UNIVERSIDADE FEDERAL DE LAVRAS

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SAMPLING TRENDS AND NON-NATIVE FISH SPECIES IN BRAZILIAN STREAMS: A SYNTHESIS OF THE ECOLOGICAL KNOWLEDGEMENT

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Tese apresentada à Universidade Federal de Lavras, como parte das exigências do Programa de Pós-Graduação em Ecologia Aplicada, área de concentração Ecologia e Conservação de Recursos em Paisagens Fragmentadas e Agrossistemas, para a obtenção do título de Doutora.

Prof. Dr. Luiz Fernando Silva Magnago
Orientador

Prof. Dr. Paulo dos Santos Pompeu
Coorientador

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2019

Dedico ao amigo Ângelo Monteiro

(in memoriam)

“Você tem que criar a confusão sistematicamente;
isso liberta a criatividade.
Tudo o que é contraditório cria vida.”
Salvador Dalí

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RESUMO GERAL

Riachos possuem características que os tornam importantes tanto para a biodiversidade quando para o bem-estar humano, mas também são ambientes altamente ameaçados pelas atividades humanas, o que tem atraído à atenção dos pesquisadores. Entretanto, apesar da elevada proporção de espécies de peixes ameaçadas de extinção, poucas ações de manejo e conservação têm sido efetivamente estabelecidas. No entanto, estudos argumentam que a comunicação entre pesquisadores, políticos e a sociedade em geral pode ser significativamente melhor através de revisões sistemáticas e sínteses de pesquisas já existentes. Nesta tese, nós realizamos uma revisão sistemática de artigos científicos sobre assembleias de peixes de riachos no Brasil, a fim de detectar tendências em protocolos amostrais e avaliar o esforço amostral nos riachos estudados (Capítulo 1). Além disto, nós avaliamos o tamanho do efeito de espécies não nativas de peixes nas assembleias de riachos da bacia do Paraná (Capítulo 2). Nós mostramos que os estudos de assembleias de peixes empregam uma grande variedade de métodos, mas apontamos para uma tendência de uso de pesca elétrica, trechos amostrais de 50 metros e tempo amostral de 60 minutos. Nossos resultados destacam que o pool regional de espécies é ainda subestimado em todas as bacias que analisamos. Os resultados do segundo capítulo mostram que espécies de peixes não nativos coexistem com espécies nativas em riachos com maior riqueza de espécies nativas na bacia do Paraná. Nós também encontramos que a grande diversidade funcional em riachos com espécies não nativas foi explicada pela riqueza de espécies nativas ao invés da riqueza de espécies não nativas, corroborando com os resultados de alta sobreposição de nichos entre esses dois grupos de espécies. Este estudo destaca que pesquisas futuras e revisões taxonômicas são ainda primordiais para a maior parte das bacias e aponta para a importância de mais estudos sobre esforço amostral de peixes em riachos brasileiros. Nós reforçamos a importância da padronização de protocolos amostrais entre os estudos a fim de que haja o avanço necessário para a conservação desses sistemas aquáticos. Além disto, concluímos que áreas com maiores riquezas de espécies de peixes nativos deveriam ser foco de manejo de espécies invasoras e esforços de conservação. Finalmente, nós acreditamos que as pesquisas em colaboração, através do compartilhamento de dados, projetos interinstitucionais e intercâmbio de profissionais é o caminho para superarmos os desafios da pesquisa ictiológica no Brasil.

Palavras-chave: Peixes de água doce. Esforço amostral. Revisão sistemática. Diversidade funcional. Sobreposição de nicho.

ABSTRACT

Stream environments have characteristics which make them at once both important for biodiversity and human wellbeing, and highly threatened by human activities, which has attracted the attention of researchers. But, despite the high proportion of endangered freshwater fish species, very few management and conservation actions explicitly for Brazilian stream fishes have been effectively established. However, studies have argued that communication among scientists, policy-makers and society at large can be significantly improved through systematic review and synthesis of existing studies. Here we did systematic review of papers about fish stream assemblages in Brazil to detect trends in sampling protocolos and evaluated sampling effort in studied basins (Chapter 1). Further, we assessed the effect-size of non-native fish species on fish assemblages on Paraná basin (Chapter 2). We showed that studies of assemblage fish stream deployed a variety of sampling methods, but we found a growing trend towards the use of the electrofishing, sampling stream lengths of 50 meters, and over duration of 60 minutes. Our results highlight that regional species pools (gamma diversity) are still underestimated in all analyzed basins. The results of manuscript 2 found that non-native fish species are coexisting with native species at higher native richness streams in Paraná basin. We also show that the greater functional diversity in streams with non-native species was explained by the richness of native species rather than the richness of non-native species, corroborating with the clear results of niche overlap between native and non-native fish species. This study highlights that further surveys and taxonomic revisions of stream fishes are still paramount in most regions and point out to importance of more fish sampling effort surveys in Brazilian streams. We reinforce the importance of standardization in sampling protocols among studies to needed a make the necessary stream conservation advances in Brazil. Moreover, we also conclude that area with higher native fish species at Paraná basin could be focus of management of fish invasion and conservation efforts. Finally, we agree that researches should work collaboratively through data sharing, interinstitucional projects and exchange of researches to overcome challenges in the freshwater fish research in Brazil.

Keywords: Freshwater fish. Systematic review. Sampling effort. Functional diversity. Niche overlap.

SUMÁRIO

PRIMEIRA PARTE

SEGUNDA PARTE

PRIMEIRA PARTE

1 APRESENTAÇÃO GERAL

O Brasil é um país com extensões continentais (8.515.759 km^2, BRASIL, 2017) e possui uma das maiores e mais diversificadas redes de drenagens do mundo (BRASIL, 2018). De acordo com o Ministério do Meio Ambiente (BRASIL, 2018), sua rede fluvial está divida em 12 regiões hidrográficas (Bacias Amazônica, Tocantins-Araguaia, Paraguai, São Francisco, Atlântico Leste, Atlântico Sudeste, Atlântico Sul e Uruguai) inseridas em biomas com características distintas. Diante de sua ampla rede hidrográfica, apresenta uma rica diversidade de peixes de água doce, com cerca de 3200 espécies descritas (DIAS et al., 2016), as quais representam aproximadamente 55% das espécies de água doce registradas na região Neotropical (REIS et al., 2003) e aproximadamente 22% daquelas registradas mundialmente (BUCKUP; MENEZES; GHAZZI, 2007). Estima-se que no Brasil, no mínimo 50% destas espécies ocorram em riachos (CASTRO, 1999).

Nas últimas décadas, os pesquisadores da região Neotropical começaram a voltar suas atenções para riachos de pequena ordem (cabeceiras e altos cursos), atraídos pelo potencial registro de novas espécies (DIAS et al., 2016) e por proverem bem-estar humano (ex.: abastecimento e lazer). Apesar de sua importância, esses sistemas são vulneráveis às ações em diferentes escalas- bacia hidrográfica, zona ripária e canal- e estão entre os mais ameaçados pela degradação humana (MEYER; WALLACE, 2001). Dentre os principais impactos sofridos está a introdução de espécies (SALA et al., 2000), um tema ainda pouco explorado nos estudos de peixes de riachos do Brasil (DIAS et al., 2016) e tido como um dos maiores desafios em ecologia da conservação (DUDGEON et al., 2006).

O sucesso da invasão das espécies exóticas deve-se a diferentes características ambientais e bióticas dos sistemas (DAVIS et al., 2000; HIERO et al., 2004; EMERY, 2007; CATFORD et al., 2009) e muitos estudos apontam que tal sucesso está associado a ambientes impactados **(**MEADOR et al., 2003; KENNARD et al., 2005; MACDOUGALL; TURKINGTON, 2005**).** Este fato é notavelmente observado na bacia do Paraná (ex.: peixes, GARRONE NETO et al., 2007; JÚNIOR et al., 2009; mexilhão dourado, TAKEDA et al., 2004; zooplanctons, ROCHA et al., 2011), a mais populosa (32,2% da população) e também a mais urbanizada (90,5% de taxa de urbanização) do Brasil, com concentração populacional notadamente nas áreas de cabeceiras e nos altos cursos (BRASIL, 2006).

Apesar do crescente número de estudos sobre peixes de riachos no Brasil nos últimos 20 anos, observa-se uma lacuna de conhecimento desde questões básicas nas áreas de taxonomia (identificação e revisão) (BUCKUP et al., 2007; AZEVEDO et al., 2010) até o teste de hipóteses ecológicas (DIAS et al., 2016). Entre os temas pouco frequentes em estudos de riachos Neotropicais encontram-se aqueles sobre protocolos amostrais (ANJOS; ZUANON, 2007; TERRA et al., 2013; MOJICA et al., 2014), fundamentais para estimativas de riqueza de espécies e estudos sobre suas distribuições. Além, o conhecimento sobre esforço amostral (ex.: tamanho, duração e técnicas amostrais) tem um caráter altamente aplicado, pois contribui para uma melhor alocação de recursos financeiros e para melhores desempenhos de inventários biológicos, biomonitoramentos e estabelecimentos de estratégias de conservação para a ictiofauna e recursos hídricos (MENDONÇA et al., 2005; HUGHES; PECK, 2008; HUGHES et al., 2012)

Observa-se também, nas duas últimas décadas, que os estudos ecológicos têm passado por processos de transformações. Conceitos baseados em estabilidade e clímax dão lugar a uma visão dinâmica ligada a processos locais e regionais (RICKLEFS, 1987). Pesquisas em pequenas escalas envolvendo uma perspectiva reducionista abrem espaço para pesquisas em larga escala, possibilitando uma visão mais holística sobre os sistemas (KEITH et al., 2012). Dentre essas mudanças e, associado ao crescente número de estudos empíricos, autores apontam para a necessidade e importância da realização de sínteses quantitativas (REICHMAN et al., 2011; PULLIN; STEWART, 2016). As revisões sistematizadas fornecem base para sínteses de conceitos, formulação de teorias, identificação de problemas e de evidências, provém conclusões gerais e orientações para pesquisas futuras (LORTIE, 2014). Autores ainda sugerem que revisões sistemáticas/ sínteses são ferramentas significativas para apoiar a prática de política de conservação e gestão ambiental (FAZEY et al., 2004; SUTHERLAND et al., 2004; LORTIE, 2014), pois facilitam o acesso à informação científica de uma forma mais objetiva e rápida por parte dos tomadores de decisões.

2 ESTRUTURA DA TESE

“O que sabemos sobre peixes de riachos no Brasil?” Este questionamento surgiu durante meu mestrado, em 2009. E esta tese constata que este questionamento ficou cuidadosamente armazenado na minha memória, ressurgindo então em um momento propício para florescer. Essa simples pergunta foi base para o desenvolvimento do meu projeto de tese. Constatei a importância de compilar dados sobre esse tema a fim de apresentar resultados quantitativos sobre como e onde são realizadas as pesquisas de peixes de riacho no Brasil. Para tanto, utilizei as bases ISI Web of Knowledge, Scielo e Scopus usando os termos *fish* e *stream* e *Brazil* para levantamento dos estudos feitos até 2016. À medida que fui trabalhando os dados me surpreendi com a presença de espécies de peixes não nativas, especialmente na bacia do Paraná: 42% dos riachos amostrados continha pelo menos uma espécies não nativa. A partir disso, estruturei minha tese em dois capítulos, apresentados em formato de artigos científicos: 1º) Assessing fish sampling effort in studies of Brazilian streams; 2º) Coexistence of native and non-native fish species in megadiverse streams. No primeiro artigo apresento uma revisão de literatura para detectar tendências de métodos amostrais utilizados em pesquisas de peixes de riachos no Brasil. Meu objetivo inclui: (i) identificar a distribuição espacial das pesquisas e as bacias hidrográficas onde os estudos são escassos; (ii) identificar os diferentes métodos amostrais utilizados, bem como (iii) analisar o nível de esforço amostral nas diferentes bacias. No segundo artigo, utilizo os dados dos riachos amostrados na bacia do Paraná para responder as questões: riachos com espécies de peixes não nativas e sem diferem na riqueza de espécies de peixes nativos? Qual o efeito da presença das espécies não nativas na diversidade funcional das assembleias de peixes?

3 CONCLUSÃO GERAL

Apesar do crescente número de estudos sobre peixes de riachos no Brasil, principalmente a partir de 2008, esses se concentram nas bacias mais populosas (Paraná e Atlântico Sul), ocasionando grandes lacunas de conhecimento sobre o tema. Observamos que o pool regional de espécies (diversidade gama) ainda é subestimado em todas as bacias hidrográficas que estudamos. Identificamos uma tendência das características de protocolos amostrais em estudos de assembleias de peixes de riachos. Porém, enfatizamos o uso de uma ampla variedade de métodos amostrais e a falta de padronização dos protocolos entre os estudos. Apontamos nesta tese a efetividade da técnica de pesca elétrica na amostragem da riqueza de espécies de peixes. Técnica esta que muitas vezes é negligenciada por falta de evidências sobre sua efetividade nos riachos Neotropicais. A partir desses resultados concluímos sobre a necessidade de um maior número de estudos sobre esforço amostral de peixes de riachos no Brasil. Desta forma, proporcionaríamos uma gama de evidências científicas para apoiar nossas decisões em relação à escolha do protocolo amostral mais adequado a pesquisa. Acreditamos que padronizações dos protocolos de amostragem são pontos iniciais para um avanço na área de conservação desses sistemas aquáticos.

Apresentamos também um primeiro passo para entender as organizações das assembleias de peixes em riachos com a presença de espécies de peixes não nativas na bacia do Paraná. Embora nossos resultados apontem uma relação positiva entre a presença de espécies não nativas e a riqueza de espécies nativas, nossa pesquisa se limita aos dados de riqueza de espécies. Sendo assim e frente ao avanço dos impactos antrópicos na bacia, outras características devem ser analisadas em pesquisas futuras: abundância, variável temporal e características ambientais (ex. presença de impactos humano e características físicas nos riachos e suas bacias de drenagem) em diferentes escalas.

Ictiólogos que trabalham com peixes de riachos no país esbarram em desafios inerentes à própria natureza da pesquisa (ex. muitas espécies endêmicas e dificuldade de acesso a determinadas localidades), desafios financeiros e carência de recursos humanos em algumas regiões (ex. Norte). Diante desses obstáculos, concluímos que o caminho para se seguir é o de colaboração entre os pesquisadores, através do compartilhamento de dados, projetos interinstitucionais e intercâmbio de profissionais.

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SEGUNDA PARTE

ARTIGO 1

ASSESSING FISH SAMPLING EFFORT IN STUDIES OF BRAZILIAN STREAMS

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Assessing fish sampling effort in studies of Brazilian streams

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Running title

Fish sampling of Brazilian streams

Abstract

Neotropical stream fish assemblages have attracted much scientific attention in recent decades. However, spatial and temporal patterns in sampling, and the types of methodologies being used in studies, remains unclear. Here, we conducted an in-depth review of the published literature on Brazilian stream fish assemblages, recording where and how these surveys were conducted. We found that the number of studies has increased over time, but significant geographic biases remain, with most studies having been conducted in southeastern and southern Brazil. Studies deployed a variety of sampling methods, but we found a growing trend towards the use of the electrofishing, sampling stream lengths of 50 meters, and over a duration of 60 minutes. Rarefaction curves showed that sampling effort is low in relation to the number of streams sampled in all basins studied, especially in the Amazon, Tocantins Araguaia, São Francisco and Uruguay basins, and northeast Brazil. Thus, we emphasize the importance of further surveys in these regions, since even basic parameters for ecological studies and conservation, such as species richness, remain critically underestimated. In light of the recent reductions in public funding for scientific research in Brazil, we also highlight the importance of networks and collaborations between Brazilian ichthyologists to add more value to scientific production and contribute to the greater standardization of data.

Keywords

Neotropical streams; freshwater fish; spatial scale; sampling methods; rarefaction curves.

