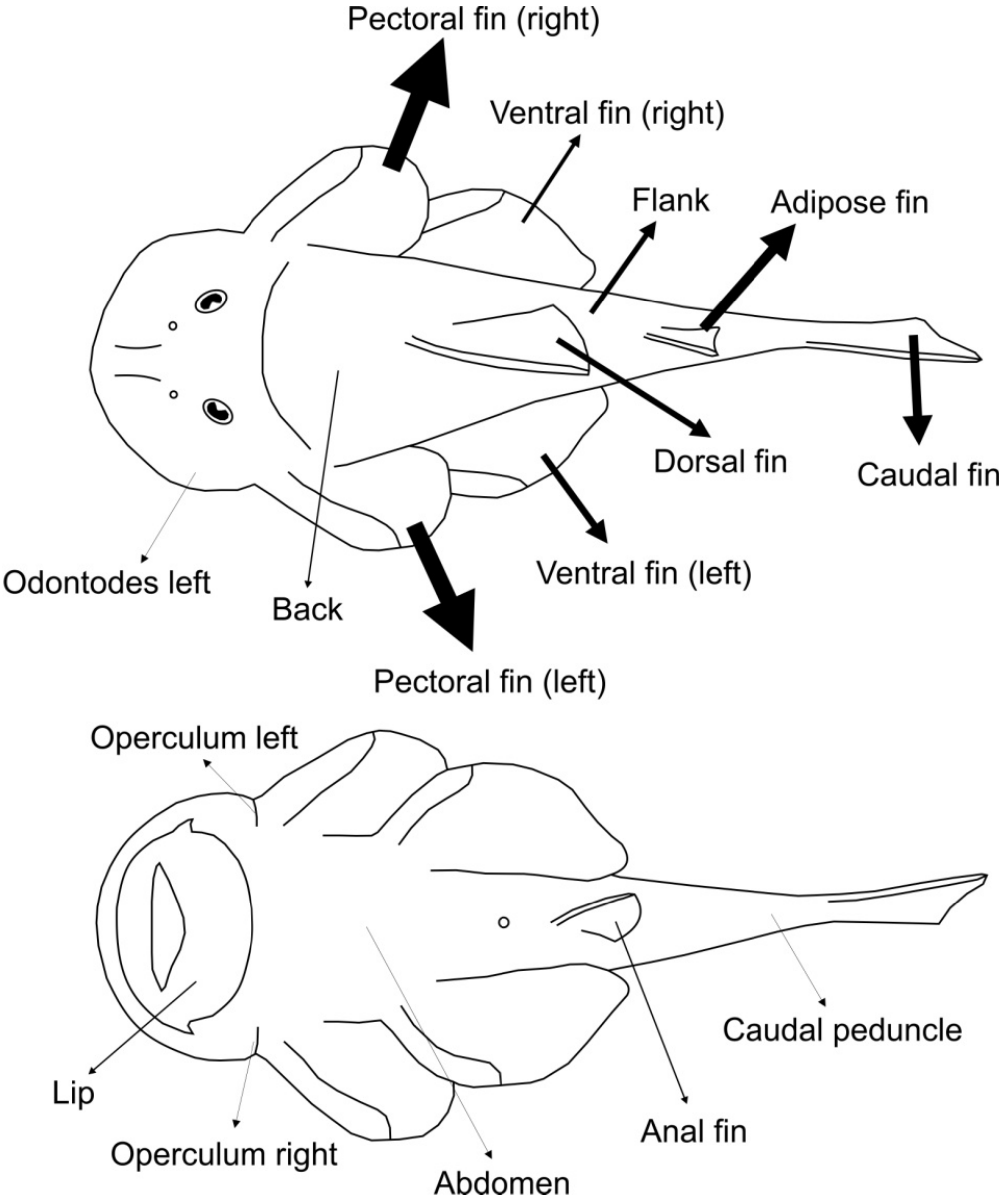

Figure 1. Body sites of the armored catfish *Pareiorhaphis hypselurus* where chironomid larvae *Ichthyocladius* sp. were found. The thickness of the arrows is proportional to the frequency of chironomids in 80 individuals.

