CECÍLIA GONTIJO LEAL

MULTISCALE ANTHROPOGENIC IMPACTS ON STREAM CONDITION AND FISH ASSEMBLAGES IN AMAZONIAN LANDSCAPES

LAVRAS-MG
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Thesis submitted for the degree of Doctor of Philosophy as a Dual PhD between the Lancaster Environment Centre, Lancaster University, United Kingdom and the Applied Ecology Postgraduate Program, Universidade Federal de Lavras, Brazil.

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LAVRAS-MG

2015

Ficha catalográfica elaborada pelo Sistema de Geração de Ficha Catalográfica da Biblioteca Universitária da UFLA, com dados informados pelo(a) próprio(a) autor(a).

Leal, Cecília Gontijo.
Multiscale anthropogenic impacts on stream condition and fish assemblages in Amazonian landscapes / Cecília Gontijo Leal. – Lavras: UFLA, 2015.
224 p. : il.

Tese(doutorado)–Universidade Federal de Lavras, 2015.
Orientador(a): Paulo dos Santos Pompeu.
Bibliografia.

1. Forest-agriculture frontier. 2. Water quality. 3. Physical habitat. 4. Human-modified tropical forests. 5. Ichthyofauna. I. Universidade Federal de Lavras. II. Título.

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APPROVED on the 27th of March 2015.

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2015

Declaration

I herewith declare that this work has been originally produced by myself for the present thesis, and it has not been previously presented to obtain a degree in any form. Collaborations with other researchers, as well as publications or submissions for publication are properly acknowledged throughout the document.

Cecília Gontijo Leal,
Lavras, Brazil, February 2015.

Para minha mãe e para o meu pai por tanto amor sempre
To the Amazon, where the powers of life are predominant

DEDICATION

Charles Darwin, Beagle Diary, on his visit to the Brazilian rainforest:

“The delight one experiences in such times bewilders the mind, — if the eye attempts to follow the flight of a gaudy butterfly, it is arrested by some strange tree or fruit; if watching an insect one forgets it in the stranger flower it is crawling over, — if turning to admire the splendour of the scenery, the individual character of the foreground fixes the attention. ***The mind is a chaos of delight, out of which a world of future & more quiet pleasure will arise****.”*

ACKNOWLEDGEMENTS / AGRADECIMENTOS

If I find enough words maybe I'll be able to express my upmost gratitude to my supervisors Paulo Pompeu, Jos Barlow and Toby Gardner. You taught me (attention to the correct spelling!) much more than I could ever have written in this thesis. The science behind samples, graphs and results are a small detail besides all feelings, memories and experiences I will take with me irrespective of the next steps in my life. You were just fundamental since the beginning. Ao Paulo que como sempre, desde o mestrado, transborda positividade e incentivos à liberdade, às tentativas, aos pensamentos (às vezes megalomaníacos) e sempre está lá vibrando orgulhoso pelas conquistas ou pronto pra dividir qualquer preocupação. Paulo, com a sua orientação eu nunca me senti sozinha. Esse eu já tinha um orientador que entende das angústias, inseguranças e desafios ao longo dos caminhos e que concorda que ser feliz vale mais do que qualquer linha no currículo, então ganhei outros dois durante o doutorado. Jos e Toby obrigada por terem mergulhado tão profundamente comigo nas águas dessa tese sempre mostrando muito além do que meus olhos enfeitiçados pelos peixes poderiam enxergar. Toby thanks for being so friendly and helpful 24h per day and cheerful with every small achievement. Jos your guidance was fundamental; in every single meeting I came out much more inspired than when I entered. This made me confident that it was worth carrying on. A vocês três: thanks for showing me that there is a big wide world out there… and to support me to go for it.

À toda Equipe Aquática do RAS, especialmente os companheiros de campo, sempre na busca incansável por igarapés "amostráveis" que por incrível que pareça não eram tão fáceis de encontrar na Amazônia de tantas águas. Manoel Nascimento (Nego), Renilson de Freitas (Graveto), Martinez de Oliveira, Elbin da Silva, Milton, Francisco Cunha (Pita) e ValderirNascimento

por tanta dedicação em campo e por me passarem um pouquinho da sabedoria naturalista nata. Aos amigos novos e antigos que no dia a dia do campo entraram nos igarapés sem medo de se molhar e ser feliz:Rafael Leitão, Bob Hughes, Débora de Carvalho, Lucas Pires, Douglas Bastos, Ceceo Chaves, Leandro Brasil, Janaína de Brito, Ceceo Chaves, Lenise da Silva, Túlio Franco, Leandro Juen, Vivian de Oliveira, José Max de Oliveira-Silva, Karina Silva, Miriam de Almeida, Marcos Vinícius da Silva e Rafael Duarte. Érika Berenguer e Bob Solar pelas trocas de figurinha inter-equipes.

Aos coordenadores do RAS Toby Gardner, Joice Ferreira, Luke Parry, Alex Lees e Jos Barlow pelo suporte constante em todas as fases do projeto. Especialmente Toby e Joice pelo apoio logístico fundamental em campo, fazendo o máximo para tudo acontecer da forma mais harmoniosa, eficiente e obviamente divertida.

To all farmers and workers unions of Santarém, Belterra, and Paragominas and all collaborating private landowners for their support. I am are also grateful to the following institutions for financial support of the RAS project: Instituto Nacional de Ciência e Tecnologia – Biodiversidade e Uso da Terra na Amazônia (CNPq 574008/2008-0), Empresa Brasileira de Pesquisa Agropecuária – Embrapa (SEG: 02.08.06.005.00), the UK government Darwin Initiative (17-023), The Nature Conservancy, Natural Environment Research Council (NERC) (NE/F01614X/1 and NE/G000816/1).

To CAPES for my scholarship in Brazil (2943-13-1) and UK (Science without Borders - PDSE-2943/13-1).

To my coautors in the thesis manuscripts Rafa Leitão, Jansen Zuanon, Bob Hughes, Phil Kaufmann, Benito, Silvio Ferraz, Jim Thomson and Ralph Mc Nally for the endless patience, help and reasonable contributions trying to decrypt my thoughts.

Ao Rafa Leitão, inseparável parceiro do início ao fim da tese, companheiro Amazônia adentro e Inglaterra afora! Incontáveis momentos divertidos juntos. Dividindo nossos desesperos com os peixes “inidentificáveis”, variáveis sem explicação biológica, planilhas com milhares de linhas e colunas sem fim, tive mais fôlego pra seguir em frente!

Aos programas de pós-graduação em Ecologia Aplicada da Universidade Federal de Lavras and Science of Tropical Environments at Lancaster University pelo apoio durante todo o doutorado. Aos professores de ambas instituições queinvestiram tanta energia em fazer a dupla titulação acontecer, principalmente Júlio Louzada e Jos Barlow.

To the tropical group, the most “vibrant research group (Barlow, 2014)” ever. Our family bond was a constant relief and inspiration to carry on: Anne, Jos, Luke, Mari, Natalie, Macaco-porco, Lisi, Careca-Cabeludo, Hannah, Sujo-safado, Érika, Rachael Carrie, Sâmia, Bobão and Fabinho.

Aos pupilos dos Pompeu do Laboratório de Ecologia de Peixes da UFLA, que desde o mestrado deixam o dia-a-dia no laboratório e as viagens de campo mais interessantes e divertidas: Débora, Dani, Marcela, Tatau, Nina, Fábio, Lud, Nara, Ivo, Rafa e toda a trupe que de alguma parte contribuem para esse cardume.

To myvery best special special friends in Lancaster who showed me that love and glitter are never too much and can cross any transoceanic barrier: Mari Piva, my husband Natalie Swan, Hannah Griffiths, Anne Toomey, Lisi Zanella, Dany Tregidgo, Guy Crawford, Olivia Havercroft, Beth Brocket, Luke Parry, Tom Walker, Al Campbell, Ali Birket, Victor Careca-cabeludo, Filipe França, Bob Solar, Fabinho, Gina Frauzin, Victoria Frausin, Érika Berenguer, Chris Rhodes, Andrezão, Grazyna Monvid, Ainara Penalver, Artzai Jauregui and so many others from the Lancaster Environment Centre and the Ecology and Conservation Group!!!Aos queridos Lisi, Toru, Filipe e Andrezão que em

Lancaster deram à dupla titulação um aconchego de família: sou duplamente grata à dupla amizade de vocês. I am also deeply thankful to the "Spanish house" team (Mari, Maria and Begoña), Érika and Darren, Grazyna and Al Campbell for hosting me and helping with the six house moving during my "one year" in Lancaster. --- Thank you all for bringing constant joy to my life in Lancaster!

Aos amigos brasileiros de sempre e que são muitos e importantes demais em todos os momentos. Os de infância, os de família, os da biologia UFMG, os do mestrado UFLA, as da Manacá, os que foram aparecendo e os que reapareceram ao longo do caminho, os da família Sá Fortes Leite. Especialmente aos biólogos que desde o começo me inspiraram a amar expedições, coletas, história natural, essa coisa de embrenhar no campos das gerais e ser meio bicho do mato mesmo: Felipe Leite, Andréa Mesquita, Fernanda Barros, Flávia Pezzini, Letícia Garcia, Pedro Vianna e Thaís Elias.

À minha família linda, especialmente pai e mãe, irmãos, cunhadas e sobrinhos sempre tão presentes, positivos, interessados em saber pra onde vou e o que tenho pra contar.Tão unicamente incentivadores do que quer que eu faça onde quer que eu esteja.

To the deepest blue eyes I've ever seen… who showed me that an ocean away means nothing and taught me to don't wash spiders down the plughole. Fitter, happier. Obrigada Al.

RESUMO

Mudanças no uso da terra e degradação florestal têm resultado em severas alterações aos ambientes tropicais no mundo, entretanto as consequências aos cursos d'água permancem pouco conhecidas. Isto é ainda mais crítico para a bacia Amazônica, particularmente sua complexa rede de igarapés. Estes igarapés fazem a conexão entre os ecossistemas terrestre e aquático através da paisagem. Além disso abrigam grande parte da diversidade de peixes, se não a maioria, da bacia que por si só é a mais diversa do mundo. Apesar da incontestável relevância dos igarapés, as consequências das mudanças no uso da terra para seu habitat aquático e fauna de peixes permanece uma grande lacuna de conhecimento. Esta tese tem como objetivo preencher parte desta lacuna investigando os efeitos dos distúrbios antrópicos em diferentes escalas espaciais na condição biológica dos igarapés em paisagens antropicamente modificadas do estado do Pará, Brasil.

O estudo começa investigando como o habitat aquático (representado por características de qualidade da água e habitat físico) responde aos distúrbios antropogênicos da paisagem (Capítulo 2). Em seguida são avaliadas mudanças na riqueza de espécies, abundância e composição das comunidades frente a alterações tanto do habitat aquático quanto da paisagem (Capítulo 3). Por último verificou-se a importância relativa das mesmas variáveis ambientais preditoras usadas no Capítulo 3 nas respostas espécie-específicas, avaliando a potencial efetividade da legislação ambiental brasileira em levá-las em consideração (Capítulo 4). Foram amostrados 99 igarapés distribuídos em cinco bacias hidrográficas e duas regiões (Santarém e Paragominas) na Amazônia oriental, região de desenvolvimento agrícola.

Foram registrados 25,526 exemplares de peixes pertencentes a 143 espécies, 27 famílias e sete ordens; sendo os igarapés altamente heterogêneos em suas características bióticas e abióticas. Por exemplo, em todas bacias hidrográficas a diversidade beta entre igarapés foi mais representada pela substituição de espécies (ca. 90%) do que pelo aninhamento. De forma geral os resultados encontrados enfatizam a importância de diversos usos da terra e escalas espaciais em influenciar o habitat aquático dos igarapés, incluindo associações entre por exemplo cobertura florestal na drenagem e temperatura da água, ou dos impactos de cruzamentos de estradas na morfologia do canal. Ambos, paisagem e habitat aquático também influenciaram as comunidades de peixes, porém o habitat aquático mostrou-se particularmente importante em explicar os padrões de abundância das espécies quando comparado a características da paisagem geralmente consideradas mais propícias ao manejo (e.g. proteção da floresta ripária). Entretanto os resultados também ressaltam a complexidade dos igarapés e as dificuldades de desvendar os efeitos de indicadores de distúrbios antrópicos em múltiplas escalas espaciais sustentados por uma inerente heterogeneidade ambiental – tanto o habitat aquático quanto as comunidades de peixes foram influenciados por uma ampla gama de variáveis que diferiram nas diferentes bacias hidrográficas e regiões.

Os resultados encontrados são utilizados para discutir os desafios e recomendações ao manejo e conservação desses sistemas amazônicos em paisagens antropicamente modificadas. Enfatizando particularmente a necessidade de estratégias coletivas planejadas em escala de drenagem, ou seja, que incorporem mais que a zona ripária dentro de propriedades rurais individuais como priorizado pela legislação ambiental brasileira vigente.

Palavras-chave: Fronteira de desenvolvimento agrícola. Qualidade da água. Habitat físico. Florestas tropicais antropicamente modificadas. Ictiofauna. Desmatamento. Cruzamento de estradas.

ABSTRACT

Land use change and forest degradation are resulting in pervasive changes to tropical ecosystems around the globe, however consequences for freshwater ecosystems remain poorly understood. This is especially true for the Amazon basin, in particular for its complex network of low-order streams. These streams connect terrestrial and aquatic ecosystems throughout landscapes and host much, of the freshwater fish fauna of the Amazon basin. Despite the biological significance of these stream networks, the consequences of land use change for the condition of instream habitat and fish fauna remain very poorly studied and understood. This thesis aims to address part of this knowledge gap by investigating the effects of anthropogenic disturbances occurring at multiple spatial scales on stream condition and fish assemblages from human-modified Amazonian forests in the state of Pará, Brazil.

The thesis starts by asking how instream habitat (composed of both water quality and physical habitat features) responds to landscape-scale anthropogenic disturbances and natural features (Chapter 2). Chapter 3 then investigates changes in fish species richness, abundance and composition following changes in both instream habitat and landscape-scale anthropogenic disturbance. Last, in Chapter 4 I attempt to disentangle the relative importance of those multiscale environmental predictor variables on species-specific disturbance responses, and evaluate the potential effectiveness of the Brazilian legislation in accounting for them. A total of 99 low-order streams were surveyed from five river basins in two large regions (Santarém and Paragominas) in the eastern Brazilian Amazon agricultural-forest frontier.

I sampled a total of 25,526 fish specimens belonging to 143 species, 27 families and seven orders. Streams appeared to be exceptionally heterogeneous in their abiotic and biotic features. For instance the contribution of turnover to the beta stream site component was much higher than nestedness in all river basins. Overall these findings underscore the importance of multiple land use changes and disturbances, at multiple spatial scales, in shaping instream habitat, including links between catchment-scale forest cover and water temperature, and the impacts of road crossings on channel morphology. Both landscape and instream habitat variables were isolated as having a marked effect on stream fish, but instream habitat differences were shown to be particularly important in explaining patterns of fish species abundance compared to other landscape factors that are more amenable to management such as the protection of riparian forest strips. However the results of the thesis also highlight the complexity of Amazonian stream systems and the difficulties in disentangling the effects of multiscale environmental predictor variables underpinned by naturally heterogeneous biophysical characteristics – with instream habitat and fish assemblages affected by a broad suite of drivers that often varied across river basins and regions.

I use the findings of the thesis to discuss challenges and recommendations for the management and conservation of low-order streams in Amazonian human-modified landscapes. In particular I emphasize the need for catchment-wide collective management approaches that go beyond the protection of riparian forests within individual properties as prioritized by existing Brazilian environmental legislation.

Keywords: Forest-agriculture frontier. Water quality. Physical habitat. Human-modified tropical forests. Ichthyofauna. Deforestation. Road crossings.

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1 GENERAL INTRODUCTION

1 GENERAL INTRODUCTION

1.1 Freshwater biodiversity

Freshwater ecosystems occupy less than 1% of the Earth’s surface, make up 0.01% of all water, and provide many vital services relevant to human well-being and poverty alleviation (MILLENNIUM ECOSYSTEM ASSESSMENT, 2005). They also host a large proportion of global biodiversity including ca. 10% of all known species and ca. 33% of all vertebrates (STRAYER; DUDGEON, 2010). Freshwater fish are both highly diverse, with estimates of up to 13,000 fish species in total, and have high levels of endemism (LÉVÊQUE et al., 2008). The Neotropical region alone is responsible for 5,600 recognised species of fish, equivalent to10% of all vertebrate species, which are distributed across some of the most diverse river basins in the world(ALBERT; BART; REIS, 2011; LÉVÊQUE et al., 2008).

Furthermore freshwater ecosystems are considered highly threatened, more so than terrestrial and marine equivalents. The main drivers of these threats are linked to anthropogenic activities leading to habitat degradation, pollution, flow regulation and water extraction, fisheries, overexploitation, and alien species introductions (STRAYER; DUDGEON, 2010). However, the scientific knowledge about freshwater systems is incomplete, and human-induced changes remain poorly understood and may be underestimated. This situation is likely to be more critical in tropical systems than temperate ones. First because fish species composition is poorly known for most tropical river basins (DUDGEON et al., 2006). Second, it is in the megadiverse tropics where landscapes are under rapid and penetrating pressure from intensive and rapid development of urban and agricultural lands with irreversible widespread consequences for natural ecosystems (FAO, 2011; MALHI et al., 2014).

Most of our understanding of human-impacts on tropical forests has been developed from the study of terrestrial systems. In contrast, aquatic systems have received far less research attention, with the majority of work to date being concentrated in a small number of well-studied regions, such as Costa Rica, Puerto Rico, and Australia (DUDGEON, 2008). Moreover, work tends to focus on lakes and large rivers that are of interest for navigation and power generation, and which host fish species of commercial value (e.g. RIBEIRO&PETRERE JUNIOR 1990; BATISTA&PETRERE Jr. 2003; ARDURA et al. 2010). As a result, tropical low-order streams have been largely neglected by scientific research, and remain poorly understood. An example of this can be seen in a recent review of 62 studies assessing faunal responses to land use change in Amazonia (PERES et al., 2010) that included only one study investigating freshwater systems, in this case, stream fish (see DIAS et al. 2010).

Effective conservation strategies should be built on robust scientific information, and the lack of research on tropical low-order streams means they generally carry little weight in management and conservation planning (BENSTEAD et al., 2003). Global conservation planning initiatives aim to prioritise conservation efforts to areas that have unique biological richness (irreplaceability) and high vulnerability to threats (e.g. biodiversity hotspot regions, protected areas). Almost all are defined based on terrestrial parameters (BROOKS et al., 2006) which are unlikely to match priorities set for freshwater systems (ABRAHAM; KELKAR, 2012; HERBERT et al., 2010). The available initiatives that account for freshwater systems are far more rudimentary than their terrestrial counterparts: for example, an attempt to categorize global freshwater units based on fish species distribution and composition, the Freshwater Ecoregions of the World, FEOW (ABELL et al., 2008), is too coarse to assist regional management strategies for low-order streams. Furthermore in Brazil, the IUCN Red Species List only started including freshwater fish in

2004, whereas similar information was created for other biotic groups in 1968 and has been refined since then. Furthermore, data availability means that it is much easier to estimate a threat status for large sized and commercially valuable fish species than typical low-order stream fish fauna (MMA, 2014).

Given the lack of information outlined above, it is critical to investigate how changes in tropical forest landscapes translate into changes in stream systems, as this will help guide effective interventions in watershed management and biodiversity conservation (MOULTON; WANTZEN, 2006).

1.2 Tropical streams

"There is no such thing as a 'typical' tropical stream"(DUDGEON, 2008)Tropical streams share some broad similarities in natural features, such as having a high water temperature for a given elevation, and often having high levels of hydrological periodicity with intense rainfall and runoff (BOULTON et al., 2008). However, beyond these broad generalities, tropical streams, like temperate streams, are extremely heterogeneous in their biotic and abiotic characteristics (DUDGEON, 2008), making it very difficult to draw further generalizations. In addition to any environmental distinctions, the high levels of land use change that characterise much of the tropics in recent decades mean that tropical streams can often differ markedly in socio-economic aspects (MOULTON; WANTZEN, 2006).

Both temperate and tropical streams networks do have one important defining feature that has profound implications for their conservation and management: their hierarchical spatial organization that determines how local conditions are highly dependent on their regional context (FRISSELL et al., 1986). This network connectivity is not restricted to the watercourses themselves; stream habitats, water quality and aquatic biota are all influenced by nested landscape scales factors through complex and varying pathways (ALLAN; ERICKSON; FAY, 1997; TOWNSEND; DOLE; ARBUCKLE, 2003; WANG; SEELBACH; HUGHES, 2006). For this reason streams and the surrounding lands are increasingly seen as “riverscapes” or riverine landscapes (ALLAN, 2004; FAUSCH et al., 2002; SCHLOSSER, 1991).

1.2.1 Streams in human-modified tropical forests

In tropical rainforests conversion of natural habits for agriculture and major infrastructure development continues to be the major driver of environmental change (FAO, 2011), with additional perturbations from widespread timber and wood extraction, changes in fire regimes, landscape fragmentation, expansion of second-growth forests, faunal extinction, species invasion, increasing CO_2 and climate change (MALHI et al., 2014). Alongside tropical forests encompass the most diverse fish streams yet the poorest known group among vertebrates (ALBERT et al. 2011a). While our understanding of anthropogenic impacts on tropical forest ecosystems has increased in the last decade (GARDNER et al., 2009) many challenges remain in disentangling their effects and understanding the combined effect of multiple disturbances. Different activities can operate in synergy, resulting in cascading effects that can be manifest over larger spatial and time scales. Moreover different landscapes are distinguished by distinct regional contexts (e.g. history of colonization) leading to divergent environmental responses (GARDNER et al., 2009).

The understanding of streams hydrological and biogeochemical responses to anthropogenic disturbances has improved in recent decades (NEILL et al. 2006; DAVIDSON et al. 2004; FIGUEIREDO et al. 2010; MACEDO et al. 2013).Where the effects of tropical deforestation on stream systems have been investigated it is evident that there are myriad consequences for changes in stream condition. Terrestrial-aquatic links occur through multiple pathways (e.g. groundwater flow, surface runoff; NEILL et al 2006) and impacts on small watercourses can result in cascading effects on larger river networks (NEILL et al., 2013). Vegetation removal, particularly in the riparian zone, can lead to alterations in runoff from upstream areas, resulting in increased erosion and sedimentation, a rise in light incidence and consequently water temperature, and a loss of organic matter inputs that are a fundamental source of nutrient for aquatic biota in heterotrophic tropical streams (DAVIDSON et al., 2004; FIGUEIREDO et al., 2010; MACEDO et al., 2013; NEILL et al., 2001, 2006, 2011, 2013). However the vulnerability of the physical stream environment and fish fauna to land use change elsewhere in the catchment remains as a major knowledge gap for streams in human-modified tropical forests.

Profound changes in freshwater ecosystems can often have a negative impact on the provision of key ecosystem services, such as the buffering of flood waters, the maintenance of water flow during dry periods, and maintenance of water quality through natural filtration and treatment (BRAUMAN et al., 2007; GREGORY et al., 1991; MILLENNIUM ECOSYSTEM ASSESSMENT, 2005) often increasing environmental vulnerability and hazard to human populations. For instance in Rio de Janeiro metropolitan region, Brazil, land use change together with natural resource exploitation and a mountainous relief has resulted in frequent landslides events in the last decade (SMYTH; ROYLE, 2000).

Although far less studied than abiotic responses, changes in stream biota following anthropogenic disturbance are likely to be pervasive. Alterations in energy input from the adjacent vegetation (e.g. leaves, large wood debris, small branches, fruits, flowers) in combination with an increase in primary production due to a loss in channel shading (e.g. algae and macrophytes) can result in shifts in trophic groups and assemblage composition. In Madagascan rainforests, for instance, endemic stream insects have been shown to be particularly vulnerable to changes in food resources and declined in abundance and biomass in deforested landscapes (BENSTEAD; PRINGLE, 2004; BENSTEAD et al., 2003). The composition of fish assemblages responded to deforestation in Ecuadorian Amazonian streams with shifts from omnivorous and insectivorous Characiformes in forested areas to periphytivorous Loricariidae in deforested sites (BOJSEN; BARRIGA, 2002). Rises in water temperature due to increased light incidence resulted in changes in the taxonomic composition of fish and benthic macroinvertebrates in Costa Rican forest streams (LORION; KENNEDY, 2008; ORION, 2009). In African streams, the fish-based index of biotic integrity (IBI) followed changes in stream physical-chemical condition, which in turn reflected a loss in catchment forest cover (KAMDEM TOHAM; TEUGELS, 1999). Moreover human disturbance such as deforestation can also result in invasion by exotic fish species (PUSEY & ARTHINGTON 2003).

1.3 The study system: the eastern Brazilian Amazon

The Amazonian rainforest is the largest and most biodiverse expanse of tropical forest on Earth and covers 4.5 of the total 7 million km^2 of the Amazon River basin drainage area. Spreading across nine South American countries, the Amazonian rainforest is of local and global relevance for the provision of myriad ecosystem services (e.g. biodiversity conservation and climate regulation; MALHI et al. 2008; PERES et al. 2010). The Amazon River basin is the largest in area and discharge in the world being responsible for 1/5 of the world’s freshwater that reaches the oceans (JUNK, 1983). Brazil holds 60% of the river basin, representing 50% of its territory, and has a comparatively large responsibility for its management and conservation. Until 2012 20% of the original Brazilian Amazon forest extent had already been deforested (INPE, 2013). Deforestation has been particularly intense across the eastern and southern regions of the Amazon that experienced a colonization boom in the 1970s. For instance the eastern state of Pará, the second largest state in the Amazon, accounted alone for 34% (ca. 138.000 km^2) of the total loss of Amazonian primary forest between 1998 and 2014 (INPE, 2015).

Since 2004 deforestation in Pará has been following a declining trend similar to the pattern observed across the Brazilian Amazon as a whole in response to an array of different strategies and dynamics, including policy interventions, private sector initiatives and changes in market conditions (NEPSTAD et al., 2014). Moreover the coverage of protected areas (PA) has rapidly expanded in the last few decades, especially during the late 90s and early 2000s; Brazil now has the largest PA system of all countries in the world covering 12.4% of its territory (WDPA, 2012). However the future of the Brazilian Amazon remains uncertain in the context of ongoing pressures and management challenges. For instance forest degradation caused by selective logging, fire and edge effects, long overlooked in both science and conservation planning and policy, are increasingly recognised as being of comparable importance to deforestation (BARLOW et al., 2012; BERENGUER et al., 2014; LAURANCE et al., 2002). In addition, Brazilian Amazon PAs are now facing new threats, including expansion of the mining and hydropower sectors (FERREIRA et al., 2014), reflected in a region and global trend of downgrading, downsizing and degazettement (BERNARD; PENNA; ARAÚJO, 2014; MASCIA et al., 2014; WATSON et al., 2014).

1.3.1 Amazonian streams and their fish assemblages

Beyond the Amazonas river and its main tributaries, the Amazon basin encompasses an immense and complex network of low-order streams – with 1st to 3rd order streams representing up to 90% of the total river length (MCCLAIN;

ELSENBEER, 2001) – connecting terrestrial and aquatic ecosystems across the region (JUNK, 1983). These streams drain large portions of upland (*terra firme* in Portuguese) forest areas that are dependent on local rainfall (compared to the annual flood pulse associated with large floodplain rivers; CARVALHO et al. 2007). Unlike some of the main river channels that originate in the Andes, these small streams are typically nutrient poor, and depend on the adjacent forest for the input of nutrients, organic material flow and for regulation of sediment input (LOWE-MCCONNELL, 1987). Moreover their channels are shaded by dense vegetation, resulting in low primary production and low coverage by algae and macrophytes outside of disturbed areas (Figure 1.1).

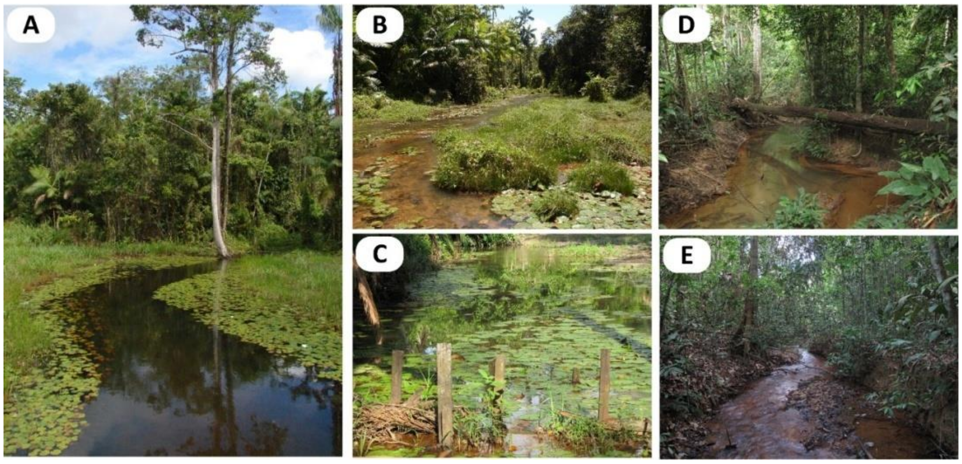

Figure 1.1 Macrophytes in low-order Amazonian streams due to disturbances to the riparian vegetation (A, B, C; Santarém) in contrast to typically shaded channels (D, E; Paragominas). All pictures taken by Sustainable Amazon Network (*Rede Amazônia Sustentável*, RAS) Aquatic Team.

The Amazon basin hosts an exceptionally diverse freshwater fish fauna, with some 2,200 species currently known (REIS; KULLANDER; CARL J. FERRARIS, 2003), and estimates suggesting that the true value is maybe twice this. The basin is the most biodiverse in South and Central America, which in turn has the most diversified freshwater fish fauna in the world, representing some 10% of all vertebrate species (LÉVÊQUE et al., 2008; LUNDBERG et al., 1998; VARI; MALABARBA, 1998). Although there is not an assessment of the extent to which low-order streams contribute to the total fish diversity of the Amazon basin, there is mounting evidence that they are highly diverse, and host a distinct ichthyofauna, including rare and locally specialised species (CARVALHO; ZUANON; SAZIMA, 2007; MENDONÇA; MAGNUSSON; ZUANON, 2005; ZUANON; BOCKMANN; SAZIMA, 2006). For instance, up to 45 fish species can be registered in a single 50m stream segment (Jansen Zuanon and Rafael Leitão personal communication). Moreover recent studies suggest a high level of species turnover between adjacent Amazonian low-order streams and river basins (ALBERT; BART; REIS, 2011; ALBERT et al., 2011; BARROS et al., 2013; MENDONÇA; MAGNUSSON; ZUANON, 2005).

1.3.2 Conservation of Amazonian streams

Conservation planning for tropical freshwater systems that are characterized by a daunting knowledge gap is challenging. Information from better studied freshwater systems, such as temperate streams, may only partly assist. While the basic principles of ensuring catchment-scale protection of native vegetation, maintenance of hydrological and natural flow regimes, and biodiversity conservation are general to all freshwater systems, regional planning and management need to rely on studies tailored to regional conditions (MOULTON; WANTZEN, 2006).

Many tropical countries have some type of environmental legislation to protect freshwater systems against deforestation (DUDGEON, 2008). Usually they include restrictions in use of the riparian zone along stream and river networks. However it is long recognized that a catchment-based management and conservation planning system is needed that can account for the importance of different disturbances at different spatial scales (MACEDO et al., 2014; MARZIN et al., 2012; SÁLY et al., 2011; WANG; SEELBACH; LYONS, 2006).

The two Brazilian legal instruments directly concerned with freshwater systems are the Fisheries Code (Federal Law Nº 11.959 June 29th 2009; BRASIL 2009) and the Water Resources Regulation (Federal Law Nº 9.433 January 8th 1997; BRASIL 1997). The first focuses on aquaculture and fishing activities, and the second on water quality parameters relevant to human consumption. However, both only permit a narrow legal perspective of stream condition and mask the importance of other degradation processes resulting in potentially misleading conclusions about the biotic integrity of stream systems (CASATTI; LANGEANI; FERREIRA, 2006; CASATTI et al., 2006; KARR; DUDLEY, 1981; PAULSEN et al., 2008).

The paramount piece of legislation regarding the protection of the broader stream environment, including adjacent native vegetation, is the Forest Code (Federal Law Nº 12.651, May 25th 2012; BRASIL 2012). The Forest Code prescribes the majority of environmental regulations for private properties, which in turn together encompass approximately 50% of the country’s native vegetation (SOARES-FILHO et al., 2010). It stipulates that 80% of the native vegetation in properties in the Amazon (reduced to 50% in areas that have been zoned for agricultural activities) should be protected in Legal Reserves, with an obligation to restore the forest area back to 50% for areas that were illegally cleared prior to 2008. The law further requires that, depending on the property size, a minimum buffer of riparian vegetation must be protected alongside all water courses – although the revised Forest Code reduced the extent of riparian vegetation that is mandated to be restored to 5 m for areas that have been declared for agricultural use. Our lack of a comprehensive understanding on how different spatial scales and distinct activities interact and affect Amazonian stream condition hinders our ability to inform adequate management and conservation strategies, and evaluate the effectiveness of the available regulations.

Brazil is now facing an enormous window of opportunity regarding conservation of the Amazon. Successful efforts from the last decade (e.g. expansion of protected areas and multiple actions to curb deforestation) are threatened by current proposals that would undermine the protection of the biome; it would include for instance allowing mining activities to occur in protected areas (FERREIRA et al., 2014). Furthermore the planned construction of additional hydropower plants will make irreversible and widespread changes in the Amazon freshwater networks. These threats are superimposed by a recent revision of the Forest Code, that clearly meant several steps back on the environmental protection on private properties (GARCIA et al., 2013; SOARES-FILHO et al., 2014) together with a long history of weak law enforcement in this vast biome. The trade-offs between economic development and environmental conservation can still run towards a more sustainable management of the Amazon but it will depend first on the current government environmental attitude (FERREIRA et al., 2014).

1.3.3 The Sustainable Amazon Network

This thesis is part of the Sustainable Amazon Network (*Rede Amazônia Sustentável*, RAS, www.redeamazoniasustentavel.org), a multidisciplinary research initiative focused on assessing social and ecological dimensions of land use sustainability in the eastern Brazilian Amazon (see GARDNER et al 2013 for details). Different from much of the existing work in the Brazilian Amazon, RAS adopted a mesoscale spatial experimental design (i.e. covering hundreds of kilometres and corresponding with the scale of individual municipalities in the country). Studies were conducted in two regions, Santarém (including parts of the municipalities of Santarém, Belterra and Mojuí dos Campos; hereafter STM) and the municipality of Paragominas (PGM), which encompass approximately 1 and 1.9 million ha respectively.

The two regions have distinct histories of human land use and occupation. STM has been occupied by Europeans since 1661, whereas PGM was formally established in 1959. However, there are also many similarities. Both regions are relatively consolidated with regards to land use change, with decreasing rates of deforestation of primary vegetation, although planned highways mean that Santarém will probably experience both increased human colonization and agricultural expansion in the near future. Large-scale, mechanized agriculture became established in both regions only in the early 2000s and has increased rapidly in recent years (usually at the expense of both pastures and secondary forest), currently occupying approximately 40,000 and 60,000 ha in Santarém and Paragominas, respectively. Today they are both characterized by a diverse patchwork of well-established mechanized agriculture, extensive and intensive cattle pastures, silviculture (mostly *Eucalyptus* spp. and *Schizolobium amazonicum*, especially in Paragominas), densely populated small-land holder colonies and agrarian reform settlements, and large areas of undisturbed and disturbed primary forests and regenerating secondary forests (GARDNER et al. 2013; Figure 1.2).