1. Introduction

Lotic ecosystems are among the most degraded natural systems on the planet (Sala et al., 2000), especially those located in tropical regions (Boyero & Bailey, 2001). Changes in landscape structure from human activities directly affect these systems, in particular, small order streams (Wohl, 2017). Stream environments have characteristics which make them at once both important for biodiversity and human wellbeing, and highly threatened by human activities (Meyer & Wallace, 2001), which has attracted the attention of researchers (Dias et al., 2016). Small order streams contain high biotic diversity, including a wide array of fish species, for which streams provide important and heterogeneous habitats and resources (Meyer et al., 2007). In addition, small order streams are fundamental to the maintenance of other connected ecosystems, including terrestrial and downstream aquatic habitats, because they are important sources of resources (Progar & Moldenke, 2002).

The Neotropical region contains over 4000 species of freshwater fishes (Toussaint et al., 2016), with around 3200 species present in Brazil (Dias et al., 2016), and at least 50% of these species occurring in streams (Castro, 1999). Stream fish assemblages are excellent indicators of environmental conditions, providing an integrated view of overall habitat quality (Chovanec et al., 2003), and reflect the impacts of medium/long term environmental stressors (Karr, 1981), as found in several Brazilian biomes (e.g. Carvalho et al., 2017; Chen et al., 2017). But, despite the high proportion of endangered fish species (IUCN, 2017), very few conservation actions explicitly for Brazilian stream fishes have been effectively established (Collares-Pereira & Cowx, 2004). Environmental regulations in Brazil on streams and adjacent riparian habitats have important limitations (Leal et al., 2017), as they do not consider impacts on aquatic biota (Castello et al., 2013), and are instead geared towards aquaculture, fishing activities and provision of water for human use (Law 11,959, Brazil, 2009; Law 9,433, Brazil, 1997). Additionally, there is limited participation by the scientific community in environmental policy, which could provide high-quality information to governments and decision makers (Azevedo-Santos et al., 2017). This limited participation also may arise from the nature of scientific research and scientists, loaded in theories, methods and data difficult to be understood by non-specialists (Brosnan & Groom, 2006; Briske, 2012; Azevedo-Santos et al., 2017). However, studies have argued that communication among scientists, policy-makers and society at large can be significantly improved through systematic review and synthesis of existing studies (Pullin et al., 2004; Sutherland et al., 2004; Pullin & Stewart, 2006; Hampton et al., 2013; Lortie, 2014).

Dias et al. (2016), through a compilation of data published up to 2012, showed that the number of studies on stream fishes in Brazil has greatly increased in recent decades. In general, most of these studies were descriptive studies of local fish fauna (ex. spatial distribution and diversity) and investigations of anthropogenic impacts on fish assemblages and sampling techniques were scarce.

Knowledge on sampling effort and protocols provide better quality data and also a better allocation of financial resources (Smith & Jones, 2005) This is because the ability to show patterns like species richness, diversity and abundance of individual species and to assess the influence of human impacts on those assemblages varies with sampling effort (Kennard et al., 2006). Sampling effort refers to the size of the sample, the number of people onsite, the techniques employed, and the sampling duration (Reynolds et al., 2003). The existence of multiple protocols for collecting data generates different results (Rabeni et al, 2009), and since the interpretations and conclusions depend on how well the assemblages of interest were sampled, it is difficult to compare relationships and patterns among ecological communities and assemblages (Cao et al., 2002).

In the present study, we present a review to detect trends in sampling methods used in published research of Brazilian stream fish assemblages. Our objectives include: (i) to identify the spatial distribution of surveys and basins with a gap of studies; (ii) to identify the different sampling methods used, as well as (iii) to analyze the level of sampling effort across different spatial scales. We believe that this general overview allows informed choice of methodology for future studies and may be a first step to overcome barriers of limited time and financial resources.

2. Methods

2.1 Publication selection and data extraction

We searched for potential papers in three online academic databases: ISI Web of Knowledge, Scielo and Scopus using the terms *fish* AND *stream* AND *Brazil*. Searches were conducted from January to July 2017 and we considered studies published until December 2016.

The first step was to examine titles and abstracts to include only fish assemblage papers with fish species occurrence data. Papers involving only single species or populations were excluded. Second, we conducted a full-text inspection of sampling design to include only papers related to sampling in wadeable (first to third order) streams. Some papers encompassing other aquatic environments (e.g. rivers, lakes, reservoirs and floodplains), but also wadeable streams were also considered when it was possible extract fish species data by sampling site. Third, we removed duplicates from the three academic databases used. Finally, when a relevant paper lacked information (coordinates and/or fish species list), we contacted authors directly to request the missing data.

For each paper, we extracted the year of publication, affiliation of the first author, institution and their geographic location (state and region), journal, river basin (ANA, 2017), geographic coordinates of sampling sites, number of streams, number of sample sites (inserted in the same or distinct streams), number of times each site was re-sampled (number of sampling occasions), temporal scale (difference between first and last sampling year), and fish species list. We also recorded the sampling methods: sampling gear (i.e., electrofishing, seine and dip net), sampling size (sample distance in stream in meters) and sampling time (duration of each sampling in minutes). When the sampling time was an interval we calculated the mean. If one paper presented two or more sampling methods, information was extracted separately for each method. The sampling density of each basin was calculated dividing the number of sampled sites by the area (1,000 km^2) of the respective watershed.

2.2 Statistical analyses

We built rarefaction/extrapolation curves (Colwell et al., 2012) to assess the sampling effectiveness and if the species richness could significantly increase with increased sampling effort (i.e. number of streams sampled per basin). We considered unidentified species (i.e. *Astyanax* sp., *Bryconamericus* sp., *Characidium* sp., *Trichomycterus* sp.) as different species, but only when occurrences were registered for different basins. All species richness rarefaction/extrapolation curves were generated based on Hill numbers for sampling-unit-based incidence data and non-parametric bootstrapping based on 100 randomizations to display the confidence intervals (±95% CI). The endpoint value (an integer specifying the sample size that is the endpoint for R/E calculation) was double the reference sample size for each basin. For these analyses, we used the *iNEXT* package (Hsieh et al., 2016).

To evaluate if surveys and sampling effort are growing continuously over time we used an exponential model (non-saturating function) to analyze six cumulative datasets: number of published papers, number of streams, number of samples sites and the most frequent sampling techniques, lengths of the samples points and sample duration times.

All analyses were performed in the *R* programming environment (R Core Team, 2017).

3. Results

3.1 Surveys in time and space

Literature searches revealed 1,152 potential papers that after filtering resulted in 120 valid references. However, we did not have access to full information (coordinates and fish species list) for 32 papers, resulting in a final list of 88 papers (Figure 1) (Table S1). Approximately 76% of the studies were led by 19 institutions located in southeastern (44%) and southern (32%) Brazil. These institutions are represented by 17 Universities, one State Research Institute and one Museum (Table S2).

Fish surveys were not well distributed among basins (Figure 2). We found 599 sampled streams in the Paraná River basin and 203 in the Amazon River basin, together representing 90% of total sampled streams (Table 1). We did not find studies in five basins: Uruguay, Northeastern Atlantic (western and eastern basins), Eastern Atlantic and the Parnaíba. Moreover, 50% of total sampled streams were conducted by only two universities, both located in southeastern Brazil (Universidade Estadual Paulista-UNESP and Universidade Federal de Lavras-UFLA). Surveys took on average 3.7 years (median=3; SD=2.08) from the collection of data to publication of findings in scientific journals, with 85% of surveys published within five years of the last field survey.

The number of papers (growth rate=1.27) increased considerably after 2008 and we did not find evidence that it is stabilizing (Figure 3a). The same was true to number of streams (growth rate=1.41) (Figure 3b) and number of sites sampled (growth rate=1.42) (Figure 3c).

3.2 Sampling effort

Most studies had a low number of sampled streams (mean=3.38; median=1; SD=5.36) with almost 50% of papers having ≤ 3 (Figure 4a). Trends for sampled points (mean=3.14; median=1; SD=3.89) were quite similar, of which 40% of studies having ≤3 (Figure 4b). Most of studies (86%) were conducted with one year of sampling or less (mean=12.57; median=1; SD=19.84) and 33% of studies sampled each site twice or only once (mean=6.77; median=5; SD=6).

Rarefaction curves for sampled streams (Figure 5) in each basin showed similar patterns and did not reach an asymptote (Table S3). When we extrapolated the number of streams required to double the reference sample size in each basin, we observed the Paraná (obs=461; est=1012.7; effort=45.52%) and South Atlantic (obs=142; est=216.12; effort= 65.7%) basin curves tended towards stabilization. However, the same was not true for the Southeast Atlantic (obs=106; est= 296.95; effort=35.7%), Paraguay (obs=91; est=189.25; effort= 48.1%) and Amazon (obs=420; est=864.55; effort=48.6%) basins. It is important to note that unidentified species represented 19% of total species. Similar patterns were obtained when we analyzed only fish that had been formally identified to species (Figure S1).

We found 11 sampling techniques, and most of surveys used one or two fishing gears (61% and 25% of total studies, respectively). We recorded 23 different lengths of sample points and 14 different duration times, but 50% of total studies did not present such information (Figure 6). Most studies employed electrofishing (53% of total studies, followed by metallic sieve- 38% and seine- 29%), sampled 50 meters of stream length (40%) and lasting 60 minutes (15%). Our results suggested that the use of electrofishing is growing continuously over time (Figure 7a), as well as 50 meters of stream lengths (Figure 7b) and sampling duration of 60 minutes (Figure 7c). The two fishing gears most frequently used in combination were metallic sieves with seine, reported in ten papers. Other methods, such as skin diving with dip net, observations from stream margins and traps, were much less common (Table S4).

4. Discussion

Here we present an in-depth review of current spatial and temporal trends in ecological research on Brazilian stream fish assemblages. Our results show that the number of studies has increased over time, but they are spatially poorly distributed, highlighting major research gaps in the Amazon, Tocantins Araguaia, São Francisco and Uruguay basins, and northeast Brazil. We observed clear trends towards the use of sampling intervals of 50 m, sampling times of 60 min and electrofishing. Our results also emphasize the wide variety of sampling methods used and the lack of standardization in sampling protocols among studies. This implies that much of the existing data on stream fish in Brazil is of limited value for use in cross study comparison.

4.1 Spatial and temporal patterns

The greatest concentration of studies was in the southeast and southern regions, and was mainly conducted by public universities (89.5%). This pattern, common to other research areas and other taxonomic groups (Brito et al., 2009, for mammal species; Campos et al., 2014, for amphibian species; Lima et al., 2015, for trees species; Silva & Perbiche-Neves, 2016, for microcrustaceans species), may be related to the unequal distribution of human, financial, scientific and technological resources at national level (Leta et al., 2006; Azevedo et al., 2010; Chiarini et al., 2014). In Brazil, scientific production is generated in almost its entirety in public universities (Leite et al., 2011), through graduate programs (Kimura, 2010). In this way, the southeast and southern regions are favored by the high concentration of universities (64.5%, Inep, 2016) and post-graduate programs (66.1%, but only 2.8% of programs in biodiversity research, Capes, 2017), in addition to the greater financial investment in research through policies implemented by regional funding agencies (Fapesp, 2011). Moreover, these regions concentrate much of Brazil’s Gross Domestic Product (GDP) (IBGE, 2017), which is reflected in the greater investment in research.

We also observed that these regions contain several historically consolidated and nationally and internationally recognized research groups in the area of stream fish ecology (see Cnpq, 2016). In this sense, 50% of the points were sampled by research groups from UNESP (São José do Rio Preto campus) and UFLA. This large data input from these two universities occurred due to financial resources linked to three major projects (1 - Biota-FAPESP; 2 - IBI - CEMIG; 3 - Biotaminas-FAPEMIG) implemented with financial resources of the states of São Paulo (FAPESP) and Minas Gerais (CEMIG and FAPEMIG). This result clearly illustrates that investment in scientific research determines the quality of data that can be generated by academic institutions in Brazil. Greater investment, besides being motivation for researchers, allows for greater structural and logistical support for the development of research and access to equipment and scientific tools that may have a higher cost, enabling the production of a science of high impact (Helene & Ribeiro, 2011) and covering larger geographical areas.

The Paraná basin was the most studied region, both in number and density (0.76) of sampled sites (670). In addition to the greater financial and scientific resources available in the regions in which this basin is inserted, research in this basin is also favored by the easy access to streams due to the high level of urban development and associated infrastructure (Dias et al., 2016). The Amazon basin holds the second largest number of publications, but with a reduced number of sampling points and sample density. Although harboring the greatest diversity of freshwater fish in Brazil (Winemiller et al., 2007), the region presents several factors that hinder research. According to Capes (2017), the region has only 5% of graduate programs in Brazil and presents a deficit of researchers. In addition, there are challenges inherent to the locality, such as difficulties in communication and transport, whereby large distances are travelled almost exclusively on navigable waterways (Becker, 2004), which makes research expensive and, probably, less attractive for postgraduate students. Among the five basins that do not appear in our studies, four are located in the Northeast region of Brazil (Parnaíba, Eastern Northeast Atlantic**,** Western Northeast Atlantic and Eastern Atlantic), corroborating with other studies that point to the scarcity of ichthyofaunal studies in these regions (Rosa, 2004). Because they are totally or partially under the dominion of the Caatinga biome, with semi-arid climate, most water courses in these regions are intermittent and seasonal (Abílio et al., 2007). This makes it difficult to conduct research on stream fish in this biome, as field work is commonly conducted in dry seasons, to facilitate access to aquatic environments and fish collection (Rosa et al., 2003). However, these basins have great potential for recording new and endemic species (Rosa et al., 2003; Nascimento et al., 2011).

We observed an exponential increase in the number of publications over time in the studied basins, with the peak of increase in 2008. Two important factors are associated with this result: (i) firstly, the number of students involved in postgraduate programs has grown since 2000 (Helene & Ribeiro, 2011). The increase is mainly associated with PhD students, who are responsible for most of Brazilian scientific production (Capes, 2014). The number of PhD graduates in 2016 (20599) represents an increase of 287% over the year 2000 (5318) (Geocapes, 2018). These results are related to the expansion of post-graduate programs, which had a 201% increase in the number of doctoral programs in public institutions in the country between 1999 (731) and 2017 (2202) (Capes, 2017). However, to better understand this relationship, new statistical analyses would be necessary to correlate the increase in the number of doctorate students with the increase of the publications targeted by this study. (ii) Second, it is due to the larger number of national journals indexed in the Web of Scince-ISI database, with a significant increase of 205% between 2002 and 2008 (Castro, 2009). We attribute this importance to the fact that 70% of the scientific papers collected are published in national journals (Table S5), probably because of the regional nature of the issues raised in these surveys.

4.2 Sampling effort

Given the low investment in research, considering the share of the total economy (Koeller et al., 2017), and the sharp spending cuts in this area (Angelo, 2016), it was expected that most studies would be conducted over short time periods and with low numbers of streams sampled, as seen in our results. According to the literature, these patterns are observed in sampling of freshwater biodiversity in general, which are often performed in the short term and without replication over time (Jackson & Fureder, 2006). Such characteristics may be associated with lack of logistics, resources and the very nature of the study. In Brazil, the main development agencies usually invest in short-term research, from one to three years (Dias et al., 2016). However, it is important to note that such restricted temporal scales can lead to the underestimation of diversity, compromising the results and, consequently, the conclusions of the published articles (Huttunen et al., 2012). These results suggest that the long-term research about stream fishes in Brazil practically does not exist, or at least, remains unpublished. Therefore, it is clear that scientific funding agencies and universities must start to invest in long-term research projects.

