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AMANDA CANTARUTE RODRIGUES

Co-occurrence patterns in invaded communities: what drives the spatial
distribution of native and non-native species?

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Tese apresentada ao Programa de Pós-Graduação em Ecologia de Ambientes Aquáticos Continentais do Departamento de Biologia, Centro de Ciências Biológicas da Universidade Estadual de Maringá, como requisito parcial para obtenção do título de Doutora em Ecologia e Limnologia.

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Orientador: Prof. Dr. Luiz Carlos Gomes
Coorientadora: Prof.^a Dr.^a Natália Carneiro Lacerda dos Santos

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Padrões de coocorrência em comunidades invadidas: o que direciona a distribuição espacial de espécies nativas e não nativas?

RESUMO

Avaliou-se comunidades invadidas sob dois contextos: 1) como comunidades nativas respondem a eventos de introdução em massa e 2) como comunidades invadidas mudam no tempo. Definiu-se um tema comum para representar as modificações nas comunidades após a invasão, a coocorrência entre espécies. O objetivo desta tese foi responder três questões: 1) se o impacto causado pela espécie não nativa é determinado pelo nível de similaridade entre espécies nativas e não nativas; 2) se o impactoteria o mesmo padrão no tempo; e 3) se existe algum atributo da população de espécies não nativas que determina o nível do impacto. Utilizou-se o conjunto de dados do projeto Pesquisas Ecológicas de Longa Duração (PELD) realizado na planície de inundação do Alto Rio Paraná (Sítio PELD/PIAP) para responder essas questões. Primeiro, avaliou-se os efeitos de espécies não nativas na coocorrência entre espécies após a invasão de mais de 30 espécies de peixes não nativos de uma só vez. Testou-se se a dissimilaridade funcional entre espécies nativas e não nativas impulsiona os padrões de coocorrência entre elas. Após, estimou-se a coocorrência em uma série temporal de 30 anos. As espécies não nativas avaliadas são originárias de diversos vetores de introdução. Testou-se se a abundância de espécies não nativas e o tempo desde a introdução impulsionam a coocorrência entre espécies nativas e não nativas. A abundância foi utilizada como atributo da população das espécies não nativas para testar se o efeito da abundância é mediado pela distância filogenética entre as espécies. Os resultados encontrados mostraram que a coocorrência entre as espécies nativas e não nativas é afetada pela distância funcional e filogenética. Portanto, integrar diversidade funcional e filogenética para entender a distribuição espacial dos organismos tem potencial para melhorar a compreensão de padrões de coocorrência entre espécies nativas e não nativas. Os resultados também mostraram que os padrões de coocorrência podem ser mais sensíveis à variabilidade temporal nos atributos da população não nativa (i.e., abundância) do que o tempo desde a introdução. Mostrou-se que a avaliação de padrões de várias espécies não nativas fornece uma compreensão mais ampla de toda a comunidade após invasões.

Palavras-chave: Coocorrência. Distância filogenética. Ictiofauna. Invasão de espécies. Planície de inundação do alto rio Paraná. Similaridade funcional. Variação temporal.

Co-occurrence patterns in invaded communities: what drives the spatial distribution of native and non-native species?

ABSTRACT

Invaded communities were evaluated under two contexts: 1) how native communities respond to massive introduction events and 2) how invaded communities change through time. A common topic was defined to represent the modifications in communities after invasion, the co-occurrence between species. The objective of this thesis was to answer three questions: 1) whether the impact caused by non-native species is determined by the level of similarity between native and non-native species; 2) whether the impact would have the same pattern through time; and 3) whether there is any attribute of the non-native species population that determines the level of the impact. The data set from the project Pesquisas Ecológicas de Longa Duração (PELD) performed at the Upper Paraná River floodplain (Sítio PELD/PIAP) was used to answer these questions. First, it was evaluated the non-native species effects on the co-occurrence between species after the invasion of more than 30 non-native fish species at once. It was tested if the functional dissimilarity between native and non-native species drives the co-occurrence patterns between them. After, it was estimated the co-occurrence in a time series of 30 years. The non-native species evaluated were introduced by several introduction vectors. It was tested if the non-native species abundance and time since introduction drive the co-occurrence between native and non-native species. The abundance was used as the attribute of non-native species population to test if the effect of abundance is mediated by the phylogenetic distance between species. The results found showed that the co-occurrence between native and non-native species is affected by functional and phylogenetic distance. Therefore, integrating functional and phylogenetic diversity to assess the spatial distribution of organisms has potential to improve the understanding of co-occurrence patterns between native and non-native species. The results also showed that co-occurrence patterns may be more sensitive to the temporal variability in non-native population attributes (i.e. abundance) than time since introduction. It was showed that evaluating patterns of several non-native species may provide a broader understating of the entire community after invasions.

Keywords: Co-occurrence. Ichthyofauna. Functional similarity. Phylogenetic distance. Species invasion. Temporal variation. Upper Paraná River floodplain.