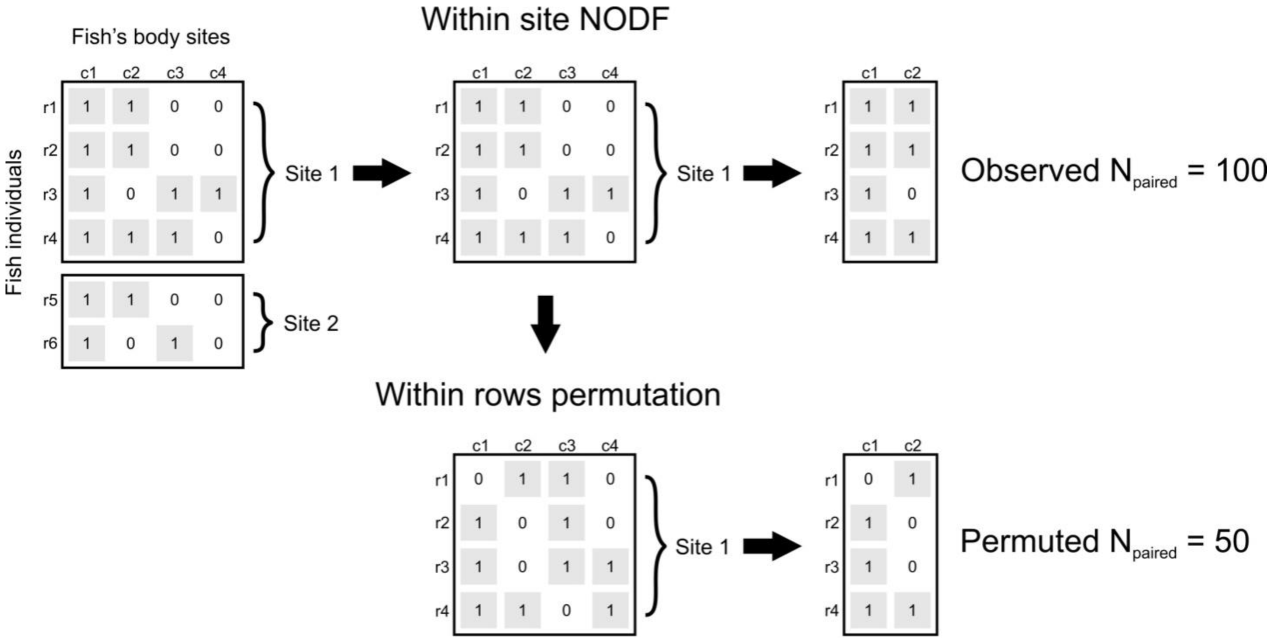

Figure 2. Illustration of the procedure used to calculate nestedness of Chironomidae larvae occupation on the body surface of the armored catfish *Pareiorhaphis hypselurus*. N_{paired} were computed separately for each stream site and then combined to obtain an average ($NODF_{columns}$). A null model was used to generate 999 values under an expected distribution of $NODF_{columns}$ values. The null model employed maintained row sums and thus all fish individuals retained the original number of symbiont chironomids, but columns (attachment sites on the fish body) were allowed to receive incidence at random.