Rarefaction curves showed that sampling effort was low in relation to the number of streams sampled in all basins. These results can be explained by a combination of factors that continue to limit basic knowledge on Brazilian fish streams. The Brazilian basins analyzed here have considerably different ichthyofauna compositions (Rosa & Lima, 2008) and, due to the natural limitation of fish dispersion, most species have relatively localized distributions (Rosenfeld, 2002). For example, Nogueira et al. (2010) found 540 small-scale watersheds with restricted-range freshwater fish species in Brazilians basins, which together represented 32% of Brazilian freshwater fish fauna. In addition, there are many extensive and heterogeneous basins located in remote and largely inaccessible areas, such as the Amazon (3,869,953 km²) and Tocantins Araguaia (918,822 km²) basins (ANA, 2017). Ultimately, according to CNPq (2016), there are just 30 freshwater fish research groups in Brazil. From these data, we can make three conclusions: (i) that results from the Tocantins-Araguaia and São Francisco basins are extremely biased as they include a very small number of sampled streams (2 and 7 streams, respectively) and sample density. (ii) The basins of Paraná and South Atlantic were the best sampled. These efforts are a consequence of what we have previously discussed in relation to the concentration of human, financial, and structural resources in these basins (Leta et al., 2006, Azevedo et al., 2010; Chiarini et al., 2014). (iii) Amazon, Tocantins Araguaia and São Francisco basins (inserted in the biomes Amazon, Cerrado and Cerrado/Caatinga respectively) are regions that remain critically undersampled and are where future research efforts should be directed.

Research on stream fish assemblages in Brazil has deployed a wide variety of sampling methods (11 techniques, 23 reach sampling lengths, 14 sampling times), but there is a growing trend towards the use of the electrofishing, sampling intervals of 50 m and sampling times of 60 minutes. The use of electrofishing is a commonly used method for collecting quantitative data on fish assemblages, in both temperate and tropical regions (Bozzetti & Schulz, 2004; Rabeni et al., 2009; Kanno et al., 2009; Price et al., 2011). This technique has been routinely used since the beginning of last century in temperate countries (Hartley, 1990) but was introduced in Brazil for fish studies only in the mid-1990s (Lobón-Cerviá et al., 1994). One of the advantages of this technique is that it is considered to be a non-destructive sampling procedure with the lower mortality rates of fish (Pusey et al., 1998). Although its efficacy in waters with low conductivity and the selectivity of the method are questioned, its efficiency in Neotropical streams is demonstrated in several studies and no general rules are possible (Mazzoni et al., 2000; Esteves & Lobón-Cerviá, 2001; Motta Buhrnheim & Cox Fernandes, 2003; Allard et al., 2014). However, we believe that the most disadvantage of the electrofishing is the high cost of the equipments. Studies have shown that the commonly appropriate sampling range is the one in which most of the species expected for that location is captured (about 90%) (Hughes et al., 2002; Reynolds et al.,2003). In general, there are few studies of fish sampling effort in Brazilian streams (Mazzoni et al., 2000; Esteves & Lobón-Cerviá, 2001; Dos Anjos & Zuanon, 2007; Terra et al., 2013) and that should be better explored. For Amazonian *terra firme* forest streams, dos Anjos and Zuanon (2007) found values from 200 m (1st order) to 280 m (3rd order) characterized local fish fauna. Terra et al. (2013) in the survey of Atlantic Forest streams showed that 40 times the means wetted channel width (from 100 up 500 m) were not sufficient to estimate fish richness in these systems, but sufficient for applying metrics of assemblages conditions (e.g. dominance, number of common species, Shannon diversity). Studies using minimum sampling intervals of 150 m yielded stabilizing fish rarefaction curves in streams of the Grande (alto Paraná basin) and São Francisco basin, although the same was not found in the Araguari and Paranaíba basins (alto Paraná basins) (Leal et al., 2014). These studies have in common higher values of stream length compared to the sampling interval most used in studies of fish streams in Brazil (50 m). Thus, future research should use reference sampling intervals values that are appropriate for knowledge and conservation of Brazilian fish stream.

Although we know the importance of delineating specific protocols according to the environmental characteristics and the objective of studies, it is necessary to begin to establish a standardized sampling pattern to increase the comparative power and the cross-information of the data obtained for fishes from Brazilian streams. The lack of standardization of these data certainly hinders the quantitative comparisons between the studies (Cao et al., 2002). In addition, uncertainties about sample effectiveness may generate erroneous results on species distribution and richness patterns (Schneck & Melo, 2010). Finally, our results showed that in half of the papers we evaluated, basic information on sampling protocols was either absent or insufficient to replicate experiments.

In a country with a continental extension of 8,515,759 km^2 (IBGE, 2017); and with one of the largest networks of drainage in the world (MMA, 2018), our study highlights that further surveys and taxonomic revisions of stream fishes are still paramount in most regions, since even basic parameters for ecological studies and conservation, such as regional species pools (gamma diversity), are still underestimated. In particular, basins located in areas of the Cerrado, Caatinga and Amazon Forest. However, global environmental issues (e.g. water scarcity, climate change, food production) also demand that researchers work collaboratively to address effects of multiple threats and environmental stressors on biodiversity, at larger scales, and using long-term experiments (Hampton et al., 2013). We believe that the Paraná and South Atlantic basins (best sampled) represent the areas with the greatest potential for such advances in research with stream fishes. However, when faced with funding difficulties, it is essential to promote the networks of collaborations between Brazilian ichthyologists to add more value to the scientific production (Leta & Chaimovich, 2002), and contribute to the greater standardization of data. However, the lack of information on sampling protocols in the included articles, as well as the resistance that we met when procuring the missing data for this study, makes us believe that a change of attitude among researchers, i.e., a greater willingness to exchange data and overall transparency (Palmer et al., 2005), is also needed to a make the necessary scientific advances.

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6. Data Availability Statement

The data that support the findings of this study are available from the corresponding author, NTJ.

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Tables

Table 1. Number of papers, institutions, sampled streams, sampled sites (n), sample density (n/1,000 km^2) and richness by Brazil river basins found in this study.

River basin	Paper	Institution	Stream	Sample site (n)	Sample density (n/1,000 km^2)	Richness
Amazon	10	9	203	217	0.06	380
Southeast Atlantic	3	2	35	51	0.14	102
South Atlantic	6	5	23	23	0.12	136
Paraguay	3	2	33	36	0.10	89
Paraná	62	17	599	670	0.76	309
São Francisco	2	2	7	9	0.01	21
Tocantins Araguaia	2	2	2	19	0.02	26
Total	88	30	893	1025		834[a]

[a] number of total fish species found in all papers. Here, the species richness presented is the original taxonomic identification of the papers

Figures

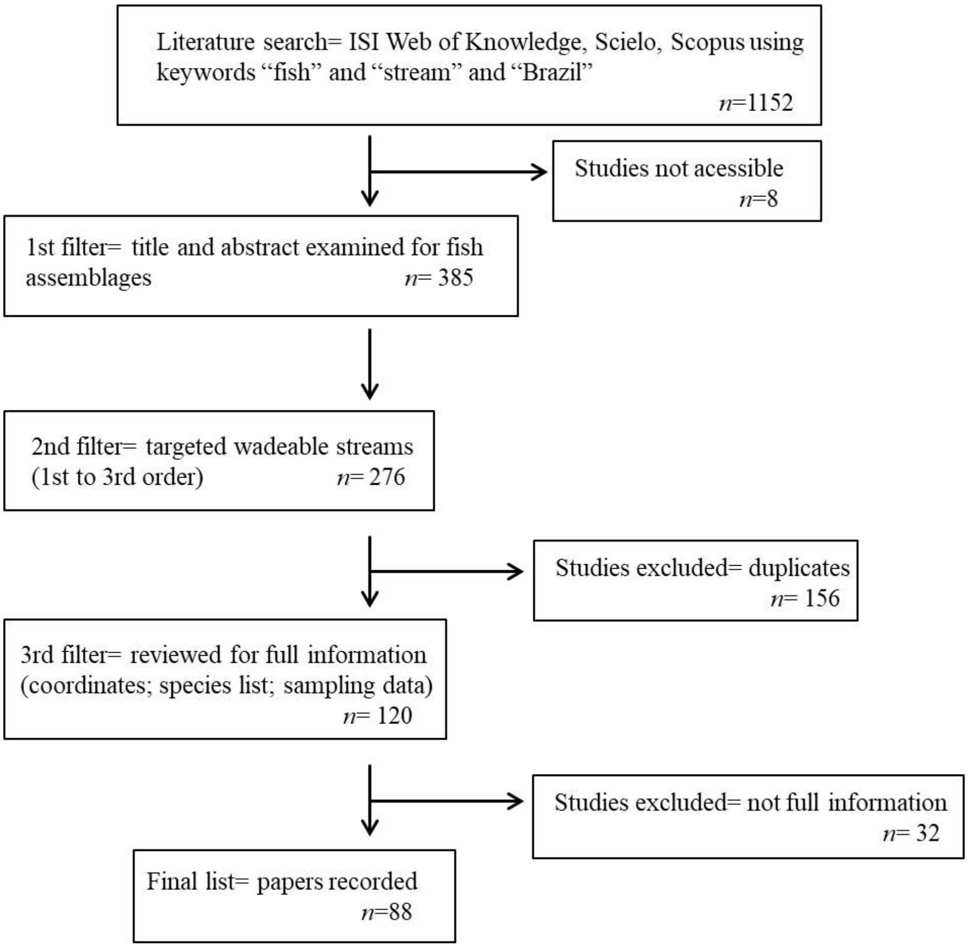

Figure 1. Flow chart detailing the process of publication selection and the number of studies from each stage of the review

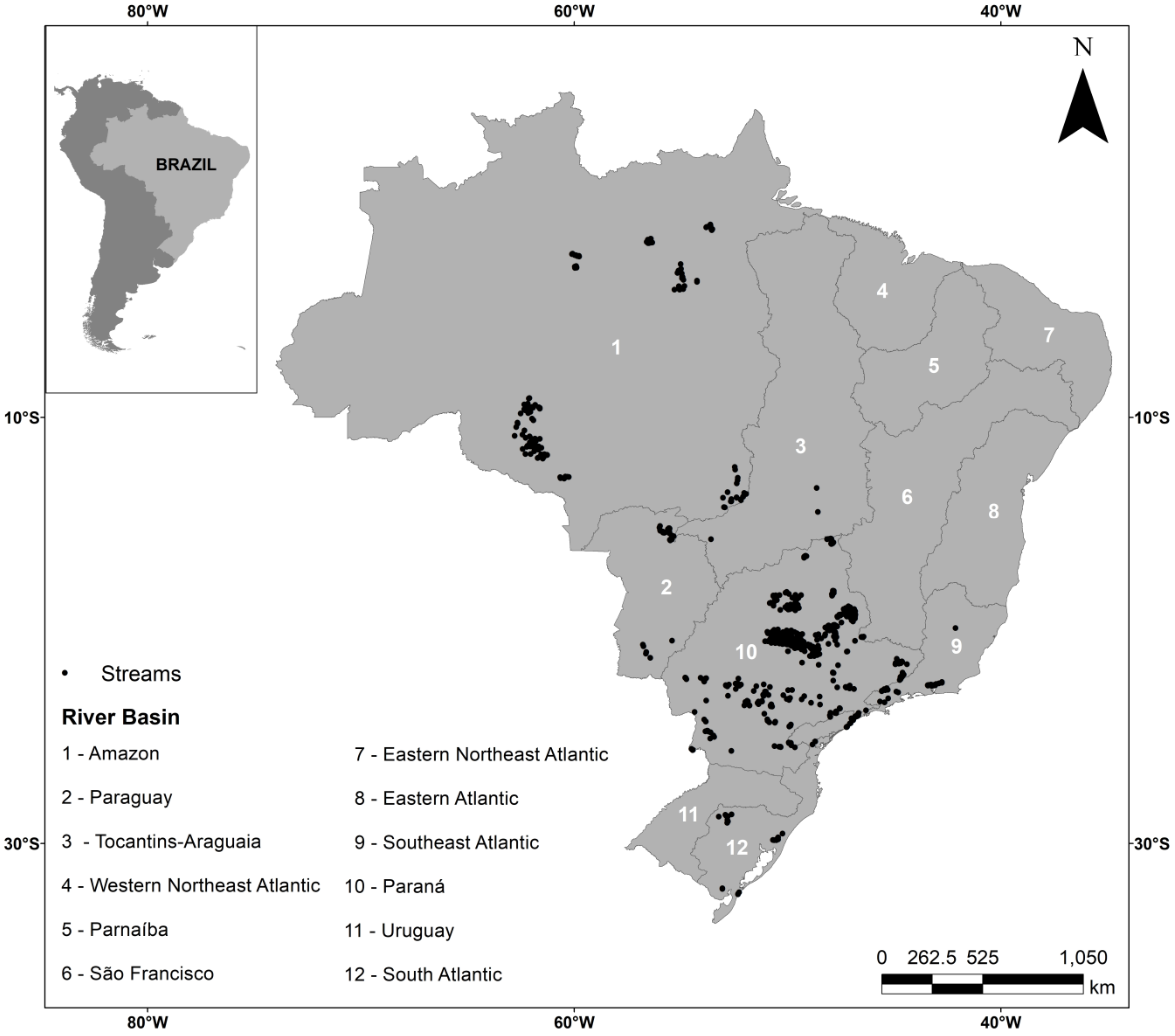

Figure 2. The location of all sampled streams found in this study

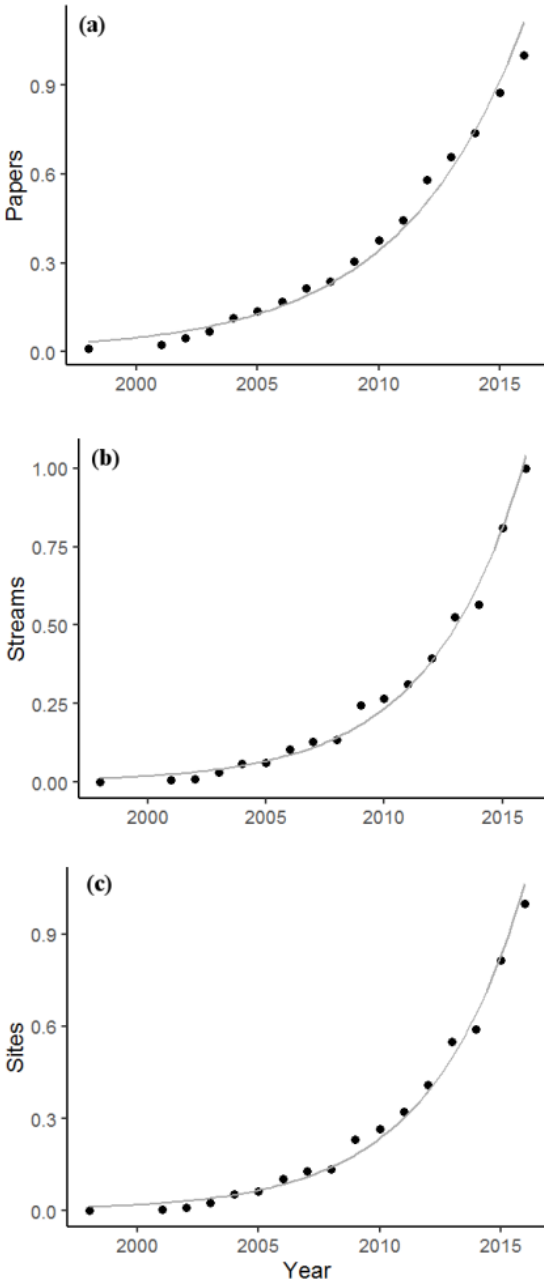

Figure 3. The growing number of surveys in fish stream assemblages in Brazil over time considering the number of papers (a), streams (b) and sampled sites (c) found in this study. The number of papers, streams and sites are cumulative and proportional to the total numbers