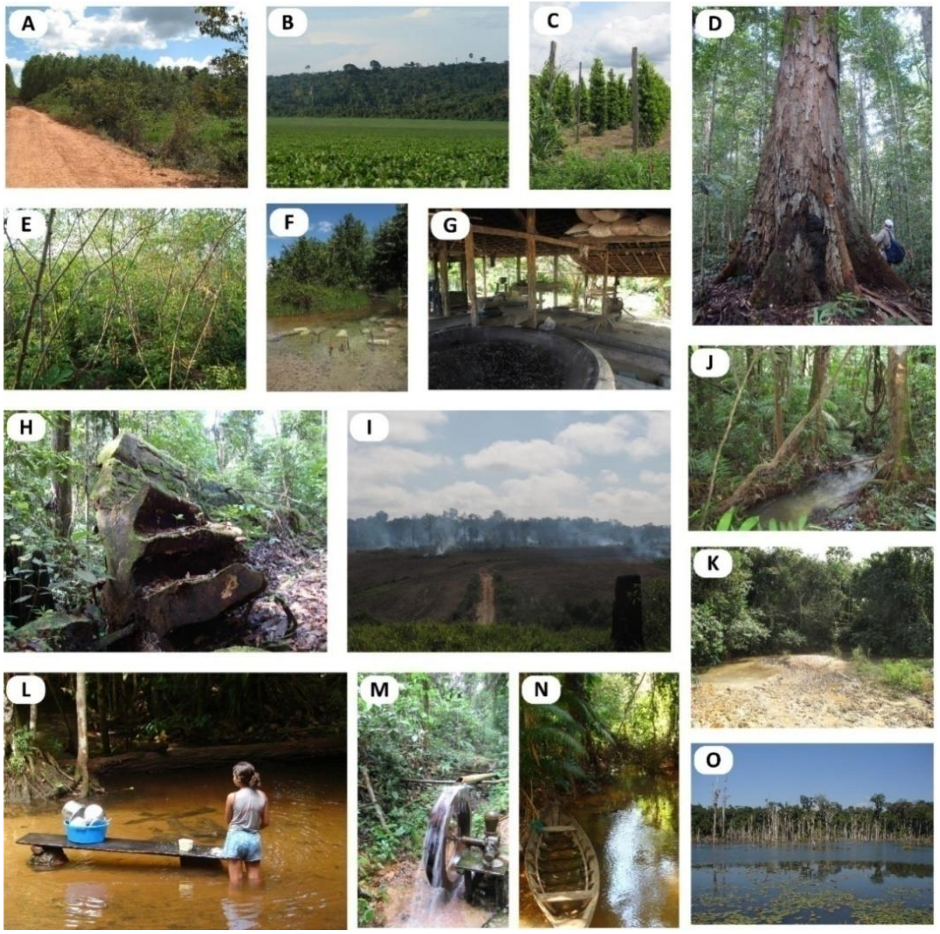

Figure 1.2 Santarém and Paragominas mosaic of land-uses: *Eucalyptus* sp. monoculture (A), soya plantation (B), small black peppercorn crop (C), primary forest (D) with preserved streams (J), manioc plantation (E) with associated use of streams (F) for manioc flour preparation (G). Logging (H) and fire associated to pasture (I) are also common. Moreover streams are largely used by rural families (L, M, N) including for small-scale hydropower generation (O), as well as cattle watering (K). Pictures D, E, H taken by Érika Berenguer, the other ones by RAS Aquatic Team.

The RAS initiative includes a diverse group of research and non-research partners from Brazil and other countries, allowing us to perform a comprehensive assessment of the aquatic condition of low-order streams. For this reason, I am the first author of the three papers to be submitted from this thesis but they will also include other co-authors. My supervisors Dr. Paulo Pompeu (UFLA), Dr. Jos Barlow (Lancaster University) and Dr. Toby Gardner (Stockholm Environment Institute) gave equal indispensable contributions to all steps of this thesis; they were involved since the first plannings of the study (definition of research questions, methodological design, implementation of the field work) through data analysis, results structuring and discussion, and prof reading of all chapters. Moreover all following partners participated one way or another in the data chapters planning and results discussion. MSc. Rafael Leitão (from INPA, Brazil) helped coordinating the aquatic field work, and conducted fish and instream habitat sampling with me. Dr. Jansen Zuanon (INPA) helped in the planning and implementation of the field work and was responsible for overseeing the identification of all fish specimens. Dr. Robert Hughes (Amnis Opes Institute and Department of Fisheries & Wildlife, USA) and Dr. Phil Kaufmann (EPA, USA) have a large amount of experience in planning stream condition assessments, and the analysis of instream habitat and fish responses; RH also participated in field work. MSc. Felipe Rossetti de Paula (ESALq/USP at the time of the study) and Dr. Sílvio Ferraz (ESALq/USP) were responsible for processing the satellite imagery and obtaining the landscape predictor variables. Dr. Jim Thomson and Dr. Ralph Mac Nally assisted with the statistical analysis. Dr. Joice Ferreira, one of RAS coordinators, played a critical role in planning the experimental design and providing support for all aspects of the field work.

1.4 Data sampling

The field work was carried out during the dry season (June to August) in two consecutive years, STM in 2010 and PGM in 2011. In each region we sampled a total of 99 stream sites (all 1st to 3rd Strahler order on a digital 1:100,000 scale map) spread across five river basins: Curuá-Una, Tapajós and Amazonas in STM, Capim and Gurupi in PGM, and a gradient of anthropogenic disturbances (Figure 1.3). We had two teams of five people each working simultaneously, resulting in two stream sites sampled per day. Usually each team was composed by three postgraduate students each responsible for instream habitat and fish data; benthos; or adult Odonata and aquatic Heteroptera, and two local field assistants.

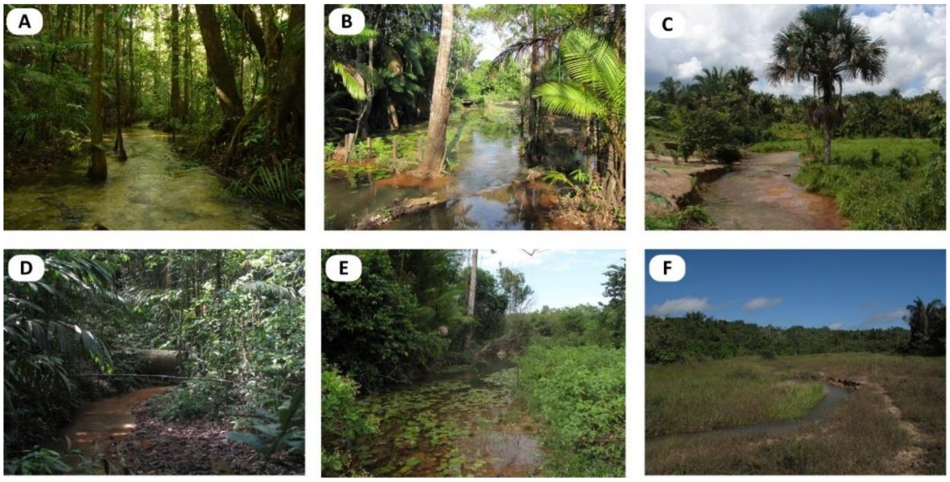

Figure 1.3 Stream sites from Santarém (A, B, C) and Paragominas (D, E, F) spread across a gradient of anthropogenic disturbances including preserved (A, D), intermediate (B, E) and degraded (C, F) conditions. All pictures taken by RAS Aquatic Team.

In both regions, sampling started after two or three weeks of planning, reconnaissance of prospective sampling areas, and training to make sure all teams were familiar with the methods and the region (Figure 1.4). This preparatory work was very important to ensure that we had a complete set of stream samples that encompassed a broad disturbance gradient and to ensure that the time in the field was managed efficiently. Our intensive sampling method required at least 6 to 8 hours in the site, meaning that previous knowledge of the local area, estimated time needed to reach the site, and established contact with landowner were essential.

Figure1.4 Aquatic team in the field: reconnaissance of prospective sampling areas and preparatory work for field sampling. All pictures taken by RAS Aquatic Team.

Stream sites were chosen based on three main criteria: (i) only one site per stream; (ii) wadeable streams (with a maximum of approximately 1.5 m depth)to ensure an effective sampling; (iii) spread across the entire region to encompass the mosaic of land uses and a gradient of disturbance. A preference was also given to select study sites from the same areas as the RAS terrestrial sampling, although this was not always possible because some areas lacked in low-order streams.

1.4.1 Landscape environmental variables

Landscape environmental variables were measured at three different spatial scales (Figure 1.5): 1) the whole catchment upstream from the stream site (‘catchment’), 2) the 100 m buffer along the entire drainage network upstream from the stream site (‘riparian network’), and 3) a 100 m riparian buffer adjacent to the stream site itself (‘local riparian’). Catchment boundaries, mean elevation, and slope were obtained through use of digital elevation models for Santarém (SRTM images with 90 m resolution; NASA - National Aeronautics and Space Administration) and for Paragominas (TopoData with 30 m resolution; INPE - National Institute for Space Research). The drainage network was constructed using the hydrological model ArcSWAT (Soil and Water Assessment Tool extension for ArcGis) for both regions.

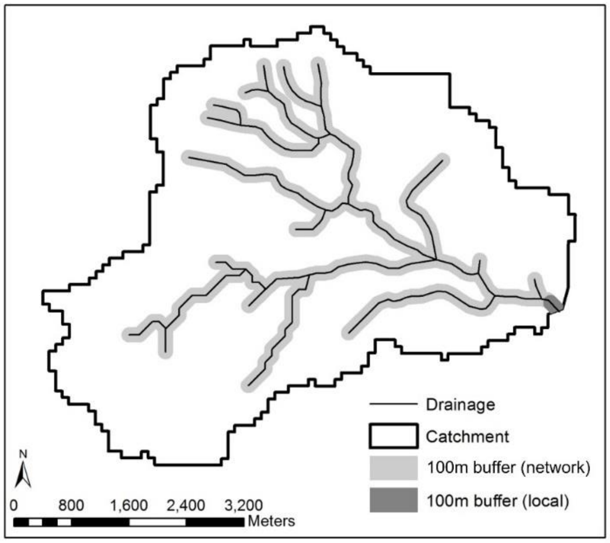

Figure 1.5 Schematic of the spatial scales considered to obtain the landscape environmental variables. Riparian buffers are referred as network and local.

Percentage of forest cover in each of the three spatial scales was obtained from a land use map (Landsat TM and ETM+ images, 30 m resolution, year 2010) (GARDNER et al 2013; Table 1 for a summary of landscape predictor variables). The history of mechanized agriculture was calculated from annual MODIS data from 2001 to 2010 (see details in GARDNER et al 2013). Finally, rivers cape fragmentation was measured using the number of upstream and downstream road crossings within a 5 km circular buffer from the stream site. The road crossings in the drainage network were identified by photo interpretation using georeferenced colour Rapideye images (2010 for STM and 2011 for PGM, 5 m resolution).

1.4.2 Instream habitat

Instream habitat is composed of both the physical and chemical characteristics of streams and can be grouped into water properties (hereinafter "water quality") and physical habitat properties (e.g. substrate type, channel morphology, sinuosity, slope, discharge, wood and cover). To assess the instream habitat we used a protocol first proposed by PECK et al (2006) and HUGHES;PECK (2008), which provides a standardized, replicable and complete assessment of the physical and chemical characteristics of wadeable streams. The resulting dataset enables the calculation of several instream variables representing key aspects of instream habitat such as stream size, stream gradient, substrate size and stability, instream cover complexity, and stream-floodplain connectivity.

Instream habitat was sampled prior to fish sampling, in a 150m segment. The stream site was subdivided into 10 continuous sections, 15 m long, by 11 cross-sectional transects (Figure 1.6). Quantitative and qualitative measurements were repeated across transects and along sections according to the method described in the thesis' chapters and GARDNER et al. (2013; Figure 1.7).

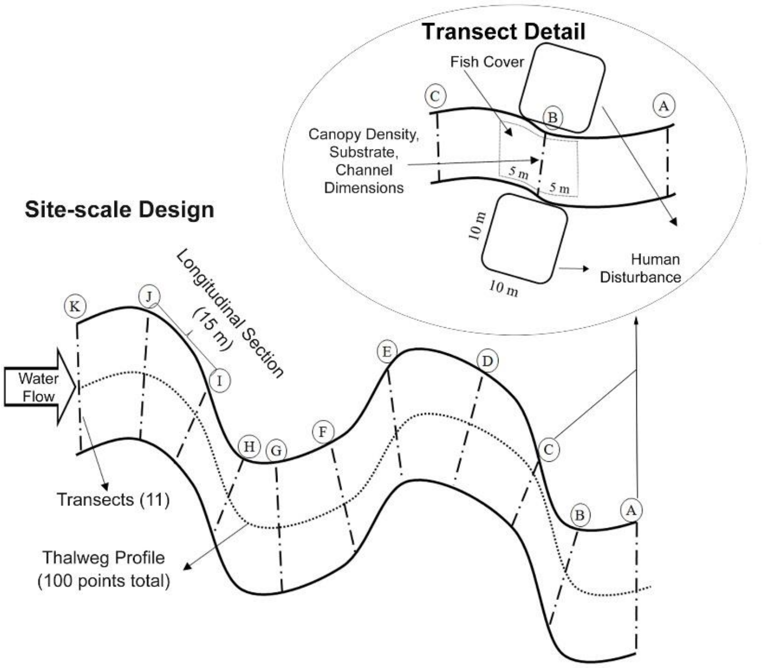

Figure 1.6 Schematic of the sampling design of the instream habitat of Amazonian sites.

Figure 1.7 Measuring instream habitat characteristics in stream sites: substrate type (A), water properties (B), channel slope (C), depth (D), and canopy density (E). All pictures by RAS Aquatic Team.

1.4.3 Fish

Following the instream habitat assessment, three people sampled the 150 m stream segment for 120 min (12 min per section). Each section was isolated using block nets to prevent fish escaping during sampling. Fish were sampled using seines (6 x 1.5 m, 5 mm stretched mesh size) and semi-circular hand nets (0.8 m in diameter, 2 mm stretched mesh size; Figure 1.8). The use of different equipment and collection techniques was applied to encompass all kinds of meso and microhabitats (e.g., riffles, pools, undercut banks, open waters, wood debris, leaf packs, sand, marginal vegetation), and consequently fish groups. All catches were made during daylight hours. Specimens were killed in an anesthetic solution of Eugenol and then fixed in 10% formalin. In the laboratory, all sampled fishes were transferred to 70% alcohol and identified to species level. Voucher specimens are deposited at INPA (Instituto Nacional de Pesquisas da Amazônia) and UFLA (Universidade Federal de Lavras) fish collections, Manaus and Lavras respectively, Brazil.

Figure 1.8 Fish sampling in low-order Amazonian streams. All pictures by RAS Aquatic Team.

1.5 Objectives and structure of the thesis

The main objective of this thesis is to disentangle and understand the role played by multiple scale anthropogenic disturbances and natural landscape features in changing the condition of Amazonian streams. I used fish and instream habitat field data, and landscape data from analyses of satellite imagery integrated into a single framework of analysis to investigate three inter-related sets of objectives (Figure 1.9). First, to investigate how instream habitat condition changes in response to past local and catchment level anthropogenic disturbances (Chapter 2). Second, to understand the effects of changes to instream habitat, as well as riparian and catchment-scale disturbances on the richness, abundance and composition of fish assemblages (Chapter 3). Third, to understand species-specific responses to the disturbances at different scales, and the implications of scale-dependent responses for current Brazilian environmental legislation for the management or private lands (Chapter 4). All three chapters are prepared for submission to Landscape Ecology (Chapter 2), Ecography (Chapter 3) and Conservation Biology (Chapter 4).

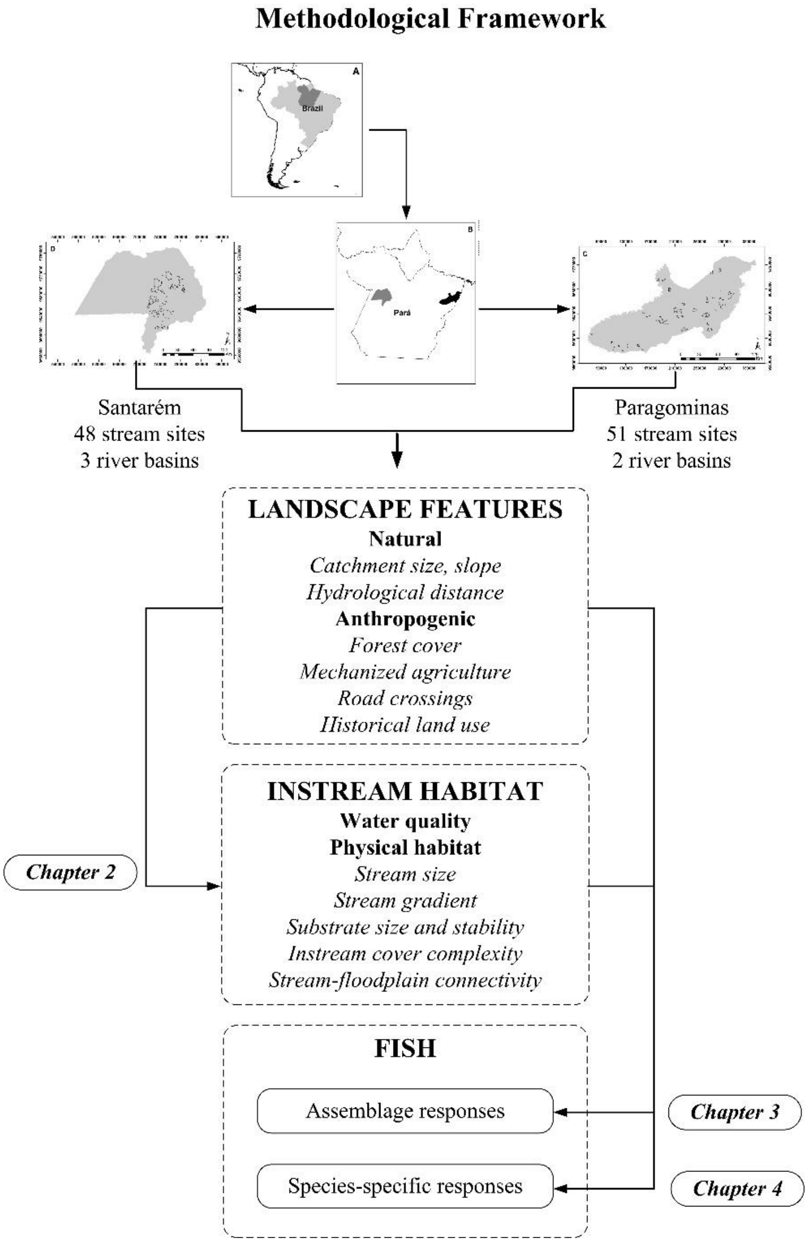

Figure 1.9 Methodological framework representing overall thesis structure and links between chapters.

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SECOND PART- MANUSCRIPTS

MANUSCRIPT 1

MULTI-SCALE ASSESSMENT OF HUMAN-INDUCED CHANGES TO AMAZONIAN INSTREAM HABITATS

(Prepared for submission to Landscape Ecology)

ABSTRACT

Context. Land use change and forest degradation have myriad impacts on tropical ecosystems. Yet their consequences for low-order streams remain very poorly understood, including in the world´s largest freshwater basin, the Amazon.

Objectives. We investigated how the physical and chemical characteristics of the instream habitat of low-order Amazonian streams change in response to past local and catchment level anthropogenic disturbances.

Methods. We used field data on instream habitat and surrounding landscapes of 99 streams from two regions in the eastern Brazilian Amazon. We conducted random forest regression trees to assess the relative importance of different predictor variables in determining changes in instream condition.

Results. Multiple drivers, operating at different spatial scales, were important in determining changes in the physical habitat and water quality of small Amazonian streams. While we found few similarities in modelled relationships between the two regions we did find strong support for non-linear responses of specific instream characteristics to landscape change, including a potential threshold effect of catchment deforestation on water temperature, with a loss of more than 20-30% resulting in consistently warmer streams.

Conclusions. Our results highlight the importance of local riparian and catchment-scale forest cover in shaping instream habitat, but also underscore the importance of other land use changes and activities, such as road crossings and upstream agriculture intensification. In contrast to the local and property-scale focus of the Brazilian Forest code, governing environmental regulations on private land, our results reinforce the importance of a rigorously enforced catchment-wide management strategy to protect the integrity of stream ecosystems.

Keywords: Anthropogenic impacts.Water quality.Physical habitat.Random forest.Watershed management.Deforestation.Land use change.Freshwater. Amazon basin. Tropics.

1 INTRODUCTION

Land use change (LUC) is one of the most important factors altering Earth’s ecosystems (Vörösmarty and Shagian 2000; Foley et al 2005; Ellis 2011) and affecting global biodiversity (Butchart et al 2010) and the conservation of ecosystem services (Millennium Ecosystem Assessment 2005; Russi et al 2013). The impacts of LUC are of greatest concern in many parts of the tropics, where major agricultural and infrastructure development are still undergoing rapid expansion, usually at the expense of natural habitats (Davidson et al 2012; Ferreira et al 2014). While our understanding of the impacts of LUC on terrestrial tropical systems has improved significantly in recent decades (Malhi et al 2014), tropical aquatic systems have received far less research attention than terrestrial systems, with the majority of existing work concentrated in a small number of well-studied regions, such as Costa Rica, Puerto Rico, and Australia (Dudgeon 2008).

Recent studies in the Amazon provide mounting evidence that LUC impacts to stream hydrobiogeochemistry can occur far beyond the adjacent forest. Terrestrial-aquatic links occur through multiple pathways (e.g. groundwater flow, surface runoff; Neill et al 2006) and impacts on small watercourses can result in cascading effects on larger river networks (Neill et al 2013). The conversion of forests into pasture and croplands is leading to manifold consequences for stream environments, such as changes in water quality (temperature and concentration of nutrients), transport of dissolved and particulate materials, and stormflow (Neill et al 2001; Davidson et al 2004; Neill et al 2006; Figueiredo et al 2010; Neill et al 2011; Macedo et al 2013; Neill et al 2013). Such changes can have marked impacts on the biotic communities of streams, such as the negative impacts of temperature increases on many aquatic groups (Lorion and Kennedy 2009; Orion 2009; Isaak et al 2011; Thomson et al 2012).

However one major knowledge gap in our understanding of the ecology of tropical aquatic systems remain virtually unstudied for the Amazonian low-order streams; the vulnerability of the physical stream environment to land use change (Casatti et al 2006a; Dudgeon 2008). Together, physical habitat and water properties constitute the lotic environment of streams (hereinafter called instream habitat), and are frequently used to detect and monitor anthropogenic changes to stream condition (Kaufmann et al 1999).

Although changes in the instream habitat have profound effects on biological assemblages and stream condition, our current knowledge of LUC effects on stream physical environments is mostly confined to temperate zones (Hughes et al 2006; Kaufmann and Hughes 2006; Beschta et al 2013) where impacts include bank erosion and sedimentation, alterations in discharge, reduced amount of wood and increases in light incidence (Gregory et al 1991; Allan et al 1997; Sutherland et al 2002; Allan 2004; Hughes et al 2006; Beschta et al 2013; Yeakley et al 2014). Increases in the concentration of fine sediments can reduce the availability of food resources and habitat for fish and invertebrates by covering hard substrates and filling interstitial spaces (Nerbonne and Vondracek 2001). In addition, the loss of riparian vegetation that often accompanies stream degradation can have a negative impact on the provision of key ecosystem services, such as the buffering of flood waters, the maintenance of water flow during dry periods, and maintenance of water quality through natural filtration and treatment (Gregory et al 1991; Millennium Ecosystem Assessment 2005; Brauman et al 2007).

In general terms, the responses of tropical instream habitat to LUC are likely to mirror those of temperate streams, because key processes are governed by similar hydraulic mechanisms. For example, changes in channel substrate are influenced by a combination of stream slope, geology, discharge, river bedform, and the presence of large wood and other organic materials. However, the specific nature of such relationships may be different in tropical regions characterized by recent deforestation, rapid increases in mechanization, and high levels of river fragmentation from poorly planned infrastructure developments. These anthropogenic differences are overlain upon the distinct natural characteristics of many tropical streams (e.g. high water temperature at a given elevation, high levels of hydrological periodicity with intense rainfall and runoff, distinct structural features of tropical vegetation) and high natural heterogeneity (Junk and Wantzen 2004; Ortiz-Zayas et al 2005; Boulton et al 2008; Boyero et al 2009). A major research challenge therefore, is to untangle how rapidly changing disturbance processes interact with high levels of natural environmental heterogeneity to influence the structure and diversity of tropical stream habitats in different regions and over gradients of land use change (Ramírez et al 2008; Boyero and Ramírez 2009).

To address these issues, we used field data from 99 stream sites distributed across two large regions in the eastern Brazilian Amazon to conduct a multi-scale assessment of the effects of deforestation and land use change on instream habitat for a low-order tropical stream system. We recorded differences not only in physical and chemical water properties, but also aimed for a comprehensive set of physical habitat characteristics of streams, including substrate type and channel morphology among others. The Amazon is the world´s largest remaining area of continuous tropical forest, but is severely threatened by myriad human activities including agricultural expansion, increases in the frequency and intensity of fire, large infrastructure developments (particularly dams and mining), the unsustainable extraction of timber and other forest products, and an unknown number of small dams in small streams resulting from road construction or built to provide water for cattle

(Asner et al 2005; Morton et al 2006; Peres and Palacios 2007; Fearnside and Pueyo 2012; Castello et al 2013; Macedo et al 2013; Ferreira et al 2014). Over the past several years, there has been a decrease in annual deforestation in the Brazilian Amazon resulting from, among other factors, several initiatives led by the government with support from NGOs and the private sector, including an increase in law enforcement and punitive actions, an increase in the protected areas network, and the establishment of moratoria on soya and beef from recently deforested areas (Boucher et al 2013; Nepstad et al 2014). However, despite these positive changes, management strategies have largely failed to address the environmental damage caused by deforestation and LUC on the hydrological connectivity of streams (Castello et al 2013). Moreover, legal protection of stream environments and associated riparian vegetation has been diminished following the revision of the Brazilian national Forest Code in 2012 (Federal Law Nº 12.651; May 25th 2012; Brasil 2012; Garcia et al 2013; Soares-filho et al 2014). The conservation status of small streams is of particular concern because they receive much less research attention and conservation action compared to major river channels and the impacts of large infrastructure developments such as dams. Yet small streams are thought to be the most diverse and extensive ecosystem type in the Amazon basin (Junk 1983; Castello et al 2013). For instance in the Cuieiras River basin, in the central Amazon, first to third order streams represent ca. 92% of the total stream length for the entire basin (Mcclain and Elsenbeer 2001).

This study is part of the Sustainable Amazon Network (Rede Amazônia Sustentável, RAS), a multidisciplinary research initiative focused on assessing both the social and ecological dimensions of land use sustainability in the eastern Brazilian Amazon (see Gardner et al 2013). We collected a detailed dataset on instream habitat characteristics of 99 stream sites covering a wide disturbance gradient in two independent regions (Figure 2.1) to answer three specific questions. 1) What are the relationships among natural and anthropogenic characteristics that may influence instream habitat? (e.g., natural controls such as catchment size and slope, and anthropogenic disturbances such as road crossings, mechanized agriculture, and deforestation). 2) Which of these predictor variables explain most of the observed variation in instream habitat condition? 3) Are relationships between landscape-level predictor variables and differences in instream habitat condition consistent between regions?

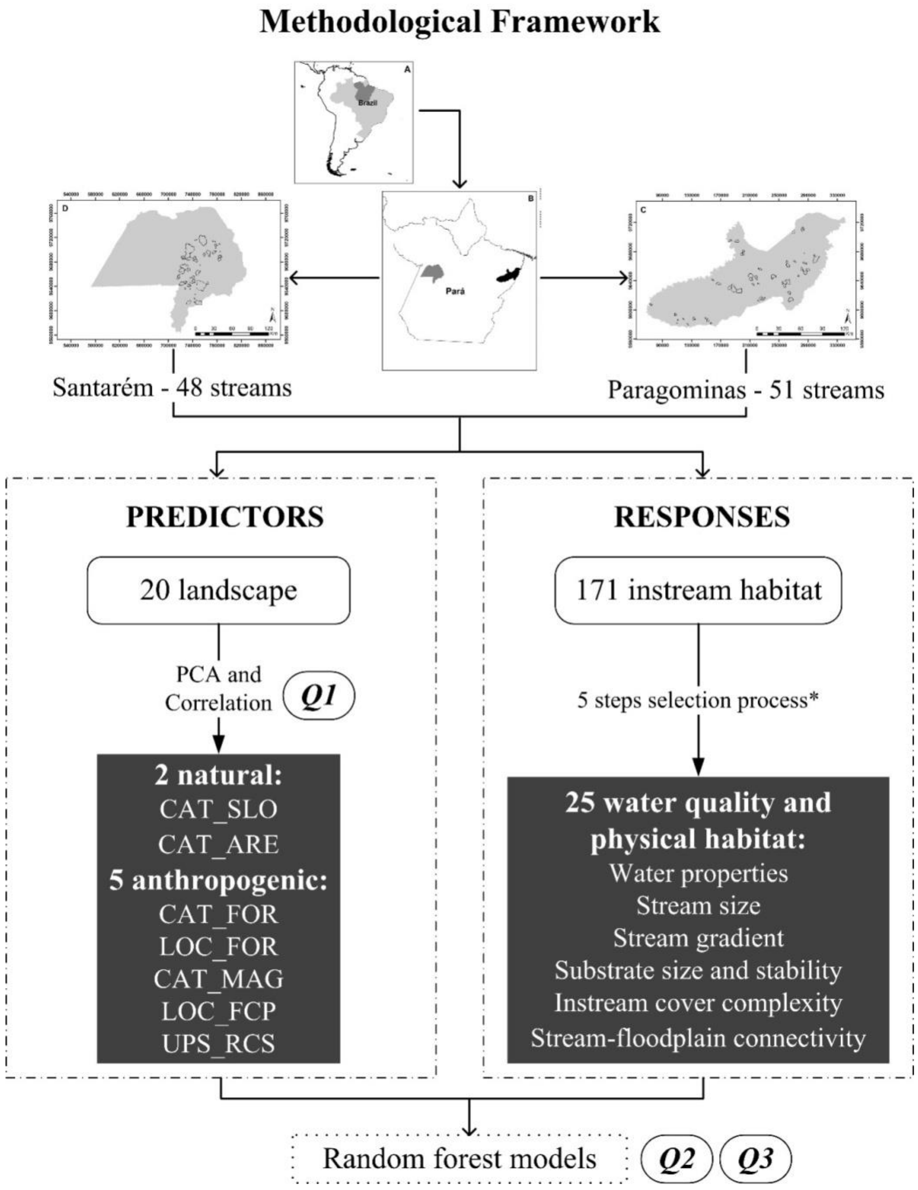

Figure 2.1 Methodological framework to investigate the response of instream habitat of low-order Amazonian stream sites to local and landscape-scale human disturbances (see Table 1). Q1, Q2 and Q3 are the research questions referred to in the Introduction; * see section "Selection of response variables" for detailed steps.

2 METHODS

2.1 Study system

We studied two regions in the eastern Brazilian Amazon state of Pará: Santarém (composed by the municipalities of Santarém, Belterra and Mojuí dos Campos; hereinafter 'STM'), located southeast of the Amazonas and Tapajós Rivers confluence; and Paragominas ('PGM'), in the lower Amazon basin. Our sampling design included 48 small stream sites (1st to 3rd Strahler order on a digital 1:100,000 scale map) in STM, draining to the Curuá-Una River basin (36 streams) or directly to the Amazonas (6) or Tapajós Rivers (6); and 51 in PGM, encompassing the Gurupi (24) and Capim (27) River basins (Figure 2.1).

The two regions have distinct histories of human land use and occupation. Santarém (ca. 1 million ha) has been occupied by Europeans since 1661, whereas PGM (ca. 1.9 million ha) was formally established in 1959. Both regions exhibit decreasing rates of primary vegetation deforestation since 2005 and have been bisected by federal highways first established in the 1960s and 70s, with cascading influences on regional development. Today both regions can be characterized as a diverse patchwork of well-established mechanized agriculture, local and regional centres for cattle markets, silviculture (mostly *Eucalyptus* spp. and *Schizolobium amazonicum*, especially in Paragominas), densely populated small-holder colonies and agrarian reform settlements, as well as undisturbed and disturbed primary forests and regenerating secondary forests (Gardner et al 2013). Stream samples were distributed along a gradient of previously known anthropogenic impact based primarily on the amount of remnant forest cover in the upstream catchment of each stream (Gardner et al 2013).

2.2 Sampling

2.2.1 Landscape predictor variables

We conducted land use assessments at three different spatial scales (Figure A2.1a): 1) the whole catchment upstream from the stream site (‘catchment’), 2) the 100 m buffer along the entire drainage network upstream from the stream site (‘riparian network’), and 3) a 100 m riparian buffer adjacent to the stream site itself (‘local riparian’). Catchment boundaries, mean elevation, and slope were obtained through use of digital elevation models for Santarém (SRTM images with 90 m resolution; NASA - National Aeronautics and Space Administration) and for Paragominas (TopoData with 30 m resolution; INPE - National Institute for Space Research). The drainage network was constructed using the hydrological model ArcSWAT (Soil and Water Assessment Tool extension for ArcGis) for both regions.

Percentage of forest cover in each of the three spatial scales was obtained from a land use map (Landsat TM and ETM+ images, 30 m resolution, year 2010; seeGardner et al 2013; Table 2.1 for a summary of landscape predictor variables). Forest included primary forest (whether undisturbed or showing signs of disturbance from fire or logging), and secondary forest older than 10 years (considered sufficiently developed to provide significant hydrological services based on our expert assessments). To calculate forest cover at different hydrological distances from the stream site, we first standardized the distances by the maximum distance in each catchment to account for differences in catchment size. Then we assigned all pixels in each catchment into near, intermediate or distant categories and then calculated the percent forest cover in each of the distance categories (Paula et al 2013; Figure A2.1b).

The history of mechanized agriculture was calculated from annual MODIS data from 2001 to 2010 (see details in Gardner et al 2013). Two historical land use indicators were calculated for catchments using a time-series of land use maps for the last two decades (following Ferraz et al 2009): forest change curvature profile (FCCP; the deforestation profile curvature) and land-use intensity index (LUI; the mean time since deforestation). FCCP is the maximum deviation of the forest change curve relative to the linear model between initial and final forest amount over time. These indicators were calculated using Land Use Change Analysis Tools (LUCAT), an open source ArcGIS extension (Ferraz et al 2011; Ferraz et al 2012).

Table 2.1 Landscape variables, natural and anthropogenic, used to predict Amazonian instream habitat condition. Selected variables are highlighted in bold.

	Landscape	**Acronym**	**Spatial scale**	**Definition**
Natural	Area	**CAT_ARE**	**Catchment**	**Catchment area**
	Slope	**CAT_SLO**	**Catchment**	**Catchment slope**
Anthropogenic	Land use	**CAT_FOR**	**Catchment**	**% forest**
		NET_FOR	Riparian network	% forest
		LOC_FOR	**Local riparian**	**% forest**
		CAT_MAG	**Catchment**	**% mechanized agriculture**
	Hydrological distance to forest	CAT_FOR_N	Catchment	% forest "near" the stream site
		CAT_FOR_I	Catchment	% forest "intermediate" distance to the stream site
		CAT_FOR_D	Catchment	% forest "distant" to the stream site
		NET_FOR_N	Riparian network	% forest "near" the stream site
		NET_FOR_I	Riparian network	% forest "intermediate" distance to the stream site
		NET_FOR_D	Riparian network	% forest "distant" to the stream site
	Historical land use indicators	CAT_FCP	Catchment	Forest change curvature profile index (FCCP)
		CAT_LUI	Catchment	Land-use intensity index (LUI)
		NET_FCP	Riparian network	Forest change curvature profile index (FCCP)
		NET_LUI	Riparian network	Land-use intensity index (LUI)
		LOC_FCP	**Local riparian**	**Forest change curvature profile index (FCCP)**
		LOC_LUI	Local riparian	Land-use intensity index (LUI)

"Table 2.1 conclusion"

	Landscape	Acronym	Spatial scale	Definition
Anthropogenic	Stream network fragmentation	**UPS_RCS**	**Catchment**	**Number of road crossings within a 5 km circular buffer upstream of the stream site divided by catchment area**
		DWS_RCS	Catchment	Number of road crossings within a 5 km circular buffer downstream of the stream site divided by catchment area

We estimated riverscape fragmentation using the number of upstream and downstream road crossings within a 5 km circular buffer from the stream site. The road crossings in the drainage network were identified by photo interpretation using georeferenced colour Rapideye images (2010 for STM and 2011 for PGM, 5 m resolution) for the study regions. To map these crossings, we identified features in the images related to the road crossings (linear lines crossing the drainage network; Jensen 2000). A subset of about half of these identified crossings were validated using Google Earth images. All landscape analyses were conducted in ArcGis 9.3© (Environmental Systems Research Institute, Redlands, CA, USA).

2.2.2 Instream habitat response variables

For each site we sampled stream physical habitat and water quality variables during the Amazonian dry season in STM (July-August 2010) and PGM (June-August 2011). Each 150 m long stream site was subdivided into 10 continuous sections, 15 m long, by 11 cross-sectional transects (Figure A2.2). We measured dissolved oxygen, conductivity, pH, and temperature with a digital portable meter placed below the water surface in the centre of the site before taking measurements inside the channel to prevent disturbance.

We measured physical habitat at the sites following Peck et al (2006) and Hughes and Peck (2008). For each section we took 10 longitudinal equidistant measurements of thalweg depth; visual quantification of bars, backwaters, side channels, and channel type (pool, glide, riffle, rapid, cascade, waterfall or dry channel); channel slope (measured with a flexible, water-filled plastic tube); and sinuosity (measured with compass bearings). We also recorded the presence of large wood of different size classes in or above the bankfull channel of the site.

For each of the 11 cross-sectional transects we measured depth and visually estimated cover of substrate type (bedrock, concrete, boulder, cobble, coarse gravel, fine gravel, sand, silt and clay, hardpan, fine litter, coarse litter, wood, roots, macrophyte, and algae) along five equidistant points transverse to the long axis of the stream. Transect characterization also included bankfull width and depth, mean wetted width and depth, incision height, undercut bank distance, and bank angle. We assessed habitat complexity at each transect in 10 m plots inside the stream channel, using visual estimates of the areal cover of filamentous algae, aquatic macrophytes, leaf packs, roots, large wood >30cm diameter, brush and small wood, overhanging vegetation <1 m above the water surface, undercut banks, boulders, and artificial structures. We measured vegetation canopy cover above the channel with a densiometer at the centre of each transect by facing upstream, downstream, left and right, as well as by facing both banks near the banks. We calculated discharge from mean current velocity (estimated from the travel time of a floating object along three known distances) and mean cross-sectional area (measured as mean depth times mean width of the three known distances) of the site.