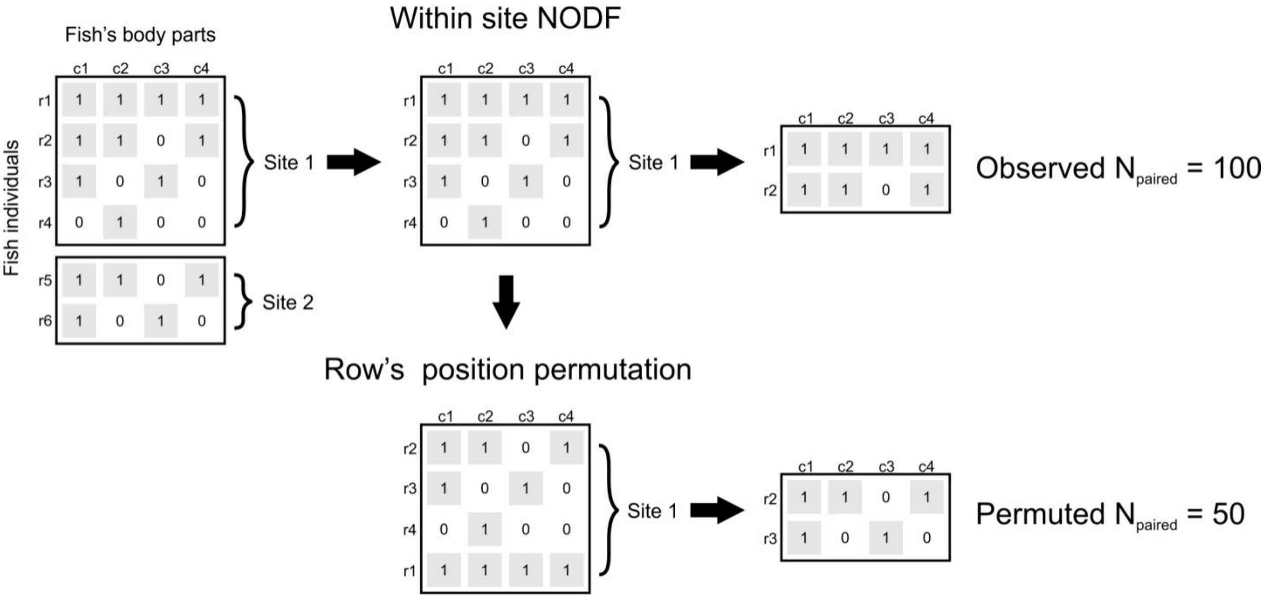

Figure 3. Illustration of the procedures used to calculate nestedness of chironomidae larvae occupation according to the sizes of individuals of the armored catfish *Pareiorhaphis hypselurus*. $NODF_{rows}$ were computed separately for each stream site as the average of all N_{paired} values between all pairs of rows. Assessment of the hypothesis was done using a permutation test which maintained the matrix intact, but randomized the order of rows, deconstructing our hypothesized ordination of largest to smallest individuals.

Results

We found associations between the chironomid larvae *Ichthyocladius* sp. and the armored catfish *Pareiorhaphis hypselurus* in 16 of the 22 sampled stream sites of the Maquiné river basin. A single fish was found in three sites and, as they cannot be used for nestedness assessments, were removed from analyses. The remaining 13 stream sites included a total of 77 fishes with 172 chironomid larvae. The smallest fish individual with larvae had 36 mm of total length and the largest individual had 93 mm (mean 65 mm). Body sites most frequently occupied were the right and left pectoral fins with 40 and 34 occurrences respectively. Other body parts with high frequency of chironomids were adipose (22), caudal (19) and dorsal (13) fins, the left and right ventral fins (14 and 8) and the body flank (10). The remaining fish's body sites were rarely occupied by chironomid and in all cases with three or less larvae (Fig. 1).

We found a nested pattern in the distribution of chironomid larvae on body sites of the *P. hypselurus*, with some fish's body parts being occupied only when others more frequently occupied body parts were already occupied (observed $\mathrm{NODF}_{\mathrm{columns}} = 32.4$; z-value = 4.2; p = 0.001). The observed NODF value for columns (our hypothesis for body site occupation) was higher than all 999 NODF values obtained using the fixed-equiprobable null model (Fig. 4A).

We also found nestedness among fish individuals differing in size (observed $\mathrm{NODF}_{\mathrm{rows}} = 6.2$; z-value = 2.3; p = 0.005; Fig. 4B). Thus, body sites occupied by chironomids in small-sized fishes were a subset of the body sites occupied by chironomids in large fishes, supporting our hypothesis regarding occupancy of hosts differing in size.