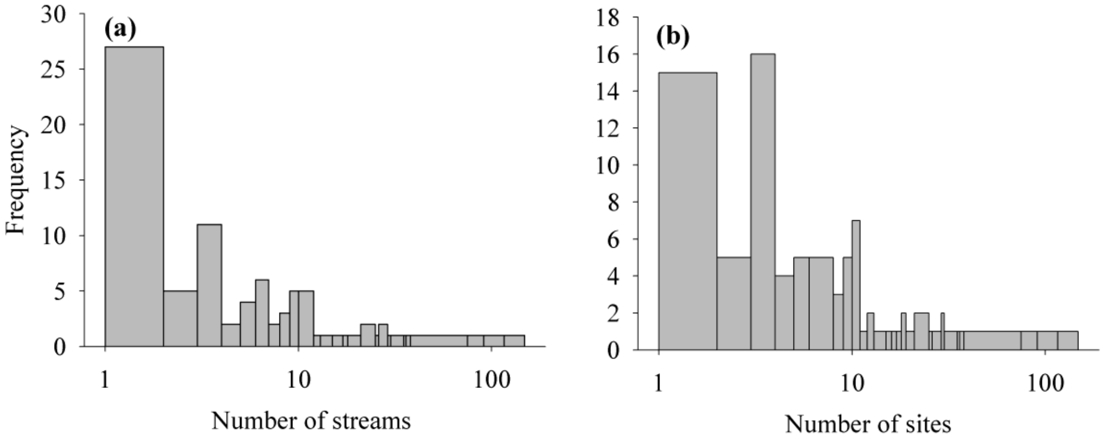

Figure 4. Frequency distribution of (a) sampled streams and (b) sampled sites of each paper found in this study. Both x- axes are in long-scale

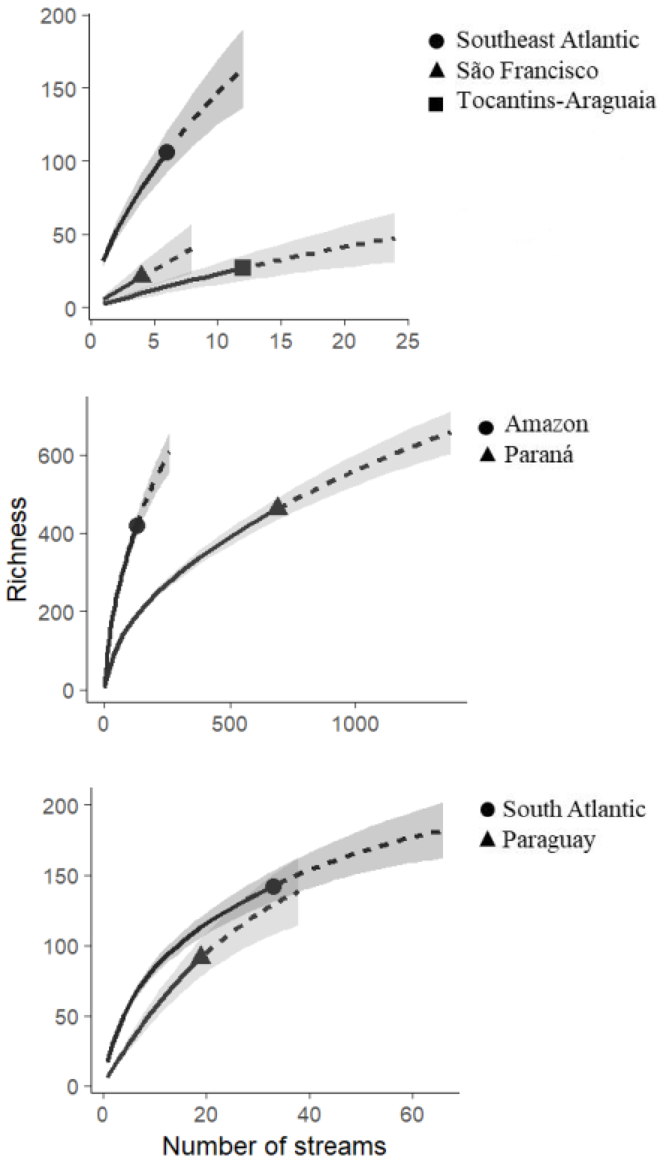

Figure 5. Rarefaction (solid) and extrapolation (dashed) curves from sampled streams of each basin found in this study. The curves were generated by converting raw species richness data to Hill numbers for sampling-unit-based incidence data with double-bounded 95% confidence intervals. We considered unidentified species (i.e. *Astyanax* sp., *Bryconamericus* sp., *Characidium* sp., *Trichomycterus* sp.) as different species, but only when occurrence was registered for different basins. The endpoint = double the reference sample size in each basin

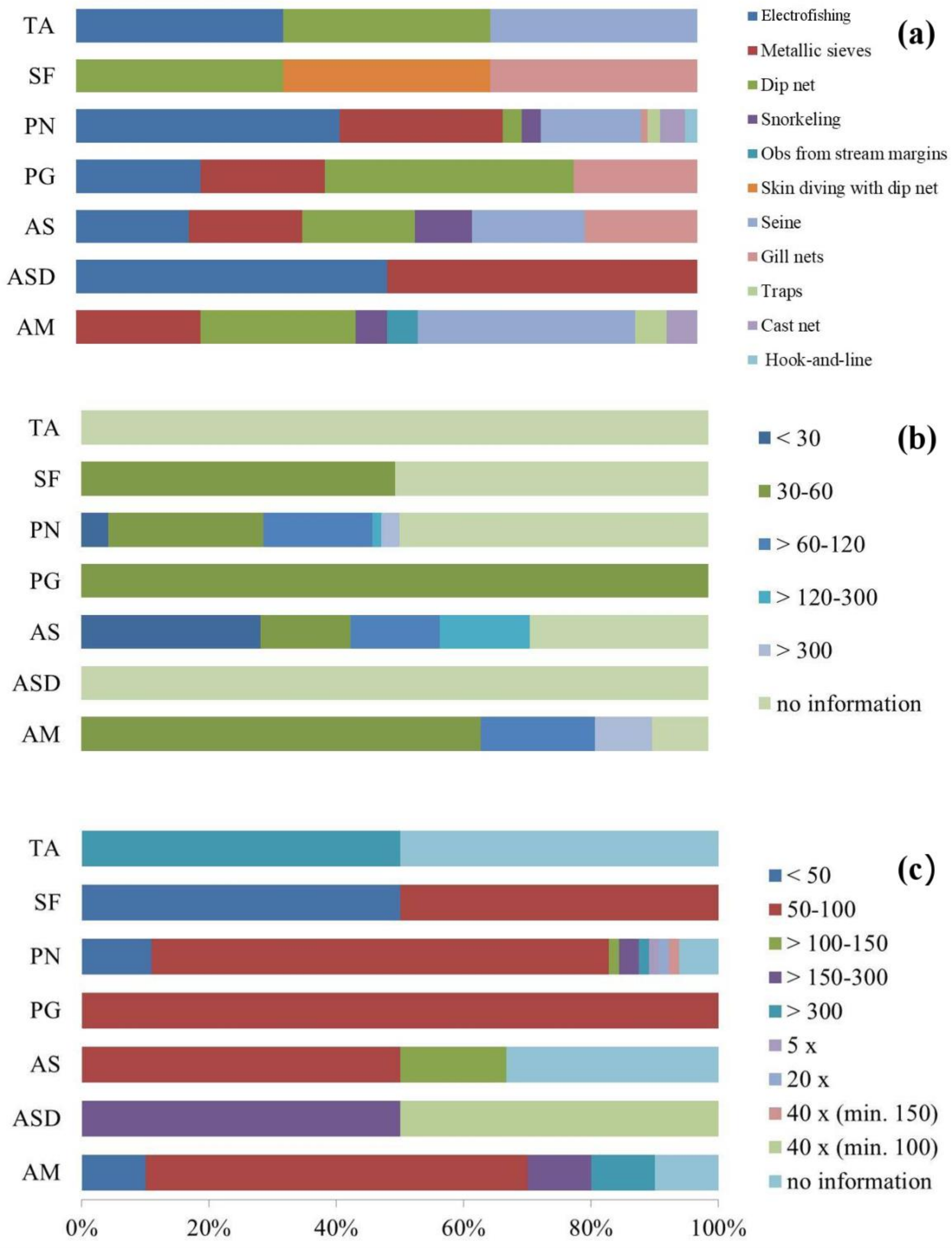

Figure 6. Percentage of fishing gear (a), times intervals of sampling in minutes (b) and lengths intervals of the samples points in meters (c) for each basin of papers found in this study. TA= Tocantins- Araguaia; SF= São Francisco; PN= Paraná; PG= Paraguay; AS= South Atlantic; ASD= Southeast Atlantic; AM= Amazon; x= multiplied by wetted width

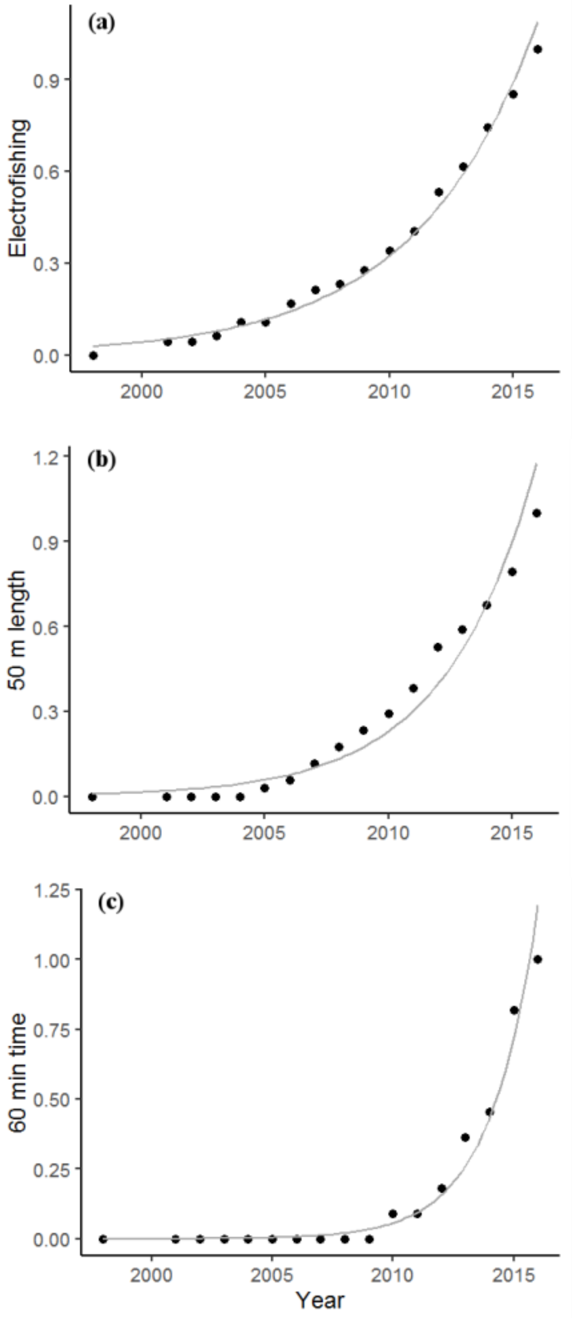

Figure 7. Main trends in sampling methodologies of Brazilian fish assemblages over time considering the fishing gear (a), sampling lengths of 50 meters (b) and 60 minute sampling duration (c). Electrofishing, 50 m length and 60 min time are cumulative and proportional to the total numbers

Electronic Supplementary Material 1

Table S1. Data of web searches and number of papers obtained from each different online database

Database	Data of search	Retrieved studies	Selected studies
ISI Web of Knowledge	16/01/2017	147	66
Scielo	19/01/2017	78	14
Scopus	13/07/2017	160	8
Total		385	88

Table S2. The institutions and the total number of studies, sampled streams and sites found in this study. UF=Federal University, UE= State University

Institution	Papers	Streams	Sample sites
UE Paulista Júlio de Mesquita Filho (UNESP)	20	321	347
UE do Oeste do Paraná (Unioeste)	7	18	28
Instituto de Pesca, Secretaria da Agricultura e Abastecimento do Estado de São Paulo (IP)	5	14	35
Universidade de São Paulo (USP)	4	37	40
UE de Maringá (UEM)	4	17	51
UE de Mato Grosso do Sul (UEMS)	4	11	13
UF do Paraná (UFPR)	4	7	17
Pontifícia Universidade Católica de Goiás (PUC GO)	3	20	29
Universidade de Brasília (UnB)	3	13	23
UE de Londrina (UEL)	3	5	13
UF de Lavras (UFLA)	3	153	155
UF de São Carlos (UFSCAR)	3	16	20
Instituto Nacional de Pesquisas da Amazônia (INPA)	2	34	40
UE de Goiás (UEG)	2	7	144
UE do Norte do Paraná (UFNP)	2	39	45
UF de Pelotas (UFPel)	2	5	10
UF do Pará (UFPA)	2	15	26
UF do Rio Grande do Sul (UFRGS)	2	12	14
UF Rural do Rio de Janeiro (UFRRJ)	2	33	38
Centro Universitário de Maringá (UniCesumar)	1	1	3
Museu de História Natural Capão de Imbuia, Prefeitura de Curitiba	1	1	3
Instituto Federal de Mato Grosso (IFMT)	1	26	26
Universidade do Amazonas (UFAM)	1	3	24
Universidade do Estado do Rio de Janeiro (UERJ)	1	1	1
Universidade do Vale do Rio dos Sinos (UNISINOS)	1	5	6

UF de Goiás (UFG)	1	21	21
UF de Rondônia (UNIR)	1	9	18
UF do Oeste do Pará (UFOPA)	1	21	21
UF do Rio de Janeiro (UFRJ)	1	26	26
UF do Rio Grande (FURG)	1	3	6

Table S3. Observed (obs) and estimated (est) richness (S) with their respective standard error (SE) for fish assemblages streams by Brazil drainage basins found in this study

Basin	S obs	S est	SE	Effort (%)
Amazon	420	864.55	81.37	48.6
Southeastern Atlantic	106	296.95	74.56	35.7
South Atlantic	142	216.12	26.51	65.7
Paraguay	91	189.25	33.8	48.1
Paraná	461	1021.7	106.42	45.52
São Francisco	21	178.5	70.49	11.76
Tocantins-Araguaia	27	115	63	23.48

Table S4. Samplings used in the fish assemblages streams surveys found in this study. Fishing gear: 1= electrofishing; 2= metallic sieves; 3= dip net; 4= snorkeling; 5= observations from stream margins; 6=skin diving with dip net; 7=seine; 8=gill nets; 9=traps; 10= cast net; 11= hook-and-line

		Basin							
Samplings		Amazônica	Atlântico Sudeste	Atlântico Sul	Paraguai	Paraná	São Francisco	Tocantins Araguaia	Total
Fishing gear	1		1	2	1	42		1	47
	2	4	1	2	1	26			34
	3	5		2	2	3	1	1	14
	4	1		1		3			5
	5	1							1
	6						1		1
	7	7		2		16		1	26
	8			2	1	1	1		5
	9	1				2			3

	10	1				4			5
	11					2			2
Lenghts (meters)	19					1			1
	20	1					1		2
	30					3			3
	40					2			2
	50	4		1	1	29			35
	60			1		1			2
	70					2			2
	75					5			5
	80	1			1	1	1		4
	95					1			1
	100	1		1	1	7			10
	125			1					1
	150					1			1
	200	1	1			1			3
	300					1			1
	350	1							1
	400					1			1
	1500							1	1
	16 a 50					1			1
	20 times its mean wetted width (ww)					1			1
	40 times its mean ww (min 150)					1			1
	40 times its mean ww (min 100)		1						1
	5 times its mean ww					1			1
	papers no information	1		2		4		1	8
Duration of each sampling (minutes)	15			2					2
	20					3			3
	30	1		1		4			6
	40					5			5
	45	1			1	2			4
	60	5			2	6	1		14
	75					1			1
	80					1			1
	90			1		6			7
	120	2				4			6
	240					1			1
	300			1					1
	390	1							1
	1020					2			2
	papers no information	1	2	2		34	1	2	42