We calculated an initial set of 171 instream habitat response variables from the field data based on Kaufmann et al (1999), including 25 channel morphology, 16 channel unit, 5 channel sinuosity and slope, 28 substrate size and composition, 33 habitat complexity, 60 large wood, and 4 stream canopy cover variables. Geometric mean substrate diameter and relative bed stability were calculated as described by (Kaufmann et al 2008).

2.3 Data analysis

2.3.1 Selection of landscape predictor variables

Given the hierarchical nature of the land use predictor variables (catchment scale encompasses riparian network which encompasses local riparian scale), we expected high levels of correlation among them. To limit redundancy and to produce a smaller set of the most representative variables of human-associated disturbances, we first conducted a principal components analysis (PCA) to identify which variables contribute the most towards distinguishing the landscape disturbance characteristics of different streams; then we excluded the variables highly correlated with those metrics ($r_{\text{Pearson}} > 0.7$).

2.3.2 Selection of instream habitat response variables

From the total set of 171 instream habitat response variables, we selected 21 for further analysis, ensuring that we included metrics for each key aspect of stream physical habitat (according to Kaufmann et al 1999): stream size, stream gradient, substrate size and stability, instream cover complexity, and stream-floodplain connectivity. Our selection process involved eliminating variables that had more than 90% of zero values (n= 25) (i), that were highly correlated with other variables ($r_{\text{Pearson}} > 0.7$) (ii), that represented the similar underlying information included in other variables (e.g. number and volume of wood or proportion and count of an individual substrate) (iii), combining those that represented closely related features (e.g. % sand substrate and % fine combined into % sand+fine) (iv), and finally using our specialist judgement to select from the remaining variables the ones to represent the stream physical habitat (v). Together with the 21 physical variables we considered four water quality variables to yield a total of 25 instream habitat response variables (Table 2.2 and A2.1).

Table 2.2 Acronyms and definitions of instream habitat (water quality and physical habitat features) response variables of Amazonian streams.

Instream habitat		Definition
Water quality	TEMP	Water temperature – °C
	DO	Dissolved oxygen – mg/L
	PH	pH
	COND	Electrical conductivity – µS/cm
Physical habitat	**Substrate**	
	FINE	Streambed surficial fines < 0.6 mm diameter – % areal cover
	SAFN	Streambed surficial sand + fines < 2 mm diameter – % areal cover
	FNGR	Streambed surficial fine gravel 2 to 16 mm diameter – % areal cover
	BIGR	Streambed surficial substrate coarse gravel and larger (> 16 mm diameter) – % areal cover)
	D_{gm}	Log_{10} Streambed substrate particle geometric mean diameter – mm – (Kaufmann et al 2008)
	Cover and wood	
	AMCV	In-channel algae and macrophytes – % areal cover
	NTCV	In-channel natural cover (wood, live trees and roots, leaf packs, overhanging vegetation, undercut banks, boulders) – % areal cover
	WOOD	Wood volume – m3/m^2 wetted channel area
	Channel morphology	
	WDDP	Mean (wetted width x thalweg depth) – m^2
	DPTH	Standard deviation of thalweg depth – cm
	THDP	Thalweg depth ratio at bankfull/low flow – dimensionless
	BKAN	Standard deviation of bank angle – %
	BKWD	Ratio: Bankfull width to bankfull thalweg depth – dimensionless
	RP100	Mean residual depth at thalweg – (m^2/m)/cm
	SINU	Channel sinuosity – dimensionless
	SLOP	Channel slope – %
	FAST	Channel fast water (% riffle + rapid + cascade + waterfall)
	Other	
	DSCH	Low flow season discharge measured in the field – m3/s
	LRBS	Log_{10} of relative bed stability estimated at bankfull flow conditions (Kaufmann et al. 2008, 2009)
	LDMB	Log_{10} of critical substrate diameter (maximum mobile diameter) at bankfull flow conditions (Kaufmann et al. 2008, 2009)
	SHAD	Canopy density (shading) measured at mid-channel – %

2.3.3 Relationships between land use change and instream habitat

To evaluate how neighbouring land use and land use change influence the physical and chemical habitat conditions we modelled instream habitat variables as functions of anthropogenic (land use and land use change) and natural (catchment area and slope) predictor variables. We used random forest (RF; from Breiman 2001) models, which allow complex interactive and non-linear response-predictor relationships, and have excellent predictive performance (Prasad et al 2006; Smith et al 2011). Random forests produce an ensemble of regression trees, where each tree is fitted to a bootstrap sample of the data, and each partition within a tree is split on a random subset of the predictor variables (Ellis et al 2012). The data not used to build a tree in each bootstrap sample, called out-of-bag (OOB) sample, is used to calculate cross-validation performance statistics and measures of variable importance (Ellis et al 2012). We calculated a pseudo-r^2 value as 1- MSE/Var(y), where MSE is the mean squared error of the out of bag predictions (Ellis et al 2012). This value estimates the proportion of variation that can be reliably predicted by the ensemble model. The relative importance (RI) of individual variables was calculated as the mean percentage increase in MSE when a variable was randomly permuted, using the conditional permutation method in the R ‘extendedForest’ library (Smith et al 2011), which reduces bias when predictors are correlated. Conditional RI values were computed from the conditional permutation distribution of each variable, permuted within three partitions of correlated ($r_{Pearson} > 0.5$) variables (see Ellis et al 2012). All models were fitted with 10,000 trees, with one third of variables randomly sampled as candidates at each split (one variable selected if total variables < 3). Variables with negative relative importance values were excluded from final models.

We fitted three RF models for each instream habitat response variable in each region: one model using all candidate predictor variables, one using natural variables only (catchment area and slope) and one using anthropogenic (LUC) variables only. Comparisons of pseudo-r^2 values for the three models, together with the RI values for individual variables, provide insights into the relative influence of anthropogenic and natural predictors, and their interactions, on instream habitat variables. All analyses were performed in R statistical environment (R Core Team 2013).

3 RESULTS

3.1 Variation in landscape characteristics of stream sites

The first two PCA axes accounted for 65% (STM) and 57% (PGM) of the variation in landscape predictors of stream site conditions (Figure 2.2, Table A2.1 and A2.2). For both regions, high correlations ($r_{\text{Pearson}} > 0.7$) among predictor variables and PCAs were consistent, allowing the selection of the same set of relatively uncorrelated variables. Correlations were particularly high between catchment and riparian network scale variables, for instance r_{Pearson} between catchment forest (CAT_FOR) and riparian network forest (NET_CAT) was 0.91 for STM and 0.83 for PGM (Table A2.3 and A2.4). We decided to consider the catchment scale throughout the study as it encompasses the riparian network zone and provides a broader representation of the landscape.

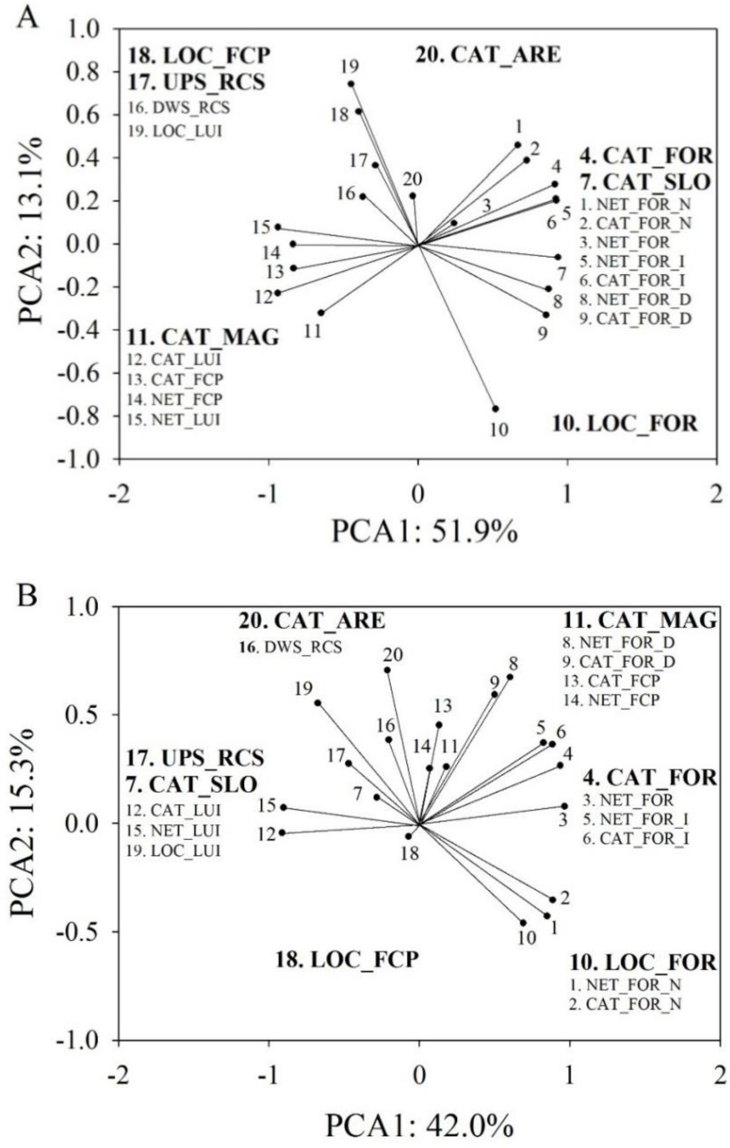

Figure 2.2 Contribution of landscape predictor variables to the first two PCA axes for Santarém (A) and Paragominas (B). Variables in bold were selected for further analysis, with excluded highly correlated metrics listed below each of them.

The subset of variables with low correlations with other predictor variables in both regions (Figure A2.3) were: catchment slope (CAT_SLO), catchment area (CAT_ARE), percentage of catchment forest (CAT_FOR), percentage of local riparian forest (LOC_FOR), local forest change curvature profile (LOC_FCP), catchment mechanized agriculture (CAT_MAG) and number of upstream road crossings (UPS_RCS). These variables were used as the subset of both natural (catchment area and slope) and anthropogenic predictors of variability in the instream habitat response variables.

3.2 LUC influences on stream site condition

Random forest models explained some of the observed variance (1.7 to 49.2% in STM and 2.1 to 34.7 in PGM) in 14 out of the 25 instream habitat variables in each region when all landscape predictors, anthropogenic and natural, were included in the models (Table 2.3, Table A2.5). In general, the inclusion of all predictor variables resulted in better model fits than when only natural or only anthropogenic where included, indicating that LUC effects can depend on differences in the natural characteristics of a given region. The anthropogenic variables alone accounted for 0.8 to 27.6% of the variance in the instream habitat responses for STM and 1.1 to 34.7% for PGM. Response variables that were partly explained by LUC characteristics in each region included variables from all major instream habitat categories: water quality, substrate, cover and wood, channel morphology, and other features.

Table 2.3 Performance of random forest (RF) models showing the percentage of variation of the instream habitat response variables explained (pseudo-R2) by models that included all predictor variables (All), only the anthropogenic (Ant) and only the natural variables (Nat). Note that strong interactions between anthropogenic and natural predictor variables can result in pseudo-R2 values for the combined (All) model that exceed the sum of values for anthropogenic and natural models (e.g. D_{gm} in STM; highlighted in light grey). Conversely, the combined model can have lower pseudo-R2 values than anthropogenic (medium grey) or natural (dark grey) models because the random inclusion of weaker predictors in individual trees may lower the overall mean predictive performance (e.g. OD in STM and COND in PGM respectively). Values in parentheses in "All" columns show the % contribution of anthropogenic variables to total variance explained in combined models.

Instream habitat		**STM**			**PGM**		
		All (% Ant)	Ant	Nat	All (% Ant)	Ant	Nat
Water	TEMP	5.5 (100)	5.3	0	34.7 (100)	35.2	0
	DO	1.7 (88)	7.7	0	0	0	0
	COND	2.3 (35)	0	0	6.8 (28)	0	16.23
Substrate	FINE	0	0	9.3	6.6 (53)	3.4	0.7
	SAFN	0	0	0	13.5 (44)	6	3.6
	FNGR	6.6 (55)	1.7	6.9	0	0	0
	D_{gm}	14.6 (55)	8.2	2.8	0	0	0
Cover and wood	WOOD	12.5 (100)	9.5	0	11.7 (62)	1.7	0
Channel morphology	WDDP	31 (47)	17.0	9.6	3.2 (50)	0	0
	DPTH	28.6 (59)	18.9	12.4	9.7 (30)	0	0
	BKAN	0	0	0	6.7 (75)	0	3.9
	BKWD	5.1 (78)	2.0	0	27.9 (45)	3.1	0
	RP100	37.2 (40)	16.5	44.6	2.1 (52)	0	8.2
	SLOP	35.8 (37)	12.4	40.0	0	0	0
	FAST	0	0	1.9	8.1 (96)	12.3	0
Other	DSCH	49.2 (56)	33.1	40.2	3.8 (87)	8.7	0
	LDMB	9.2 (67)	14.4	0	2.8 (28)	3.1	0.1
	SHAD	18.8 (59)	25.8	0	33.7 (100)	34.3	0

Variability in ten instream habitat variables were at least partly explained in both regions: temperature (TEMP), conductivity (COND), wood (WOOD), bankfull width-to-depth ratio (BKWD), standard deviation of thalweg depth (DPTH), wetted width * thalweg depth (WDDP), residual depth at thalweg (RP100), discharge (DSCH), critical diameter of substrate (LDMB), and mid-channel shading (SHAD) (Table 2.3). Among those, five had the same main predictor in both regions: local riparian forest cover for SHAD, WOOD and LDMB, road crossings for COND, and local forest change curvature profile for DSCH (Table A2.5).

The degree to which the predictor variables explained the instream habitat metrics differed between regions. For STM, seven response variables had more than 10% of their variation explained by anthropogenic predictors: discharge (27.6%), standard deviation of thalweg depth (17.0%), residual depth at thalweg (14.7%), wetted width x thalweg depth (14.6%), slope (13.1%), wood (12.5%), and mid-channel shading (11.0%; Table 3). For PGM, temperature (34.7%), mid-channel shading (33.7%), and bankfull width-to-depth ratio (12.5%) were the instream habitat response variable best explained by the anthropogenic predictors.

3.3 Influence of region and landscape scale on instream habitat condition

In both regions, variation in instream habitat response variables was driven by many predictors, with each explaining small amounts (Figure 2.3). In PGM, forest cover-related predictors were more important than other variables in explaining variability in instream habitat. On the other hand, in STM road crossings appeared to be the most important influence on many instream habitat response variables. Also in STM, mechanized agriculture was retained in most of the predictive models, but in PGM, only one instream habitat response variable was explained by CAT_MAG.

Partial contributions of single predictors were smaller in STM than in PGM. Local riparian forest cover (LOC_FOR) was positively related to wood and accounted for 11% of observed variance, with a marked increase in the volume of wood observed when forest cover exceeded 80% (Figure 2,4A). Time since local deforestation (LOC_FCP) was responsible for 9.5% of the variation in discharge, without a clear directional association (Figure 2.4B). Road crossings (UPS_RCS) were negatively related to wetted width x thalweg depth (WDDP), an indicator of wetted channel volume, and explained 10.8% of its variance (Figure 2.4C).

For PGM, local forest accounted for 20.2% of the variance in mid-channel shading, showing a consistent positive association (Figure 2.4D). Temperature had a negative relationship with forest cover at the catchment scale, explaining 20.0% of the observed variance (Figure 2.4E). The partial plots suggest there is a potential threshold at ca. 20-30% of catchment forest loss above which water temperature is consistently warmer than in more forested areas. For bankfull width-to-depth ratio (BKWD), local riparian forest cover was the most important predictor, explaining 10.6% of its variance (Figure 2.4F), with wider or shallower channels associated with stream sites having deforested adjacent areas.

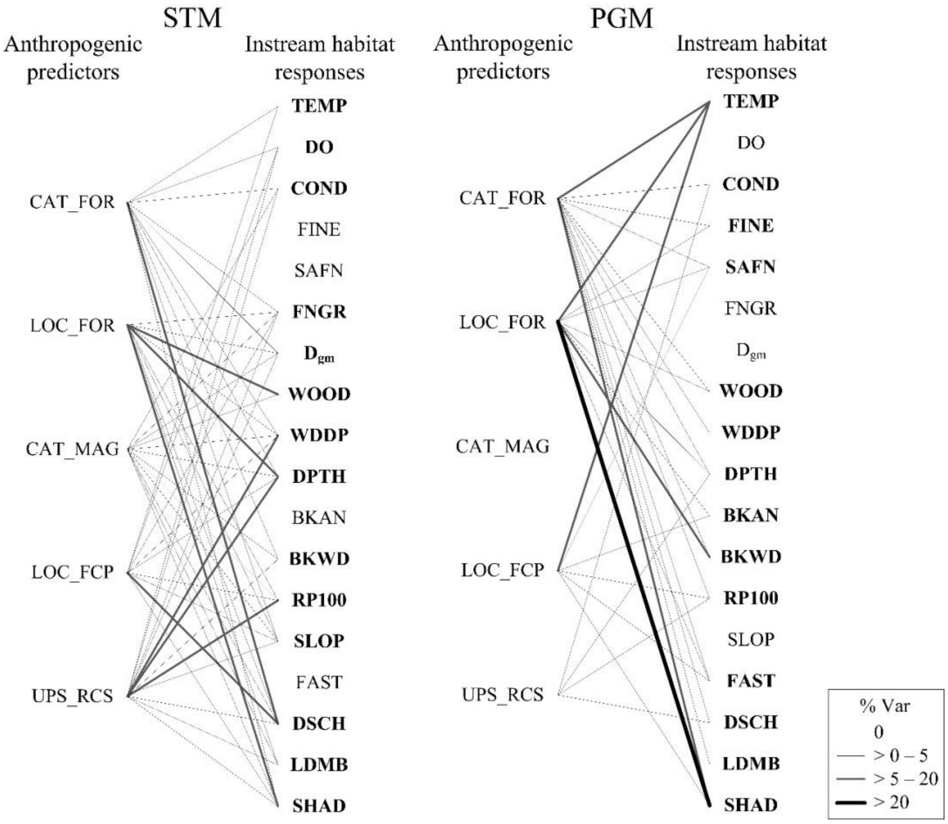

Figure 2.3 Representation of random forest (RF) models showing the percentage of variation of the instream habitat response variables explained (pseudo-R2) by anthropogenic predictor variables in Amazonian stream sites. Results are from models that included both anthropogenic and natural predictor variables (‘All’ models shown on Table 2.3).

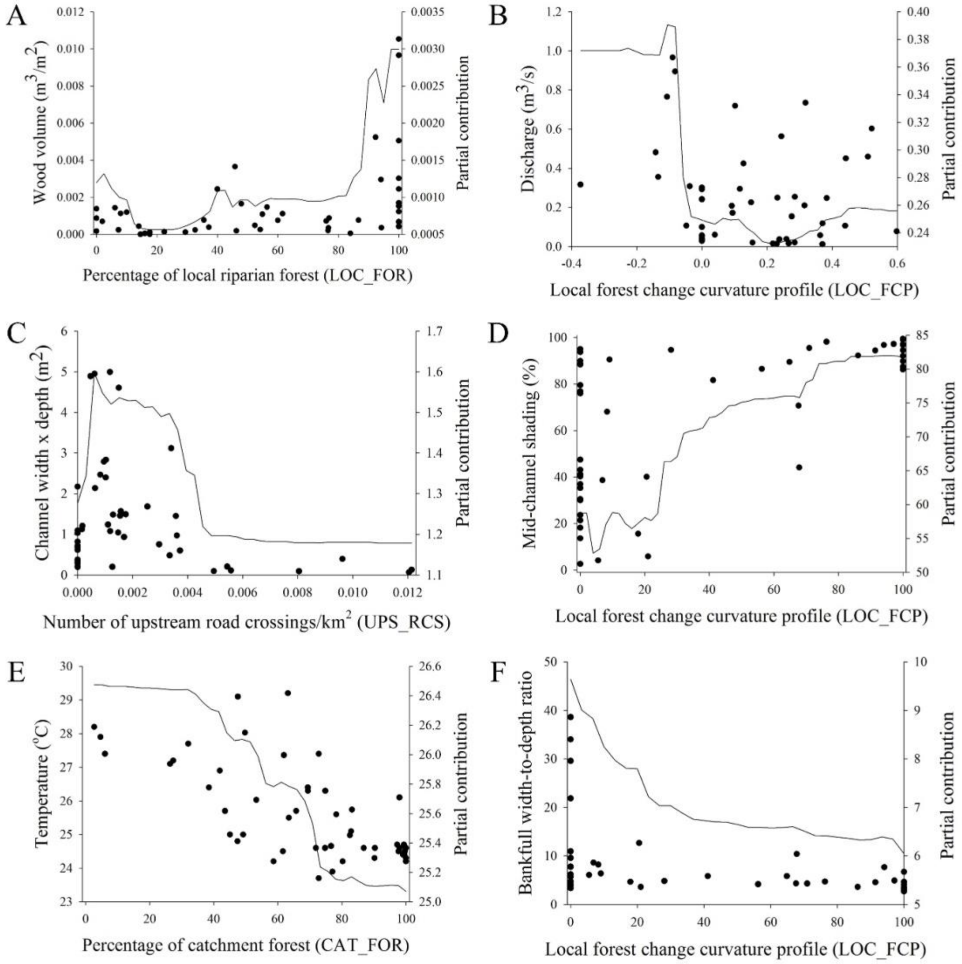

Figure 2.4 Raw data distribution (dots) and partial contribution of landscape predictor variables (lines) to instream habitat in Santarém (A, B, C) and Paragominas (D, E, F).

4 DISCUSSION

Our study is the first comprehensive, quantitative, multi-scale assessment of the consequences of LUC on the instream habitat of small Amazonian streams, including both water quality and physical habitat characteristics. Drawing on detailed landscape and habitat data from 99 Amazonian stream sites we confirm the importance of linkages between human activities and some key instream habitat response variables such as water temperature, discharge, and the volume of wood, with evidence of associations following upstream deforestation. However, our data also highlight the heterogeneous nature of such stream systems and the difficulties of identifying specific predictor variables; i.e., most habitat variables were affected by a broad suite of correlated predictors that varied between regions. We discuss our findings by first comparing them to *a priori* expectations and then by assessing some of the significant challenges involved in understanding the links between anthropogenic disturbances and the instream habitat of tropical streams. We draw on the relationships observed in our data to suggest priorities for the management of land and stream systems to improve the condition of small streams in human-modified tropical forest landscapes.

4.1 Do human-induced disturbances influence tropical instream habitats as expected?

The importance of landscape change in determining changes in instream habitat has been the focus of far more research in temperate than tropical streams (Allan 2004; Hughes et al 2006; Beschta et al 2013; Yeakley et al 2014). In our assessment of small Amazonian streams we found clear evidence that human-induced landscape disturbances were associated with noticeable changes in all types of instream variables - including water quality (e.g. temperature and conductivity in both regions), substrate (e.g. mean substrate diameter in STM and sand+fines in PGM), fish cover and wood (e.g. volume of wood in both regions), channel morphology (e.g. residual pools and standard deviation of thalweg depth in both regions), and other attributes (e.g. discharge and channel shading in both regions).

The strongest effects that we observed are consistent with other findings for temperate and tropical streams. Water temperature was strongly and positively related to increases in upstream deforestation, with a potential threshold observed in PGM where streams with less than 80% upstream forest cover had higher water temperatures. Increases in water temperature in response to deforestation have already been documented for rainforest streams (Figueiredo et al 2010; Macedo et al 2013), as have temperature-related changes in the taxonomic composition of fish and benthic macroinvertebrates (Lorion and Kennedy 2008; Orion 2009). Observed temperature increases in temperate streams have also been known to affect several aspects of the life-histories of aquatic species such as development, metabolism, growth and survival (Gillooly et al 2001; Gillooly et al 2002; Isaak et al 2011; Neuheimer et al 2011).

We also found that local riparian forest cover was particularly important in determining the volume of wood, although a number of other drivers also appeared to influence this habitat feature (e.g. mechanized agriculture in STM; catchment forest cover and road crossings in PGM). A positive link between vegetation and wood input is not surprising but the influence of catchment level drivers highlights that these effects can occur across multiple scales. Paula et al (2013) also found that local riparian forests were important sources of instream wood in tropical streams, but noted the importance of forests along the upstream riparian network zone and the entire upstream catchment because of downstream transport of wood. Large wood is a critical factor in determining long-term channel structural complexity (e.g. by forming pools and cascades), substrate composition for faunal colonization, and sediment and leaf litter accumulation (Wright and Flecker 2004; Milner and Gloyne-Phillips 2005; Kaufmann and Faustini 2012).

Effects of land use change on instream habitat were not always consistent between studied regions. Some changes in instream habitat that were evident in one region were not in the other (e.g. response of temperature to forest cover and land use change effects on substrate size and percentage of fine gravel). These differences emphasize the complexity of relationships between land use change and instream habitat, and serve to illustrate the variability in responses for regions that have different land use histories. Moreover, this complexity highlights the difficulty of any *a priori* process to select candidate variables to describe both drivers and responses to disturbance, and hence the need to survey a wide range of measures.

4.2 Challenges in understanding the influences of anthropogenic disturbances on instream habitat in tropical streams

Identifying key landscape-level drivers of environmental change in freshwater systems, and their spatial and temporal scales of influence is critically important for informing management and conservation strategies (Wang et al 2006a). This is particularly the case in areas such as the frontier regions of the Brazilian Amazon that are experiencing rapid changes in land use, such as the conversion from native vegetation to agriculture. Here we examine some of the key challenges in drawing these linkages, and discuss implications for both policy development and the design of future studies.

4.3 Disentangling the effects of anthropogenic disturbance from natural variation among Amazonian streams

The high level of multi-collinearity between natural stream characteristics and anthropogenic disturbance underlines the difficulties of disentangling the relative importance of individual factors in determining changes in the physical and chemical attributes of streams (Allan 2004). Moreover, we found that Amazonian streams are highly heterogeneous in their natural physical and chemical characteristics, hindering our ability to detect the effects of specific disturbance signals. This is especially the case regarding stream sediment characteristics, which are strongly influenced by both natural landscape features and the loss of native vegetation. For example, we found that the percent of sand and fines at stream sites varied substantially within both completely forested and largely deforested (< 10% forest cover) catchments in both regions. This may have resulted from at least three factors: 1) our definition of forest, as primary forests and second-growth forests that are over 10 years in age may be too broad; 2) land use change itself is not random, and forests on sandy soils were avoided for agriculture; and 3) both regions are underlain by paleo-lake bed sediments with high levels of sand and fines. In Santarém, stream sites draining forested catchments had 31-40% of sand and fines compared to 10-31% for deforested catchments. However in Paragominas, the variation was greater for forested catchments (22-63%) and encompassed the range observed in deforested catchments (36-47%). For relative bed stability (LRBS), an indicator of anthropogenic sedimentation (Kaufmann and Hughes 2006; Kaufmann et al 2009), the values were not consistent between regions regarding response to disturbance. Deforested streams in Paragominas showed similar LRBS values (-1.4 to -2.9) as forested streams (-1.1 to -2.5), whereas in STM LRBS appeared to be reflecting disturbance as it was markedly different for forested (-1.4 to -2.2) and deforested (-3.5 to -3.7) streams. Increasingly negative LRBS values indicate greater instability and increased erosion, accumulation, and/or movement of sediments (Kaufmann et al 1999; Kaufmann et al 2009).

4.4 Cumulative effects of multiple drivers

Identifying and understanding specific landscape level disturbances that influence instream habitats across different land uses and spatial scales is of particular importance for guiding stream conservation and management strategies (Heitke et al 2006; Wang et al 2006b). We found that multiple drivers, at multiple spatial scales, were consistently linked to changes in the stream environment. Our findings highlight the importance of considering catchment forest cover as well as local riparian vegetation and that of the entire upstream channel network to understand changes in the instream habitat conditions.

The loss of riparian vegetation has a long known detrimental impact for several physical and chemical stream processes, such as sediment filtration, bank and flow stability, and channel shading with resultant changes in temperature and primary production regulation (Karr and Schlosser 1978; Peterjohn and Correll 1984; Osborne and Koviacic 1993; Sweeney 1993). However, there are contrasting results regarding whether forest loss at the catchment (Roth et al 1996; Marzin et al 2013) or local riparian (Nerbonne and Vondracek 2001; Wang et al 2006b; Sály et al 2011; Macedo et al 2014)scales is more important in influencing instream habitat and aquatic biota. As Wang et al (2006) argued, the relative importance of catchment versus site scale depends at least partly on the range of variability in habitat and disturbance occurring at each scale.

It is very difficult to separate riparian from catchment-scale effects (Richards et al 1996; Burnett et al 2006; Sály et al 2011; Marzin et al 2013; Paula et al 2013; Macedo et al 2014), especially in forested systems like

Amazonia where changes can be highly correlated. We demonstrated that other non-forest catchment-wide impacts were also associated with significant changes in instream habitat, and should therefore be considered in the management and planning of stream conservation strategies. Drivers not linked to forest cover, such as mechanized agriculture and road crossings, accounted for an important part of the instream habitat change, and thus are additional important considerations for conserving and managing Amazon stream systems. Mechanized agriculture explained over 2% of the variation in instream habitat response variables in STM (standard deviation in thalweg depth, bankfull width to depth ratio, substrate critical diameter; Table A2.5). Road crossings upstream of the stream sites explained up to 7.5% of the variation in instream habitat response variables in STM (e.g. wetted channel width x depth, standard deviation of thalweg depth, and mean residual thalweg depth; Table A2.5).Road crossings on small streams for private access and water use are considered as low environmental impacts by the Brazilian Environmental Council (CONAMA 2006; resolution #369) in contrast to our results and those of Macedo et al (2013) and Neill et al (2013). In our study, roads often crossed streams using undersized and perched culverts, creating small reservoirs upstream of the road.

We also found small dams built to provide water for cattle, small-scale fish production, and local hydroelectric power generation, all of which are commonly overlooked as serious disturbances to habitat and biota (Castello et al 2013; Macedo et al 2013; Neill et al 2013). Macedo et al (2013) estimated that there are about 10,000 small impoundments in the Upper Xingu basin, and demonstrated that together with deforestation, their density accounted for 43% of the basin’s water temperature increase. Because such dams are prone to failure, hazardous to humans, expensive to repair, and often hinder or preclude fish migrations, they are targets for removal throughout the USA (Hughes 2012). Our results lend support to similar efforts in tropical regions.

4.5 Accounting for the full gradient of landscape disturbance

Both study regions are characterized by a complex mosaic of land uses: undisturbed and varyingly disturbed primary forests (affected by fragmentation, logging and fire), regenerating secondary forests, and a continuously varying patchwork of different agricultural systems (e.g. cattle ranching, large-scale soybean plantations, and small-scale manioc and black pepper plantations). Moreover, because both regions have retained a relatively high level of catchment forest cover (69% in Paragominas and 60% in Santarém), we sampled few heavily deforested catchments (only two catchments with < 10% forest cover in STM and three in PGM), and none of the study catchments were dominated by urban areas. The fact that severely degraded streams were absent from the catchments we surveyed suggests that environmental regulations may have helped avoid the most extreme degradation from occurring, e.g. through total removal of riparian network vegetation. We also found that instream habitat variables exhibited distinctly non-linear responses to disturbance and that the potential threshold for change in some variables occurred only at high levels of disturbance. This might be the case for variables that had similar response between the two regions yet had a weak response to disturbance (e.g. water conductivity in response to road crossings and critical diameter of stream substrate in response to changes in local riparian forest cover). For example, Biggs et al (2004) detected changes in nutrient levels only when deforestation was higher than 66%; and Casatti et al (2006) found a stronger decline in physical habitat quality than water quality in degraded tropical savannah streams.

We also could have included more detailed information on land use change and natural predictors, because in both regions we found a considerable amount of unexplained variance in instream habitat. For example, the highest explained variance for a single instream habitat response variable was 49% in STM and 35% in PGM, whereas it was less than 15% for most of them. However the level of detail in predictor and response variables is always under cost and time constraints (Hughes and Peck 2008). Moreover, there are inherent difficulties associated with describing stream-relevant anthropogenic disturbance across landscapes with such high levels of environmental heterogeneity within and between different land-uses than we were able to capture. For example, we did not account for forest degradation (i.e. fire, fragmentation and selective logging), which is widespread in the study region, results in changes in vegetation structure (Berenguer et al 2014), and contributes to instream habitat degradation. Although we accounted for deforestation and mechanized agriculture, other types of forest and pasture management and crop types could have been described in more detail if more accurate GIS data had been available. For example, water quality in Amazonian streams can respond differently according to different land uses, such as forest clearing followed by slash and burn, pasture, or soybean fields (Neill et al 2001; Biggs et al 2006; Biggs et al 2008; Macedo et al 2013; Neill et al 2013).

4.6 Time-lags in disturbance responses

The ecological consequences of anthropogenic disturbances may take years to become fully apparent in ecosystems (Hylander and Ehrlén 2013). Our results indicate that the time since deforestation was an important predictor of instream habitat change in streams (as indicated by the importance of the local deforestation curvature profile index, LOC_FCP, in explaining the response of several habitat variables, see Table A2.5). Moreover, many of the more severe land use changes in both regions are relatively recent, the effects of which may yet to be manifested. Paragominas was founded in 1965 but timber extraction only intensified in the 1980s, when it was considered the largest center of timber extraction in Brazil and one of the largest in the world (Verissimo et al 1992), and mechanized agriculture only grew significantly in the last ten years. Despite being founded in 1754 Santarém also has experienced a rapid increase in human population and rates of forest conversion only since the 1970s (Amorim 1999), with mechanized agriculture becoming relatively common in the 2000s.

Some instream habitat and ecosystem metabolism features are known to exhibit much slower responses to disturbance than others (McTammany et al 2007). We would expect water temperature to increase rapidly as a result of the clearance of riparian vegetation, but changes in channel morphology usually respond more slowly. For example, the Willamette River, Oregon was snagged and channelized mostly between 1870 and 1910 and converted from an anastomosed, 2-9 km wide floodplain river to the present mostly single-thread system, resulting in the loss of over 75% of the upper river shoreline (Sedell and Froggatt 1984). Beschta et al (2013) showed how long-term livestock grazing in the western USA resulted in loss of stream riparian vegetation, bank erosion, channel incision and widening. Because such changes typically occur slowly over multiple human generations, many observers often fail to recognize them as resulting from land and channel uses.

Finally, we also found that anthropogenic metrics explained less than 10% of the variability in all substrate and wood metrics. Working in southwestern Oregon, USA, Burnett et al (2006) reported that time delays in the decomposition of wood from prior tree mortality and upstream wood input can obscure potentially important linkages between changes in wood and logging disturbance. These effects may be even greater in the tropics, where many trees have very high wood densities and very slow rates of decomposition. Such time lags in stream physical habitat responses help explain why historical land use of temperate catchments may account for more biological variability than current land use (Harding et al 1998; Brown et al 2009).

4.7 Implications for the conservation management of Amazonian streams

Our results highlight some of the inadequacies of current Brazilian legislation in protecting stream environments and point to ways in which their management and conservation could be improved. Two Brazilian legal instruments directly concerned with instream habitats are the Fisheries Code (Federal Law Nº 11.959, June 29th 2009; Brasil 2009) and the Water Resources Regulation (Federal Law Nº 9.433, January 8th 1997; Brasil 1997). The first focuses on aquaculture and fishing activities, and the second on water quality properties relevant to human consumption. However, both only permit a narrow legal perspective of stream condition and mask the importance of other degradation processes resulting in potentially misleading conclusions about the biotic integrity of stream systems (Karr and Dudley 1981; Casatti et al 2006a; Casatti et al 2006b; Paulsen et al 2008).

The most important piece of legislation regarding the protection of broader stream environment, including adjacent native vegetation, is the Forest Code (Federal Law 12.651, May 25, 2012; Brasil 2012) that prescribes the majority of environmental regulations for private properties that together encompass approximately 50% of the country’s native vegetation (Soares-Filho et al 2014). The Forest Code stipulates that 80% of the native vegetation in properties in the Amazon (reduced to 50% in areas that have been zoned for agricultural activities) should be protected in Legal Reserves, with an obligation to restore the forest area back to 50% for areas that were illegally cleared prior to 2008. The law further requires that, depending on the property size, that a minimum buffer of riparian vegetation must be protected alongside all water courses – although the revised Forest Code reduced the extent of riparian vegetation that is mandated to be restored to 5 m for areas that have been declared for agricultural use. Our results highlight two important limitations in the effectiveness of this legislation to conserve stream environments. First, we have identified the importance of upstream forests – and not just riparian forests - in determining local stream habitat conditions, demanding a more collective-action (versus individual) approach to achieving compliance across neighbouring landowners to protect blocks of forest in individual catchments. Second, identifying the strong influence of up and downstream habitat fragmentation from road crossings, as well as mechanized agriculture, on instream physical environments highlights the need for legislation to go beyond the protection of only riparian forests and address the management of entire drainage networks (Abell et al 2007; Castello et al 2013).