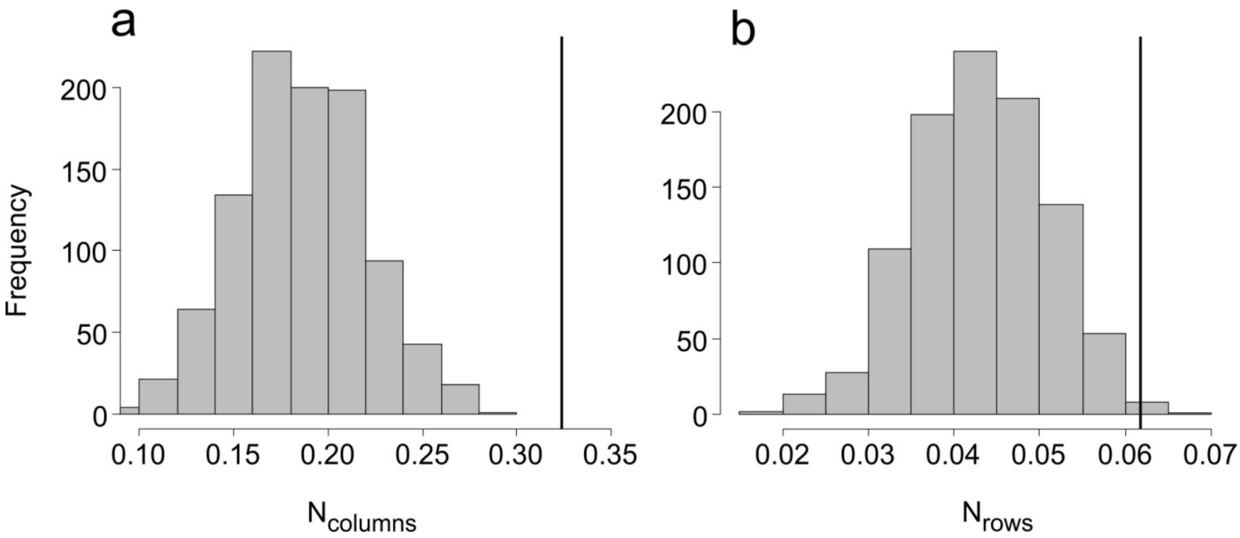

Figure 4. Expected distributions of NODF values derived from 999 permutations of the original matrix to test two hypotheses of nested occupation of the chironomid *Ichthyocladius* sp. on the body surface of its fish host *Pareiorhaphis hypselurus*. Columns of the matrix were body sites occupied by chironomid larvae and rows were fish individuals. The hypothesis for columns (A) was that less frequently occupied body sites are a subset of the more frequently occupied body sites. The hypothesis for rows (B) was that sites occupied in small fishes are a proper subset of those occupied in large fishes. Vertical black lines indicate the observed NODF values for both hypotheses.

Discussion

We found that the less frequently occupied sites by larvae on the fish host were a subset of the more frequently occupied sites, indicating an ordered attachment from optimal to less favorable sites. This finding supports our first hypothesis and suggests an ordered preference of larvae for specific sites. Preference of ectosymbiotic chironomids for specific body parts of their hosts have been suggested by several studies (*e.g*. Freihofer & Neil, 1967; Tokeshi, 1986; Sydow *et al*., 2008), but the benefits of preferential sites for larvae and the existence of intraspecific competition for sites were rarely demonstrated. Pennuto (2000) found that chironomid larvae residing farthest away from the megalopteran host's thorax were less likely to emerge than those residing on or near the thorax. In another study, Pennuto (1998) found that most of chironomid larvae that occurred singly on their megalopteran hosts emerged successfully as adult midge (70%) but emergence success declined dramatically (30%) when the density of larvae per host was high, suggesting strong competition for pupation sites on hosts. The author observed aggressive interactions between larvae during spring and suggested that larvae may compete for hosts and for the optimal sites on the host's body for pupation (Pennuto, 2000). Although we lack information on the behavior of the larvae of *Ichthyocladius* sp., our results of nested occupation suggests an ordered quality of sites and, thus, that the chironomid larvae are more benefited by occupation of a few specific sites.

Pectoral fins of *P. hypselurus* were the body sites more frequently occupied by chironomid larvae. This preference of *Ichthyocladius* larvae for the pectoral fins of *P. hypselurus* was also suggested by Sydow *et al*. (2008) study, which reported a high number of occurrences of larvae on these fins. However, we highlight that our nestedness analyses indicated that the preference for pectoral fins belongs to an ordered chain of preferences. Thus, we suggest that individuals of *I. liliane* benefit most from being attached to the pectoral fins of *P. hypselurus* and that a decreasing benefit is obtained by occupying empty but less favorable sites.