Temporal scale (years)	0	3		1	1	16		1	22
	1	6	3	4	1	39	1		54
	2			1		6		1	8
	3				1				1
	4					1			1
	6	1							1
	7					1			1
Number of sampling occasions	1	3				9		1	13
	2	2	2	1	1	9		1	16
	3				1	5			6
	4	2			1	16			19
	5			1		4	1		5
	6	1		2		7			10
	7			1					1
	8			1		2			3
	9					2			2
	12	2	1			4			7
	72 (observations sessions)					1			1
	papers no information					4			4

Table S5. Number of papers of fish assemblage streams per journals found in this study

Journals	Papers
National journals	
Neotropical Ichthyology	23
Biota Neotropica	16
Check List	7
Revista Brasileira de Zoologia	5
Brazilian Journal of Biology	4
Acta Limnologica Brasiliensia	2
Acta Scientiarum	1
Iheringia	1
Interciência	1
Studies on Neotropical Fauna and Environment	1
Zoologia	1
International journals	
Hydrobiologia	10
Community Ecology	2
Acta Ichthyologica et Piscatoria	1
Applied Ecology and Environmental Research	1
Environmental Biology of Fishes	1

Environmental Management	1
Environmental Monitoring and Assessment	1
Fisheries	1
Freshwater Biology	1
Ichthyological Exploration of Freshwaters	1
International Review of Hydrobiology	1
Journal of Applied Ichthyology	1
Landscape and Urban Planning	1
Limnologica	1
Urban Ecosystems	1
ZooKeys	1

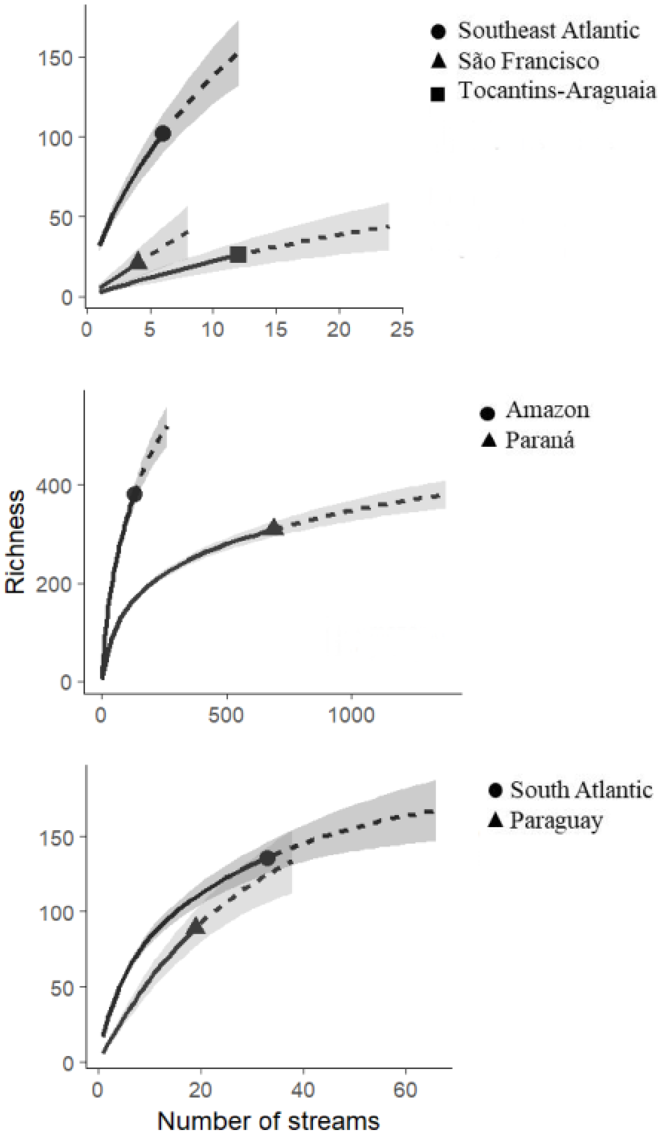

Figure S1. Rarefaction (solid) and extrapolation (dashed) curves from sampled streams of each basin found in this study. The curves were generated used species richness to Hill numbers for sampling-unit-based incidence data with doubly-bounded 95% confidence intervals. For the above results we considered the original taxonomic identification describe in the papers. The endpoint =double of reference sample size to each basin

Electronic Supplementary Material 2

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ARTIGO 2

COEXISTENCE OF NATIVE AND NON-NATIVE FISH SPECIES IN MEGADIVERSE STREAMS

Escrito em formato de artigo científico, de acordo com as normas do periódico científico Biological Invasions (versão preliminar).

Abstract

The introduction of non-native fish species is considered one of the greatest threats of freshwater biota. Brazil is has more than 3.300 freshwater fish species and a significant number occur in streams which are particularly vulnerable to invasion due the complex patterns of species endemism and rarity. The relationship between establishment of non-native species and native diversity and functionality has been debated and studies shown contrasting results. Moreover, few studies have been conducted in freshwater systems of Neotropic region. Here, we examined the presence of 20 non-native species in streams of the second largest basin in Brazil and evaluated their effect-size on fish native richness and functional diversity of assemblage. We used data of fish assemblages of 352 sampled streams in Paraná basin, which were available in 37 empirical papers published from 2004 to 2016. Our findings show that non-native fish species are coexisting with native species at higher native richness streams in Paraná basin. We also found that the greater functional diversity in streams with non-native species was explained by the richness of native species rather than the richness of non-native species, corroborating with the clear results of niche overlap between native and non-native fish species. These results imply that area with higher native fish species at Paraná basin could be focus of management and conservation efforts.

Keywords: invasion ecology; freshwater; meta-analysis; functional diversity; niche overlap

1. Introduction

Biological invasions represent a major component of global environmental change (Pyšek and Richardson, 2010) which included restructuring populations, compositions changes of communities, alterations of ecosystems process and loss of biodiversity (Simberloff and Rejmánek, 2011; Mollot et al., 2017). According to IUCN (2019), 8,000 species are threatened by invasive non-native species around the world. Once established, non-native species could affect biodiversity and community functionality through competition, predation, diseases, changes in food webs, hybridization, habitat alteration (i.e. modification of foraging and spawning habitats), biotic homogenization and nutrient cycling (Alvarez and Ward, 2019; Gallardo et al., 2015; Baiser, et al., 2012; Ehrenfeld et al., 2010; Bampfylde and Lewis, 2007; Gozlan et al., 2005; Tejerina-Garro et al., 2005).

The relationship between establishment of non-native species and native diversity has been debated and related to spatial scale of investigation (Fridley et al., 2007; Santos & Gomes, 2018; Peng et al., 2019; Tomasetto et al., 2019). In general, patterns of negative correlation of native and non-native species richness have often documented in studies on local scale (Stachowicz et al., 2002; Levine et al., 2004; Herben et al., 2004). In contrast, studies conducted at large scales have been reported the opposite pattern of positive correlation between native and non-native species richness (Fridley et al., 2004; Davis et al., 2005; Brummer et al., 2016). One of hypothesis that is thought to govern the positive native and non-native richness relation at broad-scales is theory of biotic acceptance (Stohlgren et al., 2006). The theory suggests that this positive pattern is result of similar responses of native and non-native species to environmental factors (Stohlgren et al. 2003). Thus, if sites are “good” environmental conditions for native species (e.g. available resources) it can also be good for non-native species (Gilbert & Lechowicz, 2005).

In addition to species richness, an increasing number of studies have pointed out functional diversity (FD) as an important component of biodiversity, allowing a more complet approach to understand ecosystems process and ecological interactions (Mouillot et al., 2011; Cadotte et al., 2011; Laureto et al., 2015). FD is a measurement of the range of biological traits of organisms, and therefore the functions of organisms in a given ecosystem (Petchey and Gaston, 2002; Violle et al., 2007). In invasion ecology, "limiting similarity" hypothesis predicts that successful non-native species are functionally dissimilar from the species already present in the community, so the coexisting species undergo minimal competition possibility (Abrams, 1983; Hiero et al., 2005; Catford et al., 2009). Thus, the establishment of non-native species is expected to cause traits overdispersion and increase overall assemblage FD (Catford et al., 2009; Matsuzaki et al., 2013).

These concepts and hypothesis of invasion biology have been developed and evaluated on the temperate regions (Moyle and Light, 1996; Olden et al., 2006; Mitchell and Knouft, 2009), mainly for plant community (Levine, 2000; Naem et al., 2000; Sax, 2002; Hierro et al., 2005; Emery et al. 2007). However, few studies have been conducted in freshwater systems of megadiverse neotropics regions, such as Brazil (Frehse et al., 2016; Santos and Gomes, 2018).

Brazil has extraordinary freshwater fish fauna with more than 3,300 species (Froese and Pauly, 2016) and a significant number occur in streams (Castro et al., 1999) which are particularly vulnerable ecosystem to invasion due the complex patterns of species endemism and rarity (Meyer et al., 2007). Non-native fish species in Brazil are a huge threat (Pelicice et al., 2014) and most introductions are intentional for improving aquaculture, enhancing sport fishing, stocking reservoirs and ornamental fish or even biological control projects (Alves et al., 2007; Pelicice et al., 2016). Here, we examined the presence of 20 non-native species in streams of Paraná river basin and evaluated their effect on fish assemblages. Paraná river basin is the second largest Brazilian river basin (879.860 km²) and the most populous drainage of the country (PNRH-DBR, 2005). Paraná basin streams have been highly impacted by human disturbance, suffering drastic alterations on fish communities (Casatti et al., 2009; Teresa et al., 2015; Santos et al., 2015), but also have less impacted areas (Pompeu et al., 2009).

First, we tested whether streams with non-native fish species and without differ in native fish species richness. Since our analysis involved a large scale, we hypothesize that streams with presence of non-native fish species have in average more richness of native species than non-invaded streams. Still, we expected that this relation is positively influenced by richness of non-native fish. Second, we examined the effect of the presence of non-native fish species on overall fish assemblages FD. Based on the "limiting similarity" theory, we expect that in average the overall FD will be higher in streams with non-native species and that such increase will be proportional to richness of these species. Studies suggest that native freshwater fish assemblages are shaped primarily by the niche partitioning process (Mason et al., 2008, Comte et al., 2016), therefore we also hypothesize lower niche overlap between native and non-native species on invaded streams, corroborating with the "limiting similarity".

2. Materials and Methods

2.1 Database

We used data of fish assemblages of 352 sampled streams in Paraná basin, which were available in 37 empirical papers published from 2004 to 2016. This database was originally assembled to address trends in sampling on published research of stream fish assemblages in Brazil (spatial distribution of surveys, sampling methods used and level of sampling effort) (see Supplementary material). The database provided information of river basin (ANA, 2017), geographic coordinates of sampling sites, number of streams and fish species list.

The first steps was select surveys of Paraná basin in the database and then remove duplicates from papers with the same sampled site (priori from more recent papers). Second, we included only papers with fish species data extracted by sampling site (Figure 1). The taxonomic classification of species was update through Eschmeyer's Catalog of Fishes (Fricke et al., 2018). We only considered fish species that have a current valid scientific name and unidentified species were excluded (i.e. *Astyanax* sp., *Bryconamericus* sp., *Characidium* sp., *Trichomycterus* sp.). Non-native species were regarded as those with established reproducing populations registered outside their natural range follows Buckup et al. (2007), FishBase (Froese & Pauly, 2011), Eschmeyer's Catalog of Fishes (Fricke et al., 2018), Ota et al. (2018) Finally, we categorized each sampled site in Paraná sub-basins according to ANA (2017).

The total of 149 streams have the presence of non-native species (n=20 species) (Table 1) and 203 streams have only native species (n=182) (see Suplementary material).

2.2 Traits collection

For each species we recorded five functional traits classes: body size, trophic guilds, migratory behavior, fecundation and parental care. These ecological attributes are most commonly available and were obtained by specific literature (Supplementary material). The traits classes were chosen because they represent well-known characteristics of habitat use, feeding and life history strategies which are commonly used in assessment of fish functional diversity (Table 2) (Goldstein and Meador, 2004; Ibañez et al., 2007; Pease et al., 2012).

2.3 Statistical analyses

2.3.1 Functional diversity

Functional diversity was assessed with the functional richness index (FRic) for each sampling site (Villeger et al., 2008). FRic reflects functional trait-space occupation by each local fish assemblage and are independent of specie richness (Villeger et al., 2008). This multidimensional measure was estimated using the convex hull volume proposed by Cornwell et al. (2006). According this method, the convex hull algorithm determines the most extreme points and links them to build the convex hull for calculates the volume inside (Cornwell et al., 2006; Villeger et al. 2008). For these, we created one matrix of presence-absence for each stream and a second matrix of functional traits. For the second matrix, we used Gower's general coefficient of distance to transformer in a matrix of distance among streams. The Gower´s (1971) measure is indicates for mixed variables (numeric, ordered or factor) and standardized all the traits by the range. For these analyses, we used the *FD* package (Laliberté & Legendre, 2010).

2.3.2 Meta-analysis

We used the standardized difference between control (streams with non-native fish species) and treatment (streams without non-native fish species) groups to quantify and summarize the effect sizes of the presence of non-native fish species on richness of native assemblages and functional diversity of streams. We calculated mean, standard deviation and sample size (number of stream) for each invaded (treatment group) and un-invaded (control group) assemblages for each sub-basin. For each pair of treatment and control assemblages per sub-basin, we calculated *Hedges'd* as for estimates the magnitude of effect and its direction, according to: $d=(X_t - X_c/S)*J$, where X_t is the response of the treatment group, X_c is the response of the control group, S is the pooled standard deviation and J a weighting factor based on the number of replicates per treatment to remove small sample size bias (Rosenberg et al., 2000). These analyses were performed using the "*escalc*" function in the "*metafor*" package (Viechthbauer 2010).

After measuring effect size we have employed mixed-effects model to explaining the data heterogeneity by moderators. By doing it, we were interested in the degree to which effect size on richness and functional diversity may be influenced by. We have developed two models: first model assessed whether richness of non-native fish is potentially predictor of the effect size on richness native assemblages; and second model attempted whether richness of native and non-native species are predictor on functional diversity. The model selections were performed using "*rma.glmulti*" function in the "*metafor*" package (Viechthbauer 2010). We used maximum likelihood estimation to test and compare all possible combinations of the variables and null model. The best models were determined by an information-theoretical approach based on the Akaike Information Criterion of Second Order (AICc) and it is indicated by the lowest AICc or delta ≤ 2 (Burnham & Anderson, 2002). According the authors, this estimator is indicated to remove small sample bias. If model selection pointed out null model as the best then fixed-effect model was built instead of random model. We choose evaluated fixed-effect model because it makes a conditional inference only about the streams included in the analysis, providing perfectly valid inferences of heterogeneity to the set of our data (Hedges and Vevea, 1998). For the fixed-effect model we calculated the weighted mean effect size ($d+$) of all sub-basins and their respective 95% bootstrap-confidence intervals. Confidence intervals that did not overlap zero were considered significant (Rosenberg et al. 2000). Positive and negative values of $d+$ indicate a general trend of positive and negative effects of the presence of non-native fish on the variables measures, respectively. According to Cohen (1992), d+ values around 0.2 are considered weak effects, 0.5 are considered moderate effects, and 0.8 are considered strong effects. We also employed Q test to detect heterogeneity of effects sizes among sub-basins (Q) and used X^2 distribution to evaluate the significance. Significant Q indicated the variance among effects sizes differ by more than sampling error, such as characteristics of the samples, variations in the designs quality and variations of treatments (Hunter and Schmidt, 2000; Brockwell and Gordon, 2001).