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APPENDIX CHAPTER 2 (A2)

The tables and figures presented here will be submitted as supplementary material to the manuscript.

Table A2.1 Mean, range, and standard deviation of landscape predictors and instream habitat variables of Santarém and Paragominas stream sites.

Predictor and response variables	Unit	Santarém				Paragominas			
		Mean	Min	Max	SD	Mean	Min	Max	SD
Landscape									
CAT_ARE	km^2	2871.67	83.02	22726.0	4710.08	1253.99	44.30	5045.32	1236.25
CAT_SLO	%	7.22	3.96	14.80	2.95	4.64	1.55	9.49	1.83
CAT_FOR	%	60.15	4.80	100.00	31.18	68.86	2.71	100.00	27.02
LOC_FOR	%	55.16	0	100.00	36.40	37.87	0	100.00	42.95
CAT_MAG	%	7.67	0	59.45	13.87	2.52	0	44.04	7.39
LOC_FCP	-	0.15	-0.37	0.60	0.20	0.05	-0.27	0.74	0.18
UPS_RCS	n/km^2	0.0021	0	0.0121	0.0030	0.0031	0.00	0.0226	0.0040
Instream habitat									
Water									
TEMP	ºC	25.06	23.50	27.70	0.93	25.61	23.70	29.20	1.43
OD	mg/L	6.12	3.22	8.10	1.13	4.65	1.96	6.83	1.20
PH	-	4.71	3.08	7.40	0.70	5.34	3.02	7.75	0.95
COND	µS/cm	17.02	7.00	23.90	3.75	32.58	15.40	76.20	13.06
Substrate									
FINE	%	18.81	0	91.43	22.80	9.05	0	40.95	9.07
SAFN	%	39.23	1.90	91.43	21.70	31.86	3.81	63.81	12.98
FNGR	%	3.47	0	36.19	6.48	7.89	0	50.48	12.33
BIGR	%	2.90	0	54.29	8.57	2.45	0	33.33	6.33
D_{gm}	mm	-0.96	-2.11	1.75	0.82	-0.57	-2.11	1.27	0.67
Cover and wood									
AMCV	%	5.21	0	52.27	13.35	12.93	0	76.36	20.24
NTCV	%	50.64	2.27	113.64	26.69	76.35	0	223.86	45.87
WOOD	m^3/m^2	0.0032	0	0.0755	0.0110	0.0031	0	0.0110	0.0032
Channel morphology									
WDDP	m2	1.35	0.07	4.99	1.33	1.09	0.15	3.44	0.72
DPTH	cm	14.75	4.22	33.81	7.12	17.42	5.14	41.10	6.96
THDP	-	2.81	1.22	13.21	2.29	2.57	1.38	4.82	0.95
BKAN	%	17.58	3.18	35.67	7.50	19.10	3.20	34.35	6.13
BKWD	-	17.97	0.81	85.95	16.25	7.93	2.68	38.62	8.09
RP100	(m2/m)/ cm	0.47	0.01	0.90	0.16	0.49	0.25	0.80	0.10
SINU	-	1.18	1.02	1.35	0.08	1.16	0	1.49	0.19
SLOP	%	0.01	0.001	0.03	0.01	4.73	0.31	24.00	4.08
FAST	%	15.89	0	90.00	20.56	14.73	0	70.00	18.09
Other									
DSCH	m3/s	0.25	0.01	0.97	0.25	0.19	0.01	0.79	0.18
LRBS	-	-0.26	-1.74	2.22	0.83	0.32	-1.99	2.25	0.74
LDMB	-	-0.70	-1.81	0.08	0.39	-0.89	-2.06	-0.12	0.36
SHAD	%	81.89	8.16	99.33	25.97	66.75	2.67	99.47	32.02

Table A2.2 Landscape variables contributions to the first two PCA axes.

Landscape predictor variables	STM		PGM	
	PCA1	PCA2	PCA1	PCA2
CAT_ARE	-0.03	-0.22	-0.21	0.71
CAT_SLO	0.24	-0.10	-0.28	0.12
CAT_FOR	0.93	-0.20	0.94	0.27
NET_FOR	0.94	0.06	0.96	0.08
LOC_FOR	0.52	0.77	0.69	-0.46
CAT_FOR_N	0.87	0.21	0.89	-0.35
CAT_FOR_I	0.92	-0.28	0.88	0.36
CAT_FOR_D	0.67	-0.46	0.50	0.59
NET_FOR_N	0.86	0.33	0.85	-0.43
NET_FOR_I	0.92	-0.21	0.82	0.37
NET_FOR_D	0.73	-0.39	0.60	0.67
UPS_RCS	-0.29	-0.36	-0.47	0.28
DWS_RCS	-0.37	-0.22	-0.20	0.38
CAT_FCP	-0.40	-0.61	-0.07	-0.06
NET_FCP	-0.84	0.00	0.07	0.25
LOC_FCP	-0.83	0.11	0.13	0.45
CAT_MAG	-0.65	0.32	0.18	0.26
CAT_LUI	-0.45	-0.74	-0.91	-0.04
NET_LUI	-0.94	-0.08	-0.90	0.07
LOC_LUI	-0.94	0.23	-0.67	0.55
Eigenvalue	10.38	2.61	8.41	3.07
Variance explained (%)	51.92	13.06	42.04	15.34
Cumulative variance (%)	-	64.99	-	57.38

Table A2.3 Pearsoncorrelations among Santarém landscape predictor variables. Values higher than 0.7 are highlighted in bold.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1. CAT_ARE	1.0	-0.3	0.0	0.0	-0.2	0.0	0.0	0.1	0.0	0.0	0.1	0.5	0.3	-0.1	0.0	-0.1	0.1	0.0	0.1	0.0
2. CAT_SLO	-0.3	1.0	0.2	0.1	-0.1	0.2	0.3	0.1	0.1	0.2	0.0	-0.3	-0.2	-0.3	-0.3	0.0	-0.4	-0.3	-0.2	0.0
3. CAT_FOR	0.0	0.2	1.0	**0.8**	0.3	**0.8**	**0.9**	**0.8**	**0.7**	**0.9**	**0.7**	-0.2	-0.3	**-0.8**	**-0.7**	-0.3	-0.6	**-0.9**	**-0.8**	-0.3
4. NET_FOR	0.0	0.1	**0.8**	1.0	0.5	**0.8**	**0.8**	0.6	**0.9**	**0.9**	**0.7**	-0.3	-0.3	**-0.7**	**-0.8**	-0.3	-0.5	**-0.8**	**-1.0**	-0.4
5. LOC_FOR	-0.2	-0.1	0.3	0.5	1.0	0.6	0.3	0.1	0.7	0.4	0.1	-0.4	-0.3	-0.3	-0.4	-0.6	-0.1	-0.3	-0.5	**-0.8**
6. CAT_FOR_N	0.0	0.2	**0.8**	**0.8**	0.6	1.0	**0.8**	0.4	**0.9**	**0.7**	0.5	-0.3	-0.4	-0.6	-0.7	-0.4	-0.6	**-0.8**	**-0.8**	-0.5
7. CAT_FOR_I	0.0	0.3	**0.9**	**0.8**	0.3	**0.8**	1.0	**0.7**	**0.7**	**0.9**	**0.7**	-0.1	-0.2	**-0.8**	**-0.7**	-0.2	**-0.7**	**-0.9**	**-0.8**	-0.2
8. CAT_FOR_D	0.1	0.1	**0.8**	0.6	0.1	0.4	**0.7**	1.0	0.4	**0.7**	**0.8**	0.0	-0.2	-0.5	-0.5	-0.1	-0.5	**-0.8**	-0.5	0.0
9. NET_FOR_N	0.0	0.1	**0.7**	**0.9**	**0.7**	**0.9**	**0.7**	0.4	1.0	**0.7**	0.5	-0.4	-0.4	-0.6	**-0.7**	-0.4	-0.4	**-0.7**	**-0.8**	-0.6
10. NET_FOR_I	0.0	0.2	**0.9**	**0.9**	0.4	**0.7**	**0.9**	**0.7**	**0.7**	1.0	**0.8**	-0.2	-0.2	**-0.8**	**-0.8**	-0.2	-0.6	**-0.9**	**-0.9**	-0.2
11. NET_FOR_D	0.1	0.0	**0.7**	**0.7**	0.1	0.5	**0.7**	**0.8**	0.5	**0.8**	1.0	-0.1	-0.1	-0.6	-0.6	-0.1	-0.4	**-0.7**	**-0.7**	-0.1
12. UPS_RCS	0.5	-0.3	-0.2	-0.3	-0.4	-0.3	-0.1	0.0	-0.4	-0.2	-0.1	1.0	0.4	0.2	0.2	0.1	0.0	0.2	0.2	0.1
13. DWS_RCS	0.3	-0.2	-0.3	-0.3	-0.3	-0.4	-0.2	-0.2	-0.4	-0.2	-0.1	0.4	1.0	0.2	0.2	0.2	0.3	0.3	0.4	0.1
14. CAT_FCP	-0.1	-0.3	**-0.8**	**-0.7**	-0.3	-0.6	**-0.8**	-0.5	-0.6	**-0.8**	-0.6	0.2	0.2	1.0	**0.9**	0.3	0.5	**0.8**	**0.7**	0.3
15. NET_FCP	0.0	-0.3	**-0.7**	**-0.8**	-0.4	**-0.7**	**-0.7**	-0.5	**-0.7**	**-0.8**	-0.6	0.2	0.2	**0.9**	1.0	0.4	0.4	**0.7**	**0.8**	0.3
16. LOC_FCP	-0.1	0.0	-0.3	-0.3	-0.6	-0.4	-0.2	-0.1	-0.4	-0.2	-0.1	0.1	0.2	0.3	0.4	1.0	0.0	0.3	0.3	**0.7**
17. CAT_MAG	0.1	-0.4	-0.6	-0.5	-0.1	-0.6	**-0.7**	-0.5	-0.4	-0.6	-0.4	0.0	0.3	0.5	0.4	0.0	1.0	**0.8**	0.6	0.1
18. CAT_LUI	0.0	-0.3	**-0.9**	**-0.8**	-0.3	**-0.8**	**-0.9**	**-0.8**	**-0.7**	**-0.9**	**-0.7**	0.2	0.3	**0.8**	**0.7**	0.3	**0.8**	1.0	**0.8**	0.3
19. NET_LUI	0.1	-0.2	**-0.8**	**-1.0**	-0.5	**-0.8**	**-0.8**	-0.5	**-0.8**	**-0.9**	**-0.7**	0.2	0.4	**0.7**	**0.8**	0.3	0.6	**0.8**	1.0	0.5
20. LOC_LUI	0.0	0.0	-0.3	-0.4	**-0.8**	-0.5	-0.2	0.0	-0.6	-0.2	-0.1	0.1	0.1	0.3	0.3	**0.7**	0.1	0.3	0.5	1.0

Table A2.4 Pearson correlations among Paragominas landscape predictor variables. Values higher than 0.7 are highlighted in bold.

	1	2	3	4	5	6	7	8	9	10	11	12	13	16	15	14	17	18	19	20
1. CAT_ARE	1.0	0.0	0.0	-0.1	-0.3	-0.4	0.0	0.2	-0.4	0.0	0.2	0.5	0.5	0.2	0.1	0.0	0.2	0.1	0.2	0.4
2. CAT_SLO	0.0	1.0	-0.2	-0.2	-0.2	-0.3	-0.2	-0.2	-0.3	-0.2	-0.1	0.1	0.3	0.3	0.2	-0.1	-0.1	0.3	0.2	0.2
3. CAT_FOR	0.0	-0.2	1.0	**0.9**	0.5	**0.7**	**0.9**	**0.7**	**0.7**	**0.8**	**0.7**	-0.3	-0.1	0.2	0.0	-0.1	0.2	**-0.9**	**-0.8**	-0.5
4. NET_FOR	-0.1	-0.2	**0.9**	1.0	0.6	**0.8**	**0.9**	0.4	**0.8**	**0.9**	**0.7**	-0.4	-0.1	0.1	0.1	0.0	0.2	**-0.9**	**-0.9**	-0.6
5. LOC_FOR	-0.3	-0.2	0.5	0.6	1.0	**0.8**	0.4	0.0	**0.8**	0.3	0.1	-0.3	-0.1	0.0	0.0	-0.1	0.1	-0.5	-0.6	**-0.9**
6. CAT_FOR_N	-0.4	-0.3	**0.7**	**0.8**	**0.8**	1.0	0.6	0.2	**1.0**	0.5	0.3	-0.5	-0.2	0.0	0.0	-0.1	0.1	**-0.8**	**-0.8**	**-0.8**
7. CAT_FOR_I	0.0	-0.2	**0.9**	**0.9**	0.4	0.6	1.0	0.6	0.5	**0.9**	**0.8**	-0.4	-0.1	0.3	0.1	-0.1	0.2	**-0.8**	**-0.8**	-0.4
8. CAT_FOR_D	0.2	-0.2	**0.7**	0.4	0.0	0.2	0.6	1.0	0.2	0.6	**0.7**	0.0	0.1	0.2	-0.1	-0.1	0.2	-0.5	-0.4	0.0
9. NET_FOR_N	-0.4	-0.3	**0.7**	**0.8**	**0.8**	**1.0**	0.5	0.2	1.0	0.5	0.2	-0.5	-0.3	0.0	0.0	0.0	0.1	**-0.7**	**-0.8**	**-0.8**
10. NET_FOR_I	0.0	-0.2	**0.8**	**0.9**	0.3	0.5	**0.9**	0.6	0.5	1.0	**0.8**	-0.3	-0.1	0.2	0.1	-0.1	0.1	**-0.8**	**-0.7**	-0.2
11. NET_FOR_D	0.2	-0.1	**0.7**	**0.7**	0.1	0.3	**0.8**	**0.7**	0.2	**0.8**	1.0	-0.1	0.0	0.3	0.2	-0.1	0.2	-0.5	-0.4	0.0
12. UPS_RCS	0.5	0.1	-0.3	-0.4	-0.3	-0.5	-0.4	0.0	-0.5	-0.3	-0.1	1.0	0.5	-0.1	-0.2	0.2	0.0	0.3	0.3	0.3
13. DWS_RCS	0.5	0.3	-0.1	-0.1	-0.1	-0.2	-0.1	0.1	-0.3	-0.1	0.0	0.5	1.0	0.0	-0.1	0.0	0.0	0.1	0.1	0.1
14. CAT_FCP	0.2	0.3	0.2	0.1	0.0	0.0	0.3	0.2	0.0	0.2	0.3	-0.1	0.0	1.0	**0.7**	-0.3	0.2	0.0	0.0	0.0
15. NET_FCP	0.1	0.2	0.0	0.1	0.0	0.0	0.1	-0.1	0.0	0.1	0.2	-0.2	-0.1	**0.7**	1.0	0.2	0.3	0.0	0.1	0.1
16. LOC_FCP	0.0	-0.1	-0.1	0.0	-0.1	-0.1	-0.1	-0.1	0.0	-0.1	-0.1	0.2	0.0	-0.3	0.2	1.0	0.0	-0.1	-0.1	0.0
17. CAT_MAG	0.2	-0.1	0.2	0.2	0.1	0.1	0.2	0.2	0.1	0.1	0.2	0.0	0.0	0.2	0.3	0.0	1.0	-0.1	-0.1	0.0
18. CAT_LUI	0.1	0.3	**-0.9**	**-0.9**	-0.5	**-0.8**	**-0.8**	-0.5	**-0.7**	**-0.8**	-0.5	0.3	0.1	0.0	0.0	-0.1	-0.1	1.0	**1.0**	0.6
19. NET_LUI	0.2	0.2	**-0.8**	**-0.9**	-0.6	**-0.8**	**-0.8**	-0.4	**-0.8**	**-0.7**	-0.4	0.3	0.1	0.0	0.1	-0.1	-0.1	**1.0**	1.0	0.6
20. LOC_LUI	0.4	0.2	-0.5	-0.6	**-0.9**	**-0.8**	-0.4	0.0	**-0.8**	-0.2	0.0	0.3	0.1	0.0	0.1	0.0	0.0	0.6	0.6	1.0

Table A2.5 Performance of random forest (RF) models showing the percentage of variation of the instream habitat response variables explained (pseudo-R2) by each predictor variable (partial contribution) considering models that included anthropogenic and natural variables.

	Instream habitat	Predictor variables							Total variance explained (%)
		Natural		Anthropogenic					
		CAT_ARE	CAT_SLO	CAT_FOR	LOC_FOR	CAT_MAG	LOC_FCP	UPS_RCS	
Santarém	TEMP			1.3			4.3		5.5
	OD	0.2		0.7				0.8	1.7
	COND	0.7	0.8	0.1		0.1		0.6	2.3
	FNGR	2.4	0.5	1.1	0.2	0.6	0.3	1.5	6.6
	D_{gm}	2.3	4.2	0.5	3.1	1.6		2.8	14.6
	WOOD				11.0	1.4			12.5
	WDDP	15.1	1.3	1.3	1.1	0.3	4.5	7.5	31.0
	DPTH	10.7	0.8	1.0	7.4	2.4	0.7	5.6	28.6
	BKWD	1.1				2.5		1.4	5.1
	RP100	18.8	3.7		4.5	1.7	2.1	6.5	37.2
	SLOP	13.7	9.1	2.3	3.4	1.6	1.7	4.1	35.8
	DSCH	20.4	1.2	5.9	4.9	1.5	9.5	5.8	49.2
	LDMB	1.3	1.7		2.8	2.1		1.4	9.2
	SHAD	4.0	3.8	0.6	5.6		4.3	0.4	18.8
Paragominas	TEMP			20.0	7.6		7.1		34.7
	COND	3.2	1.7	0.8				1.1	6.8
	FINE		3.2	1.1	2.4				6.6
	SAFN	7.6		5.0	0.3		0.6		13.5
	WOOD	4.5		1.6	4.6			1.0	11.7
	DPTH	5.9	1.0	2.0	0.7			0.3	9.7
	BKAN	0.5	1.1		4.2		0.7		6.7
	BKWD	6.5	8.9	2.0	10.6				27.9
	WDDP	1.6		1.6					3.2
	RP100	0.9	0.1	0.7			0.3	0.1	2.1
	FAST	0.3		2.1	2.3		3.4		8.1
	DSCH	0.5		0.3			3.0		3.8
	LDMB	0.4	1.6	0.8					2.8
	SHAD			10.4	20.2		3.1		33.7

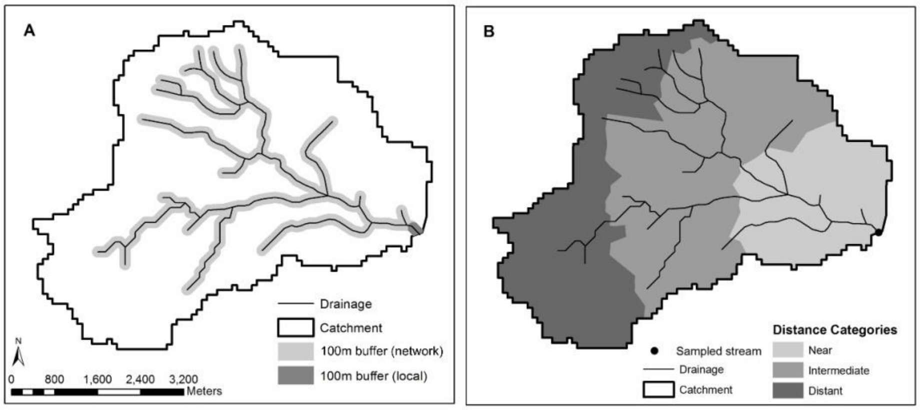


Figure A2.1 Schematic of the spatial scales (a) and hydrological distances (b) considered to obtain the landscape predictor variables of instream habitat of Amazonian stream sites.

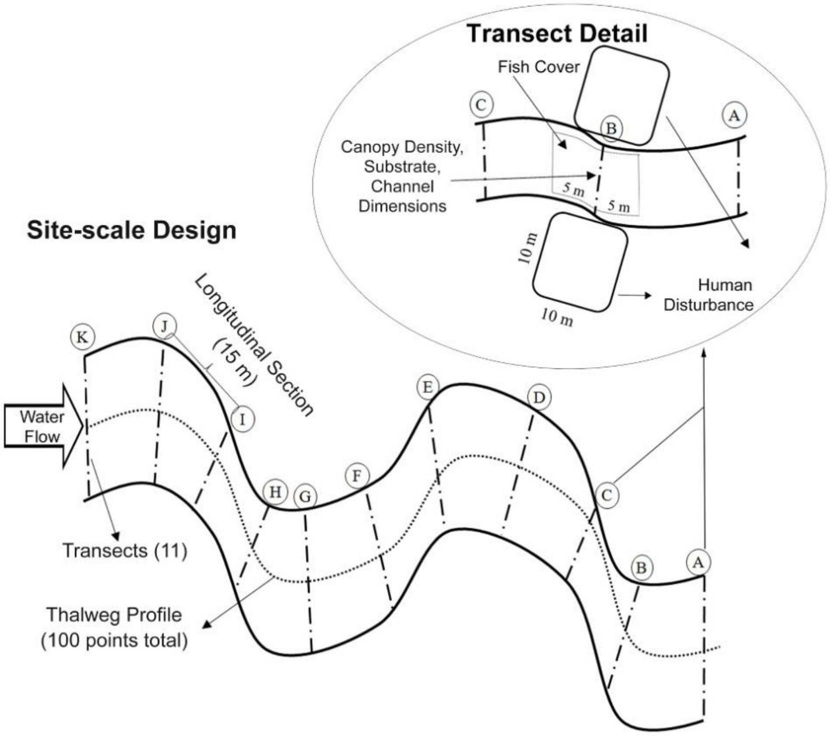


Figure A2.2 Schematic of the sampling design of the instream habitat of Amazonian stream sites.

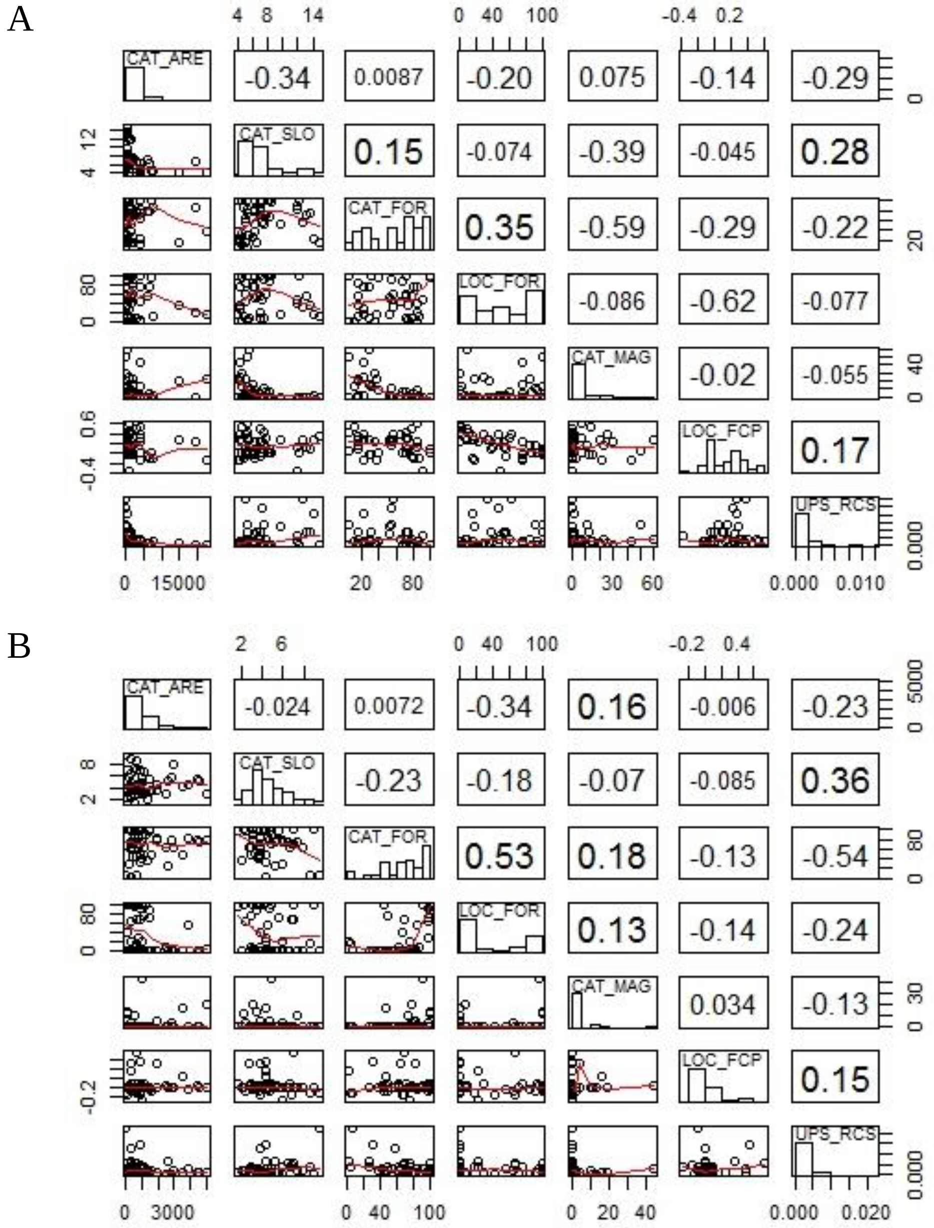
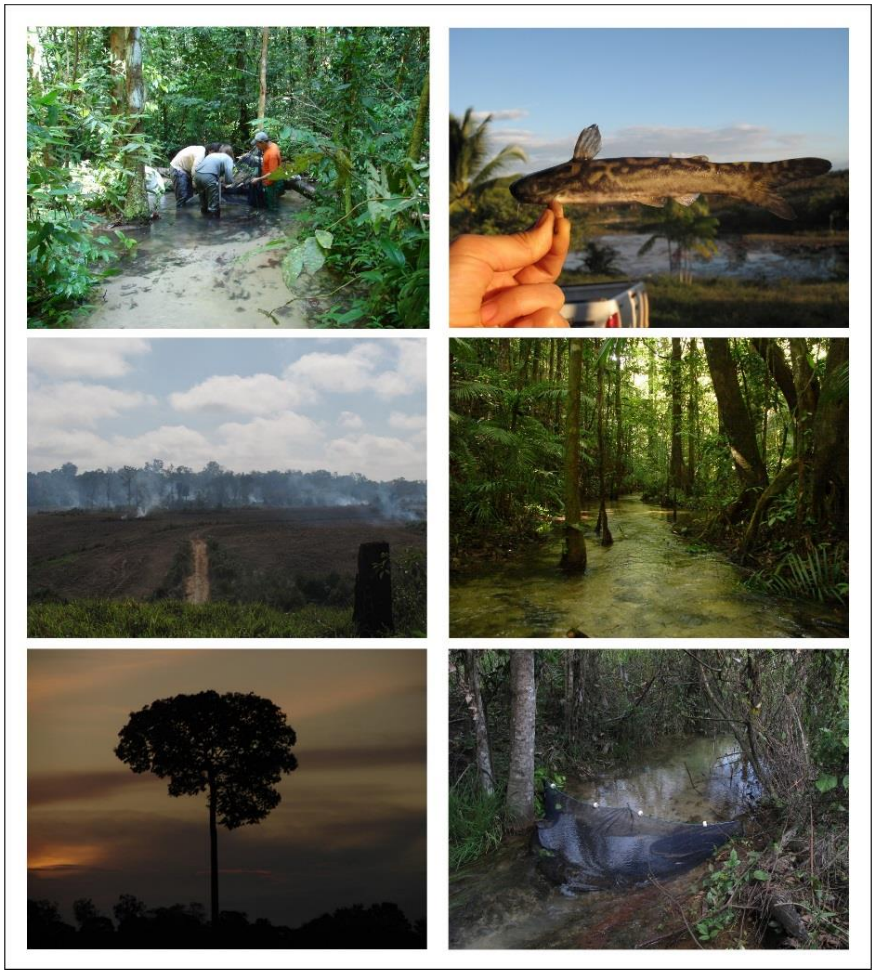


Figure A2.3 Pearson correlation and scatterplot of the landscape predictor variables for Santarém (a) and Paragominas (b) stream sites.

MANUSCRIPT 2

A LARGE-SCALE ASSESSMENT OF FISH DIVERSITY IN SMALL STREAMS ACROSS HUMAN-MODIFIED AMAZONIAN LANDSCAPES

(Prepared for submission to Ecography)



ABSTRACT

The Amazon basin encompasses an extremely diverse freshwater fish fauna which is threatened by mounting impacts from land use change (LUC). Yet there is hardly any information on the patterns of stream fish diversity in areas of the basin that have already been modified by human activity. We sampled fish in 94 low-order stream sites across five river basins and two large regions including a wide range of land uses to investigate the effects of anthropogenic activities on the richness, abundance and composition of fish assemblages. To examine the proximate drivers of fish diversity in varyingly disturbed streams we sampled differences in both instream habitat (water quality and physical habitat such as substrate, channel morphology etc), and LUC at multiple scales in the surrounding landscape (e.g. forest cover, riverscape fragmentation, mechanized agriculture) as well as natural stream features (e.g. catchment slope and size). We sampled a total of 25,526 fish specimens belonging to 143 species, 27 families and seven orders. Our findings highlight an exceptionally high beta diversity between stream sites (helping to explain more than 70% of the total diversity in each of the river basins) and between river basins, showing that these low-order streams are very heterogeneous in their vertebrate biota. Alpha diversity was comparable to what had already been reported for relatively undisturbed stream sites in other Amazonian systems. Fish assemblage structure and composition were influenced by a broad set of environmental variables related to both natural features as well as differences in the disturbance of the local instream habitat and the surrounding landscape, with relationships varying markedly between different river basins. We use these findings to discuss conservation and management challenges and recommendations for Amazonian stream systems in human-modified landscapes. In particular we emphasize the need for collective-action management approaches at both landscape and regional levels to address the high levels of heterogeneity and species-environment relationships between basins and regions.

Keywords: Land use change. Species turnover. Deforestation. Watershed management. Freshwater ecology. Tropics. Ichthyofauna.

1 INTRODUCTION

The Neotropical region hosts the most diversified freshwater fish fauna in the world, representing some 10% of all vertebrate species (Lundberg et al 1998; Vari and Malabarba 1998; Lévêque et al 2008). There are currently 5,600 known Neotropical freshwater fish species (Albert et al 2011a) and estimates of up to 8,000 (Vari and Malabarba 1998) indicate that many species have yet to be discovered. The Amazon basin, the largest in area and discharge in the world (Junk 1983), accounts for a significant part of this number by hosting an unparalleled richness of 2,200 species (Reis et al 2003). The conservation of such an important portion of the world's fish diversity presents significant challenges (Thieme et al 2007), with threats from rapid and intensive agricultural and infrastructure development affecting much of the Amazon (Davidson et al 2012; Castello et al 2013; Ferreira et al 2014). These challenges are further confounded by a lack of knowledge about the distribution and diversity of freshwater fish in the region, hindering the development of appropriate management strategies and conservation planning.

Most existing research on Amazonian fish has focussed on species and populations within the Amazon River itself, or its main tributaries (e.g. Fernandes et al. 2004). These waters host most of the species of commercial interest (e.g. Ribeiro & Petrere Junior 1990; Barthem et al. 1991; Batista & Petrere Jr. 2003; Ardura et al. 2010) and are increasingly affected by hydropower plants (Ferreira et al 2014; Tundisi et al 2014). However, the 7 million km^2 of drainage area of the Amazon basin encompasses an immense and complex network of low-order streams (1st to 3rd order streams represent up to 90% of the total river length; Mcclain & Elsenbeer 2001), that connects terrestrial and aquatic ecosystems across the region (Junk 1983). Unlike some of the main river channels that originate in the Andes, such streams are typically nutrient poor, and depend on the adjacent forest for the input of nutrients, organic material flow and regulations of sediment input (Lowe-McConnell 1987).

Although small streams are recognized as marking an important contribution to fish diversity in the Amazon basin, the fish fauna that inhabit these systems remains very poorly documented(Albert et al 2011b; Albert et al 2011c). The majority of research to date has been carried out in a small number of well-studied regions in undisturbed stream systems, with small-scale studies focussing on population dynamics, habitat use, reproductive strategies, feeding habits or other aspects of species natural history (Sabino and Zuanon 1998; Bührnheim and Fernandes 2003; Zuanon et al 2006; Espírito-Santo et al 2009; Rodrigues et al 2012; Espírito-Santo et al 2013). Whilst useful, there are two main reasons why these small-scale studies in relatively undisturbed regions are limited in their ability to inform freshwater conservation challenges in the Amazon. First, low-order streams are naturally highly heterogeneous in substrate cover, channel morphology, water physico-chemical properties, and water flow.

These differences result in a high level of natural environmental heterogeneity of micro and meso habitats for fish (Junk 1983; Carvalho et al 2007), with a resultant high level of species turnover between adjacent streams and river basins (Mendonça et al 2005; Albert et al 2011a; Albert et al 2011b; Barros et al 2013). Second, in contrast to studies on terrestrial systems (Gardner et al 2009; Malhi et al 2014) there is very little information on patterns of fish diversity from frontier regions of the Amazon, where the integrity of stream systems is impacted by pervasive land use change and forest degradation. These anthropogenic disturbances have resulted in dramatic changes to instream habitat of low-order streams in human-modified landscapes (Chapter 2), but the effects on fish assemblages remain very poorly documented and understood (but see Bojsen & Barriga 2002; Dias et al. 2010).

The present study aims to address this knowledge gap by presenting the first assessment of patterns in fish diversity for low-order streams across multiple landscapes and land use systems that are typical of the agricultural-forest frontier region of the Brazilian Amazon. We sampled 94 low order streams across five major river basins and two large regions that are characterized by a heterogeneous mosaic of land uses and history of human occupation. This comprehensive sample provided us a unique opportunity to investigate (i) what are the patterns of fish diversity across a very broad range of human-modified Amazonian streams, including differences in species richness, abundance, composition and the partitioning of diversity into alpha and beta components, and (ii) how these diversity metrics are influenced by environmental variables related to human disturbance, such as land use change and cover, instream habitat characteristics and natural features. We draw on these findings to discuss the status of low-order streams in Amazon frontier regions and identify priorities for conservation management strategies of stream systems.

2 METHODS

2.1 Study region

We studied two regions in the eastern Brazilian Amazon state of Pará: Santarém (composed by the municipalities of Santarém, Belterra and Mojuí dos Campos) (hereinafter 'STM'), located southeast of the Amazonas and Tapajós Rivers confluence; and Paragominas ('PGM'), in the lower Amazon basin. The study regions belong to two of the 426 Freshwater Ecoregions of the World (FEOW) proposed as biogeographic units to assist global freshwater biodiversity conservation planning (Abell et al 2008), STM in 'Amazonas Lowlands' and PGM in 'Amazonas Estuary and Coastal Drainages'. Our sampling design included five main river basins and 44 stream sites in STM and 50 in PGM (all 1st to 3rd Strahler order on a digital 1:100,000 scale map). In STM, 33 streams belong to the Curuá-Una River basin, five flow directly to the Amazonas River and six to the Tapajós River; PGM stream sites encompass the Gurupi (24) and Capim (26) River basins (Figure 3.1).

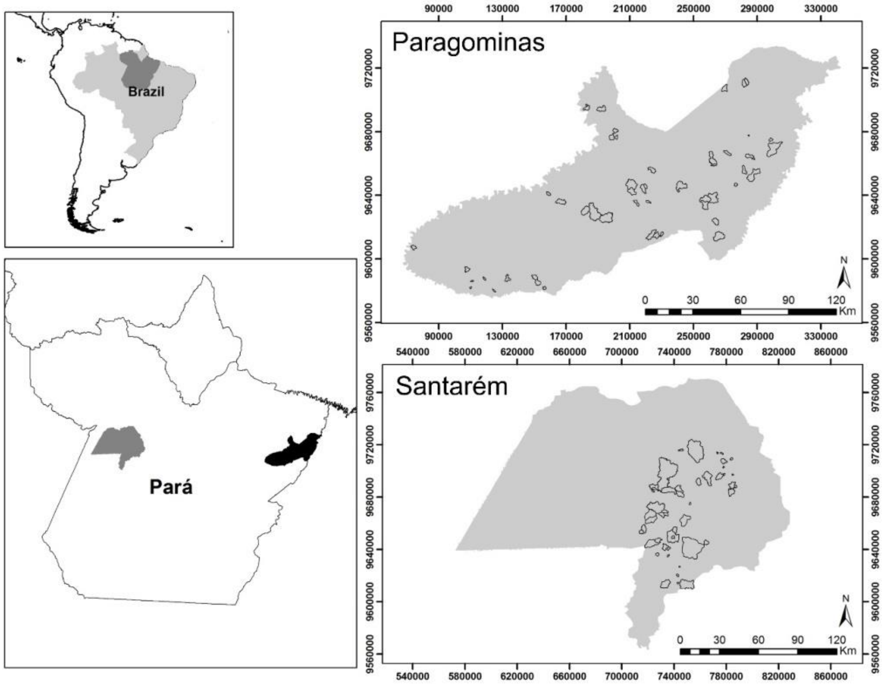


Figure 3.1 Location of stream site catchments in Paragominas (ca. 1.9 million ha) and Santarém (ca. 1 million ha) regions, Pará state, eastern Brazilian Amazon.