According to Tokeshi (1993), chironomids may benefit from living on host's body in four ways: (i) increased mobility, (ii) better feeding opportunity, (iii) protection from disturbances and (iv) reduced risk of predation. Preferential or ordered site occupation may be not explained by increased mobility because sites are in the same fish individual. Regarding feeding opportunity, there is some indications supporting that differential quality and quantity of food available for chironomids may differ depending on the attachment site location. The host armored catfish *P. hypselurus* inhabits mountain streams riffles with low turbidity and fast flowing waters (Dala-Corte, pers. observ.). Several riffle-dwelling loricariids, as well as the species studied by us, have benthic habits and usually feed on the peryphitic biofilm by grazing rock surfaces (Buck & Sazima, 1995; Delariva & Agostinho, 2001; Casatti & Castro, 2006), suspending a cloud of detritus with diatoms and other microorganisms while it forages (*e.g.* Leitão, Caramaschi & Zuanon, 2007). In fact, Sydow *et al.* (2008) reported that diatom algae were the main food item found in preliminary analysis of *Ichthyocladius* diet. Once pectoral fins of *P. hypselurus* constitute a flat surface located immediately posterior to the head and close to the mouth, it is plausible that larvae preference for these sites is due to the greater availability of suspended food (Freihofer & Neil, 1967). This explanation, however, is weakened by the fact that posterior sites were also frequently occupied by larvae (*e.g.* adipose and caudal fins). We have no evidence to evaluate the two remaining mechanisms suggested by Tokeshi (1993), that is, protection from disturbance or predation benefits. Furthermore, two additional plausible mechanisms to explain the larvae preferential occupation on the body of fishes are that sites may differ in quality for pupation or attachment suitability. Regarding site preference for pupation, there are supporting evidences for chironomids living on megalopteran larvae (de la Rosa 1992; Pennuto, 2000; 2003). For attachment suitability, Freihofer & Neil (1967) suggest that some sites on fishes are more easily occupied by larvae due to the presence of anchor structures such as interopercular tuft and odontodes (dermal teeth). *Pareiorhaphis hypselurus* individuals have the body covered by odontodes, which are hypertrophied on pectoral-fin and on the cheeks of nupitial males (Pereira & Reis 2002). Regardless sex or size of individuals, we observed that pectoral and pelvic fins of *P. hypselurus* are covered by a dense layer of more developed odontodes, which may also influence the attachment suitability for larvae. It is possible, however, that the ordered occupation by chironomid larvae on the body of fishes found by us indicates that one or multiple of the mentioned mechanisms are acting to determine this pattern. Specific manipulative experiments would be necessary to shed light on the relative importance of these mechanisms.

We found that size of the fishes was also an important factor for chironomid larvae occupation, with small-sized hosts harboring not only less chironomid larvae but also in a subset of body sites of those sites occupied in large fishes. Several previous studies have shown that the number of chironomid larvae per host increases in large hosts, either for freshwater macroinvertebrates (*e*.g. Svensson, 1980; Tokeshi, 1986; Pennuto 1997; Hayashi & Ichiyanagi, 2005) and armored catfish (Sydow *et al*. 2008). Differing from these studies, however, we asked whether the site occupation by larvae follows a nested pattern, whereas sites usually not unoccupied in small fishes tend to be used in larger fishes. A necessary condition for the nested pattern, supported by our nestedness analyses, is that large individuals harbor more larvae than small individuals. A plausible explanation is that large host individuals of the armored catfish *P. hypselurus* can support larvae on more body sites due to their behavior, since large fishes cover a wider swimming area and may suspend more food to chironomid larvae while foraging by scrapping the periphyton layer. Thus, more sites could become suitable to larvae occupation in large fishes. Also, large hosts have large surface area and are older, increasing the probability and time available for chironomid colonization (*e.g.* Tokeshi, 1986; Giberson *et al*., 1996; Sydow *et al*. 2008). Although these two plausible mechanisms explain the presence of more larvae on large fishes, they do not explain the nested occupation of body sites according to fish size. The nested pattern, however, could be explained by competition among chironomid larvae for attachment sites on hosts. Hence, in larger hosts with higher larval densities, larvae could occupy only vacant and/or unsaturated sites on hosts or even be displaced from good sites by other larvae. For example, Pennuto (2003) found that the largest larva occupied the thoracic position on megalopteran hosts when more than one chironomid larvae was present. The author also found that when a single larva occurred on a host, even if it was a small one, it was always located in the anterior region of the host's body, indicating preferential attachment site (Pennuto 2003). However, we did not evaluate the differences between larvae size occupying different body sites and thus more evidences are necessary to support this hypothesis of competition for preferential sites on armored catfishes.