Models where moderators were significant explaining the heterogeneity were analyzed applying "*rma.uni*" function. Moderators test (i.e., QM, Omnibus test) was also applied to reject null hypotheses pointing the moderators' significance. Finally, we predict average relative risks for the increases of the absolutes moderator values and their 95% confidence intervals through "*predict*" function. All functions are contained in the "*metafor*" package (Viechthbauer 2010).

Sampling bias were assessed creating funnel plots (Sterne and Egger, 2001) of the standard error of the log ratio of means versus the log ratio means effect size to models without moderators and versus residuals to models involving moderators. In the absence of sampling bias we would expect the points falling inside de pseudo-confidence region, forming a funnel shape (Sterne and Egger, 2001). We used the "*funnel*" function in the "*metafor*" package (Viechthbauer 2010).

2.3.3 Niche overlap

For each functional sub-trait we calculated niche overlap between native and non-native species following the kernel distributions estimators of Mouillot et al. (2005). For this, we used species richness of each functional sub-trait as character measurement for each sampled stream. These non-parametric method estimates a density distribution to each individual measurement using a kernel function, which take into account the value of character measurement, the number of data points (number of streams) and the bandwidth (defined as 1·06σn–1/5 with σ being standard deviation). After, species density at any character was calculated as the sum of kernel density of each data point. The niche overlap is calculated as the area between the resulting species density and ranges from 0 to 1. All the analyses were performed in R program (R Core Team 2017).

3. Results

Overall, the presence of non-native fish species had a moderate positive effect size on native fish richness (d+ = 0.47, CI = 0.35- 0.6, df = 5) and varied from significant and positive to non-significant among the basins (Figure 2). This means that streams with presence of non-native fish species in Paraná, Paranaíba and Grande sub-basins have in average more richness of native species than non-invaded stream. Also, the magnitudes of these effects differ significantly among the sub-basins (Q= 12.54, p = 0.03).

The effect of presence of non-native species on functional diversity also varied from positive to nonsignificant among the basins (Figure 3). However, these results were positively influenced by the richness of native species (z_teste = 4.04, $p < 0.0001$) and suggested native richness as principal source of heterogeneity among these effects (QM = 16.34, $p < 0.0001$), contradicting our hypothesis. Our prediction showed increasingly larger effects of presence of non-native species on functional diversity in sub-basins more richness (Figure 4). These effect is twice as large for native assemblages with 30 species, four times larger and six times larger for assemblages with 60 and 80 species, respectively (Supplementary material; Table 3). The evidence of coexistence between native and non-native fish species on streams was reinforcing through results of higher niche overlap for all functional sub-traits varying from 0.8 to 0.96 (Figure 5).

We did not observe marked indication of sampling bias (Supplementary material; Figure 1). The exception was Iguaçu basin data for fixed-effect model of presence of non-native fish species on native species richness.

4. Discussion

Our findings showed that non-native fish species are coexisting with native species at higher native richness streams in Paraná basin. We also found that there are differences of functional diversity between streams with and without non-native species in half of the sub-basins. This was explained, in part, by the richness of native species rather than the richness of non-native species, corroborating with the clear results of niche overlap between native and non-native fish species. These results imply that area with higher native fish species at Paraná basin could be focus of management and conservation efforts.

The results showed that presence of non-native fish species are positively related to native species richness, suggesting that biotic acceptance could occurs for fish in streams at Paraná basin. It implies that areas with high native species richness indicate good habitat for non-native species (Stohlgren et al., 2003). In this way, it is expected that both native and non-native species respond similarly to a group of environmental factors (Stohlgren et al., 2006). Our finding suggests that native fish species do not competitively exclude non-native from successfully establishing (biotic resistance). Thus, our result is consistent with proposition of Moyle and Light (1996) that non-native species are mainly limited by their tolerance to abiotic conditions at invaded sites and that biotic resistance plays only a minor role in fish invasions. Therefore, at appropriated abiotic conditions non-native fish species will establish regardless of the already present biota (Ricciard and Mottiar, 2006). However, our study has important limitations due the unable to incorporate environmental data and this mechanism should be test in future researches. Moreover, the results of Iguaçu, Paranapanema and Tietê sub-basins probably were affected by the lower number of sampling sites and sampling bias at Tietê by the lower richness too. We believe that environmental data also would contribute to explain significantly differences of the magnitude of effect-sizes among the sub-basins, since the studied streams are within-unit with large range of conditions, various levels of landscape preservation and different land uses (high landscape preservation, Pompeu et al., 2009; pasture, Fagundes et al., 2015; agriculture, Ribeiro et al., 2016; urban, Cunico et al., 2012), reinforcing the importance to of environmental variables in studies of non-native species in fish streams at Paraná basin.

The greater richness of native fish species explained the greater functional diversity in streams with non-native species in tree sub-basins. In this context, regions with support higher pool of species allow that more functional strategies coexist and, in turn, increase functional diversity (Comte et al., 2016). However, we expected that non-native species richness would be functionally distinct from native species but the results contradicted or hyphothesis. These results and high niche overlap between native and non-native species support to rejecting “limiting similarity” hyphothesis. The level of functional redundancy among species can derive for the balance between limiting similarity versus niche filtering process (Mouillot et al., 2007). Following from this concept, coexisting non-native fish species more similar to native species in Paraná, Paranaíba and Grande sub-basins would be mainly driver by environmental factors acting as niche filters (Poff, 1997; see Pearson et al., 2018). Despite, there are some concepts about success of similar species at the same environment. Non-native species may present degrees of pre-adaptation to the local environment that allowing species invasion in sites with functional similar native species (Darwin, 1859; Duncan and Williams, 2002). Moreover, another niche dimensions might be contributing to accommodate non-native species functionally similar with native species. Studies of functional traits of fish require gathering difficult data and many of them rely only on categorical traits of functional guilds or groups (e.g. Ibañez et al., 2007; Matsuzaki et al., 2013) such as ours. However, measure broad quantitative traits such as morphological measures could characterize more multiple niche dimensions (Pease et al., 2012).

Although our results point out to a positive relationship between the presence of non-native species and native species, we agree that other important questions are important to evaluated and predict possible impacts of non-native fish species on assemblages. Here, we assume our data as static, since we do not analyzed temporal scale, also important variable in invasion ecology (Hastings, 1996; Richardson and Pysek, 2006; Sax et al., 2007). However, non-native species have different introductions histories and the time since introduction could be affects the dynamic of species niche (Comte et al., 2016) and, in turn, functional diversity of assemblages. Furthermore, interactions of coexisting species may cause changes in population size, even if richness remains unchanged (Gotelli and Arnett, 2000; Sagouis et al., 2015).

Although challenges remain, this study is an important step to understand the assemblage organizations of invaded streams in Neotropical region. Our found suggest that presence of native fish species generally indicates good habitat for non-native species with similar functionality. These results imply that area with higher native fish species at Paraná basin could be focus of fish invasions management and conservation efforts. We hope that in-depth testing will vet these questions and stimulate further advances. Thus, we have proposed the following outstanding questions: are environmental variables potentially predictor of the effect size of the non-native species on richness native assemblages? Streams with non-native fish species and without differ in native fish species abundance? Finally, are environmental factors acting as niche filters to non-native fish species in streams at Paraná basin?

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Data Availability Statement

The data that support the findings of this study are available from the corresponding author, NTJ.

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Tables

Table 1. List of non-native fish species from Paraná basin found in this study.

Classification
Characiformes
Characidium lagosantense
Erythrinus erythrinus
Hollandichthys multifasciatus
Hyphessobrycon boulengeri
Hyphessobrycon eques
Hyphessobrycon reticulatus
Odontostilbe microcephala
Cyprinodontiformes
Atlantirivulus santensis
Poecilia reticulata
Xiphophorus hellerii
Gymnotiformes
Gymnotus pantherinus
Perciformes
Coptodon rendalli
Oreochromis niloticus
Satanoperca pappaterra
Salmoniformes
Oncorhynchus mykiss
Siluriformes
Heptapterus mustelinus
Ictalurus punctatus
Imparfinis longicaudus
Parancistrus aurantiacus
Rhyacoglanis pulcher

Table 2. Functional traits and sub-traits of the fish species analyzed in this study and their respective descriptions.

Trait	Sub-trait	Description
Body size	maximum body length	Maximum length of the fish, from the tip of the snout to the posterior end of the least vertebra or to the posterior end to the midlateral portion of the hypural plate.
Trophic guild	piscivorous	Species that feed predominantly on fish.
	detritivorous	Species that feed predominantly on detritus.
	omnivorous	Species that feed predominantly on animal and vegetal matter.
Fecundation	external fecundation	Species with external fertilization of eggs.
	internal fecundation	Species that fertilize eggs internally.
Parental care	without parental care	Species without parental care. It is common higher production of gametes.
	with parental care	Species with parental care (i.e. built and guard nest, attached eggs to the body, care of independently swimming young's).
Migratory	long distance migrants	Species that require long migrations to spawn.

Figures

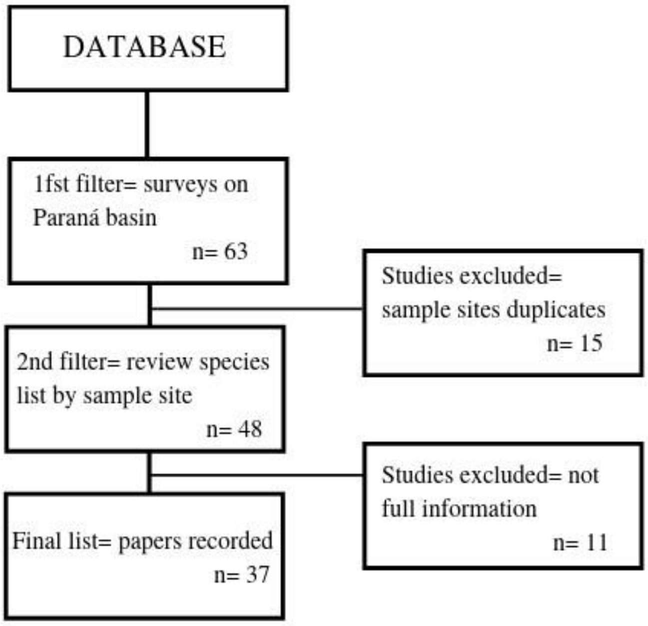

Figure 1. Flow chart detailing the process of database selection and the number of studies from each stage of the review

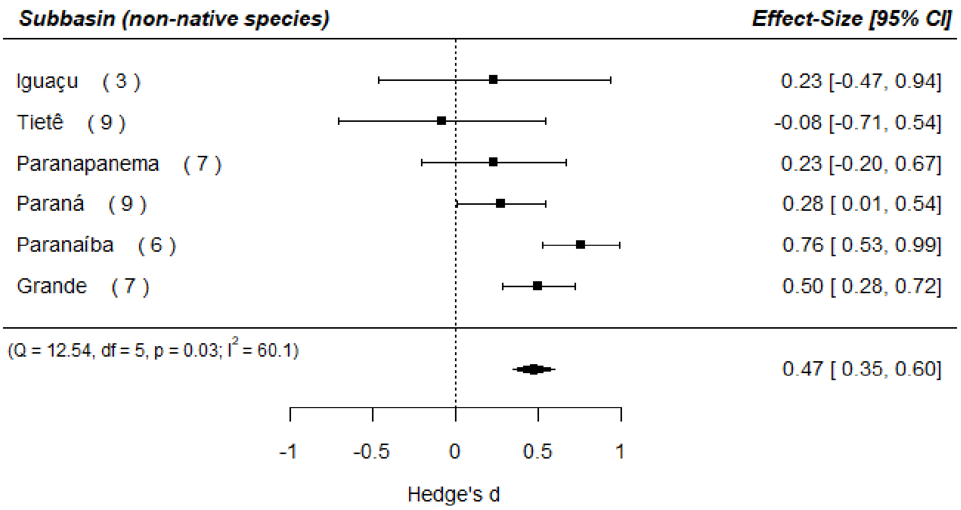

Figure 2. Effects-sizes of presence of non-native fish species on native fish richness. The effect size of each sub-basin and the cumulative effect size are reported with its 95% confidence intervals. Effects are significant if confidence intervals do not overlap with zero.

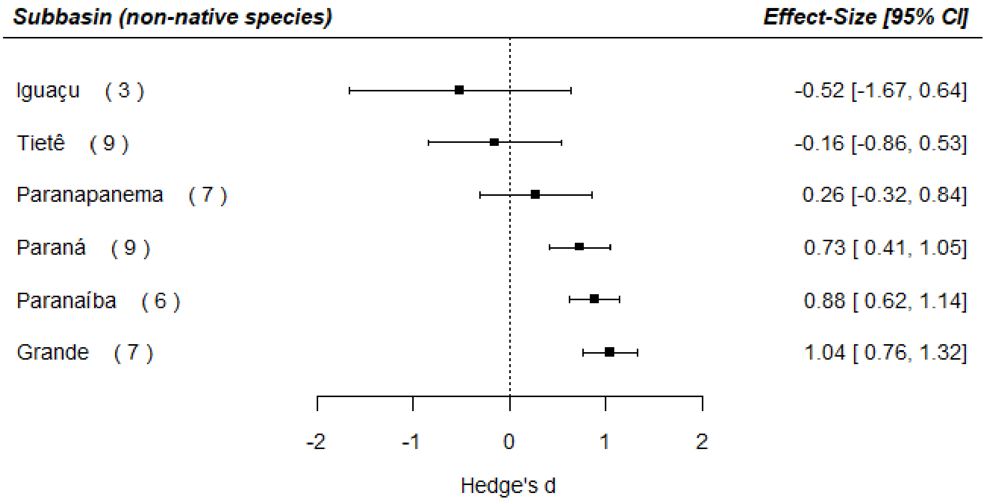

Figure 3. Effects of presence of non-native fish species on FD of overall assemblage. The effect size of each sub-basin is reported with its 95% confidence intervals. Effects are significant if confidence intervals do not overlap with zero

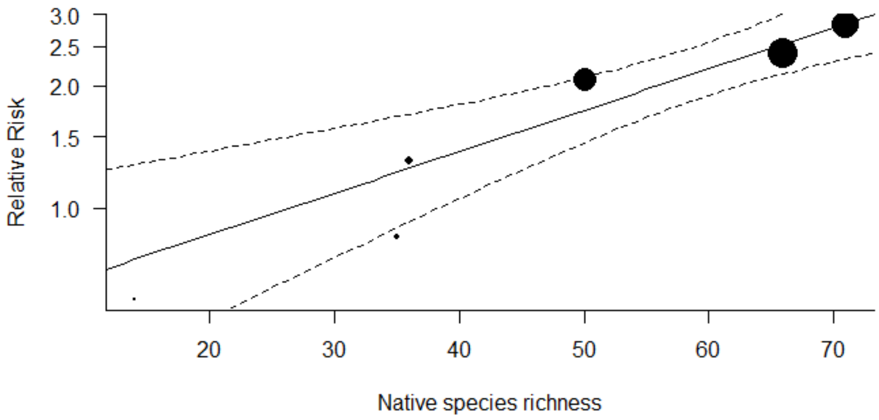

Figure 4. Relative risk of effect of presence of non-native fish species on functional diversity of overall assemblage versus native fish species richness. The predict effects on each sub-basin is represented by spheres with corresponding confidence intervals bounds.