The two regions have distinct histories of human land use and occupation. STM (ca. 1 million ha) has been occupied by Europeans since 1661, whereas PGM (ca. 1.9 million ha) was formally established in 1959. However, there are also many similarities; both regions are bisected by federal highways, mechanized agriculture arrived in the last two decades, and deforestation of primary forest has started to decrease since 2005. Today they are both characterized by a diverse patchwork of well-established mechanized agriculture, extensive and intensive cattle pastures, silviculture (mostly *Eucalyptus* spp. and *Schizolobium amazonicum*, especially in Paragominas), densely populated small-holder colonies and agrarian reform settlements, as well as large expanses of undisturbed and disturbed primary forests and regenerating secondary forests (Gardner et al 2013).

2.2 Data sampling

2.2.1 Environmental predictor variables

We measured a broad set of environmental predictor variables including both landscape-scale and instream habitat descriptors. Landscape predictor variables encompassed anthropogenic and natural characteristics of the stream sites at different spatial scales. Instream habitat predictor variables comprised differences in water quality and physical habitat characteristics that can be influenced by both anthropogenic and natural characteristics.

2.2.2 Landscape-scale

We conducted land use assessments at three different spatial scales (Figure A3.1a): 1) the whole catchment upstream from the stream site ('catchment'), 2) the 100 m buffer along the entire drainage network upstream from the stream site ('riparian network'), and 3) a 100 m riparian buffer at the stream site ('local riparian'). Catchment boundaries, mean elevation, and slope were obtained through use of digital elevation models for Santarém (SRTM images with 90 m resolution; NASA - National Aeronautics and Space Administration) and for Paragominas (TopoData with 30 m resolution; INPE - National Institute for Space Research). The drainage network was constructed using the hydrological model ArcSWAT (Soil and Water Assessment Tool extension for ArcGis) for both regions.

Percentage of forest cover in each of the three spatial scales was obtained from a land use map (Landsat TM and ETM+ images, 30 m resolution, year 2010; Gardner et al. 2013); Table 1 for a summary of landscape predictor variables). Forest included primary forest (whether undisturbed or showing signs of disturbance from fire or logging), and secondary forest older than 10 years (considered sufficiently developed to provide significant hydrological services). To calculate forest cover at different hydrological distances from the stream site, we first standardized the distances by the maximum distance in each catchment to account for differences in catchment sizes. Then we assigned all pixels in each catchment into near, intermediate or distant categories and then calculated the percent forest cover in each of the distance categories (Paula et al 2013; Figure A3.1b).

The history of mechanized agriculture was calculated from annual MODIS data from 2001 to 2010 (see details in Gardner et al. 2013). Two historical land use indicators were calculated for catchments using a time-series of land use maps for the last two decades (following Ferraz et al 2009): forest change curvature profile (FCCP; the deforestation profile curvature) and land use intensity index (LUI; the mean time since deforestation). FCCP is the maximum deviation of the forest change curve relative to the linear model between initial and final forest amount over time. These indicators were calculated using Land Use Change Analysis Tools (LUCAT), an open source ArcGIS extension (Ferraz et al 2011; Ferraz et al 2012).

We visually estimated the presence and proximity of 11 categories of human activities in the local riparian zone (i.e., annual crops, pastures, dams and revetments, buildings, pavements, roadways, pipes, landfill/trash, parks/lawns, logging and mining); and calculated an index of proximity of anthropogenic impact denoted by W1_HALL (see Peck et al. 2006; Hughes & Peck 2008).

We estimated riverscape fragmentation using the number of upstream and downstream road crossings within a 5 km circular buffer from the stream site. The road crossings in the drainage network were identified by photo interpretation using georeferenced colour Rapideye images (2010 for STM and 2011 for PGM, 5 m resolution) for the study regions. To map these crossings, we identified features in the images related to the road crossings (linear lines crossing the drainage network; Jensen 2000). A subset of about half of these identified crossings were validated using Google Earth images. Hydrological distance between each stream site and the main river downstream (4th order reaches) were calculated using Landsat images. All landscape analyses were conducted in ArcGis 9.3© (Environmental Systems Research Institute, Redlands, CA, USA).

2.2.3 Instream habitat

We sampled measures of stream physical habitat and water quality during the Amazonian dry season in STM (July-August 2010) and PGM (June-August 2011). Each 150 m long stream site was subdivided into 10 continuous sections, each 15 m long, by 11 cross-sectional transects (Figure A3.2). We measured dissolved oxygen, conductivity, pH, and temperature with a digital portable meter placed below the water surface in the centre of the stream site before taking measurements inside the channel to prevent disturbance.

We measured physical habitat at the stream sites following Peck et al (2006) and Hughes and Peck (2008). For each section we took 10 longitudinal equidistant measurements of thalweg depth; visual quantification of bars, backwaters, side channels, and channel type (pool, glide, riffle, rapid, cascade, waterfall or dry channel); channel slope (measured with a flexible, water-filled plastic tube); and sinuosity (measured with compass bearings). We also recorded the presence of large wood of different size classes in or above the bankfull channel of the stream site.

For each of the 11 cross-sectional transects we measured depth and visually estimated cover of substrate type (bedrock, concrete, boulder, cobble, coarse gravel, fine gravel, sand, silt and clay, hardpan, fine litter, coarse litter, wood, roots, macrophyte, and algae) along five equidistant points transverse to the long axis of the stream. Transect characterization also included bankfull width and depth, mean wetted width and depth, incision height, undercut bank distance, and bank angle. We assessed habitat complexity at each transect in 10 m plots inside the stream channel, using visual estimates of the areal cover of filamentous algae, aquatic macrophytes, leaf packs, roots, large wood >30cm diameter, brush and small wood, overhanging vegetation <1 m above the water surface, undercut banks, boulders, and artificial structures. We measured vegetation canopy cover above the channel with a densiometer at the centre of each transect by facing upstream, downstream, left and right, as well as by facing both banks near the banks. We calculated discharge from mean current velocity (estimated from the travel time of a floating object along three known distances) and mean cross-sectional area (measured as mean depth times mean width of the three known distances) of the stream site.

These measurements were used to calculate an initial set of 171 instream habitat predictor variables from the field data based on Kaufmann et al (1999), including 25 channel morphology, 16 channel unit, 5 channel sinuosity and slope, 28 substrate size and composition, 33 habitat complexity, 60 large wood, and 4 stream canopy cover variables. Geometric mean substrate diameter and relative bed stability were calculated as described by Kaufmann et al. (2008).

2.2.4 Fish

Following the instream habitat assessment, three people sampled fish in the entire area of the stream site for 120 min (12 min per section). During this procedure, each 15 m section was isolated by block nets. Fish were sampled using seines (6 x 1.5 m, 5 mm stretched mesh size) and semi-circular hand nets (0.8 m in diameter, 2 mm stretched mesh size). The use of different equipment and collection techniques was applied to encompass all kinds of meso and microhabitats (e.g., riffles, pools, undercut banks, open waters, wood debris, leaf packs, sand, marginal vegetation), and consequently fish groups. All catches were made during daylight hours. Specimens were killed in an anesthetic solution of Eugenol and then fixed in 10% formalin. In the laboratory, all sampled fishes were transferred to 70% alcohol and identified to species level. Voucher specimens are deposited at INPA (Instituto Nacional de Pesquisas da Amazônia) and UFLA (Universidade Federal de Lavras) fish collections, Manaus and Lavras respectively, Brazil.

2.3 Data analysis

2.3.1 Selection of environmental predictor variables

The initial set of 20 landscape and 171 instream habitat predictor variables was reduced to nove and 23 respectively (Table 3.1; and see Chapter 2). In sampling the instream habitat predictor variables we adopted a protocol that allows for a standardized, replicable and comprehensive assessment of the physical and chemical characteristics of wadeable streams. The resulting dataset enables the calculation of several variables representing key aspects of instream habitat such as stream size, stream gradient, substrate size and stability, instream cover complexity, and stream-floodplain connectivity. To reduce the complete list of variables to a smaller set of the most representative and weakly correlated environmental predictors we followed the procedure outlined in Chapter 2, with the only distinction being the use of riparian network forest cover (NET_FOR) instead of catchment forest (CAT_FOR) as the first has more relevance for freshwater conservation in the context of the Brazilian Forest Code.

Table 3.1 Environmental predictor variables (landscape-scale and instream habitat) used to predict fish diversity and composition from Amazonian stream sites.

Environmental predictor variables		Definition
Landscape		
Natural	CAT_ARE	Catchment area – ha
	CAT_SLO	Catchment slope
	DST_RIV	Distance to large river (≥ 4th Strahler order)
Anthropogenic	NET_FOR	% network riparian forest
	LOC_FOR	% local riaprian forest
	W1_HAL	Proximity weighted tally of riparian/stream side disturbances (W1_HALL; Kaufmann et al., 1999) - dimensionless
	CAT_MAG	% mechanized agriculture
	LOC_FCP	Forest change curvature profile index (FCCP; Ferraz et al., 2009)
	DEN_RCS	Number of road crossings within a 5 km circular buffer upstream and dowstream the stream site divided by catchment area
Instream habitat		
Water quality	TEMP	Water temperature – ºC
	DO	Dissolved oxygen – mg/L
	PH	pH
	COND	Electrical conductivity – µS/cm
Physical habitat	**Substrate**	
	FINE	Streambed surficial fines < 0.6 mm diameter – % areal cover
	SAFN	Streambed surficial sand + fines < 2 mm diameter – % areal cover
	FNGR	Streambed surficial fine gravel 2 to 16 mm diameter – % areal cover

"Table 3.2, conclusion"

	Environmental predictor variables	Definition
Physical habitat	BIGR	Streambed surficial substrate coarse gravel and larger (> 16 mm diameter) – % areal cover
	Cover and wood	
	AMCV	In-channel algae and macrophytes – % areal cover
	NTCV	In-channel natural cover (wood, live trees and roots, leaf packs, overhanging vegetation, undercut banks, boulders) – % areal cover
	WOOD	Wood volume – m3/m^2 wetted channel area
	Channel morphology	
	WDDP	Mean (wetted width x thalweg depth) – m^2
	DPTH	Standard deviation of thalweg depth – cm
	THDP	Thalweg depth ratio at bankfull/low flow – dimensionless
	BKAN	Standard deviation of bank angle – %
	BKWD	Ratio: Bankfull width to bankfull thalweg depth – dimensionless
	RP100	Mean residual depth at thalweg – (m^2/m)/cm
	SINU	Channel sinuosity – dimensionless
	FAST	Channel fast water (% riffle + rapid + cascade + waterfall)
	Other	
	DSCH	Low flow season discharge measured in the field – m3/s
	LRBS	Log$_{10}$ of relative bed stability estimated at bankfull flow conditions (Kaufmann et al. 2008, 2009)
	LDMB	Log$_{10}$ of critical substrate diameter (maximum mobile diameter) at bankfull flow conditions (Kaufmann et al. 2008, 2009)
	SHAD	Canopy density (shading) measured at mid-channel – %

2.3.2 Analyzing fish assemblage diversity patterns

We assessed the representativeness of our sampling design through species-based accumulation curves constructed using the analytical formula implemented in EstimateS v.9 (Colwell 2013). Next we obtained the sample coverage index (*C*) that calculates the total probability of occurrence of all observed species, wherein 1-*C*indicates the probability that some species were not sampled (Marcon and Hérault 2014).Sample coverage was obtained using 'Chao' estimator in 'Coverage' function from R 'entropart' library (R Core Team 2013).

To analyze the spatial distribution of fish diversity into multiple spatial scales we used multiplicative diversity partitioning of the Hill numbers (so-called 'effective number of species'), a mathematically unified family of diversity indices that consider both abundance and species richness and differ by the order *q* (Jost 2007; Chao et al 2012; Chao et al 2014). Diversity is calculated considering:

$$^{q}\mathrm{D}= \left(\sum_{i=1}^{S} p_i^q\right)^{1/(1-q)}$$

where p_i is the abundance of the *i*-th species in the community, *S*is the total number of species, and *q*, referred as the order of the index. The parameter *q* is related to the sensitivity to species relative abundance. When $q = 0$ abundance is not taken into account and the index give more weight to rare species, therefore diversity simply represents species richness. When $q = 1$ diversity consider each species relative abundance and is equivalent to the exponential of Shannon's entropy index, that is rare or dominant species are equally weighted (so-called a measure of the "typical" species). Lastly, when $q = 2$ dominant species are favoured being the index equivalent to the inverse of Simpson diversity.

Multiplicative diversity partitioning considered the following decompositions: $\beta_{basin}=\gamma_{region}/\alpha_{basin}$ and $\beta_{site} =\gamma_{basin}/\alpha_{site}$wherein site refers to the stream sites, basin refers to the river basin and region to STM and PGM. The analysis was performed using 'multipart' function from R 'vegan' library considering 1,000 simulations (Oksanen et al 2011; Oksanen et al 2013; R Core

Team 2013). To investigate the magnitude of variation in beta diversity in different spatial scales ($\beta_{basin}$and β_{site}) we calculated the relative compositional dissimilarity ($^{q}D_{\beta}$) between communities using the transformation of beta proposed by Jost (2006).

Next we decomposed the beta components of β_{site} diversity to investigate whether variation is species composition across stream sites in each river basin is due to turnover (species replacement) or nestedness (species loss or gain), using Sørensen (β_{SOR}) and Simpson (β_{SIM}) indices (Baselga and Orme 2012). β_{SOR} accounts for both turnover and nestedness, whereas β_{SIM} considers only turnover. Therefore the difference between both indices gives a representation of the nestedness component (β_{NES}): $\beta_{NES} = \beta_{SOR}- \beta_{SIM}$. We ran beta decomposition for richness (Hill's number of order 0) using 'beta.multi' function in R 'betapart' library (Baselga and Orme 2012; R Core Team 2013).

We used nonmetric multidimentional scaling (MDS) followed by the analysis of similarities (ANOSIM) to test for significant differences in multivariate assemblage structure and composition between the five river basins. For ordination analyses on abundance data we applied Hellinger transformation followed by Bray-Curtis index (Legendre and Gallagher 2001); and for qualitative data we used Sørenson index. Both analyses were performed in the Primer v.6 software (Clarke and Gorley 2006).

The influence of continuous environmental variables, standardized by maximum values, on the fish assemblage structure was analyzed by the 'envfit' function within the R library 'vegan' considering 1,000 permutations (Oksanen et al 2013; R Core Team 2013). This method fits environmental vectors onto ordination plots based on their Pearson correlation with the ordination axes (Oksanen et al 2013). For this approach we considered Curuá-Una, Capim and Gurupi basins separately, and discarded the Amazonas and Tapajós river basins as each were represented by very few stream sites.

To evaluate how environmental predictor variables influence fish assemblages we modelled species richness and abundance as functions of the environmental predictor variables. We used random forest (RF; from Breiman 2001) models, which allow complex interactive and non-linear response-predictor relationships, and have excellent predictive performance (Prasad et al 2006; Smith et al 2011). Random forests produce an ensemble of regression trees, where each tree is fitted to a bootstrap sample of the data, and each partition within a tree is split on a random subset of the predictor variables (Ellis et al 2012). The data not used to build a tree in each bootstrap sample, called out-of-bag (OOB) sample, is used to calculate cross-validation performance statistics and measures of variable importance (Ellis et al 2012). We calculated a pseudo-r^2 value as 1- MSE/Var(y), where MSE is the mean squared error of the out of bag predictions (Ellis et al 2012). This value estimates the proportion of variation that can be reliably predicted by the ensemble model. The relative importance (RI) of individual variables was calculated as the mean percentage increase in MSE when a variable was randomly permuted, using the conditional permutation method in ‘randomForest’ function in the R ‘extendedForest’ library (Smith et al 2011; R Core Team 2013), which reduces bias when predictors are correlated. Conditional RI values were computed from the conditional permutation distribution of each variable, permuted within 3 partitions of correlated ($r_{\mathrm{Pearson}} > 0.5$) variables (see Ellis et al. 2012). All models were fitted with 10000 trees, with one third of variables randomly sampled as candidates at each split (1 variable selected if total variables < 3). Variables with negative relative importance values were excluded from final models.

3 RESULTS

We collected a total of 25,526 fish specimens belonging to 143 species, 27 families and seven orders (Table A3.1). In STM region, 5,846 individuals and 60 species were recorded in 33 stream sites in the Curuá-Una River basin, 596 individuals and 15 species in six stream sites in the Tapajós River basin and 510 specimens and 19 species in the five Amazonas River basin stream sites. In PGM, the Capim River basin (26 stream sites) was represented by 7,421 individuals and 83 species, and the Gurupi basin (24 stream sites) produced 11,153 specimens and 83 species. The mean species richness per stream site was 12 (ranging from 3 to 20) for the Curuá-Una, 20 (6-45) for the Capim and 24 (9-43) for the Gurupi. The Amazonas and Tapajós River basin stream sites had much lower richness values 7 (4-9) and 7 (4-11), probably reflecting the fact that they were connected to fewer small streams and belongedto small catchments draining independently to the Amazonas and Tapajós rivers. Nevertheless seven species in Amazonas (*Ancistrus* sp.1, *Astyanax maculisquamis*, *Copella* sp., *Creagrutus ignotus*, *Crenicichla inpa*, *Hemigrammus stictus*, *Pyrrhulina zigzag*) and two in Tapajós (*Hemigrammus vorderwinkleri* and *Iguanodectes variatus*) were exclusive of these river basins, which represented6% of the total richness.Furthermore Curuá-Una, Capim and Gurupi presented 19 (31.7% of the total richness of this river basin), 20 (24.2%) and 25 (30.1%) exclusive species respectively (Figure 3.2). In sum species restricted to a single river basinrepresented 52% of the total registered for both regions, STM and PGM.

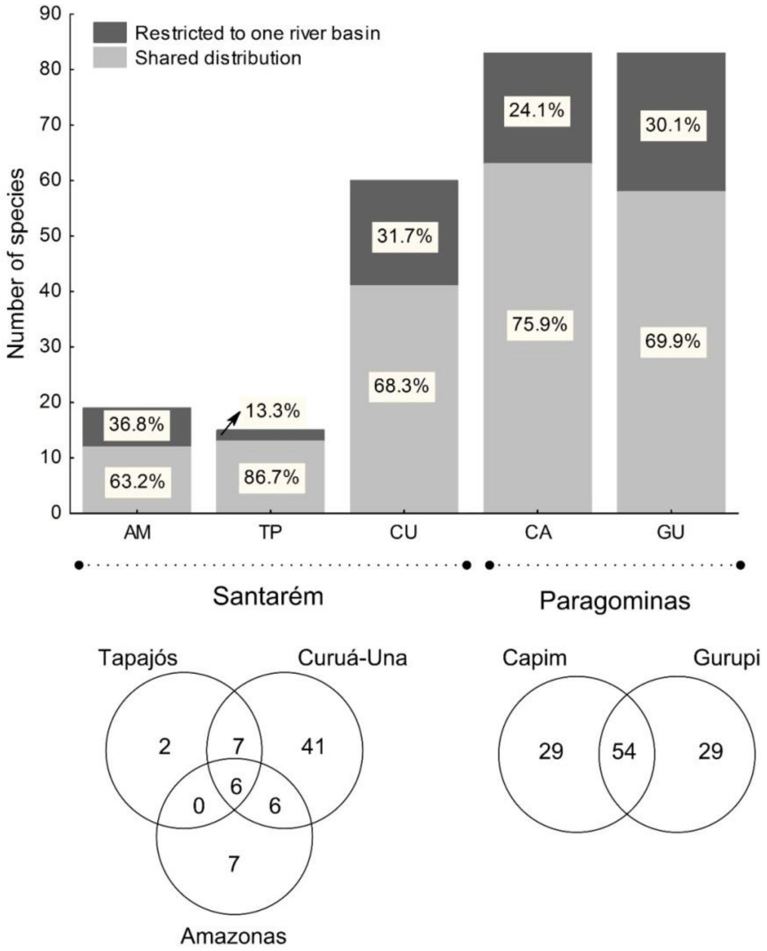


Figure 3.2 Fish species distribution in Amazonas (AM), Tapajós (TP), Curuá-Una (CU), Capim (CA) and Gurupi (GU) showing the percentage of species restricted or shared with other river basins and the number of species shared by river basins within each region (Venn diagrams).

Each assemblage was dominated by a few abundant and widespread species (Figure 3.3). In the Curuá-Una, *Moenkhausia* cf. *collettii* and *Hyphessobrycon* sp. each accounted for about 20% of the total abundance and were collected in 91% and 70% of the stream sites respectively. Together with the next three most abundant species they accounted for 70% of the total abundance of this river basin. In the Capim and Gurupi we found similar patterns of dominance, with the most abundant species (*Hyphessobrycon heterorhabdus* in the former and *Hemigrammus rodwayi* in the latter) accounting for 25% of the abundance in each basin. All the remaining species represented 7% or less of the total number of individuals, while the ten most abundant species represent 70% of the number of individuals.

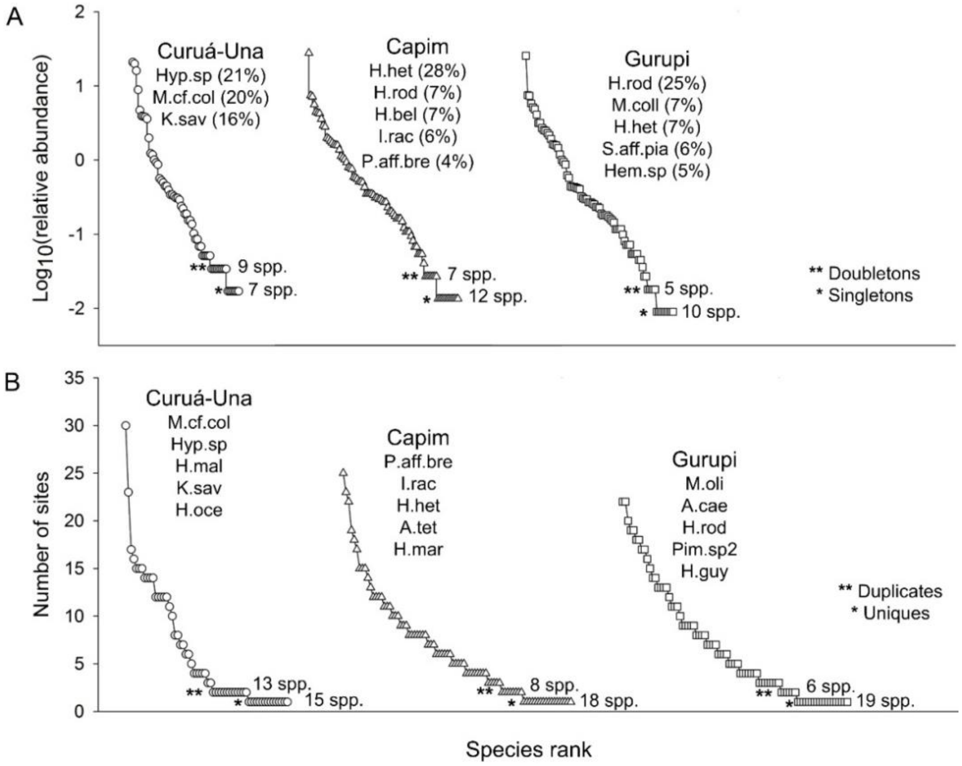


Figure 3.3 Rank of relative species abundance (A) and occurrence in stream sites (B) in Curuá-Una, Capim and Gurupi river basins. Together the indicated species represent 50% of the total abundance in each basin (A) and the five better distributed species (B). In each graph doubletons, singletons, duplicates and uniques are indicated.

Although the species accumulation curves began to level off, they did not reach their asymptote, suggesting we sampled most but not all of the richness in each basin (Figure 3.4). Nevertheless the probability of occurrence of those missed species was less than 1% in each river basin as indicated by the sample coverage indicator for Curuá-Una (*C*= 0.999), Capim (*C*= 0.9996) and Gurupi (*C*= 0.9984). The Capim and Gurupi curves were visually very similar, and indicate a higher level of species richness than in the Curuá-Una basin.

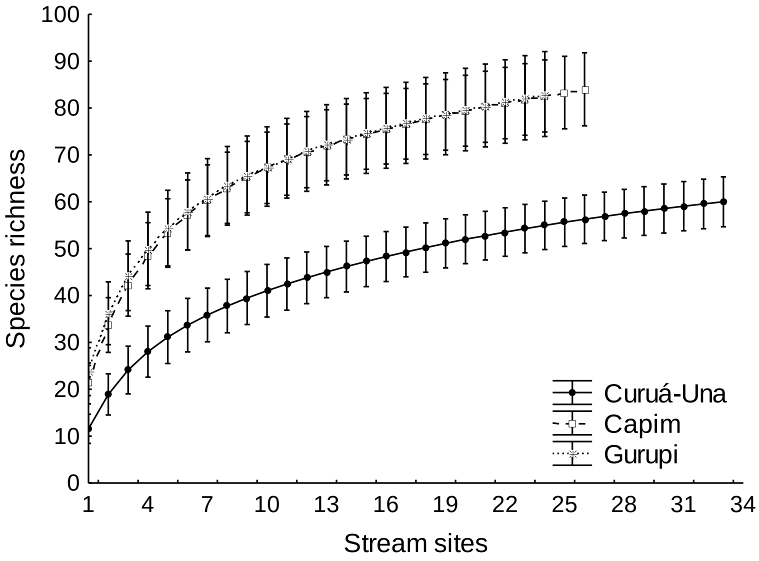


Figure 3.4 Stream site-based rarefaction curves for stream fish from Curuá-Una, Capim, and Gurupi River basins.

Alpha and gamma diversities were consistently higher when considering rare species β-diversity among stream site was consistently higher when considering rare species compared to the typical (q= 1) and dominant (q= 2) species (Figure 3.5A and B). The same pattern was observed for beta diversity between stream sites (Figure 3.5C), the contribution of beta diversity to the compositional dissimilarity between assemblages was ca. 60% higher comparing the scenario where rare species receive more weight($^{0}D_{\beta}$= 0.83, 0.78, 0.74 for Curuá-Una, Capim and Gurupi respectively) with the scenario where dominant species are more important ($^{2}D_{\beta}$=0.51, 0.47, 0.50). However beta diversity between river basins were less sensitive to changes in the order q (Figure 3.5C). All values were significantly different from those expected by chance obtained from 1000 permutations ($p<0.001$). The contribution of turnover to the β_{site} component was much higher than nestedness in all river basins (92.5% in Gurupi, 93.3% in Capim and 95.2% in Curuá-Una; Figure 3.5D).

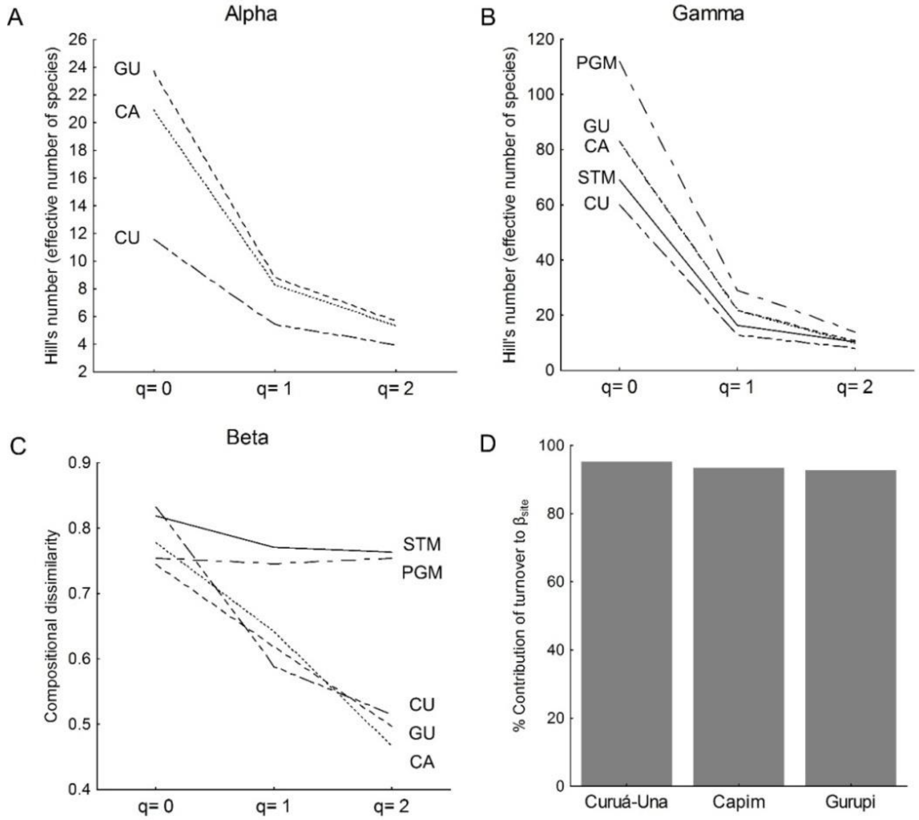


Figure 3.5 Multiplicative diversity partitioning showing the following components: α_{basin} (A), γ_{region} and γ_{basin} (B), β_{site} and β_{basin}(C) and the contribution of turnover to β_{site} (D).River basins: Curuá-Una (CU), Capim (CA) and Gurupi (GU). Regions: Santarém (STM) and Paragominas (PGM).

The fish assemblages in different river basins differed in structure (Figure 3.6A) and species composition (Figure 3.6B) (both results significant: Global *R*= 0.726 and 0.731 respectively; p< 0.001) and all pair wise river basin comparisons were highly significant (p< 0.002).

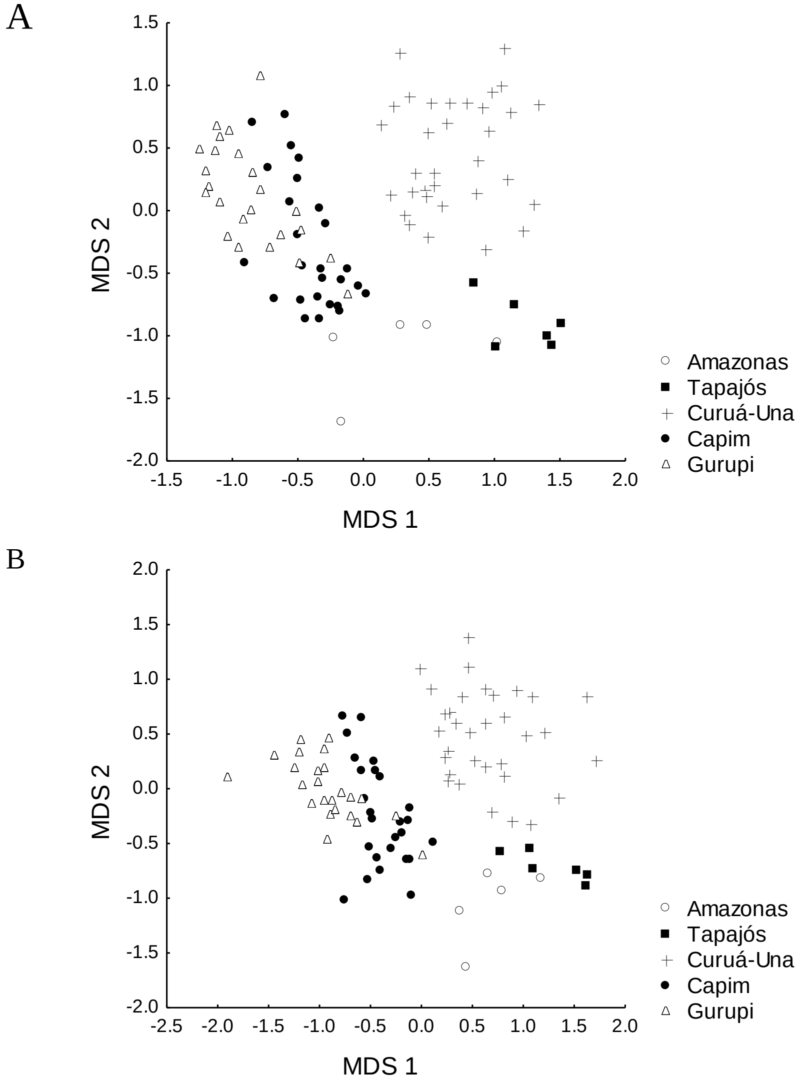


Figure 3.6 Nonmetric multidimensional scaling (MDS) of the fish assemblages from five river basins in the eastern Brazilian Amazon. Ordination analysis was based on quantitative (Bray-Curtis, stress= 0.22; A) and qualitative (Sorensen, stress= 0.21; B) dissimilarity matrices.

Fish assemblage structure was related to a varying set of instream habitat, anthropogenic and natural characteristics in each river basin (Table A3.2, Figure 3.7), without a congruent pattern of more important predictor variables among them.

Considering the non-linear random forest models, the environmental predictors together explained 34% of variability in fish species richness and 4% of abundance in the Curuá-Una, 43% and 27% of richness and abundance in the Capim, and 8% and 23% of richness and abundance in the Gurupi (Table 3.2). However, individual predictor variables contributed very little to the total variation explained: only seven predictors had a partial effect of more than 5%.

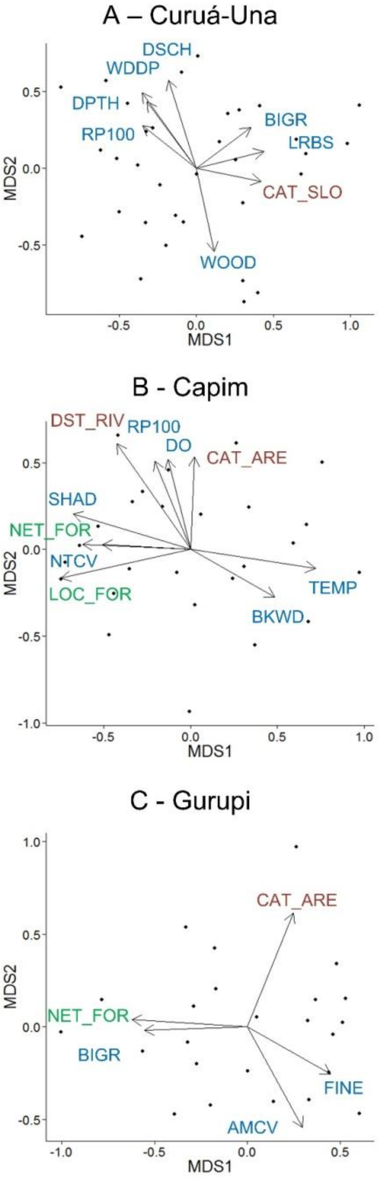


Figure 3.7 Nonmetric multidimensional scaling (MDS) of the fish assemblages from Curuá-Una (A), Capim (B) and Gurupi (C) river basins. The MDS analysis was based on Bray-Curtis dissimilarity index scores. Significant environmental vectors from ‘envfit’ represent instream habitat (blue), anthropogenic (green), and natural (red) predictor variables. See Table 1 for the codes of the predictor variables.

Table 3.3 Performance of random forest models showing the percentage of variation of richness and abundance explained by environmental predictor variables in Curuá-Una (CU), Capim (CA) and Gurupi (GU) River basins. Partial effect of single variables greater than 5% in bold.

Environmental predictor variables		**% Variance Explained**					
		Richness			**Abundance**		
		CU	**CA**	**GU**	**CU**	**CA**	**GU**
Landscape							
Natural	DIST_RIV			0.4	0.8	1.9	0.1
	CAT_ARE		2.0	0.0			0.6
	CAT_SLO						
Anthropogenic	NET_FOR	**8.5**	1.3	0.3	0.1	2.1	0.5
	LOC_FOR	0.7	0.7		0.4		
	W1_HAL	0.7	**6.2**				1.8
	CAT_MAG			0.8	0.4	4.9	
	LOC_FCP		1.1		0.2		
	DEN_RCS	1.2		0.4	0.2	2.1	
Instream habitat							
Water quality	TEMP		0.2				
	DO	0.9	1.6				2.0
	PH		4.2	0.4			0.3
	COND	0.8	4.0			2.2	
Physical habitat	*Substrate*						
	FINE		0.9	0.4		1.2	3.1
	SAFN	3.2					
	FNGR			0.5			0.8
	BIGR	0.8					
	Cover and wood						
	AMCV		2.3	1.1	0.2	3.7	**5.2**
	NTCV			0.3			2.0
	WOOD		**6.5**	1.6			1.7
	Channel morphology						
	WDDP	**5.6**			0.6		
	DPTH	**5.3**		0.2	0.3		0.5
	THDP	1.0			0.3		
	BKWD			0.4			
	BKAN		**6.5**	0.3		4.6	
	RP100	3.1					

"Table 3.4, conclusion"

Environmental predictor variables	**% Variance Explained**					
	Richness			**Abundance**		
	CU	**CA**	**GU**	**CU**	**CA**	**GU**
SINU					3.9	
FAST						
Other						
DSCH	2.2	0.6			0.5	1.4
LRBS	0.8					1.8
LDMB	1.1					
SHAD	3.3		0.4	0.4		0.8
Total	33.9	43.1	7.7	4.0	27.2	22.5

4 DISCUSSION

Our findings illustrate that Amazonian human-modified landscapes host exceptionally high levels of fish diversity. For instance, working in preserved systems Mendonça (2010) found an average of 16 (range: 12-51) species per stream (131 stream sites across the main Amazon sub-basins in Brazil) whereas we found an average of 14 (range: 3-45; 94 1st to 3rd order stream sites). We also show that these low-order streams are environmentally very heterogeneous, as indicated by the high species turnover between both individual river basins as well as between stream sites within basins (helping to explain more than 70% of the total diversity in each of the river basins). Finally, we show that fish assemblage structure and composition are influenced by a broad set of environmental variables related to both natural features as well as differences in the disturbance of the local instream habitat and the surrounding landscape, with relationships varying between different river basins. We first discuss how these results influence our understanding of the biogeography and diversity in Amazonian stream systems, before examining implications for their conservation and management.