Our results suggest that chironomid larvae show an ordered occupation on their host's body, generating a nested pattern. This result indicate that chironomid larvae have an ordered preference for attachment sites on the host's body surface, in which sites are occupied sequentially from the optimal ones to the less favorable sites. We also found a nested pattern of larvae occupation regarding fish size in which large hosts can harbor larvae at sites that usually are unoccupied in small hosts. Furthermore, our study suggests that nestedness is a distribution pattern expected to occur in cases where symbiont species have preferential sites for attachment on their host's body and thus a sequential occupation from the optimal to the least favorable site.

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Considerações finais

Específicas

1. O capítulo 1 evidencia que em bacias com distintos graus de uso agrícola, alterações antrópicas na faixa ripária tem consequências mais pronunciadas sobre o habitat de riachos, e alteram a riqueza e a diversidade funcional alfa de peixes. Isso sugere que a proteção das faixas ripárias é um ponto chave para o manejo ambiental visando à conservação da biodiversidade aquática dos Campos Sulinos do Brasil.

2. O capítulo 2 sugere que a diversidade beta em peixes de riachos responde de uma forma não linear a alterações agrícolas da paisagem, aumentando a diferenciação na composição em níveis baixos de uso agrícola na sub-bacia, e causando homogeneização da ictiofauna entre comunidades quando o uso do solo ultrapassa 40% da área total da sub-bacia a montante. Esses resultados fornecem evidências que sustentam que as consequências ambientais oriundas da agricultura modificam o balanço entre processos estocásticos e determinísticos na montagem de comunidades.

3. O estudo que eu desenvolvi no capítulo 3 indica que a posição do riacho na rede de drenagem tem grande influência nos processos que determinam a dinâmica de comunidades de peixes. A posição na rede de drenagem vai influenciar a severidade dos filtros ambientais, a distância em relação a outros trechos de riachos e, portanto, a conectividade com fontes de imigrantes, e também afeta a capacidade do habitat em termos de tamanhos. Além disso, eu constatei que as espécies diferem em suas respostas à esses processos. Esses resultados explicam por que é tão complexo predizer a dinâmica de comunidades de peixes em sistemas dendríticos.

4. No capítulo 4 eu mostrei que a utilização de espécies generalistas, abundantes e de ampla distribuição, pode ser de grande valia para entender como as alterações ambientais afetam os

ecossistemas de riachos e o papel das espécies na teia trófica. Eu encontrei que uma espécie generalista pode mudar seu papel trófico em riachos impactados por alterações na faixa ripária, e que isso pode levar a um aumento no comprimento médio do intestino da população em virtude de mudanças na qualidade do alimento consumido.

5. No capítulo 5, observei que as larvas de quironomídeo apresentam uma ocupação ordenada na superfície corporal no peixe hospedeiro, indicando um padrão de aninhamento em relação a sítios preferenciais. Também evidenciei que isso pode mudar dependendo do tamanho corporal do peixe, uma vez que algumas partes corporais podem se tornar mais favoráveis à ocupação em peixes maiores. Esses resultados sugerem que um padrão aninhado é esperado em casos de ocupação preferencial de hóspedes em sítios específicos no corpo de seus hospedeiros.

Gerais

Na medida em que os riachos são cada vez mais submetidos a pressões antrópicas, o entendimento dos processos que determinam os padrões observados nos ecossistemas aquáticos torna-se urgente e fundamental. Os resultados da minha tese apresentam diversas implicações, uma vez que eles contribuem para esclarecer quais são os processos e como eles atuam afetando as comunidades, populações e interações em peixes de riachos. Os capítulos 1 e 2 contribuem principalmente em relação a fornecer informações com base científica em como mitigar impactos de uso do solo agrícola sobre a biodiversidade de peixes de riachos. O capítulo 3 levanta diversas questões que devem ser levadas em consideração em monitoramentos e em futuros estudos. O capítulo 4 lança uma luz no entendimento das consequências das alterações ambientais para as espécies que tem a capacidade de manter populações em riachos degradados. Isto é, o que acontece em um riacho pode depender da posição do mesmo na rede dendrítica, e isso precisa ser previamente ponderando antes de desenvolver estudos e antes de traçar planos de manejo. O capítulo 5 mostra que processos pensados em escalas mais amplas (filtros ambientais) também podem ocorrer em escala de interação interespecífica, e determinar um padrão de aninhamento.

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