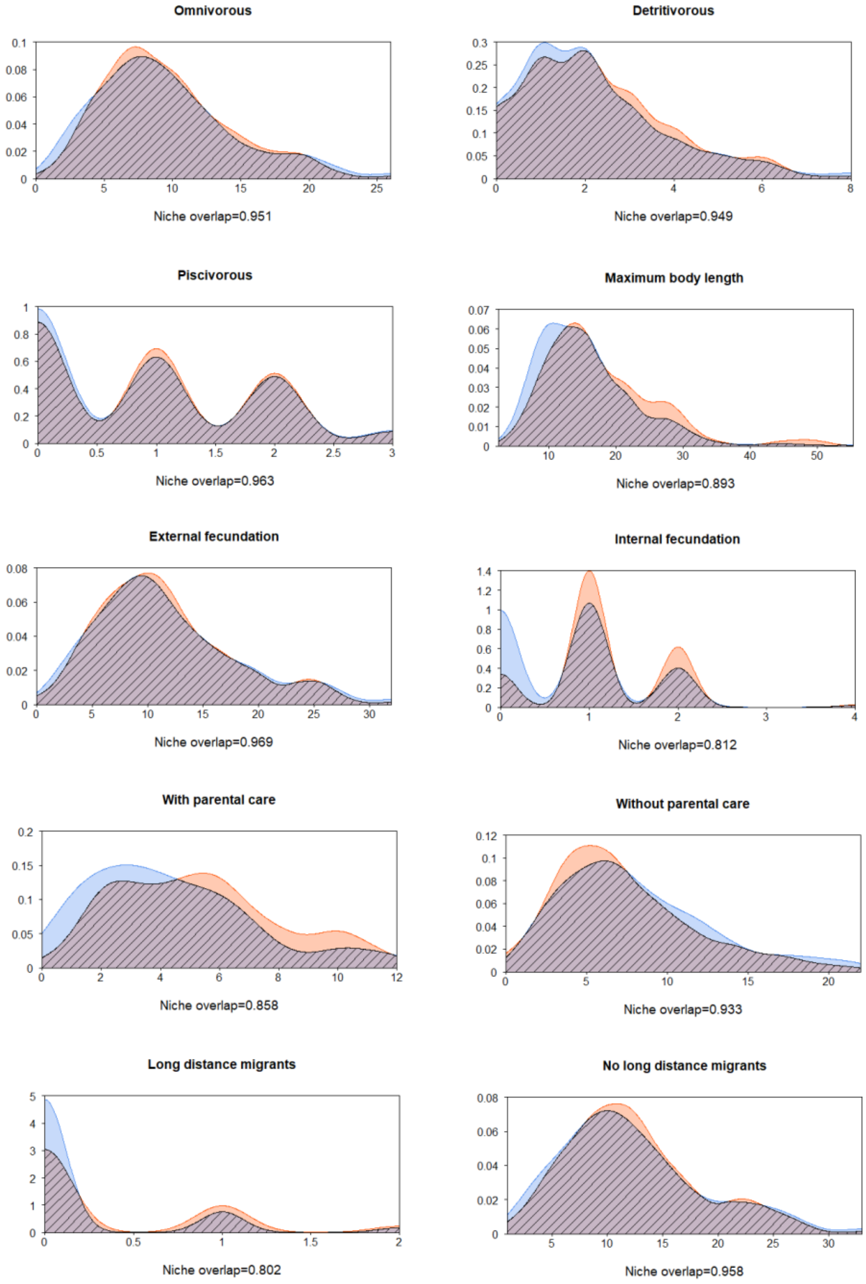

Figure 5. Niche overlap between native (blue) and non-native fish (red) richness on invaded streams analyzed in this study, for each functional sub-trait. X axis= density of each functional trait; Y axis= ln (richness species of each sub-trait per stream)/ ln(total native or non-native richness per stream).

Supplementary material 1

Publication selection and data extraction of database

We searched for potential papers in three online academic databases: ISI Web of Knowledge, Scielo and Scopus using the terms *fish* AND *stream* AND *Brazil*. Searches were conducted from January to July 2017 and we considered studies published until December 2016.

The first step was to examine titles and abstracts to include only fish assemblage papers with fish species occurrence data. Papers involving only single species or populations were excluded. Second, we conducted a full-text inspection of sampling design to include only papers related to sampling in wadeable (first to third order) streams. Some papers encompassing other aquatic environments (e.g. rivers, lakes, reservoirs and floodplains), but also wadeable streams were also considered when it was possible extract fish species data by sampling site. Third, we removed duplicates from the three academic databases used. Finally, when a relevant paper lacked information (coordinates and/or fish species list), we contacted authors directly to request the missing data.

Tables

Table 1. Number of sample streams and richness by sub-basins of Paraná river basin select on the database in this study. The number in parenthesis is the streams with presence of non-native species.

Sub-basin	Stream	Native richness	Non-native richness
Grande	129 (46)	81	7
Iguaçu	6 (2)	18	3
Paraná	67 (44)	80	9
Paranaíba	110 (31)	89	6
Paranapanema	22 (16)	56	7
Tietê	17 (10)	60	9

Table 2. List of fish species presence-absence data in streams of Paraná basin found in this study. S= maximum body length (cm); TG= trophic guild; F= fecundation; PC= parental care; M= long distance migrants; * = non-native species

Species	S[1]	TG[2]	F[3]	PC[3]	M[3]
Acestrorhynchus lacustris	27	piscivorous	external	without	0
Ancistrus mullerae	12.5	detritivorous	external	with	0
Apareiodon affinis	17	detritivorous	external	without	0
Apareiodon ibitiensis	11.3	detritivorous	external	without	0
Apareiodon piracicabae	12	detritivorous	external	without	0
Aphyocharax dentatus	7.2	omnivorous	external	without	0
Apteronotus albifrons	50	omnivorous	external	without	0
Aspidoras fuscoguttatus	3.8	omnivorous	external	without	0
Aspidoras lakoi	4	omnivorous	external	without	0
Astyanax altiparanae	7.9	omnivorous	external	without	0
Astyanax bockmanni	6.72	omnivorous	external	without	0
Astyanax eigenmanniorum	7.71	omnivorous	external	without	0
Astyanax fasciatus	6.5	omnivorous	external	without	0
Astyanax lacustris	6.69	omnivorous	external	without	0
Astyanax paranae	11.3	omnivorous	external	without	0
Astyanax rivularis	9.9	omnivorous	external	without	0

Astyanax scabripinnis	7.8	omnivorous	external	without	0
Astyanax schubarti	9	omnivorous	external	without	0
Astyanax totae	8.2	omnivorous	external	without	0
*Atlantirivulus santensis**	4.7	omnivorous	external	without	0
Australoheros facetus	19.3	omnivorous	external	without	0
Brachyhypopomus pinnicaudatus	18.6	omnivorous	external	without	0
Brycon orbignyanus	27	omnivorous	external	without	1
Bryconamericus iheringi	7.3	omnivorous	external	without	0
Bryconamericus stramineus	7	omnivorous	external	without	0
Bryconamericus turiuba	6.1	omnivorous	external	without	0
Callichthys callichthys	17	omnivorous	external	with	0
Cetopsis gobioides	10.9	omnivorous	external	without	0
Cetopsorhamdia iheringi	11.1	omnivorous	external	without	0
Characidium fasciatum	6.7	omnivorous	external	without	0
Characidium gomesi	6.5	omnivorous	external	without	0
Characidium oiticicai	6.8	omnivorous	external	without	0
Characidium xanthopterum	4.7	omnivorous	external	without	0
Characidium zebra	7.4	omnivorous	external	without	0
*Characidium lagosantense**	4.1	omnivorous	external	without	0
Cichlasoma paranaense	7.8	omnivorous	external	with	0
*Coptodon rendalli**	31	omnivorous	external	with	0
Corumbataia cuestae	3.3	detritivorous	external	without	0
Corydoras aeneus	7.5	omnivorous	external	without	0
Corydoras ehrhardti	4.7	omnivorous	external	without	0
Corydoras difluviatilis	4.7	omnivorous	external	without	0
Corydoras flaveolus	3.8	omnivorous	external	without	0
Corydoras paleatus	6.6	omnivorous	external	without	0
Crenicichla britskii	14.5	omnivorous	external	with	0
Crenicichla jaguarensis	14.8	omnivorous	external	with	0
Crenicichla niederleinii	23.5	omnivorous	external	with	0
Curculionichthys insperatus	3	detritivorous	external	with	0
Curculionichthys piracanjuba	2.7	detritivorous	external	with	0
Cyphocharax modestus	16.2	detritivorous	external	without	0
Cyphocharax gillii	10	detritivorous	external	without	0

Cyphocharax vanderi	6.8	detritivorous	external	without	0
Eigenmannia trilineata	25	omnivorous	external	without	0
Eigenmannia virescens	44	omnivorous	external	without	0
*Erythrinus erythrinus**	20	piscivorous	external	with	0
Farlowella amazonum	22.5	detritivorous	external	with	0
Galeocharax knerii	33.1	piscivorous	external	without	0
Geophagus brasiliensis	14.8	omnivorous	external	with	0
Glandulocauda melanopleura	5.5	omnivorous	internal	with	0
Gymnotus carapo	76	omnivorous	external	with	0
Gymnotus inaequilabiatus	60	omnivorous	external	with	0
Gymnotus pantanal	25.1	omnivorous	external	with	0
*Gymnotus pantherinus**	23.6	omnivorous	external	with	0
Gymnotus paraguensis	24	omnivorous	external	with	0
Gymnotus sylvius	36	omnivorous	external	with	0
Hemigrammus marginatus	4.5	omnivorous	external	without	0
Hemigrammus parana	3.1	omnivorous	external	without	0
*Heptapterus mustelinus**	26	omnivorous	external	without	0
Hisonotus depressicauda	5	detritivorous	external	with	0
Hisonotus francirochai	3.6	detritivorous	external	without	0
*Hollandichthys multifasciatus**	9.6	omnivorous	internal	with	0
Hoplerythrinus unitaeniatus	15	piscivorous	external	with	0
Hoplias intermedius	16.9	piscivorous	external	with	0
Hoplias malabaricus	49	piscivorous	external	with	0
Hoplosternum littorale	15.8	omnivorous	external	without	0
Hyphessobrycon anisitsi	6	omnivorous	external	without	0
Hyphessobrycon balbus	4.63	omnivorous	external	without	0
Hyphessobrycon bifasciatus	4.2	omnivorous	external	without	0
*Hyphessobrycon boulengeri**	2.6	omnivorous	external	without	0
Hyphessobrycon eques	4	omnivorous	external	without	0
*Hyphessobrycon reticulatus**	5.3	omnivorous	external	without	0
Hypostomus albopunctatus	40	detritivorous	external	with	0
Hypostomus ancistroides	14	detritivorous	external	with	0
Hypostomus cochliodon	23	detritivorous	external	with	0
Hypostomus derbyi	31	detritivorous	external	with	0

Hypostomus hermanni	20.18	detritivorous	external	with	0
Hypostomus iheringii	11.6	detritivorous	external	with	0
Hypostomus margaritifer	25.5	detritivorous	external	with	0
Hypostomus myersi	20.7	detritivorous	external	with	0
Hypostomus nigromaculatus	10.2	detritivorous	external	with	0
Hypostomus regain	41	detritivorous	external	with	0
Hypostomus strigaticeps	16	detritivorous	external	with	0
Hypostomus tietensis	12.5	detritivorous	external	with	0
*Ictalurus punctatus**	57	omnivorous	external	with	0
Imparfinis borodini	15.7	omnivorous	external	without	0
*Imparfinis longicaudus**	10.4	omnivorous	external	without	0
Imparfinis mirini	8.5	omnivorous	external	without	0
Imparfinis schubarti	9.3	omnivorous	external	without	0
Isbrueckerichthys calvus	9	detritivorous	external	with	0
Jenynsia eigenmanni	6.5	omnivorous	internal	with	0
Knodus moenkhausii	4.8	omnivorous	external	without	0
Laetacara araguaiae	4.2	omnivorous	external	with	0
Leporinus elongates	19	omnivorous	external	without	1
Leporinus friderici	40	omnivorous	external	without	0
Leporinus lacustris	11.1	omnivorous	external	without	0
Leporinus microphthalmus	11.8	omnivorous	external	without	0
Leporinus obtusidens	40	omnivorous	external	without	1
Leporinus octofasciatus	23.5	omnivorous	external	without	0
Leporinus paranensis	16	omnivorous	external	without	0
Leporinus piavussu	38	omnivorous	external	without	0
Leporinus striatus	7.37	omnivorous	external	without	0
Lepthoplosternum pectorale	6	omnivorous	external	without	0
Melanorivulus apiamici	3	omnivorous	external	without	0
Melanorivulus pictus	2.6	omnivorous	external	without	0
Microlepidogaster arachas	4.3	detritivorous	external	with	0
Microlepidogaster longicolla	4.6	detritivorous	external	with	0
Moenkhausia bonita	4.4	omnivorous	external	without	0
Moenkhausia intermedia	8	omnivorous	external	without	0
Moenkhausia sanctaefilomenae	7	omnivorous	external	without	0

Neoplecostomus corumba	7.8	omnivorous	external	with	0
Neoplecostomus paranensis	9.3	omnivorous	external	with	0
*Odontostilbe microcephala**	4.6	omnivorous	external	without	0
Oligosarcus paranensis	20.5	piscivorous	external	without	0
Oligosarcus pintoi	8.8	piscivorous	external	without	0
Oligosarcus planaltinae	9.9	piscivorous	external	without	0
*Oncorhynchus mykiss**	56	omnivorous	external	without	1
*Oreochromis niloticus**	60	detritivorous	external	with	1
Ossancora eigenmanni	15.2	omnivorous	external	without	0
Otothyropsis polyodon	3.7	detritivorous	external	with	0
Pamphorichthys hollandi	2.1	omnivorous	internal	with	0
*Parancistrus aurantiacus**	19.3	detritivorous	external	with	0
Pareiorhina carrancas	4.1	detritivorous	external	with	0
Parodon nasus	12.7	detritivorous	external	without	0
Phalloceros caudimaculatus	6.5	omnivorous	internal	with	0
Phalloceros harpagos	3.4	omnivorous	internal	with	0
Phalloceros reisi	2.7	omnivorous	internal	with	0
Phallotorynus jucundus	3	omnivorous	internal	with	0
Phenacorhamdia unifasciata	6.2	omnivorous	external	without	0
Phenacorhamdia hoehnei	3.8	omnivorous	external	without	0
Phenacorhamdia tenebrosa	6.7	omnivorous	external	without	0
Piabina anhembi	6.52	omnivorous	external	without	0
Piabina argentea	7.3	omnivorous	external	without	0
Pimelodella avanhandavae	12.5	omnivorous	external	without	0
Pimelodella gracilis	18	omnivorous	external	without	0
Pimelodella meeki	10	omnivorous	external	without	0
Pimelodus maculatus	36	omnivorous	external	without	1
Planaltina britskii	3.5	omnivorous	internal	without	0
Planaltina myersi	4.6	omnivorous	internal	without	0
*Poecilia reticulate**	5	omnivorous	internal	with	0
Prochilodus lineatus	49.52	detritivorous	external	without	1
Pseudopimelodus mangurus	34.5	omnivorous	external	with	0
Pseudotocinclus tietensis	6	detritivorous	external	with	0
Pyrrhulina australis	5	omnivorous	external	without	0

Rhamdia quelen	16.32	omnivorous	external	without	0
Rhamdiopsis microcephala	7.8	omnivorous	external	without	0
*Rhyacoglanis pulcher**	6.19	omnivorous	external	with	0
Rineloricaria latirostris	23.3	detritivorous	external	with	0
Rineloricaria pentamaculata	12.29	detritivorous	external	without	0
Roeboides descalvadensis	8.9	omnivorous	external	without	0
Salminus hilarii	50	piscivorous	external	without	1
*Satanoperca pappaterra**	17.4	detritivorous	external	with	0
Schizodon borellii	20.75	omnivorous	external	without	0
Serrapinnus heterodon	4.1	omnivorous	external	without	0
Serrapinnus notomelas	3.56	omnivorous	external	without	0
Serrasalmus maculatus	15.72	piscivorous	external	with	0
Serrasalmus marginatus	22.1	piscivorous	external	with	0
Steindachnerina brevipinna	10.9	detritivorous	external	without	0
Steindachnerina insculpta	10.6	detritivorous	external	without	0
Sternopygus macrurus	140	omnivorous	external	without	0
Synbranchus marmoratus	150	omnivorous	external	with	0
Tatia neivai	8.2	omnivorous	internal	with	0
Trachelyopterus galeatus	22	omnivorous	internal	with	0
Trichomycterus brasiliensis	13.5	omnivorous	external	without	0
Trichomycterus candidus	7.5	omnivorous	external	without	0
Trichomycterus castroi	14.8	omnivorous	external	without	0
Trichomycterus davisi	6.3	omnivorous	external	without	0
Trichomycterus diabolus	6.1	omnivorous	external	without	0
Trichomycterus iheringi	16.1	omnivorous	external	without	0
Trichomycterus itatiayae	6.7	omnivorous	external	without	0
Trichomycterus maracaya	5.1	omnivorous	external	without	0
Trichomycterus paolence	6.8	omnivorous	external	without	0
Triportheus nematurus	18.3	omnivorous	external	without	0
*Xiphophorus hellerii**	8.5	omnivorous	internal	without	0

[1] www.fishbase.org; Costa (2008); Graça and Pavanelli (2007). Richer-de-Forges et al. (2009); Rolla et al. (2009); Shibatta and Vari (2017); Lucena and Soares (2016); Weyl and Hecht (1998); Aquino and Couto (2016); Lima et al. (2017); Jerep et al. (2007); Birindelli and Britski (2009); Shah et al. (2011); Slobodian et al. (2017); Rodríguez and Miquelarena

(2005); Nascimiento et al. (2014); Oyakawa and Mattox (2009); Ota et al. (2018); Vera-Alcaraz et al. (2012); Zawadzki et al. (2008).