4.1 Insights into the biogeography and diversity of Amazonian streams

Some of our findings on stream fish diversity across human-modified tropical forest landscapes are consistent with work in relatively undisturbed streams in the Central Amazon. In terms of general assemblage structure, a few dominant small sized (<20 cm of standard length) species were both widely distributed and abundant, most of them belonging to the Characidae family. We also found a high level of dominance by Characiformes and Siluriformes, which make up the majority of both species and individuals in more preserved streams and our samples from more human-modified areas (Lowe-McConnell 1987; Lévêque et al 2008). Previous works have shown that small Amazonian streams host a distinct ichthyofauna, including rare and locally specialised fish species (Mendonça et al 2005; Zuanon et al 2006; Carvalho et al 2007). Our study also supports this conclusion with a high number of rare species (singletons and doubletons accounted for up to 18% of Gurupi River basin total richness, 22% of Capim and 26% of Curuá-Una) and a high level of beta diversity between streams.

In terms of species richness, methodological discrepancies (e.g. number of sites, sampled length, different Strahler order of stream sites, and scale of study) and the lack of Amazon-wide models for stream fish richness hinder our ability to make a comprehensive comparison between previous studies and ours. For instance, in more preserved Amazonian systems, previous studies report 49 species in 38 low-order stream sites (Mendonça et al 2005), 269 species in 131 sites (Mendonça 2010) and 78 species in 22 sites (Barros et al 2013). However those studies differ considerably from each other and ours in terms of spatial scale, which ranged from 10,000 ha in a single river basin to millions of hectares encompassing several basins. The only record of species richness from a human-modified system in the Amazon River basin reported 32 species from 44 stream sites from logging activityin ca. 120,000 ha (Dias et al 2010), but even these numbers remain difficult to compare to our study because of methodological differences (e.g. the referred study investigate effects of logging activity whereas we included other LUC activities).

Species turnover between river basins (STM and PGM; Figure 3.4A) and between sites inside river basins (Curuá-Una, Capim and Gurupi; Figure 3.4B) accounted for most of the total diversity. On the other hand, alpha diversity at the level of both stream sites and within-site sampling sections contributed very little to the total gamma diversity. Previous studies have indicated that Amazonian streams have a high species turnover (Bührnheim and Fernandes 2003; Mendonça et al 2005; Albert et al 2011b), although ours is the first to compare across multiple streams for an entire region and to use diversity partitioning methods. Such differences in species composition between spatially close Amazonian streams and river basins might reflect dispersal limitations imposed by geographic barriers, as well as a high degree of species habitat specialization (Mendonça et al 2005; Albert et al 2011b).

Finally, our findings illustrate how aquatic and terrestrial surveys can produce contrasting results, and conservation and management strategies motivated by terrestrial studies can be inappropriate or inadequate for freshwater ecosystems. For example, in terms of total and estimated species richness, Capim and Gurupi were both more diverse for fishes than the Curuá-Una River basin, which is the opposite of what was found for terrestrial fauna (birds, dung beetles, ants and orchid bees) and flora (tree, liana and palm together) in the same study areas (see Gardner et al. 2013), and also runs counter to Amazon-wide models of tree species richness (Ter Steege et al 2003). The only other taxa with comparable patterns of richness were the Odonata, with PGM being more diverse than STM, although the opposite was found for Heteroptera and EPT (Ephemeroptera, Plecoptera and Trichoptera together; Gardner et al. 2013).

4.2 Natural and anthropogenic drivers of fish assemblage structure and composition

Fish assemblages in tropical forest landscapes are influenced by a broad range of spatial and temporal environmental factors (Winemiller et al 2006). Pervasive anthropogenic changes to forests across the tropics suggest that human impacts may be as important as natural drivers (Malhi et al 2014). Our results support this broad conclusion by clearly demonstrating how Amazonian fish assemblages are linked to a wide range of both natural and anthropogenic variables, which can covary between themselves in often complex ways that are distinct for different river basins. These complex response patterns highlight the difficulty of identifying dominant drivers, and may be explained by the multiple linkages between land use change and instream habitat condition (see Chapter 2), the many potential interactions between human-impacts and natural stream features, the masking of responses by scale-dependent effects, and the fact that some disturbances are only evident above a certain level of intensity or after a minimum period of time (Allan 2004).

In particular, our examination of species richness and total abundance showed mixed responses to a wide set of environmental variables in different basins, with no clear association with particular predictor variables. This matches findings from other studies, that report equivocal effects of deforestation on total stream fish abundance; positive for Ecuadorian (Bojsen and Barriga 2002) and Mexican (Lyons et al 1995) streams or negative for African streams (Kamdem Toham and Teugels 1999). Although species richness is still often used to inform conservation strategies, our results provide additional evidence that it is a highly inadequate measure of disturbance given the mixed signals it can provide (see also Mackey & Currie 2001; Barlow et al. 2007; Mouillot et al. 2013).

The complex dynamics that connect aquatic and terrestrial systems mean that disturbances throughout the landscape can have manifold effects on stream systems themselves and the structure of fish assemblages (Allan 2004). Changes in both riparian vegetation and land use in up and downstream areas can lead to instream habitat alteration and homogenization (Chapter 2; Casatti et al. 2009). We found that predictor variables reflecting anthropogenic disturbances at multiple scales can influence the fish assemblages of small

Amazonian streams, but we did not find a dominant pattern of responses that was consistent across all river basins. For instance water quality predictor variables were only strongly associated with differences in Capim assemblages; substrate variables were found to be important in Curuá-Una and Gurupi but in Capim; different measurements of wood and cover exhibited differing levels of importance in each of the three river basins; and channel morphology appeared to be important only in the Curuá-Una and Capim basins (Table A3.3, Figure 3.6).

The lack of detailed information on the life history traits of Amazonian fish limits our understanding of assemblage-level responses. However examples from other tropical systems suggest that increasing human disturbances can drive species composition changes as well as taxonomic and functional homogenization. For instance in savannah streams from southeastern Brazil, disturbance-tolerant species replaced more sensitive and specialist species following changes in dissolved oxygen and substrate associated with agricultural development (Casatti et al 2012). Other studies have reported on shifts from specialized to opportunistic feeders in response to changes in stream substrate components and allochthonous input of organic matter due to deforestation in Ecuadorian Amazon (Bojsen and Barriga 2002) and other stream systems in South and Central American (Lorion and Kennedy 2008; Zeni and Casatti 2014). Finally Wright & Flecker (2004) emphasize that instream large wood plays a crucial role in structuring fish assemblages in Venezuelan streams with increases in total abundance and presence of rare species.

4.3 Challenges and opportunities for the conservation of stream fish assemblages in human-modified Amazonian landscapes

Our results demonstrate that Amazonian fish assemblages in low-order streams are both diverse and highly heterogeneous, with a high level of species turnover (and therefore biotic uniqueness) between streams and different river basins (Figure 3.4). Such heterogeneity is a key component of freshwater biodiversity (Dudgeon et al 2006; Tedesco et al 2012) and highlights the importance of biogeographic information that underpins most biodiversity conservation priority setting protocols (e.g. Brooks et al. 2006). A current approach for freshwater systems, Freshwater Ecoregions of the World, FEOW(Abell et al 2008) presents a first attempt to categorize the Earth's freshwater systems based on fish species distribution and composition. While this is a valuable step forward for prioritizing worldwide conservation strategies, our results show that it is far too coarse to assist management strategies that take account of low-order streams of the Amazon basin. For instance, Santarém and Paragominas represent a very small portion of two different FEOWs, yet the species composition of fish assemblages varied markedly among individual streams in both regions. This indicates that conservation planning work needs to start from smaller scales and be extrapolated to larger regions. Although diversity partitioning is usually not considered in management and conservation approaches (Olden et al 2010; Villéger et al 2014), we show how it can provide useful insights at the mesoscale.

The clearance and degradation of primary forest are known to have resulted in significant losses to terrestrial biodiversity in the Amazon (Peres et al 2010), yet much less is known about freshwater systems which are thought to be amongst the most threatened in the world (Dudgeon et al 2006; Strayer and Dudgeon 2010; Vörösmarty et al 2010). Furthermore, most conservation prioritisation exercises are based on criteria related to irreplaceability and vulnerability of terrestrial biota, such as the endemism or total diversity of birds or plants, and measures of past habitat loss and protection (Hoekstra et al 2005;

Brooks et al 2006). This reliance on terrestrial biota is concerning, as freshwater systems have been widely neglected by conservation priority setting processes, and our results give further support to the expectation that priorities for terrestrial systems are unlikely to match priorities set for freshwater systems (Herbert et al 2010; Abraham and Kelkar 2012).

The high level of fish species turnover across multiple scales found in our study strongly supports the need to plan management and conservation strategies at landscape and regional levels. Moreover, our results indicate that there are no simple management actions that can guarantee the preservation of stream fish fauna given the complex interactions between environmental variables and species responses. For instance strategies cannot rely on isolated protected areas (PAs) designed for terrestrial diversity, which face a new suite of threats in the Brazilian Amazon (Ferreira et al 2014) following a global trend of downgrading, downsizing and degazettement (Mascia et al. 2014; Watson et al. 2014).Similarly, the regulation of Amazonian private lands by the federal environmental legislation Forest Code (Federal Law Nº 12.651, May 25th 2012; Brasil 2012), do not properly account for stream environments. The Forest Code considers that the protection of streams and its aquatic biota is ensured by the presence of the riparian zone, whereas there is consistent evidence (e.g. Hughes et al. 2006; Wang et al. 2006), further supported by our work, that upstream forests elsewhere in the catchment as well as disturbances other than deforestation (e.g. mechanized agriculture and riverscape fragmentation from road crossings) are strongly linked to changes in instream habitat condition (Chapter 2). Therefore we emphasize the importance of collective conservation actions involving compliance across neighbouring landowners to ensure that the integrity and connectivity of stream systems are maintained across entire regions.

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APPENDIX CHAPTER 3 (A3)

The tables and figures presented here will be submitted as supplementary material to the manuscript.

Table A3.1 Stream fish species collected in Santarém (STM) and Paragominas (PGM) river basins: Amazonas (AM), Curuá-Una (CU), Tapajós (TP), Capim (CA) and Gurupi (GU).

Order	**Family**	**Species**	**STM**			**PGM**	
			AM	**CU**	**TP**	**CA**	**GU**
Beloniformes	Belonidae	*Potamorrhaphis eigenmanni*					1
Characiformes	Acestrorhynchidae	*Acestrorhynchus falcatus*		2	1	12	16
	Anostomidae	*Leporinus friderici*				23	13
	Characidae	*Astyanax* cf. *bimaculatus*				1	48
		Astyanax maculisquamis	23				
		Bario steindachneri				8	
		Bryconops caudomaculatus		227	57	314	302
		Bryconops melanurus		211		143	275
		Charax leticiae					46
		Creagrutus ignotus	22				
		Gymnocorymbus thayeri					33
		Hemigrammus bellottii				526	
		Hemigrammus guyanensis					539
		Hemigrammus levis		2		255	
		Hemigrammus ocellifer		275		202	28
		Hemigrammus rhodostomus				68	
		Hemigrammus rodwayi				547	2832
		Hemigrammus sp.1				22	573
		Hemigrammus sp.2				58	
		Hemigrammus stictus	1				
		Hemigrammus vorderwinkleri			4		
		Hyphessobrycon copelandi					6
		Hyphessobrycon heterorhabdus	47	519	1	2068	817
		Hyphessobrycon sp.		1223			
		Iguanodectes rachovii				409	

"Table A3.1, concluded"

Order	Family	Species	STM			PGM	
			AM	CU	TP	CA	GU
		Iguanodectes variatus			246		
		Jupiaba anteroides				26	
		Knodus cf. *victoriae*				1	132
		Knodus savannensis		938			
		Knodus sp.n.					183
		Microschemobrycon geisleri				1	13
		Moenkhausia celibela		9			
		Moenkhausia collettii				312	834
		Moenkhausia cf. *collettii*		1163			
		Moenkhausia comma		23		43	11
		Moenkhausia oligolepis				127	276
		Moenkhausia sp.				218	
		Phenacogaster cf. *pectinatus*				21	30
		Phenacogaster cf. *wayana*					1
		Poptella brevispina				38	18
		Poptella compressa					6
		Pristella maxillaris					69
		Serrapinnus aff. *piaba*					646
	Crenuchidae	*Characidium* aff. *pteroides*		18			
		Characidium cf. *etheostoma*				84	173
		Crenuchus spilurus	1	11	1	5	
		Melanocharacidium dispilomma		9		3	
		Microcharacidium weitzmani				44	240
	Curimatidae	*Curimatopsis macrolepis*		33		25	20
		Cyphocharax gouldingi		2		38	48
		Steindachnerina amazonica				1	213
	Erythrinidae	*Erythrinus erythrinus*	8	26		26	8
		Hoplerythrinus unitaeniatus		1		6	2
		Hoplias curupira	2	13		1	
		Hoplias malabaricus	3	31		22	25
	Gasteropelecidae	*Gasteropelecus sternicla*					13
	Lebiasinidae	*Copella arnoldi*				120	20
		Copella nigrofasciata	1	29	60		
		Copella sp.	11				
		Nannostomus beckfordi				2	453

"Table A3.1, concluded"

Order	Family	Species	STM			PGM	
			AM	**CU**	**TP**	**CA**	**GU**
		Nannostomus eques				11	
		Nannostomus marginatus		230	2		
		Nannostomus nitidus				56	
		Nannostomus trifasciatus				116	
		Pyrrhulina aff. *brevis*				328	162
		Pyrrhulina zigzag	2				
	Serrasalmidae	*Myloplus rubripinnis*		3			13
		Serrasalmus rhombeus					3
		Serrasalmus sp.		2			
		Tometes sp.		3			
Cyprinodontiformes	Rivulidae	*Rivulus* cf. *urophthalmus*		20	7	56	47
		Rivulus dibaphus	254	20	155		
Gymnotiformes	Apteronotidae	*Apteronotus albifrons*		1			
	Gymnotidae	*Gymnotus carapo*		6		13	30
		Gymnotus coropinae	34	69	2	41	33
	Hypopomidae	*Brachyhypopomus beebei*					2
		Brachyhypopomus brevirostris		5		22	
		Brachyhypopomus sp.1				14	26
		Brachyhypopomus sp.2				7	
		Brachyhypopomus sp.3					1
		Hypopygus lepturus		54		32	45
		Microsternarchus bilineatus				15	
		Steatogenys duidae				4	
	Rhamphichthyidae	*Gymnorhamphichthys petiti*		51	8	76	
		Rhamphichthys marmoratus					1
	Sternopygidae	*Eigenmannia* aff. *trilineata*		8		118	351
		Sternopygus macrurus		3		1	29
Perciformes	Cichlidae	*Acaronia nassa*		1			
		Aequidens epae		59	32		
		Aequidens tetramerus		113		130	95
		Apistogramma aff. *regani*				143	
		Apistogramma agassizii				17	
		Apistogramma caetei					351
		Apistogramma taeniata	16	223			
		Cichla kelberi					1

"Table A3.1, concluded"

Order	Family	Species	STM			PGM	
			AM	**CU**	**TP**	**CA**	**GU**
		Crenicichla aff. *lepidota*		2			
		Crenicichla aff. *menezesi*	1	72		102	105
		Crenicichla inpa	5				
		Crenicichla johanna		1			
		Crenicichla strigata		1			
		Geophagus altifrons				6	5
		Heros notatus				2	
		Hypselecara temporalis		5			
		Mesonauta festivus		1			
		Nannacara taenia				20	
		Satanoperca jurupari		14		8	21
Siluriformes	Aspredinidae	*Bunocephalus* cf. *amaurus*		4		1	2
		Bunocephalus coracoideus				2	1
	Auchenipteridae	*Tatia* aff. *dunni*				1	1
		Tatia intermedia					4
		Tetranematichthys wallacei				2	
		Trachelyopterus galeatus					17
	Callichthyidae	*Callichthys callichthys*				4	9
		Corydoras julii					26
		Corydoras sp.					8
		Megalechis picta				12	2
	Cetopsidae	*Denticetopsis seducta*		18		1	1
		Helogenes marmoratus	76	26	18	80	49
	Doradidae	*Acanthodoras cataphractus*		2		2	6
	Heptapteridae	*Brachyglanis microphthalmus*		4			
		Gladioglanis conquistador				5	
		Imparfinis sp.					19
		Imparfinis stictonotus					64
		Mastiglanis asopos		11		20	
		Pimelodella sp.1				26	5
		Pimelodella sp.2				8	256
		Rhamdia muelleri		3		12	2
		Rhamdia quelen		2		15	6
	Loricariidae	*Ancistrus* sp.1					36
		Ancistrus sp.2	2				
		Farlowella platorynchus				4	

"Table A3.1, conclusion"

Order	Family	Species	STM			PGM	
			AM	**CU**	**TP**	**CA**	**GU**
		Farlowella schreitmuelleri					34
		Hemiodontichthys acipenserinus					1
		Hypostomus cf. *cochliodon*					1
		Otocinclus hoppei					3
		Parotocinclus sp.1		2			
		Parotocinclus sp.2		19			
		Rineloricaria sp.				26	176
	Pseudopimelodidae	*Batrochoglanis raninus*		1		1	8
	Trichomycteridae	*Ituglanis amazonicus*		3		36	112
		Trichomycterus hasemani				2	
Synbranchiformes	Synbranchidae	*Synbranchus madeirae*		17	2	2	20
		Synbranchus sp.	1	2		1	

Table A3.2 Pearson correlation, r2 and p-value (from ‘envfit’ function) between environmental predictor variables and the first two multidimensional scaling (MDS) axis for Curuá-Una, Capim and Gurupi River basins. Only significant values ($p < 0.05$) are presented.

Environmental predictor variables		**Curuá-Una**				**Capim**				**Gurupi**			
		MDS1	**MDS2**	**r2**	**p**	**MDS1**	**MDS2**	**r2**	**p**	**MDS1**	**MDS2**	**r2**	**p**
Landscape													
Natural	DIST_RIV					-0.59	0.81	0.55	0.00				
	CAT_ARE					0.01	1.00	0.26	0.03	0.37	0.93	0.45	0.00
	CAT_SLO	0.98	-0.20	0.18	0.05								
Anthropogenic	NET_FOR					-0.99	0.11	0.36	0.01	-1.00	0.05	0.39	0.00
	LOC_FOR					-0.98	-0.21	0.55	0.00				
	W1_HAL												
	CAT_MAG												
	LOC_FCP												
	DEN_RCS												
Instream habitat													
Water quality	TEMP					0.98	-0.19	0.52	0.00				
	DO					-0.30	0.95	0.25	0.04				
	PH												
	COND												

"Table A3.2, conclusion"

	Environmental predictor variables	Curuá-Una				Capim				Gurupi			
		MDS1	MDS2	r2	p	MDS1	MDS2	r2	p	MDS1	MDS2	r2	p
Physical habitat	*Substrate*												
	FINE									0.85	-0.52	0.27	0.03
	SAFN												
	FNGR												
	BIGR	0.80	0.60	0.19	0.03					-1.00	-0.01	0.31	0.02
	Cover and wood												
	AMCV									0.47	-0.88	0.40	0.01
	NTCV					-1.00	0.03	0.26	0.02				
	WOOD	0.21	-0.98	0.31	0.00								
	Channel morphology												
	WDDP	-0.58	0.81	0.37	0.00								
	DPTH	-0.60	0.80	0.29	0.00								
	THDP												
	BKWD					0.86	-0.52	0.32	0.01				
	BKAN												
	RP100	-0.78	0.62	0.20	0.03	-0.35	0.94	0.32	0.01				
	SINU												
	FAST												
	Other												
	DSCH	-0.30	0.95	0.36	0.00								
	LRBS	0.97	0.24	0.20	0.04								
	LDMB												
	SHAD					-0.94	0.33	0.48	0.00				

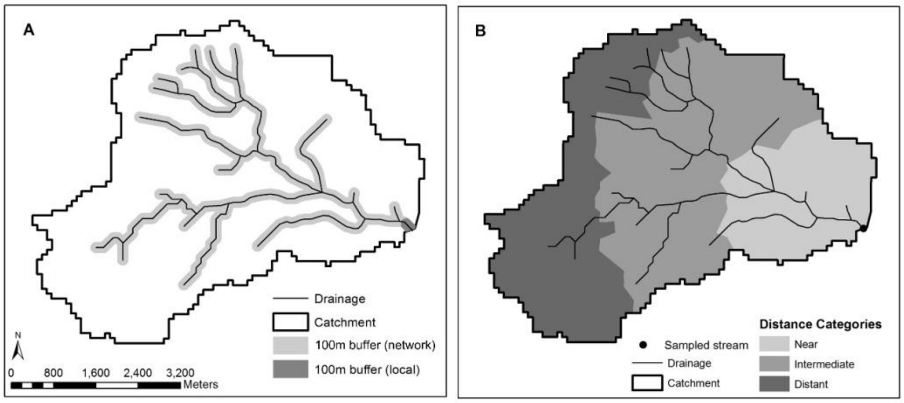


Figure A3.1 Schematic of the spatial scales (A) and hydrological distances (B) considered to obtain the landscape predictor variables of fish from Amazonian stream sites. Riparian buffers are referred as network and local.

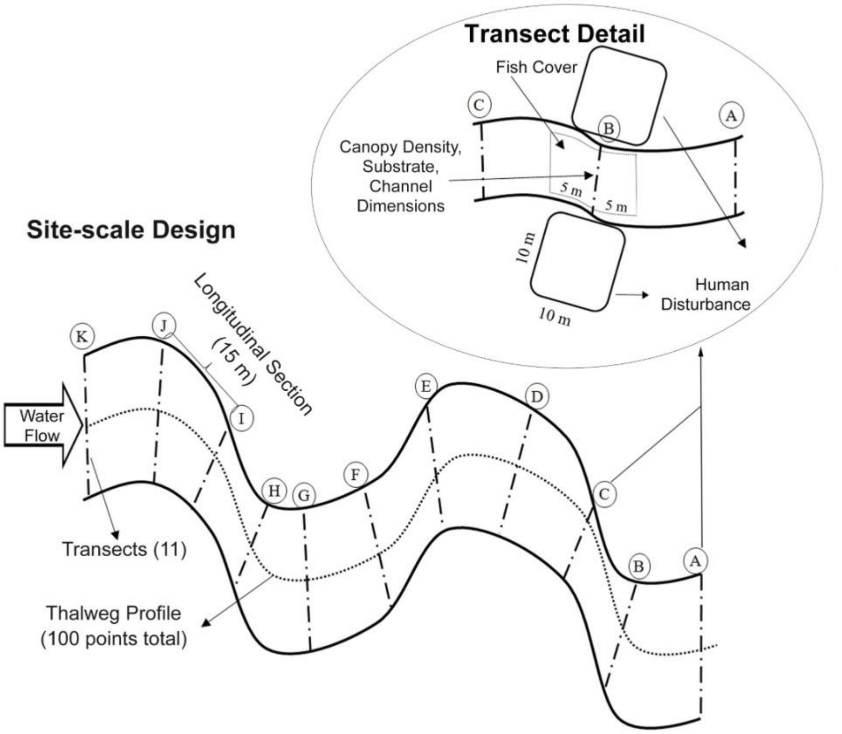


Figure A3.2 Schematic of the sampling design of the instream habitat of Amazonian stream sites.

MANUSCRIPT 3

A LARGE-SCALE ASSESSMENT OF LOCAL, RIPARIAN AND CATCHMENT-LEVEL IMPACTS ON AMAZONIAN STREAM FISH

(Prepared for submission to Conservation Biology)

ABSTRACT

Agricultural expansion in the tropics is one of the main drivers of biodiversity loss worldwide. In addition to the conversion of native vegetation for crops and livestock there is wider set of human-induced disturbances that are often neglected by environmental legislation and conservation programs in tropical countries. The consequences of human-induced disturbances to the ecological integrity of freshwater systems are far less studied than impacts on terrestrial ecosystems. Here we address this knowledge gap by investigating the relative importance of local (i.e. instream habitat), riparian and other landscape scales human impacts, as well as natural features on changes in the occupancy and abundance of stream fish in the eastern Amazon. We undertook a large and multi-scale assessment of stream environmental features from 83 sites in five river basins and two large regions distributed along a broad gradient of different land uses in the eastern Amazonian forest-agriculture frontier. We found mixed responses by stream fish from different river basins (e.g. regarding the importance of natural stream features) but consistent associations with differences in instream habitat features. Species-specific responses were not associated with differences in trophic guild for any of the river basins. We use our findings to discuss the effectiveness of the Brazilian environmental legislation which only explicitly account for a subset of human disturbances. In particular, the focus of existing legislation on the protection of riparian vegetation is insufficient to guarantee the conservation of stream environments. The focus of conservation efforts on riparian vegetation should not take away from the importance of conservation efforts at other scales (e.g. catchment and local instream habitat), and ultimately the need to shift towards the management of drainage networks if we are to effectively safeguard stream condition and fish biodiversity.

Keywords: Land use change.Tropics.Watershed management. Brazilian Forest Code.Trophic functional structure.

1 INTRODUCTION

Land use change in the tropics is one of the principal drivers of global biodiversity loss (Laurance et al 2014), and agricultural expansion in many countries can be expected to increase with population growth and shifts towards more diverse and protein-rich diets (Tilman et al 2011; Tilman and Clark 2014). The forested tropics, such as the Brazilian Amazon, hold many of the lands that could be available for agricultural expansion, which is being facilitated by new strain of crops, climatic change, and infrastructure development such as new and improved roads (Vera-Diaz et al 2008). In addition to deforestation, the remaining forests are often degraded by a suite of additional disturbances such as logging, fire and edge effects (Asner et al 2005; Broadbent et al 2008; Souza et al 2013). Understanding how these activities combine to affect biodiversity is vital for planning appropriate conservation strategies and helping countries meet their pledges to the Convention on Biological Diversity´s Strategic Action plan and biodiversity-related Aichi Targets.

Freshwater biodiversity is considered to be more imperilled than terrestrial and marine biodiversity counterparts (Dudgeon et al 2006; Strayer and Dudgeon 2010), yet most of the scientific research addressing species responses to habitat change and degradation focus on the terrestrial fauna and flora. For instance, a recent review of 62 studies assessing faunal responses to land use change in Amazonia(Peres et al 2010)included only one that investigated fish (Dias et al 2010). Moreover, where fish responses to anthropogenic pressures have been studied in the Amazon, they have been largely restricted to studies of large channels, hydropower plants projects and commercially important species (e.g. Barthem et al. 1991; Ardura et al. 2010; Fearnside 2014). In contrast, there are very fewstudies on how fish assemblages in low-order streams respond to human impact [see Dias et al. 2010 on impacts from selective logging and

Bojsen and Barriga 2002 on effects of deforestation], even though they make up the vast majority of watercourses in the Amazon basin (1st to 3rd order streams make up ca. 90% of the total river length in some Amazonian sub-basins; Mcclain & Elsenbeer 2001) and permeate across almost all agricultural landscapes.

The conservation of fish in low-order streams requires integrated approaches that include the management of both the land and the stream systems themselves, at the scale of individual sites as well as entire catchments (Allan et al 1997; Revenga et al 2005; Wang et al 2006a; Castello et al 2013). There are two key reasons why it is important to disentangle the relative importance of drivers of change across different spatial scales. First, some environmental features are more amenable to assessment and management than others, but are not necessarily key determinants of changes in aquatic condition. For instance, in streams from pasture-dominated landscapes in southeastern Brazilian savannahs Casatti et al. (2006) found fish assemblages to respond to physical habitat changes but not to water quality. However Brazilian agencies that monitor streams conservation only take into account water quality properties related to human use. Second, it is important to evaluate the extent to which management of the riparian zone can be an effective tool in watershed management when compared to land management elsewhere in the catchment, especially as the latter is often neglected or considered less important (Allan et al 1997). Wang et al (2003) demonstrated the importance of considering both riparian and catchment level features: management practices in the riparian zone improved the physical habitat condition of Wisconsin streams in USA, but were not sufficient to restore fish fauna unless paired with upland management across catchments. At present, there is no clear consensus about whether disturbances at catchment (Roth et al 1996; Marzin et al 2013) or riparian scale (Nerbonne and Vondracek 2001; Sály et al 2011; Macedo et al 2014) are the most relevant drivers of change to aquatic biota. Moreover, existing studies are unlikely to predict patterns in the Neotropics that are naturally heterogeneous and are characterized by very high levels of fish diversity and species turnover between streams and river basins (Mendonça et al. 2005; Albert et al. 2011; chapter 3).

Understanding the relative importance of local and landscape scale drivers of Amazonian stream condition is particularly important in the context of the Brazilian Forest Code (Federal Law Nº 12.651, May 25th 2012; Brasil 2012). It is the central piece of legislation governing environmental management of private lands in Brazil, and specifically regulates the extent of riparian and non-riparian forest cover that need to be protected in different sized properties. Given most watercourses extend beyond protected areas, the Brazilian Forest Code is likely to be critical for Amazonian low-order stream fish conservation, yet at present no studies evaluate the potential effectiveness of the regulations in this context.

Here we use data from a large-scale study linking species abundance with environmental features across 83 stream sites, sampled in a diverse mosaic of different land uses across two regions of the eastern Brazilian Amazon. In doing so, we provide the first assessment of the relative effect of multi-scale drivers on fish diversity in low-order Amazonian streams. The environmental predictor variables were divided into four groups. The first three groups were chosen to represent indicators of human disturbance that are amenable to management and legislation in to varying extents and at different spatial scales, and include 'instream habitat' which depicts local features, the 'riparian network' which links to legislation aimed at maintaining forest cover in the riparian zone to protect watercourses and associated biota, and 'other landscape' which includes catchment level variables associated with forest cover across landscapes. We kept riparian network separated from the other landscape scales because it is clearly linked to governance requirements therefore being more amenable to management. In addition, we add a fourth group called 'natural' which includes features of streams that existed before human influences, or are not amenable to management (Figure 4.1). Because community responses of stream fish are weak (Chapter 3) and may mask important patterns, we focused on understanding species-specific responses. Specifically we ask (i) what are the shared and independent effects of environmental characteristics more amenable to management (i.e. riparian and other landscape) on fish species abundance, and how do these compare with the effects of instream habitat and natural features of streams? (ii) Is there any evidence for associations between fish species with anthropogenic disturbance at the local or landscape levels? and (iii) Can species responses be associated with their trophic functional groups? We use insights from our findings to discuss the complexity of species-environment relationships and the adequacy of the current Brazilian environmental legislation for the conservation of Amazonian low-order stream fish.

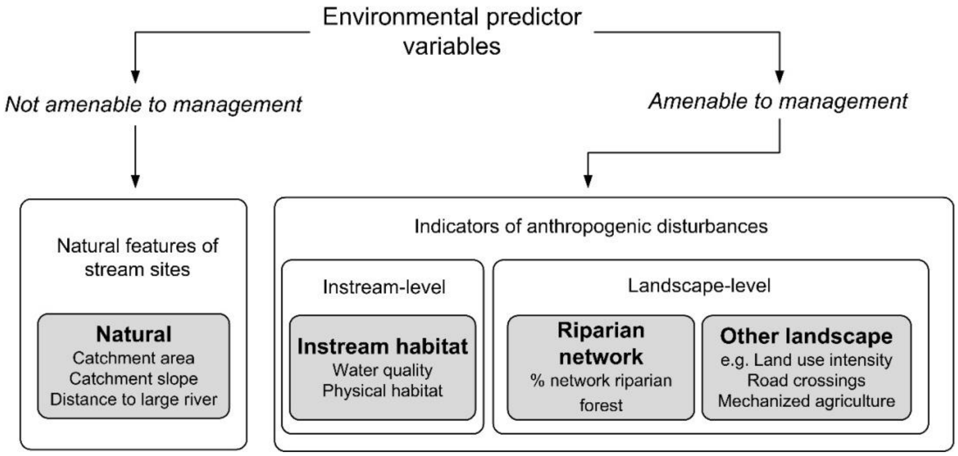


Figure 4.1 Schematic of the environmental predictor variables divided into four groups ('natural', 'instream habitat', 'riparian network' and 'other landscape') used to investigate stream fish species-specific responses in the eastern Amazon.

2 METHODS

2.1 Study region

We studied two regions in the eastern Brazilian Amazon state of Pará. Santarém (hereinafter 'STM') covers ca. 1 million ha and is composed of the municipalities of Santarém, Belterra and Mojuí dos Campos. It is located southeast of the confluence of the Amazonas and Tapajós Rivers. Paragominas ('PGM') is a single 1.9 million ha municipality in the far east of the Amazon basin. The study regions belong to two of the Freshwater Ecoregions of the World (FEOW) proposed as biogeographic units to assist global freshwater biodiversity conservation planning (Abell et al 2008), STM in 'Amazonas Lowlands' and PGM in 'Amazonas Estuary and Coastal Drainages'. Our study design included three main river basins across the two regions, and we sampled 33 stream sites in Curuá-Una (STM), 26 in Capim (PGM) and 24 in Gurupi (PGM). All wadeable streams 1st to 3rd Strahler order on a digital 1:100,000 scale map (Figure 4.2).

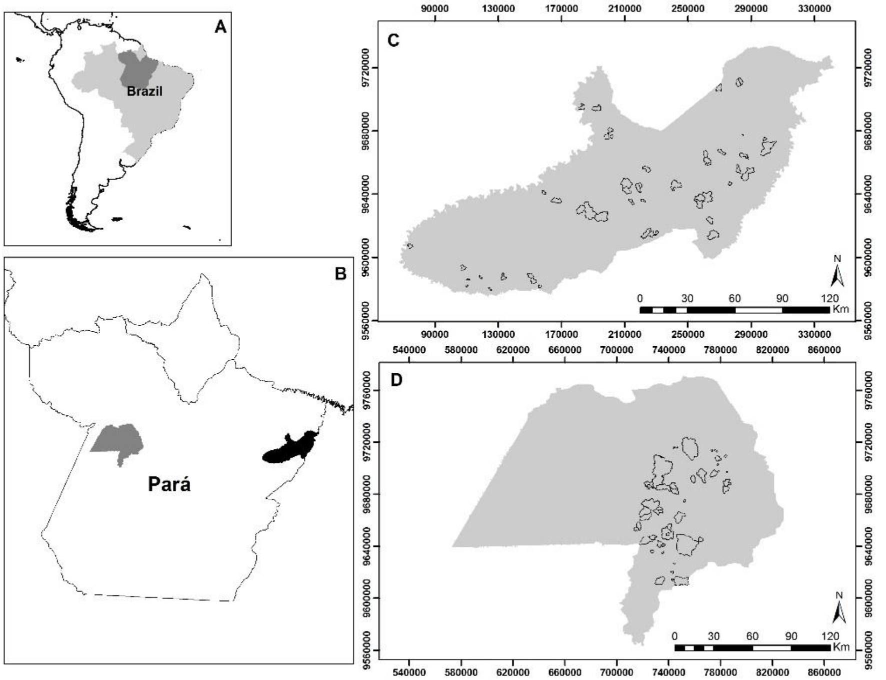


Figure 4.2 Location of stream site catchments in Paragominas (ca. 1.9 million ha) and Santarém (ca. 1 million ha) regions, Pará state, eastern Brazilian Amazon.

The two regions have distinct histories of human land use and occupation. STM has been occupied by Europeans since 1661, whereas PGM was formally established in 1959. Both regions exhibit decreasing rates of primary vegetation deforestation since 2005 and have been bisected by federal highways, with cascading influences on regional development. Native forests cover around two-thirds of both regions, and include undisturbed and disturbed primary forests and regenerating secondary forests (Gardner et al 2013). Production areas encompass a diverse patchwork of cattle ranches, well-established mechanized agriculture, densely populated small-holder colonies and agrarian reform settlements, silviculture (mostly *Eucalyptus* spp. and *Schizolobium amazonicum*, especially in Paragominas).

2.2 Data sampling

2.2.1 Fish

Field work was carried out during the Amazonian dry season in STM (July-August 2010) and PGM (June-August 2011). Three people sampled fish in a 150 m segment of the stream site for 120 min (12 min per section). Each 150 m long stream site was subdivided into 10 continuous sections (isolated by block nets), each 15 m long, by 11 cross-sectional transects (Figure A4.1). Fish were sampled using seines (6 x 1.5 m, 5 mm stretched mesh size) and semi-circular hand nets (0.8 m in diameter, 2 mm stretched mesh size). The use of different equipment and collection techniques was applied to encompass all kinds of meso and microhabitats (e.g., riffles, pools, undercut banks, open waters, wood, leaf packs, sand, marginal vegetation), and consequently fish groups. All catches were made during daylight hours. Specimens were killed in an anesthetic solution of Eugenol and then fixed in 10% formalin. In the laboratory, all sampled fishes were transferred to 70% alcohol and identified to species level.

In order to determine trophic groups we analyzed the stomach content from five to 15 specimens of each species. We combined information about frequency of occurrence (number of times that the item occurred relative to the total number of stomachs with food) and relative volume (relative to the total volume of food) of the food items in the Alimentary Index (IA*i*) proposed by Kawakami & Vazzoler (1980). Species were classified in the following trophic groups when consumed $\geq$ 60% of items: carnivore (animal with no predominance of a specific group), detritivore (particulated organic matter with associated microorganisms and non-organic matter), insectivore allochthonous (terrestrial insects), insectivore autochthonous (aquatic insects), general insectivore (insects, no predominance in their origin), perifitivore (periphyton), piscivore (fish) and herbivore (organic matter). Species were classified as omnivore when there was no predominance of any specific item.

2.2.2 Environmental predictor variables

2.2.2.1 Instream habitat

We measured dissolved oxygen, conductivity, pH, and temperature with a digital portable meter placed below the water surface in the centre of the stream site before taking measurements inside the channel to prevent disturbance.

We measured physical habitat at the stream sites following Peck et al (2006) and Hughes and Peck (2008). For each section we took 10 longitudinal equidistant measurements of thalweg depth; visual quantification of bars, backwaters, side channels, and channel type (pool, glide, riffle, rapid, cascade, waterfall or dry channel); channel slope (measured with a flexible, water-filled plastic tube); and sinuosity (measured with compass bearings). We also recorded the presence of large wood of different size classes in or above the bankfull channel of the stream site.

For each of the 11 cross-sectional transects we measured depth and visually estimated cover of substrate type (bedrock, concrete, boulder, cobble, coarse gravel, fine gravel, sand, silt and clay, hardpan, fine litter, coarse litter, wood, roots, macrophyte, and algae) along five equidistant points transverse to the long axis of the stream. Transect characterization also included bankfull width and depth, mean wetted width and depth, incision height, undercut bank distance, and bank angle. We assessed habitat complexity at each transect in 10 m plots inside the stream channel, using visual estimates of the areal cover of filamentous algae, aquatic macrophytes, leaf packs, roots, large wood >30cm diameter, brush and small wood, overhanging vegetation <1 m above the water surface, undercut banks, boulders, and artificial structures. We measured vegetation canopy cover above the channel with a densiometer at the centre of each transect by facing upstream, downstream, left and right, as well as by facing both banks near the banks. We calculated discharge from mean current velocity (estimated from the travel time of a floating object along three known distances) and mean cross-sectional area (measured as mean depth times mean width of the three known distances) of the stream site.

We calculated an initial set of 171 instream habitat predictor variables from the field data based on Kaufmann et al (1999), including 25 channel morphology, 16 channel unit, 5 channel sinuosity and slope, 28 substrate size and composition, 33 habitat complexity, 60 large wood, and 4 stream canopy cover variables. Geometric mean substrate diameter and relative bed stability were calculated as described by Kaufmann et al. (2008).

2.2.2.2 Riparian network

Riparian network (‘riparian network’) scale consisted of a 100 m buffer along the entire drainage network usptream from the stream site. We measured land use, hydrological distance to forest and indicators of historic land use at this scale as detailed below for the other landscape scales.

2.2.2.3 Other landscape

Besides riparian network scale we conducted land use assessments at the whole catchment upstream from the stream site (‘catchment’) and in a 100 m riparian buffer at the stream site (‘local riparian’; Figure A4.2A).

We measured land use with the percentage of forest cover in each spatial scale obtained from a land use map (Landsat TM and ETM+ images, 30 m resolution, year 2010; Gardner et al 2013; Table 1 for a summary of landscape predictor variables). Forest included primary forest (whether undisturbed or showing signs of disturbance from fire or logging), and secondary forest older than 10 years (considered sufficiently developed to provide significant hydrological services based on our expert assessments). The history of mechanized agriculture was calculated from annual MODIS data from 2001 to 2010 (see details in Gardner et al 2013).

To calculate forest cover at different hydrological distances from the stream site, we first standardized the distances by the maximum distance in each catchment to account for differences in catchment sizes. Then we assigned all pixels in each catchment into near, intermediate or distant categories and then calculated the percent forest cover in each of the distance categories (Paula et al 2013; Figure A4.2B).

Two historical land use indicators were calculated for catchments using a time-series of land use maps for the last two decades (following Ferraz et al 2009): forest change curvature profile (FCCP; the deforestation profile curvature) and land use intensity index (LUI; the mean time since deforestation). FCCP is the maximum deviation of the forest change curve relative to the linear model between initial and final forest amount over time. These indicators were calculated using Land Use Change Analysis Tools (LUCAT), an open source ArcGIS extension (Ferraz et al 2011; Ferraz et al 2012).

We visually estimated the presence and proximity of 11 categories of human activities in the local riparian zone (i.e., row crops, pasture, dams and revetments, buildings, pavement, roadways, pipes, landfill/trash, parks/lawns, logging, and mining); and calculated an index of proximity of anthropogenic impact (W1_HALL; Peck et al. 2006; Hughes & Peck 2008).

We estimated stream riverscape fragmentation using the number of upstream and downstream road crossings within a 5 km circular buffer from the stream site. The road crossings in the drainage network were identified by photo interpretation using georeferenced colour Rapideye images (2010 for STM and 2011 for PGM, 5 m resolution) for the study regions. To map these crossings, we identified features in the images related to the road crossings (linear lines crossing the drainage network; Jensen 2000). A subset of about half of these identified crossings were validated using Google Earth images. All landscape analyses were conducted in ArcGis 9.3© (Environmental Systems Research Institute, Redlands, CA, USA).

2.2.2.4 Natural

The drainage network was constructed using the hydrological model ArcSWAT (Soil and Water Assessment Tool extension for ArcGis) for both regions. Catchment boundaries, mean elevation, and slope were obtained through use of digital elevation models for Santarém (SRTM images with 90 m resolution; NASA - National Aeronautics and Space Administration) and for Paragominas (TopoData with 30 m resolution; INPE - National Institute for Space Research). Hydrological distance between each stream site and the main river downstream (4th order reaches) were calculated using Landsat images.

2.3 Data analysis

2.3.1 Selection of the environmental predictor variables

The initial set of 22 landscape and 171 instream habitat predictor variables was reduced to nine and 23 respectively in order to limit redundancy and produce a smaller set of the most representative environmental predictor variables (Table 4.1). Given the hierarchical nature of the landscape variables (catchment scale encompasses riparian network scale which encompasses local riparian scale), we expected high levels of correlation among them.

Among the instream habitat predictor variables, the protocol used provides a standardized, replicable and complete assessment of the physical and chemical characteristics of wadeable streams. The resulting dataset enables the calculation of several variables representing key aspects of instream habitat such as stream size, stream gradient, substrate size and stability, instream cover complexity, and stream-floodplain connectivity. However it is unlikely to include all 171 as predictor variables, for instance some of them are closely related features (e.g. the substrates % sand, % fine and % sand+fine; or wood considered as count or volume), or are redundant ($r_{Pearson} \geq 0.7$) and others were represented mostly by zeros in our study system. On the other hand, it can be difficult to select *a priori* variables to describe instream habitat and responses to anthropogenic disturbance given the complex relationship between land use change, instream habitat and biotic responses, which result in variation in responses. Therefore our choice of instream habitat predictor variables aimed for considering informative and not redundant variables following the rationale outlined in Chapter 2.

Table 4.1 Environmental variables (landscape and instream habitat) used to predict fish species-specific abundances from Amazonian stream sites.

Environmental predictor variables Group	Code	Definition
Natural		*Landscape: natural features*
	CAT_ARE	Catchment area – ha
	CAT_SLO	Catchment slope
	DST_RIV	Distance to large river (≥ 4th Strahler order)
Riparian network		*Landscape: anthropogenic disturbances*
	NET_FOR	% riparian network forest
Other landscape	LOC_FOR	% local riparian forest
	W1_HAL	Proximity weighted tally of riparian/stream side disturbances (W1_HALL; Kaufmann et al., 1999)
	CAT_MAG	% mechanized agriculture
	LOC_FCP	Forest change curvature profile index (FCCP; Ferraz et al., 2009) - dimensionless
	DEN_RCS	Number of road crossings within a 5 km circular buffer upstream and downstream the stream site divided by catchment area
Instream habitat		*Instream habitat: water quality*
	TEMP	Water temperature – ºC
	DO	Dissolved oxygen – mg/L
	PH	pH
	COND	Electrical conductivity – µS/cm
		Instream habitat: physical habitat
	Substrate	
	FINE	Streambed surficial fines < 0.6 mm diameter – % areal cover
	SAFN	Streambed surficial sand + fines < 2 mm diameter – % areal cover
	FNGR	Streambed surficial fine gravel 2 to 16 mm diameter – % areal cover
	BIGR	Streambed surficial substrate coarse gravel and larger (> 16 mm diameter) – % areal cover
	Cover and wood	
	AMCV	In-channel algae and macrophytes – % areal cover
	NTCV	In-channel natural cover (wood, live trees and roots, leaf packs, overhanging vegetation, undercut banks, boulders) – % areal cover
	WOOD	Wood volume – m3/m^2 wetted channel area
	Channel morphology	

"Table 4.2, conclusion"

Environmental predictor variables Group	 Code	Definition
Instream habitat	WDDP	Mean (wetted width x thalweg depth) – m^2
	DPTH	Standard deviation of thalweg depth – cm
	THDP	Thalweg depth ratio at bankfull/low flow – dimensionless
	BKAN	Standard deviation of bank angle – %
	BKWD	Ratio: Bankfull width to bankfull thalweg depth – dimensionless
	RP100	Mean residual depth at thalweg – (m^2/m)/cm
	SINU	Channel sinuosity – dimensionless
	FAST	Channel fast water (% riffle + rapid + cascade + waterfall)
	Other	
	DSCH	Low flow season discharge measured in the field – m3/s
	LRBS	Log$_{10}$ of relative bed stability estimated at bankfull flow conditions (Kaufmann et al. 2008, 2009)
	LDMB	Log$_{10}$ of critical substrate diameter (maximum mobile diameter) at bankfull flow conditions (Kaufmann et al. 2008, 2009)
	SHAD	Canopy density (shading) measured at mid-channel – %

2.3.2 Statistical analysis

For species-specific models, we retained species from each river basin that occurred in at least 25% of the stream sites and had a total number of individuals equal or larger than the number of stream sites (e.g. we sampled 33 stream sites in Curuá-Una, so we considered species that had $\geq$ 33 individuals and that occurred in $\geq$ 8 sites). We used variance partitioning to evaluate the shared and independent effects of the predictor variable groups on species abundance (research question 1) and random forest models to investigate the partial effect of single predictor variables on species-specific responses (research question 2). In both cases we analysed each species in each river basin separately.

To estimate how much of the variation in species abundance is explained by the four environmental predictor variable groups we used variance partitioning analysis (Borcard et al 1992). We used ‘varpart’ function of the R ‘vegan’ library that automatically calculates the adjusted redundancy analysis (R^2_a). The R^2_a correct for the number of predictor variables in each group and number of observations in the response variable, therefore is considered to produce unbiased estimates (Peres-Neto et al 2006). Even though prior to the variance partitioning analysis we performed a forward selection of the instream habitat group (originally with 23 predictor variables) to reduce the chance of overestimation. We used ‘forward.sel’ function available in the R ‘packfor’ library (Blanchet et al 2008) considering 999 Monte Carlo permutation tests and retaining variables with $p < 0.10$.

For the random forest models we included the same predictor variables retained by the forward selection and used in variance partitioning. We used random forest (RF; from Breiman 2001) models, which allow complex interactive and non-linear response-predictor relationships, and have excellent predictive performance (Prasad et al 2006; Smith et al 2011). Random forests produce an ensemble of regression trees, where each tree is fitted to a bootstrap sample of the data, and each partition within a tree is split on a random subset of the predictor variables (Ellis et al 2012). The data not used to build a tree in each bootstrap sample, called out-of-bag (OOB) sample, is used to calculate cross-validation performance statistics and measures of variable importance (Ellis et al 2012). We calculated a pseudo-r^2 value as 1- MSE/Var(y), where MSE is the mean squared error of the out of bag predictions (Ellis et al 2012). This value estimates the proportion of variation that can be reliably predicted by the ensemble model. The relative importance (RI) of individual variables was calculated as the mean percentage increase in MSE when a variable was randomly permuted, using the conditional permutation method in ‘randomForest’ function in the R ‘extendedForest’ library (Smith et al 2011), which reduces bias when predictors are correlated. Conditional RI values were computed from the conditional permutation distribution of each variable, permuted within 3 partitions of correlated ($r_{\text{Pearson}} > 0.5$) variables (see Ellis et al. 2012). All models were fitted with 10000 trees, with one third of variables randomly sampled as candidates at each split (1 variable selected if total variables < 3). Variables with negative relative importance values were excluded from final models.

After modelling we looked at the partial effect of individual predictor variables that accounted for more of the explanation of a single species. Finally we selected models that resulted in $\geq$ 10% of total explanation to run a cluster heat map using ‘heatmap.2’ function available in R ‘gplots’ library, considering Euclidean distance. With the heatmap we aimed to answer the third research question, that is, if species responses to the environmental predictor variables is associated with their trophic functional guilds.

All analyses were performed in R statistical environment (R Core Team 2013).

3 RESULTS

In total we collected 25,526 fish specimens and 143 species, being 60 species (5,846 specimens) in Curuá-Una, 83 in Capim (7,421) and 83 in Gurupi (11,153) (Chapter 3). Many species existed as singletons (e.g. 12 species in Capim) or at very few sites (e.g. 50% of Curuá-Una species occurred in three or less sites), and were too rare to model. The criteria to select species for the analysis left a shorter list of 52 species that is 14 from Curuá-Una, 28 from Capim and 35 from Gurupi (Table 4.2).

For these 52 species, the four classes of predictor variables, instream habitat, riparian network, other landscape, and natural, accounted for some of the variation in the studied river basins, but a great part of it remained unexplained (Figure 4.3). In the Curuá-Una River basin the predictors accounted for up to 58% (*Aequidens epae* and *Hyphessobrycon* sp.) whereas five species did not have any variation explained. In the Capim, *Hemigrammus rodwayi* had the highest percentage explained (74%) and seven species species did not have any variation explained. Twenty four species from the Gurupi River basin had some variance related to the predictors, *Gymnotus coropinae* was the best explained (47%), whereas 11 remained largely unexplained.

When partitioning out the relative importance of each of the four groups of predictor variables, instream habitat stood out as the most important for Curuá-Una species (Figure 4.3A). In Capim and Gurupi, there was a less pronounced difference between the variance explained by the predictor groups (Figure 4.3B, C). However, in all river basins, forest cover in the riparian network represented the smallest portion of species-specific variation.

Table 4.3 Fish species trophic groups (allo= allochthonous, auto= autochthonous, gen= generalist), number of individuals (N) and occurrence in number of stream sites (Sites) from Curuá-Una, Capim and Gurupi River basins, eastern Brazilian Amazon.

Order	**Family**	**Species**	**Code**	**Trophic group**	**Curuá-Una**		**Capim**		**Gurupi**	
					N	**Sites**	**N**	**Sites**	**N**	**Sites**
Characiformes	Characidae	*Astyanax* cf. *bimaculatus*	ast.cf.bima	omnivore					48	11
		Bryconops caudomaculatus	bry.caud	insectivorous allo			320	14	302	13
		Bryconops melanurus	bry.mela	insectivorous allo	211	12	148	13	282	12
		Hemigrammus bellottii	hem.bell	insectivorous allo			530	7		
		Hemigrammus guyanensis	hem.guya	insectivorous allo					539	19
		Hemigrammus ocellifer	hem.ocel	insectivorous allo	275	15	208	15		
		Hemigrammus rodwayi	hem.rodw	insectivore auto			551	11	2844	20
		Hemigrammus sp.1	hem.sp.1	omnivore					573	9
		Hyphessobrycon heterorhabdus	hyp.hete	insectivore general	519	14	2068	22	817	18
		Hyphessobrycon sp.	hyp.sp	insectivorous allo	1223	23				
		Iguanodectes rachovii	igu.rach	insectivorous allo			416	23		
		Jupiaba anteroides	jup.ante	insectivorous allo			26	8		
		Knodus savannensis	kno.sava	insectivorous allo	938	16				
		Knodus sp.n.	kno.sp.n	omnivore					183	7
		Moenkhausia collettii	moe.coll	insectivorous allo			315	9	834	18
		Moenkhausia cf. *collettii*	moe.cf.coll	insectivore genl	1165	30				
		Moenkhausia comma	moe.comm	insectivorous allo			43	8		
		Moenkhausia oligolepis	moe.olig	insectivorous allo			127	12	276	22
		Moenkhausia sp.	moe.sp	insectivorous allo			218	10		
		Phenacogaster cf. *pectinatus*	phe.cf.pect	insectivore auto					30	7

"Table 4.2. Continued"

Order	Family	Species	Code	Trophic guild	Curuá-Una		Capim		Gurupi	
					N	Sites	N	Sites	N	Sites
Characiformes	Characidae	*Serrapinnus* aff. *piaba*	ser.aff.piab	herbivore					646	10
	Crenuchidae	*Characidium* cf. *etheostoma*	cha.cf.ethe	insectivore auto			84	10	175	11
		Microcharacidium weitzmani	mic.weit	insectivore auto			44	12	240	14
	Curimatidae	*Cyphocharax gouldingi*	cyp.goul	detritivore					48	6
	Erythrinidae	*Hoplias malabaricus*	hop.mala	piscivore					25	13
	Lebiasinidae	*Copella arnoldi*	cop.arno	insectivorous allo			120	9		
		Nannostomus beckfordi	nan.beck	omnivore					456	14
		Nannostomus marginatus	nan.marg	insectivore general	230	15				
		Nannostomus trifasciatus	nan.trif	omnivore			117	10		
		Pyrrhulina aff. *brevis*	pyr.aff.brev	insectivorous allo			330	25	162	17
Cyprinodontiformes	Rivulidae	*Rivulus* cf. *urophthalmus*	riv.cf.urop	insectivorous allo			56	11	47	13
Gymnotiformes	Gymnotidae	*Gymnotus carapo*	gym.cara	insectivore auto					30	7
		Gymnotus coropinae	gym.coro	insectivore auto	69	12	41	12	33	9
	Hypopomidae	*Brachyhypopomus* sp.1	bra.sp.1	insectivore auto					26	9
		Hypopygus lepturus	hyp.lept	insectivore auto	54	8	32	8	45	7
	Rhamphichthyidae	*Gymnorhamphichthys petiti*	gym.peti	insectivore auto	51	14	76	15		
	Sternopygidae	*Eigenmannia* aff. *trilineata*	eig.aff.tril	insectivore auto			118	11	351	13
		Sternopygus macrurus	ste.macr	carnivore					29	6
Perciformes	Cichlidae	*Aequidens epae*	aeq.epae	omnivore	59	11				
		Aequidens tetramerus	aeq.tetr	omnivore	116	15	134	19	97	15

"Table 4.2. conclusion"

Order	Family	Species	Code	Trophic guild	Curuá-Una		Capim		Gurupi	
					N	**Sites**	**N**	**Sites**	**N**	**Sites**
Perciformes	Cichlidae	*Apistogramma* aff. *regani*	api.aff.rega	insectivore auto			143	15		
		Apistogramma caetei	api.caet	insectivore auto					352	22
		Apistogramma taeniata	api.taen	detritivore	223	12				
		Crenicichla aff. *menezesi*	cre.aff.mene	carnivore	72	14	102	17	105	17
Siluriformes	Loricariidae	*Ancistrus* sp.1	anc.sp.1	perifitívore					36	8
		Farlowella schreitmuelleri	far.schr	perifitívore					34	6
		Rineloricaria sp.	rin.sp	perifitívore					178	16
	Cetopsidae	*Helogenes marmoratus*	hel.marm	insectivorous allo			80	18	49	8
	Heptapteridae	*Imparfinis stictonotus*	imp.stic	insectivore auto					64	9
		Pimelodella sp.1	pim.sp.1	insectivore auto			26	8		
		Pimelodella sp.2	pim.sp.2	insectivore auto					257	19
	Trichomycteridae	*Ituglanis amazonicus*	itu.amaz	insectivore auto			37	8	112	11

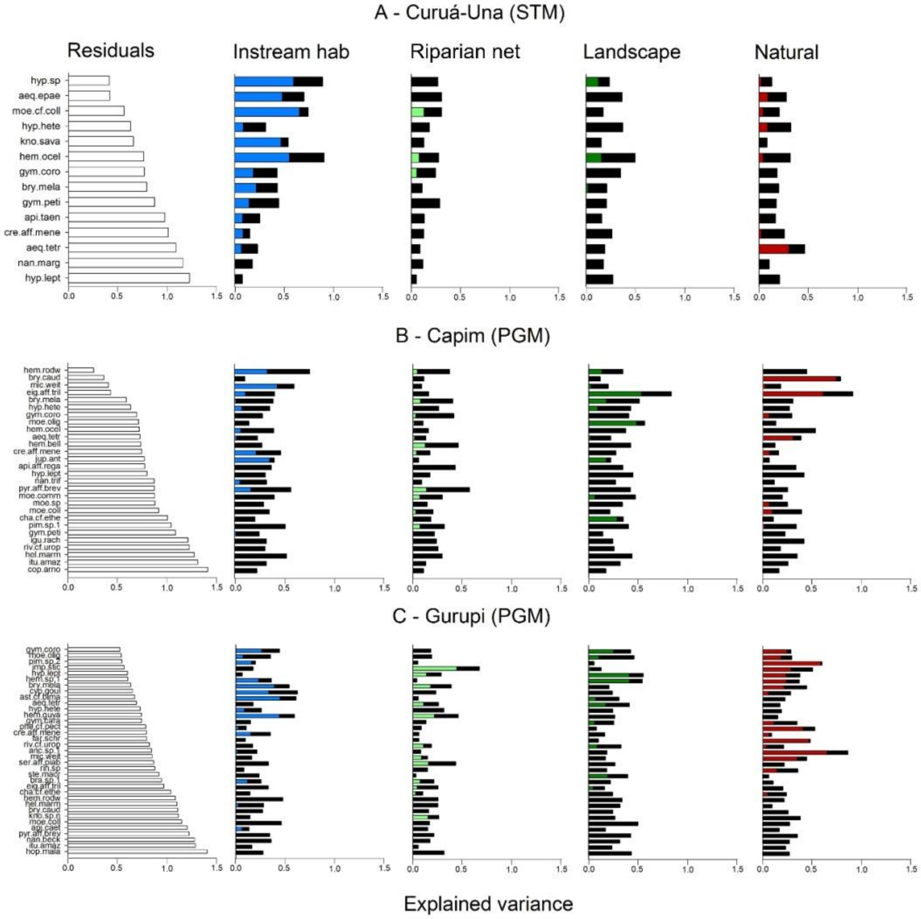


Figure 4.3 Partitioning of the variation in occupancy of stream fish species in Curuá-Una (A), Capim (B) and Gurupi (C) River basins, showing the effects of each group of predictor variables when partitioning out the effects of the other groups through redundancy analysis. Blue, light green, dark green and red represent respectively the fractions explained by instream habitat, riparian network, other landscape and natural alone; black represent all other fractions together.

Assessing the independent effects of each predictor group indicated a similar pattern of responses. In the Curuá-Una, other landscape, riparian, and natural made a small contribution (maximum of 4% all together) to the total mean explanation (22%), whereas instream habitat accounted for most of the observed variability in species abundance (21% alone; Figure 4.4A). In the Capim, instream habitat was the most important group (15% alone), but landscape (12%), natural (10%), and riparian (8%) contributed more to the total (22%; Figure 4.4B). In contrast, natural characteristics made the greatest contribution in the Gurupi (10% alone), with instream habitat (9%), other landscape (7%) and riparian network (5%) also contributing to the total mean explanation (18%; Figure 4.4C).

Assessments of the partial effect of single predictor variables from random forest models revealed 19 associations where a species abundance increased with increasing disturbance (Figure 4.5A), and 10 where abundance decreased with disturbance (Figure 4.5B). In both scenarios, instream habitat accounted for the majority of the associations (24). In addition, the analysis indicated eight partial effects where fish species abundance was linked with predictor variables that were not clearly related to stream condition (Figure 4.5C).

Algae and macrophyte cover was an important predictor variable for seven species and helped explaining up to 28% (*Microcharacidium weitzmani* in the Capim drainage) in their abundance change (Figure 4.5A). Visual assessment of the partial plots suggests that the main changes occurred at 20 to 40% of algae and macrophyte cover above which the seven species increased in abundance. *Hemigrammus ocelifer*, *Bryconops melanurus* and *Gymnotus carapo* responded to changes in mid-channel shading and decreased in abundance with more than 20 to 40% of shading (Figure 5A). Conductivity was clearly associated with a decrease in the abundance of two species(*Gymnotus coropinae* and *M. weitzmani*; Figure 4.5B). The other instream habitat predictor variables were responsible for one or two partial effects.

There were only five clear associations between landscape-level characteristics and the abundance of individual fish species (Figure 4.5). *Bryconops melanurus* abundance responded negatively to riparian network forest cover (12% partial effect) and *Moenkhausia oligolepis* to local riparian forest (10%). *Moekhausia comma* also increased in disturbed condition, showing a positive association with mechanized agriculture (15%). *Gymnotus coropinae* appeared sensitive to forest cover, and was consistently related to stream sites with ca. 80% of local riparian and riparian network forest cover with each explaining 10 and 12% respectively in its abundance increase.

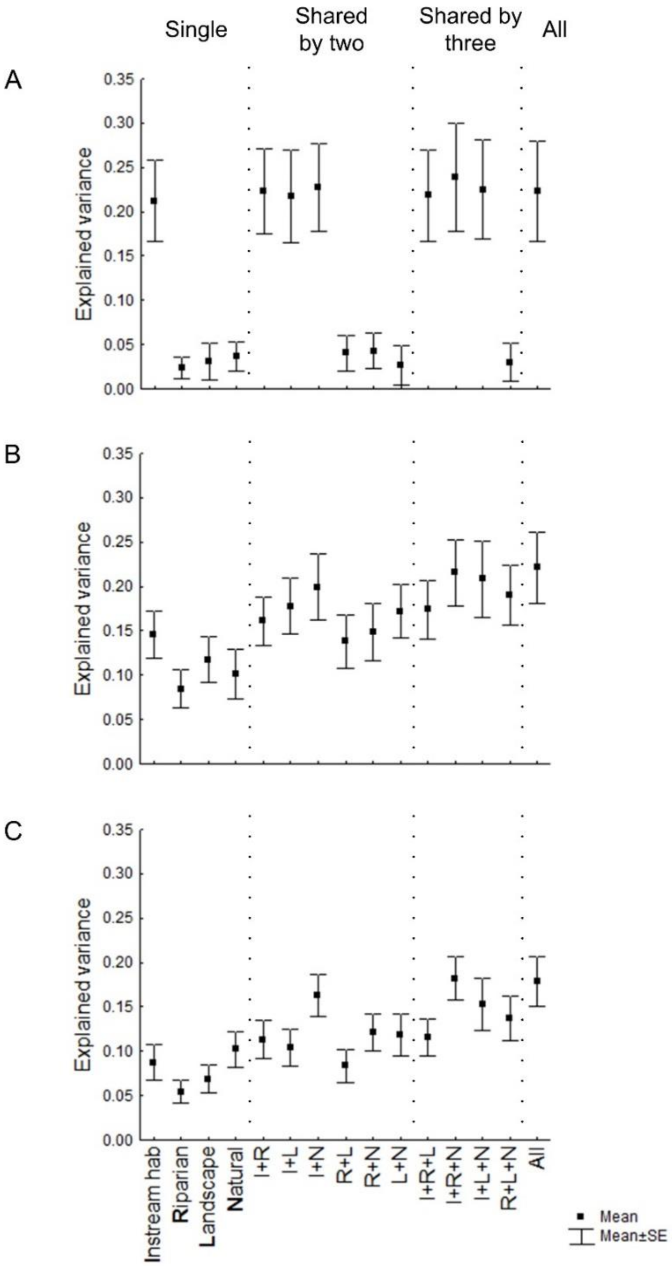
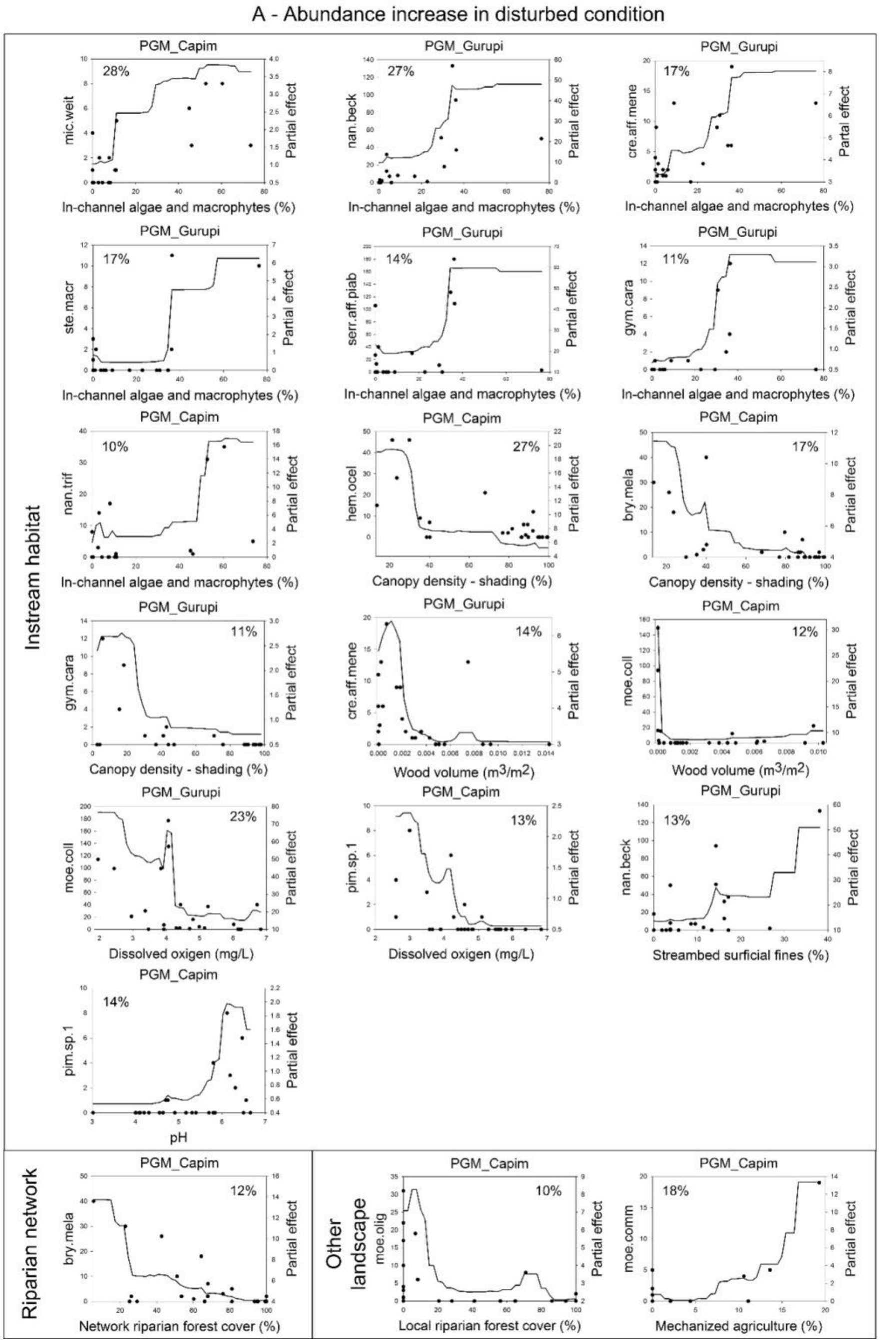
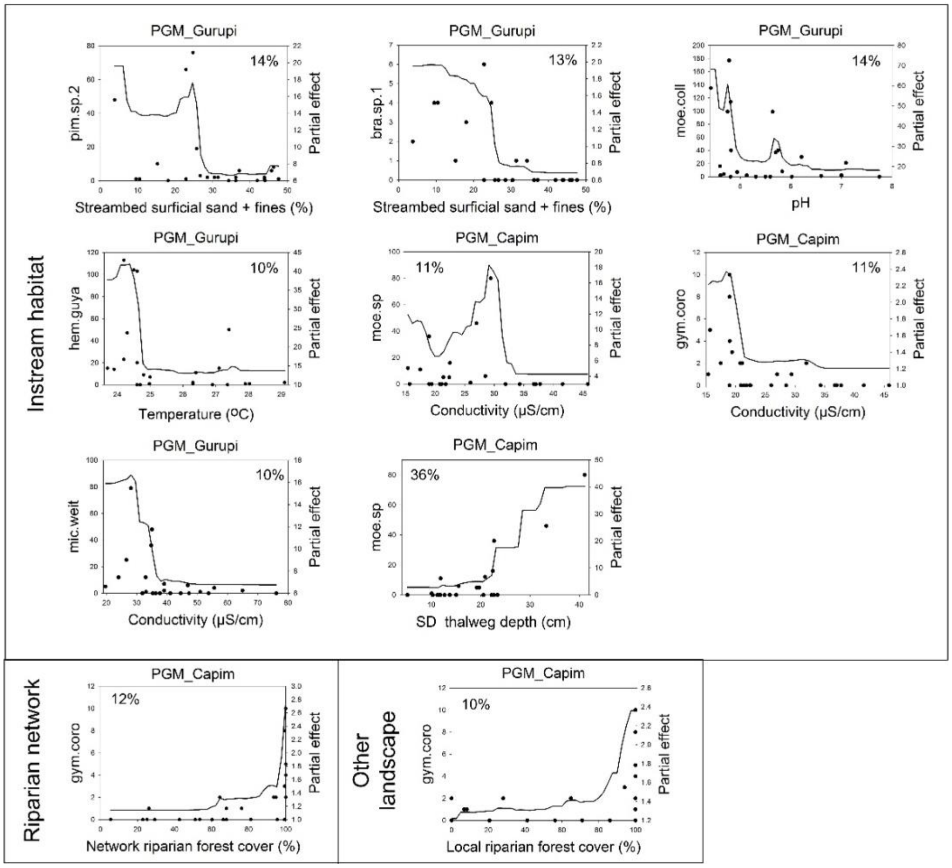


Figure 4.4 Isolated and shared effects of instream habitat (I), riparian network (R), other landscape (L), and natural (N) predictor variable groups on stream fish represented by mean and standard error (SE) for each river basin: Curuá-Una (A), Capim (B) and Gurui (C).