[2] www.fishbase.org; Abilhoa and Grein (2011); Abilhoa (2007); Abilhoa et al. (2009); Lima et al. (2008); Aranha et al. (1998); Aranha et al. (2000); Azevedo et al. (2016); Barreto and Aranha (2006); Barreto et al. (2013); Bastos et al. (2011); Bennemann et al. (2005); Braga et al. (2008); Brandão-Gonçalves et al. (2009); Buck and Sazima (1995); Carvalho et al. (2017); Casatti, L. (2002); Casatti et al. (2009); Casatti et al. (2001); Costa-Pereira and Resende (2012); Delariva et al. (2013); Durães et al. (2001); Ferreira et al. (2012); Fragoso-Moura et al. (2017); Giora et al. (2005); Graça and Pavanelli (2007). Rondineli et al. (2011); Hahn and Cunha (2005); Niloticus, (2014); Kintopp and Abilhoa (2009); Mazzoni et al. (2010); Py-Daniel et al. (2005); Rondineli et al. (2011); Severo-Neto et al. (2015); Shelton et al. (2017); Silva et al. (2012); Souza Filho and Casatti (2010); Uieda et al. (1997).

[3] www.fishbase.org; Agostinho et al. (2003); Agostinho et al. (2004); Crampton et al. (2016); Graça and Pavanelli (2007).

Table 3. Functional richness index (FRic) for each stream with their respective total richness and treatment (n= only native species; nn= presence of non-native species).

Stream	Richness	Fric	Treatment
s1	2	NA	n
s2	7	0.003	n
s3	11	0.572	nn
s4	24	0.816	nn
s5	8	0.503	nn
s6	6	0.503	nn
s7	26	0.813	nn
s8	21	0.785	nn
s9	6	0.001	n
s10	10	0.177	n
s11	10	0.335	nn
s12	23	0.581	nn
s13	28	0.815	nn
s14	2	NA	n
s15	18	0.791	nn
s16	9	0.130	nn
s17	1	NA	nn
s18	9	0.472	nn

s19	4	0.007	n
s20	1	NA	n
s21	14	0.794	nn
s22	4	0.504	n
s23	4	0.007	n
s24	14	0.648	nn
s25	3	0.123	nn
s26	1	NA	n
s27	2	NA	n
s28	6	0.504	nn
s29	5	0.006	n
s30	4	0.002	n
s31	6	0.006	n
s32	1	NA	n
s33	2	NA	n
s34	1	NA	n
s35	5	0.234	nn
s36	6	0.282	n
s37	2	NA	n
s38	22	0.791	nn
s39	2	NA	n
s40	2	NA	n
s41	5	0.003	n
s42	3	0.000	n
s43	3	0.001	n
s44	4	0.000	nn
s45	3	0.001	n
s46	4	0.000	n
s47	4	0.003	n
s48	2	NA	n
s49	4	0.003	n
s50	6	0.003	n
s51	15	0.465	n
s52	3	0.002	n
s53	3	0.117	n
s54	6	0.001	nn
s55	2	NA	n
s56	1	NA	n

s57	17	0.582	n
s58	14	0.505	nn
s59	25	0.838	nn
s60	3	0.000	nn
s61	1	NA	n
s62	1	NA	n
s63	14	0.789	nn
s64	4	0.178	n
s65	3	0.009	n
s66	16	0.854	nn
s67	11	0.644	n
s68	1	NA	n
s69	3	0.495	n
s70	15	0.799	nn
s71	19	0.595	nn
s72	22	0.899	nn
s73	12	0.581	n
s74	17	0.795	nn
s75	9	0.505	nn
s76	2	NA	n
s77	16	0.897	nn
s78	9	0.505	nn
s79	22	0.792	nn
s80	5	0.359	n
s81	4	0.493	n
s82	3	0.286	n
s83	2	NA	n
s84	2	NA	n
s85	1	NA	n
s86	5	0.000	n
s87	4	0.285	n
s88	3	0.490	n
s89	5	0.001	n
s90	2	NA	n
s91	3	0.296	n
s92	7	0.287	n
s93	3	0.004	n
s94	1	NA	n

s95	1	NA	n
s96	7	0.288	n
s97	5	0.286	n
s98	4	0.282	n
s99	1	NA	n
s100	5	0.002	n
s101	1	NA	n
s102	2	NA	n
s103	4	0.004	n
s104	2	NA	n
s105	4	0.000	n
s106	8	0.565	n
s107	5	0.565	n
s108	8	0.000	n
s109	7	0.780	n
s110	2	NA	n
s111	3	0.180	n
s112	6	0.779	nn
s113	2	NA	n
s114	2	NA	n
s115	4	0.660	nn
s116	9	0.006	n
s117	9	0.290	nn
s118	7	0.006	n
s119	27	0.815	nn
s120	9	0.511	nn
s121	24	0.796	nn
s122	7	0.363	n
s123	14	0.909	nn
s124	18	0.552	nn
s125	13	0.543	nn
s126	28	0.793	nn
s127	1	NA	n
s128	5	0.503	n
s129	8	0.504	nn
s130	17	0.617	n
s131	15	0.799	nn
s132	3	0.000	n

s133	1	NA	n
s134	14	0.575	n
s135	11	0.786	nn
s136	15	0.686	n
s137	5	0.693	nn
s138	16	0.792	nn
s139	8	0.787	nn
s140	4	0.000	n
s141	15	0.580	n
s142	10	0.787	nn
s143	8	0.534	nn
s144	3	0.001	n
s145	13	0.791	nn
s146	8	0.472	n
s147	12	0.677	nn
s148	7	0.787	nn
s149	11	0.506	nn
s150	16	0.787	nn
s151	8	0.288	n
s152	9	0.358	nn
s153	14	0.812	nn
s154	6	0.479	nn
s155	4	0.177	n
s156	9	0.787	nn
s157	7	0.008	n
s158	15	0.787	nn
s159	11	0.535	nn
s160	7	0.286	nn
s161	21	0.789	nn
s162	10	0.472	n
s163	9	0.361	n
s164	9	0.535	nn
s165	13	0.414	nn
s166	1	NA	n
s167	6	0.645	nn
s168	3	0.117	nn
s169	11	0.677	nn
s170	11	0.788	nn

s171	5	0.001	n
s172	9	0.535	nn
s173	10	0.503	nn
s174	11	0.646	nn
s175	9	0.677	nn
s176	13	0.680	nn
s177	5	0.116	nn
s178	6	0.676	nn
s179	7	0.529	nn
s180	7	0.187	n
s181	7	0.174	n
s182	5	0.177	n
s183	15	0.470	n
s184	8	0.470	n
s185	10	0.504	nn
s186	10	0.789	nn
s187	12	0.615	nn
s188	19	0.584	nn
s189	10	0.897	nn
s190	7	0.501	nn
s191	17	0.815	nn
s192	18	0.899	nn
s193	5	0.288	n
s194	2	NA	n
s195	10	0.500	nn
s196	15	0.579	n
s197	13	0.580	n
s198	20	0.584	n
s199	3	0.004	n
s200	11	0.362	nn
s201	11	0.576	n
s202	1	NA	n
s203	7	0.504	nn
s204	14	0.618	nn
s205	6	0.504	nn
s206	10	0.466	nn
s207	3	0.000	n
s208	12	0.578	nn

s209	7	0.384	nn
s210	9	0.297	n
s211	13	0.465	n
s212	11	0.504	nn
s213	3	0.003	n
s214	9	0.786	nn
s215	14	0.786	nn
s216	10	0.786	n
s217	12	0.650	nn
s218	12	0.677	nn
s219	5	0.179	n
s220	13	0.791	n
s221	13	0.544	nn
s222	8	0.357	n
s223	11	0.469	n
s224	8	0.384	nn
s225	2	NA	n
s226	11	0.504	nn
s227	11	0.587	n
s228	14	0.504	nn
s229	11	0.688	nn
s230	4	0.125	n
s231	6	0.007	n
s232	7	0.566	nn
s233	4	0.004	n
s234	1	NA	n
s235	15	0.544	nn
s236	10	0.501	n
s237	10	0.612	nn
s238	12	0.612	nn
s239	4	0.003	n
s240	3	0.000	n
s241	3	0.003	n
s242	6	0.115	nn
s243	4	0.502	n
s244	9	0.498	nn
s245	9	0.787	n
s246	12	0.504	nn

s247	2	NA	n
s248	17	0.179	n
s249	12	0.504	nn
s250	22	0.792	nn
s251	21	0.807	nn
s252	19	0.792	nn
s253	11	0.585	nn
s254	27	0.649	nn
s255	16	0.785	n
s256	10	0.504	nn
s257	8	0.006	nn
s258	4	0.004	nn
s259	1	NA	n
s260	13	0.795	nn
s261	11	0.178	n
s262	14	0.811	nn
s263	26	0.915	nn
s264	6	0.000	n
s265	4	0.572	n
s266	10	0.786	n
s267	3	0.000	n
s268	11	0.574	n
s269	18	0.286	nn
s270	7	0.108	n
s271	12	0.287	n
s272	3	NA	n
s273	8	0.286	n
s274	11	0.297	n
s275	12	0.779	nn
s276	8	0.114	n
s277	2	NA	n
s278	5	0.001	n
s279	5	0.004	n
s280	5	0.288	n
s281	10	0.003	nn
s282	3	0.000	n
s283	10	0.004	n
s284	9	0.290	n

s285	14	0.288	n
s286	9	0.788	nn
s287	14	0.294	n
s288	10	0.292	n
s289	8	0.004	n
s290	6	0.288	n
s291	5	0.288	n
s292	10	0.287	n
s293	8	0.317	nn
s294	8	0.004	n
s295	13	0.287	nn
s296	9	0.118	nn
s297	5	0.020	n
s298	10	0.577	n
s299	1	NA	n
s300	9	0.815	nn
s301	11	0.788	nn
s302	3	0.006	n
s303	4	0.003	nn
s304	8	0.361	nn
s305	11	0.647	nn
s306	7	0.397	n
s307	5	0.007	n
s308	12	0.505	n
s309	9	0.678	n
s310	11	0.678	n
s311	12	0.789	nn
s312	1	NA	n
s313	11	0.678	nn
s314	5	0.004	n
s315	1	NA	n
s316	4	0.001	n
s317	3	0.000	n
s318	4	0.004	n
s319	6	0.500	nn
s320	7	0.578	n
s321	9	0.505	n
s322	3	0.003	n

s323	4	0.290	n
s324	5	0.004	n
s325	6	0.004	n
s326	4	0.004	n
s327	5	0.285	n
s328	2	NA	n
s329	8	0.004	n
s330	4	0.503	nn
s331	9	0.306	n
s332	8	0.504	nn
s333	5	0.503	nn
s334	9	0.460	n
s335	5	0.503	nn
s336	9	0.590	n
s337	9	0.504	n
s338	6	0.287	n
s339	10	0.296	n
s340	6	0.287	n
s341	4	0.499	n
s342	4	0.004	n
s343	12	0.504	nn
s344	3	0.283	n
s345	6	0.503	nn
s346	8	0.504	nn
s347	8	0.288	n
s348	8	0.676	nn
s349	1	NA	nn
s350	19	0.793	n
s351	17	0.544	nn

Figures

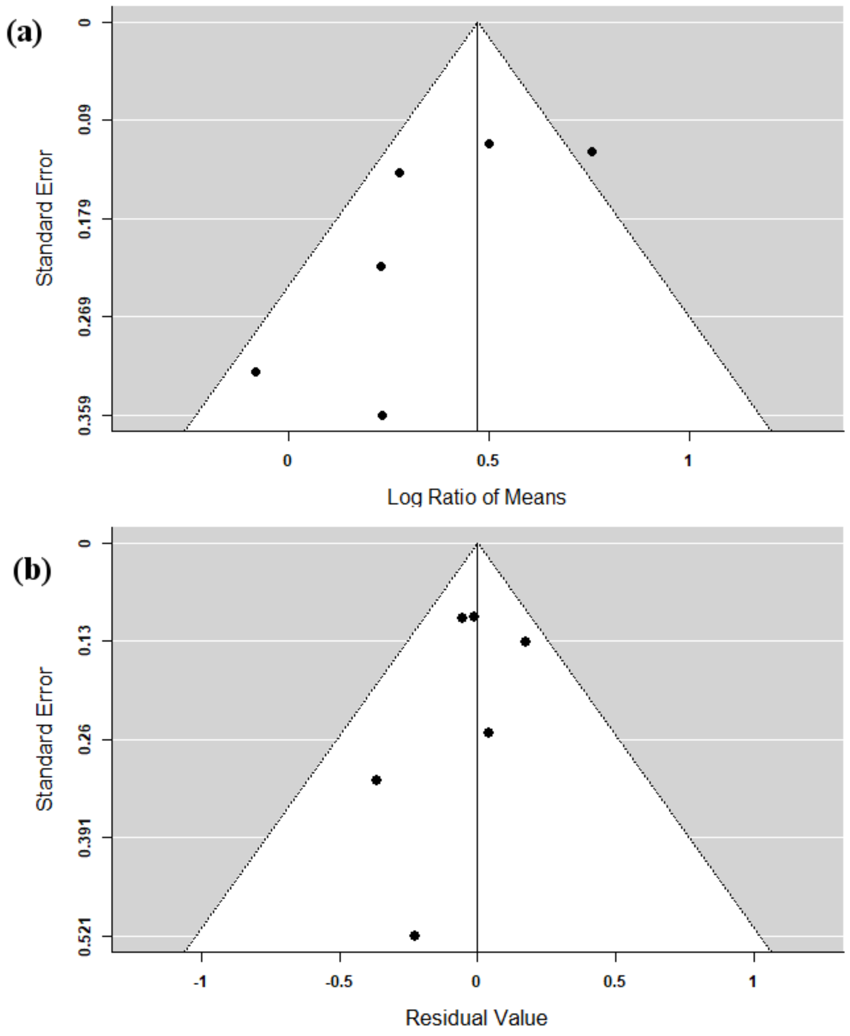

Figure 1. Funnel plots for (a) fixed-effects model for presence of non-native fish species on native fish species richness and (b) mixed-effect model for presence of non-native fish species on functional diversity. Black points represents each sub-basin.

Supplementary Material 2

Reference list of the studies included in our analysis.

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