A - Abundance increase in disturbed condition
Instream habitat
Riparian network
Other landscape
PGM_Capim
28%
mic.weit
Partial effect
In-channel algae and macrophytes (%)
PGM_Gurupi
27%
nan.beck
Partial effect
In-channel algae and macrophytes (%)
PGM_Gurupi
17%
cre.aff.mene
Partial effect
In-channel algae and macrophytes (%)
PGM_Gurupi
17%
ste.macr
Partial effect
In-channel algae and macrophytes (%)
PGM_Gurupi
14%
serr.aff.piab
Partial effect
In-channel algae and macrophytes (%)
PGM_Gurupi
11%
gym.cara
Partial effect
In-channel algae and macrophytes (%)
PGM_Capim
10%
nan.trif
Partial effect
In-channel algae and macrophytes (%)
PGM_Capim
27%
hem.ocel
Partial effect
Canopy density - shading (%)
PGM_Capim
17%
bry.mela
Partial effect
Canopy density - shading (%)
PGM_Gurupi
11%
gym.cara
Partial effect
Canopy density - shading (%)
PGM_Gurupi
14%
cre.aff.mene
Partial effect
Wood volume (m3/m2)
PGM_Capim
12%
moe.coll
Partial effect
Wood volume (m3/m2)
PGM_Gurupi
23%
moe.coll
Partial effect
Dissolved oxigen (mg/L)
PGM_Capim
13%
pim.sp.1
Partial effect
Dissolved oxigen (mg/L)
PGM_Gurupi
13%
nan.beck
Partial effect
Streambed surficial fines (%)
PGM_Capim
14%
pim.sp.1
Partial effect
pH
PGM_Capim
12%
bry.mela
Partial effect
Network riparian forest cover (%)
PGM_Capim
10%
moe.olig
Partial effect
Local riparian forest cover (%)
PGM_Capim
18%
moe.comm
Partial effect
Mechanized agriculture (%)

B - Abundance decrease in disturbed condition



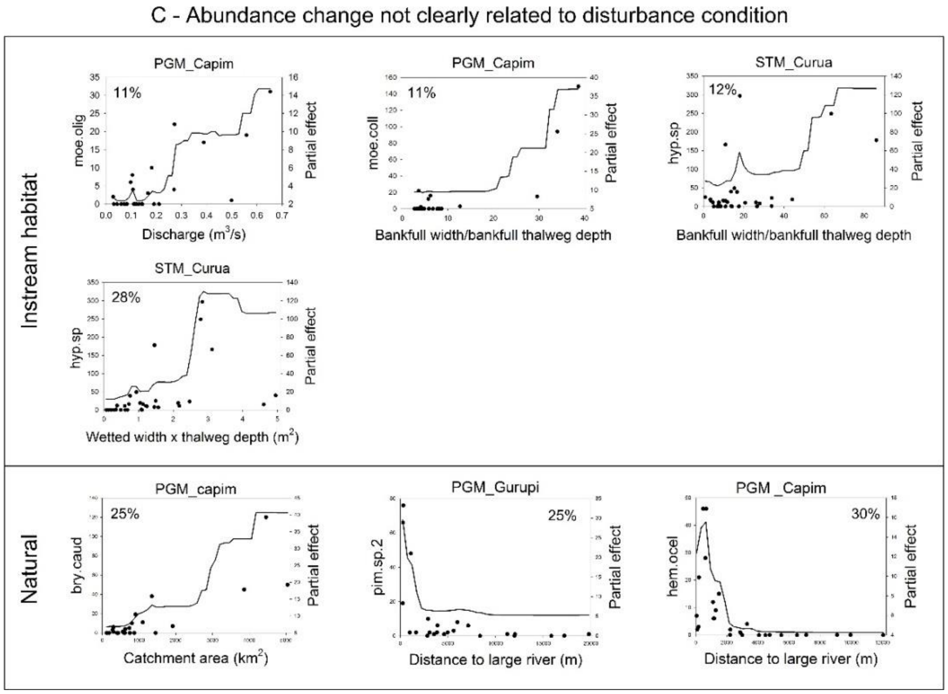


Figure 4.5 Partial effect from random forest models (lines) of physical and chemical habitat, riparian network forest cover and other landscape predictors showing positive associations (dots) with disturbed (A) or better preserved condition (B). Other partial effects were not clearly attributed to sites condition as they can be representing size as well as anthropogenic disturbance (C).

Species clustering based on random forest results did not relate to trophic functional groups (Figure 4.6). The Curuá-Una drainage was not included in this analysis because only six species had some variance explained by RF models. In the Capim, the analysis separated species with stronger partial effects responses to various predictor variables from those with lower partial responses (Figure 4.6A). In the Gurupi, species were grouped into two main clusters, according to whether they were associated with water quality and natural characteristics or wood and cover (Figure 4.6B).

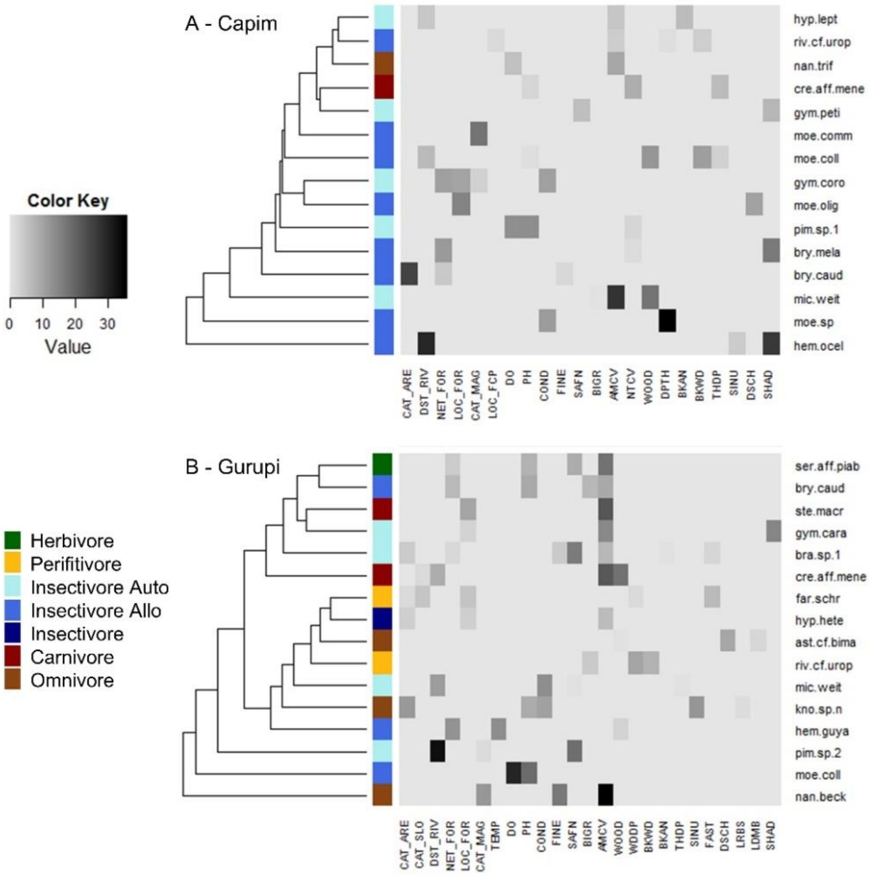


Figure 4.6 Cluster heat-map of species based on random forest (RF) models results for Capim (A) and Gurupi (B) River basins. Each cell is coloured based on the percentage of explanation values generated by RF. The cluster on the left side of each heat-map groups species with similar response patterns according to their relationship with different predictor variables, based on Euclidean distance.

4 DISCUSSION

Human-modified Amazonian landscapes are complex, and are often comprised of multiple land uses operating heterogeneously across different spatial scales. We found that such complexity results in mixed responses from stream fish species across different river basins, making it difficult to discern the relative importance of different environmental variables. Nonetheless, we observed that changes in the instream habitat condition, underpinned by gradients of anthropogenic disturbance, were associated with some changes in fish species abundance. Other environmental variables contributed less towards explaining overall variability in fish patterns, and their importance often varied across different river basins. Species-specific responses were not associated with trophic functional structure. Overall these results indicate that monitoring and conserving Amazonian stream fish requires a broad assessment of local and landscape-level disturbances and natural characteristics of streams, while the forest cover variables that form the basis of the protection for riparian habitat under the Forest Code (permanent protected area or APP in Portuguese) are often poor predictors of patterns of fish diversity. First, we focus on the scientific challenges of developing species-environment relationships in species-rich human-modified Amazonian landscapes. We then evaluate how our findings provide insights and recommendations for their management and conservation planning.

4.1 Understanding anthropogenic disturbances in megadiverse tropical systems

4.1.1 Relative importance of environmental drivers

Overall we found that stream fish species can be linked to a wide range of natural and anthropogenic predictor variables acting through several spatial scales. Although species-specific responses varied across river basins the most consistent associations were with instream habitat condition. Although feeding habits do not appear to explain fish responses to disturbance, including other life history traits in the analysis could enhance our understanding of the consequences of land use change on stream biota. Our findings highlight the difficulties in disentangling the effects of anthropogenic disturbances in a naturally heterogeneous system (i.e high fish species diversity and turnover; Chapter 3) characterized by a diverse mosaic of land uses and natural characteristics as well as little previous research.

On Chapter 2 we demonstrate that the linkages between landscape and instream habitat are complex and diverse. Here, we confirm how these complex linkages are underpinned by highly heterogeneous species-environment relationships. Anthropogenic disturbances at catchment, riparian or local scale can have varying effects depending, for example, on the preservation status of the river basin, biotic group under study, range of the disturbance gradient, and natural variability of the systems (Kaufmann and Hughes 2006; Wang et al 2006b; Sály et al 2011; Marzin et al 2012; Macedo et al 2014). For instance in degraded systems from southeastern Brazilian savanna, catchment and riparian network vegetation showed a close link with fish assemblages (Casatti et al. 2009, 2012), whereas in preserved streams in the USA, instream habitat characteristics stood out when compared to other landscape drivers (Wang et al 2006b). This study highlights the major relevance of instream habitat for Amazonian fish species, which in turn is largely determined by multiple landscape factors (Chapter 2).

4.1.2 Challenges in understanding species-environment relationships in tropical streams

The intrinsic complexity of megadiverse systems is complicated by the natural hierarchical organization of stream networks, where local conditions are under some level of regional influence (Allan et al 1997; Allan 2004). For instance we found a high-level of multi-collinearity among natural and anthropogenic disturbances at several spatial scales hindering our ability to disentangle the relative importance of individual drivers of changes in the instream habitat condition (Chapter 2).

Despite measuring a broad set of environmental predictors representing different scales of the landscape, we still found a high level of unexplained variance in our models predicting species abundance. An additional challenge involves investigating responses to disturbance by rare species, which are more difficult to model as they cannot be included in most statistical analysis. We excluded 58%, 62% and 73% of the species in Gurupi, Capim and Curuá-Una respectively, as they were absent from less than 25% of the stream sites and occurred with few individuals. However, it seems plausible that many of these rare species could be the most sensitive to anthropogenic impacts, particularly if they are only present in the relatively small number of streams that were in undisturbed catchments. Excluding these from the assessment means that we could be overlooking an important component of the assemblage, and underestimating land use effects on stream fish. The fact that most associations between fish and disturbance were positive (i.e. abundance increased with disturbance) suggests that we could be failing to model the full suite of species that decrease with disturbance (Figure 4.5).

Finally, working in a relatively preserved stream system meant that we did not account for the full gradient of anthropogenic disturbance. Both regions have retained a relatively high level of catchment forest cover, 69% in Paragominas and 60% in Santarém, and we did not sample heavily disturbed catchments. Allan (2004) for instance describe that temperate streams can remain in good condition until a threshold of 30-50% of agriculture cover above which changes occur. Legacy effects may also take play resulting in disturbance effects only appearing long after the initial disturbance (Hylander and Ehrlén 2013). Therefore many of the impacts of land use change in both regions are relatively recent, and the effects of which may take some time to be manifest (Chapter 2). Yet even under relatively small disturbance we showed that some dominant species were benefited and responded positively to anthropogenic impact.

4.1.3 Implications for management

We found that environmental predictor variables amenable to management (insofar as they are legally enforceable) in the riparian zone, as well as landscape characteristics of the wider catchment play a relatively smaller role in determining changes in fish species abundance when compared to differences in instream habitat. However, in interpreting this scale-dependent effect it is important to remember that changes to instream habitat are themselves influenced by the combined effects of multiple landscape-scale drivers (Chapter 2). Therefore a key aspect to be considered in the conservation management of Amazonian streams is the need for interventions at multiple scales. The importance of the riparian network zone to stream health and aquatic biodiversity is largely recognised (e.g. Karr & Schlosser 1978; Nerbonne & Vondracek 2001; Pusey & Arthington 2003; Lorion & Kennedy 2009) but activities and impacts elsewhere in the catchment cannot be neglected (Roth et al 1996; Allan et al 1997; Castello et al 2013; Marzin et al 2013). If critical impacts of stream disturbance on fish assemblages occur mainly through changes in instream habitat, monitoring and assessment programs that only account for changes at riparian and catchment scales may underestimate impacts. Finally it is necessary to consider that restoration of the riparian or catchment areas may only translate into changes in instream habitat over long (decadal) time-scales.

Brazilian legal instruments that could relate to fish fauna and stream condition include the Water Resources Regulation (Federal Law Nº 9.433, January 8th 1997; Brasil 1997) and the Fishery Code (Federal Law Nº 11.959, June 29th 2009; Brasil 2009). However both are clearly focused on water quality and fish resources for human consumption therefore they do not account for a wide perspective of stream condition (Chapter 2). The paramount legislation concerning riverscapes is the Forest Code (Federal Law Nº 12.651, May 25th 2012; Brasil 2012), which has been recently revised and diminished protection of stream environments and associated riparian vegetation, APP (Garcia et al 2013; Soares-Filho et al 2014). For instance, depending on the property size, the revised Forest Code reduced the extent of riparian vegetation that is mandated to be restored to 5 m for areas that have been declared for agricultural use.

A major limitation of the Brazilian Forest Code, in the conservation of aquatic systems, is that it is focussed on the scale of individual private properties, whereas, as our results have shown (and see Chapter 2), interventions are also required at the catchment-level demanding collective strategies involving different landowners working together. The FC also focuses on imposing more restrictions to land use in the riparian zone in order to preserve areas of important value for biodiversity and other ecosystem services (e.g. watercourses). This is certainly valuable if did not weakened the conservation efforts at non-riparian scale. For instance the legislation allows out-of-catchment compensation for deforestation which can result in catchments with low forest cover outside of the riparian zone. This is particularly worrying when considering that even restrictions on deforestation within riparian zones have not been shown to be effective, with evidence from Paragominas suggesting that riparian areas may be being deforested faster than non-riparian lands (Nunes et al 2014).

Despite those inadequacies, the Forest Code has some critically important provisions regarding management and conservation of the Amazon and other Brazilian environments that go far beyond those available in other tropical countries. However our results reveal two key limitations of the legislation in protecting low-order streams and fish fauna. A failure to adequately account for anthropogenic activities at the catchment scale and a failure to deal with instream habitat. Both appeared to be important in this study and in previous investigations in the same region (Chapters 2 and 3). This narrow interpretation of what constitutes the stream environment, and the way in which stream environments are connected to terrestrial environments, can serve to downplay the importance of conservation efforts at non-riparian scales which are highly relevant in determining the composition of local fish assemblages. Therefore our findings give further support to the strong need for legislation to go beyond the protection of only riparian forests addressing the management of entire drainage networks (Abell et al 2007; Castello et al 2013).

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APPENDIX

The tables and figures presented here will be submitted as supplementary material to the manuscript.

Table A.4.1 Performance of random forest models showing the percentage of species-specific variation explained by the predictors in the Curuá-Una River basin, Brazilian eastern Amazon. Values greater than 10% highlighted in bold; species codes are presented in Table 4.2.

Predictor variables	**Species / Curuá-Una River basin**					
	hyp.sp	**kno.sava**	**hyp.hete**	**bry.mela**	**hyp.lept**	**api.taen**
CAT_MAG			7.0			
TEMP				4.8		
COND		8.3				
FINE			2.6			
BIGR		2.1				
NTCV		8.5				
WDDP	**28.1**				1.9	
DPTH					3.1	
THDP					0.3	
BKAN			2.7			
BKWD	**12.1**					
RP100						2.4
SINU				1.5		
DSCH				4.2		1.7
Total variance explained (%)	40.2	19.0	12.3	10.5	5.3	4.1

Table A4.2 Performance of random forest models showing the percentage of species-specific variation explained by the predictors in the Capim River basin, Brazilian eastern Amazon. Values greater than 10% highlighted in bold; species codes are presented in Table 4.2.

Predictor variables	Species / Capim River basin																		
	hem. ocel	moe. sp	mic. weit	gym. coro	moe. coll	bry. caud	bry.m ela	pim. sp.1	moe. olig	moe. comm	cre.aff. mene	hyp. lept	nan. trif	gym. peti	riv.cf. urop	hyp. hete	hel. marm	hem. rodw	jup. ante
CAT_ARE						**25.3**													
DST_RIV	**29.6**				6.7							4.7							
NET_FOR				**10.7**		4.3	**11.7**												
LOC_FOR				**10.1**					**15.1**							3.0			
CAT_MAG				3.1						**17.7**									
LOC_FCP															1.7				
TEMP																		0.9	
DO								**13.5**					5.5						
PH					1.1			**13.5**			2.3					0.3			
COND		**11.4**		**10.7**													1.4		
FINE						2.0											1.1	0.9	
SAFN														5.9					0.1
BIGR			0.5																
AMCV			**27.6**									4.8	**9.6**		3.8				
NTCV							1.4	2.4			8.6								
WOOD			**17.7**		12.3														
DPTH		**35.7**													1.0				
THDP					3.0						6.4								
BKAN				0.1								6.4					0.5		

Predictor variables	Species / Capim River basin																		
	hem.ocel	moe.sp	mic.weit	gym.coro	moe.coll	bry.caud	bry.mela	pim.sp.1	moe.olig	moe.comm	cre.aff.mene	hyp.lept	nan.trif	gym.peti	riv.cf.urop	hyp.hete	hel.marm	hem.rodw	jup.ante
BKWD					11										3.5			0.4	
SINU	3.9																		
FAST																			0.1
DSCH									10										
LDMB																		0.1	
SHAD	27						17							7.3					
Total variance explained (%)	60.3	47.1	45.8	34.6	34.2	31.6	29.8	29.4	25.5	17.7	17.2	15.9	15.1	13.2	10.0	3.3	3.0	2.3	0.1

Table A4.4 Performance of random forest models showing the percentage of species-specific variation explained by the predictors in the Gurupi River basin, Brazilian eastern Amazon. Values greater than 10% highlighted in bold; species codes are presented in Table 4.2.

Predictor variables	Species / Gurupi River basin																					
	nan. beck	pim.s p.2	cre.aff. mene	moe. coll	kno. sp.n	ser.aff. piab	bra. sp.1	bry. caud	ste. macr	hem.g uya	gym.c ara	riv.cf. urop	far. schr	hyp. hete	mic.w eit	ast.cf. bima	anc. sp.1	phe.cf. pect	aeq.t etr	hem. rodw	pyr.aff. brev	hel. marm
CAT_ARE					9.1		3.1						1.3	2.6			2.7	0.8				
CAT_SLO			1.3										3.8									
DST_RIV		**25.3**	6.7												4.2		2.0					
NET_FOR						3.0	1.5	5.0		9.8												
LOC_FOR																			0.6	0.1		
W1_HAL																					1.5	
CAT_MAG	9.3	1.2																	0.7			
LOC_FCP									7.6		2.1		3.9	2.6								
TEMP										**10.3**										1.5		
DO				**22.7**																		
PH				**14.4**	6.8	6.0		7.1														
COND					8.1										5.1							
FINE	**12.9**						3.1															
SAFN		**14.0**				6.7	**12.5**								0.2							
BIGR								5.3				3.2							1.1	0.8		2.2
AMCV	**27.2**		**16.7**			**13.7**	5.3	7.2	**17.0**		**10.9**			4.9			1.7	0.8				
WOOD			**13.7**							2.1						0.5			0.8			
WDDP												7.6	1.3								0.8	
THDP															0.5			0.2				
BKAN							0.5															
BKWD												5.9										
SINU					9.3																	
FAST							1.8						5.0					1.6				
DSCH																7.4						
LRBS					1.0																	
LDMB																1.7						
SHAD											**11.2**											
Total variance explained (%)	49.3	40.5	38.4	37.0	34.3	29.4	27.8	24.6	24.5	22.3	24.3	16.7	15.4	10.1	10.1	9.5	6.4	3.4	3.2	2.4	2.3	2.2

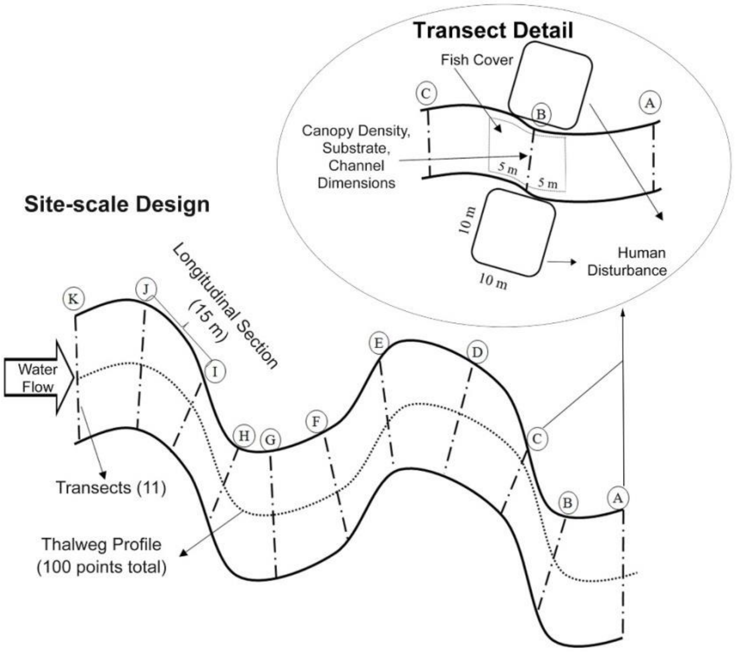

Figure A4.1 Schematic of the sampling design of the instream habitat of Amazonian stream sites.

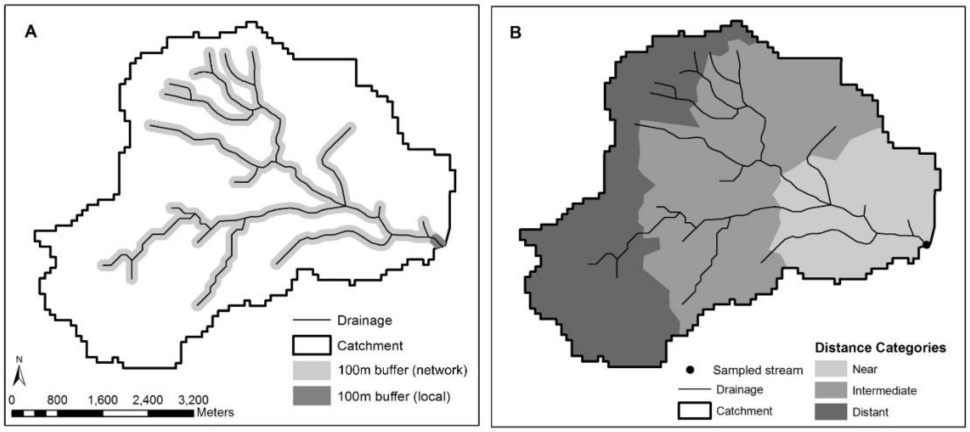

Figure A4.2 Schematic of the spatial scales (A) and hydrological distances (B) considered to obtain the landscape predictor variables of fish from Amazonian stream sites. Riparian buffers are referred as network and local.

CONCLUDING REMARKS

Pictures by Rafael Leitão and Érika Berenguer

1 CONCLUDING REMARKS

This thesis is the first comprehensive, quantitative, and multi-scale assessment of the consequences of anthropogenic disturbances to both stream fish and instream habitat condition across the forest-agriculture frontier of the eastern Brazilian Amazon. As such, it is also one of the first studies of its kind of any area of tropical forest in the world. Earlier research on freshwater systems in the Amazon basin have focused on investigating hydrological and biogeochemical processes of streams (DAVIDSON et al., 2004; FIGUEIREDO et al., 2010; MACEDO et al., 2013; NEILL et al., 2001, 2006, 2011, 2013) or on the effects of a single land use such as logging (DIAS; MAGNUSSON; ZUANON, 2010) or deforestation (BOJSEN; BARRIGA, 2002) on stream fish. The findings presented here demonstrate that multiple anthropogenic pressures can act in complex and cumulative ways, and across multi spatial scales. This thesis therefore marks an important advance on our current understanding of low-order stream systems in human-modified Amazonian forests and provides hitherto unavailable information to assist management and conservation planning at both site and landscape scales.

1.1 Synopsis of key findings

The main objective of this thesis was to investigate how stream condition and fish assemblages respond to anthropogenic disturbances occurring at several spatial scales in multiple use Amazonian landscapes. Particular emphasis was given to disentangling the associations among drivers acting at different spatial scales on both the biotic and abiotic characteristics of streams.

The instream habitat provides the environmental habitat and resources for aquatic biota and is therefore critical in determining the provision of several ecosystem services (e.g. water for human and animal use; Karr & Schlosser, 1978; Osborne & Koviacic, 1993; Peterjohn & Correll, 1984). However, to date there has been no comprehensive study of changes in physical instream environment for human-modified Amazonian stream systems. In Chapter 2 I address this gap by asking how water quality and physical habitat respond to landscape-scale anthropogenic disturbances. I found that disturbances at both riparian and catchment scales can drive marked changes in water characteristics, substrate type, in-channel cover and wood volume, channel morphology as well as other key stream features, such as seasonal discharge. Working in two regions revealed how these responses can be highly context dependent on the natural characteristics and heterogeneity of the stream systems. There were few similarities in responses of instream habitat characteristics to landscape disturbances between the two independent study regions of Santarém and Paragominas and it was not possible to identify any single variable with an overriding effect. By encompassing a range of land-use change (LUC) variables other than differences in forest cover I was able to identify the critical role also played by other characteristics of human-modified landscapes in shaping instream habitat, such as the number of upstream and downstream road crossings and the extent of upstream mechanized agriculture. The findings from Chapter 2 reinforce the need for a catchment-wide management strategy to protect the integrity of Amazonian stream ecosystems, in agreement with what is a general suggestion for freshwater systems (ABELL; ALLAN; LEHNER, 2007; CASTELLO et al., 2013; MACEDO et al., 2013; STRAYER; DUDGEON, 2010).

Building on the results of Chapter 2 I then asked how anthropogenic disturbances at both local (i.e. instream habitat) and landscape (i.e. riparian and catchment) scales as well as natural stream features can influence both the makeup of entire fish assemblages (Chapter 3) and the distribution and abundance of individual species (Chapter 4). In Chapter 3 I first examined the general patterns of richness, abundance and composition of fish assemblages across the full set of study sites and sought to isolate the relative importance of different proximate drivers of change. The most important finding from this chapter is that low-order Amazonian streams are extraordinarily heterogeneous in their fish biota with exceptionally high-levels of beta diversity between stream sites and between river basins. Furthermore both assemblage structure and composition were influenced by a broad set of environmental variables, including both natural stream features and anthropogenic-disturbances that varied in importance depending on the river basins. This high level of biological uniqueness in stream assemblages and complexity of interactions with different drivers gives further support to the findings from Chapter 2; single and isolated conservation strategies, such as focusing only on riparian vegetation, are unlikely to effectively safeguard the diversity and integrity of stream fish assemblages.

Finally I analyzed species-specific responses to the same environmental predictor variables used in Chapter 3 but divided these variables into groups according to their spatial scale and the extent to which they are amenable to management and regulation (Chapter 4). Similarly to Chapters 2 and 3, the species-specific approach revealed mixed responses to environmental predictor variables across river basins, underlining the difficulties in discerning their relative importance of any single driver. Nonetheless differences in instream habitat contributed more towards explaining overall patterns of variability in fish species abundance across different river basins than other groups of predictor variables. I used the findings of this chapter to critically examine the effectiveness of the Brazilian environmental legislation for conserving low-order stream systems. Brazilian environmental legislation on private land, determined particularly by the Forest Code, gives a strong emphasis to the conservation of riparian vegetation. However this narrow interpretation of what constitutes the stream environment, and the way in which stream environments are connected to terrestrial environments, can serve to downplay the importance of conservation efforts at non-riparian scales (i.e. catchment and local instream habitat) that my work underscores as being highly relevant in determining the composition of local fish assemblages. These findings give further to support to Chapters 2 and 3 regarding the strong need for addressing the management of the entire drainage networks to protect stream condition and fish biodiversity.

Throughout all three chapters I examined the challenges involved in disentangling the relative effects of anthropogenic impacts and natural features on these heterogeneous Amazonian stream systems – the combination of which probably contributed to the high level of unexplained variance in some of the models. Understanding the importance of specific variables was particularly cofounded by the fact that:

i. There are high levels of multi-collinearity between natural stream features and anthropogenic disturbances (Chapter 2) making it difficult to discern the effects of specific disturbance signals (ALLAN, 2004);
ii. Human-induced changes are the result of cumulative effects of multiple drivers (ALLAN, 2004), meaning that even if specific predictor variables appear to be responsible for only a small part of the variability in responses they may still be critical in determining overall effects (Chapter 2);
iii. Working in relatively preserved stream systems meant that it was not possible to account for the full gradient of anthropogenic disturbance since in our study no severely degraded (e.g. completely deforested and semi-urban) catchments were sampled (Chapters 2, 3 and 4);

iv. The absence of strong signals associated with some disturbances and species does not mean that they are necessarily absent; they may just not yet be manifested. The ecological consequences of anthropogenic disturbances may take years to become fully apparent in ecosystems (HYLANDER; EHRLÉN, 2013), and both of our study regions, Santarém and Paragominas, have only experienced a relatively recent history of more intensive land use and widespread deforestation;

v. Species life history traits can enhance the understanding of land use change on stream biota (MOUILLOT et al., 2013). However there is hardly any comprehensive information for Amazonian fish that could be used to relate life-history traits to species responses (e.g. in the work of Chapter 4 as presented here);

vi. Investigating responses to disturbance by rare fish species presents a particular challenge as they cannot be included in most statistical analyses due to their low sample sizes. However these species may be the most sensitive to anthropogenic pressures and in megadiverse heterogeneous systems like the Amazon, they can represent up to 70% of the total diversity of a single river basin assemblage (Chapter 4). Not accounting for this important portion of the freshwater biota means that anthropogenic impacts on freshwater ecosystems can be easily underestimated.

1.2 Application of research findings: recommendations for the management and conservation of Amazonian riverscapes

The results from the three chapters combine to illustrate some important findings that can be used to assist the development of management strategies and conservation plans for Amazonian streams at multiple spatial scales. The most significant conclusion of this thesis is the need for conservation strategies to go beyond the protection of only riparian forests by addressing the management of entire drainage networks. This is particularly relevant in the context of the Brazilian Forest Code (FC), which has a narrow interpretation of freshwater systems; it imposes more restrictions on land uses within a narrow riparian zone but has no explicit provision of the protection and management of upstream areas. Moreover the FC operates at the property scale (hundreds to thousands of hectares), whereas results of this thesis indicate that interventions are required at the catchment-level (thousands to tens or hundreds of thousands of hectares), demanding collective strategies that involve multiple landowners. These general conclusions are grounded by the following specific findings:

i. Human-induced landscape disturbances can be closely associated with marked changes to instream habitat (e.g. higher water temperature in more deforested catchments and less wood volume in streams where local forest have been disturbed; Chapter 2) which in turn have cascading impacts on stream fish fauna (e.g. landscape and instream habitat features resulting in cumulative effects on the distribution and abundance of individual species; Chapters 3 and 4). As such a specific recommendation is that monitoring and management programs include indicators of instream habitat integrity to ensure that the impacts of human disturbance are not underestimated. And that those indicators are surveyed in a wide range of measures;

ii. Anthropogenic activities elsewhere in the catchment (e.g. road crossings and mechanized agriculture) are important drivers of change in stream biotic and abiotic condition (Chapters 2, 3 and 4);

iii. Disturbances other than changes in forest cover, such as the introduction of mechanized agriculture or an increase in the number of road crossings over streams, can have an important effect on instream habitat (Chapter 2);

iv. Streams are environmentally heterogeneous and biologically distinct in their fish composition, evidenced by the extremely high beta diversity between different stream sites and rivers basins. Such heterogeneity is a key facet of freshwater biodiversity and demonstrates that freshwater fish conservation cannot rely on isolated protected areas;

v. Differences in results from Santarem and Paragominas suggest that signals of human disturbance are underpinned by specific regional characteristics (e.g. natural heterogeneity in geophysical properties and history of land use).

1.3 Future research priorities

In heterogeneous, megadiverse and poorly studied regions like Brazilian Amazon low-order stream networks it is challenging to outline research priorities whilst basic information on species distribution and natural history is lacking. Nevertheless further studies that expand our understanding of the relative and cumulative effects of multiscale anthropogenic disturbances on Amazonian stream system integrity are unquestionably needed. Future work should also address the specific design parameters of existing environmental legislation and current management and conservation strategies to identify and help improve potential inadequacies. Therefore, I suggest that future work seeks to account for both local as well as landscape-level patterns and processes, helping to underpin efforts to extrapolate to larger spatial scales and to draw more general conclusions. I particularly emphasize the importance of approaches that:

i. Include other land uses (e.g. urban areas) and indicators of forest degradation (e.g. effects of fire, fragmentation and logging) in order to account for the full disturbance gradient;
ii. Investigate responses from other aquatic organisms such as macroinvertebrates, to understand how different biotic groups may diverge in their responses to human pressures (MARZIN et al., 2012). Work is also needed that includes life history traits of fish species other than trophic guilds, which can help in understanding the patterns of observed responses (MOUILLOT et al., 2013);
iii. Expand the similar kind of multi spatial scale approach presented here to other river basins systems, to further understand the effects of regional context. Understanding the factors that underpin this context specificity is a vital step in scaling up these results to the rest of the Amazon and other tropical systems, and will assist with regional conservation planning;
iv. Understanding forest condition – how does the condition (as well as extent) of forests affect aquatic systems. This is particularly important with development of large-scale logging concessions, and threat of wildfires;
v. Monitoring work over time to investigate how time lags and shifting baselines in undisturbed forests may influence stream condition responses to anthropogenic disturbances.

1.4 Conclusions

The multiscale experimental approach presented here provides clear and multiple lines of evidence regarding the links between anthropogenic pressures and changes in stream condition, including both the fish fauna and instream physical habitat. Instead of highlighting the importance of a small number of human activities, this thesis demonstrates that most instream habitat features and fish species are affected by a broad set of disturbances that vary and interact across regions and river basins. In this sense, Amazonian streams have proven to be determined by multiple and complex interactions with natural and anthropogenic environmental variables that change across different spatial scales.

While this thesis has significantly expanded our understanding of tropical streams facing anthropogenic pressures, there are still many challenges to tackle and avenues for future research. Nevertheless the lack of a more detailed understanding of these impacts cannot be used as an excuse to delay improved management and conservation strategies in light of rapid rates of biodiversity loss. Those strategies need to rely on the available information as they are being refined by specific studies that can help provide important information on regional context. For instance the findings presented by this research indicate a need for the Forest Code to determine that compensation for deforestation ensure minimum levels of protection to upstream areas, and that regulations regarding roads crossing streams should be improved (e.g. increasing guidance regarding bridges and culverts). Nonetheless the precautionary principle should be followed when managing these poorly studied systems: stream conservation depend on a catchment-wide planning.

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APPENDIX: OTHER OUTCOMES

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ABSTRACT

Science has a critical role to play in guiding more sustainable development trajectories. Here, we present the Sustainable Amazon Network (Rede Amazônia Sustentável, RAS): a multidisciplinary research initiative involving more than 30 partner organizations working to assess both social and ecological dimensions of land-use sustainability in eastern Brazilian Amazonia. The research approach adopted by RAS offers three advantages for addressing land-use sustainability problems: (i) the collection of synchronized and co-located ecological and socioeconomic data across broad gradients of past and present human use; (ii) a nested sampling design to aid comparison of ecological and socioeconomic conditions associated with different land uses across local, landscape and regional scales; and (iii) a strong engagement with a wide variety of actors and non-research institutions. Here, we elaborate on these key features, and identify the ways in which RAS can help in highlighting those problems in most urgent need of attention, and in guiding improvements in land-use sustainability in Amazonia and elsewhere in the tropics. We also discuss some of the practical lessons, limitations and realities faced during the development of the RAS initiative so far.

Leitão, R.P.; Zuanon, J.; **Leal, C.G.**; Pompeu, P.S.; Gardner, T.A.; Barlow, J.; Hughes, R.M.; Kaufmann, P.R.;Kasper, D.; Ferreira, J.; de Paula, F.R.; Ferraz, S.; Villéger, S.; Mouillot, D. Disentangling the multiple effects of land use on the functional structure of fish assemblages in small Amazon streams. In preparation for *Ecological Applications*.

ABSTRACT

Agricultural land use is a primary source of impact to small streams. However, the causal processes involved in this relationship are complex, operating through multiple pathways and spatial scales; and the taxonomic structure of stream assemblages often shows contrasting responses to land use changes. This scenario reflects the difficulty to established effective management of these ecosystems, and illustrates the need to examine complementary facets of biodiversity under mechanistic causal pathway perspectives. In this study, we investigate how landscape fragmentation and deforestation, mediated by alterations in instream habitat, affect the functional structure of stream fish assemblages in two regions of the mid-eastern Amazon. We conducted standardized fish sampling in 94 headwater sites, and characterized local habitat conditions by several physical attributes such as substrate, channel morphology, bed complexity and stability. We also estimated the density of road crossings (i.e., landscape fragmentation) and the degree of deforestation at different spatial scales. Each of the 141 captured fish species was functionally characterized by 18 ecomorphological traits related to feeding, locomotion, and habitat. For each of the two regional species pools, we built a multidimensional space using these traits and then computed complementary indices to quantitatively describe the functional structure of the assemblages. By using Structural Equation Modelling we identified mechanistic causal pathways of land use on these biodiversity indicators. For instance, local riparian deforestation increased macrophyte+grass cover with subsequent reductions of the functional evenness of assemblages (i.e., increased the dominance of few trait combinations). Landscape fragmentation upstream from sample sites and deforestation at catchment and riparian scales altered the channel morphology and the structure of stream bottoms, changing the functional identity of assemblages (e.g., species that use the benthic compartment were negatively affected). Fragmentation downstream from the sites had remarkable negative effects on the functional richness (i.e., losing regional connectivity potentially reduces the range of niche occupation by assemblages), and on the functional evenness and divergence, suggesting a trend of functional homogenization of local assemblages. We believe this study offers significant insights to fruitful

future investigations concerning functional responses of stream fish assemblages to landscape alterations in the Amazon.