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1 INTRODUCTION

One of the greatest causes of global changes in biodiversity is species invasions. The invasion science has been recently developed as a research field, dedicated to detecting, understanding, and mitigating invasion impacts, since both intentional and unintentional introductions increased throughout the 20th century (SIMBERLOFF et al. 2013). A lot of studies have been made, especially regarding invasion hypotheses trying to explain the success of some non-native species (CATFORD; JANSSON; NILSSON, 2009) and the effects that a new species causes on the native biodiversity (RICCIARDI; KIPP, 2008; CUCHEROUSSET; OLDEN, 2011). However, while these topics continue to be widely studied (STRAYER, 2012; GALLIEN; CARBONI, 2017), we still face some challenges when studying the processes structuring invasion patterns. As human actions are continuously changing the landscape worldwide and destroying natural barriers, different communities are even more susceptible to massive invasion events, and most studies until now have not considered the effects of a group of non-native species on the native community. The second is monitoring non-native species over time, which offers different answers regarding their effects on communities, since several common ecological and/or evolutionary processes should modulate the effect of a non-native species over time (STRAYER et al. 2006). Therefore, this thesis was made with the expectation to contribute to the understanding of these two gaps in invasion science.

The construction of the idea of this thesis was based on previous studies and field observations. The study area, the Upper Paraná River floodplain, is one of the most threatened South American wetland (RUARO et al. 2020) and the Upper Paraná ecoregion hosts the largest number of non-native fish species in the Neotropics (105 species; GUBIANI et al. 2018). Previous studies and field observations of pioneer

researchers in the area showed that after non-native species introductions, some native species decreased in abundance and started to be less captured in the floodplain. One of the most emblematic case is regarding the rapid increase and spread of the piranha *Serrasalmus marginatus* Valenciennes, 1837 and the pronounced decrease in abundance and occurrence of its native congener *Serrasalmus maculatus* Kner, 1858 (AGOSTINHO; JÚLIO JR., 2002; AGOSTINHO, 2003; ALEXANDRE et al. 2004; ALVES et al. 2017; RODRIGUES et al. 2018), but other cases are also known (GOIS et al. 2015; GANASSIN et al. 2020). The common key between these cases is that native and non-native species are phylogenetically related, which also predicts high similarities in ecological niche use ('phylogenetic signal'; BLOMBERG; GARLAND JR.; IVES, 2003; WEBER; STRAUSS, 2016). Therefore, the apparent pattern found in the floodplain was that non-native species would negatively affect similar native species.

It is common to find studies evaluating the coexistence between similar native and non-native species. A lot of them report that the non-native species affected the spatial distribution of native species by displacing them from optimal habitats through competitive exclusion (CHENG et al. 2009; RICHTER-BOIX et al. 2013; SMITH et al. 2019; PASCUAL-RICO et al. 2020). This is based on the theory that similar species would compete to limit the exploitation of the resource by other species (the limiting similarity principle; MACARTHUR; LEVINS, 1967; TILMAN, 1982). Therefore, the objective of this thesis was to answer three questions: 1) whether the impact caused by non-native species is determined by the level of similarity between native and non-native species, since most of studies evaluated pre-determined species and species with specific shared characteristics (e.g. same trophic niche; PASCUAL-RICO et al. 2020); 2) whether the impact would have the same pattern through time; and 3) whether there

is any attribute of the non-native species population that determines the level of the impact.

To answer each question, the co-occurrence between native and non-native fish species was estimated and used as the response variable, and the project Pesquisas Ecológicas de Longa Duração (PELD), performed in the Upper Paraná River floodplain provided the data. First, it was assessed the non-native species effects on the co-occurrence between species after a massive invasion event. More than 30 non-native fish species started to occur in the floodplain after the construction of the Itaipu dam, which eliminated a geological barrier and allowed the introduction and spread of species in areas located upstream the barrier (JÚLIO JÚNIOR et al. 2009). It was tested if the functional dissimilarity between species drives the co-occurrence patterns and if the co-occurrence is affected by species status (i.e. native and non-native status). After, it was estimated the co-occurrence in a time series of 30 years, in order to assess the co-occurrence pattern through time. The non-native species evaluated were introduced by several introduction vectors. It was tested if the non-native species abundance and time since introduction drive the co-occurrence between native and non-native species. Finally, it was tested if the effect of abundance is mediated by the phylogenetic distance between species. It is expected that our objectives will improve the understanding of invasion processes in the invasion science, allowing researchers to make predictions and extrapolations for other communities.

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2 FUNCTIONAL DISSIMILARITY DETERMINES THE CO-OCCURRENCE OF NATIVE AND NON-NATIVE SPECIES

ABSTRACT

Aim: Species coexistence is predicted to be driven by competitive interactions. In invaded communities, non-native species start to co-occur with the native community, but this novel interaction may result in increased competition, as they do not share a coevolutionary history. If native and non-native species share similar resources, then competition is intensified and this may result in modifications in the spatial distribution of native populations. We aimed to understand the drivers of co-occurrence between native and non-native species.

Location: Upper Paraná River floodplain, Southern Brazil

Methods: We sampled fish and estimated the co-occurrence between pairs of species and the functional dissimilarity between them using morphological traits. We used Linear Mixed Models to test if the functional dissimilarity drives co-occurrence patterns and if co-occurrence is affected by species status.

Results: Functional diversity between assemblages of native and non-native species did not differ significantly. We found that co-occurrence was affected by functional dissimilarity between species: more similar native and non-native species tended to co-occur less than expected by chance in the floodplain. The co-occurrence was also affected by species status: it was higher between pairs of native species than between pairs of native and non-native species.

Main conclusions: Biotic interactions such as competition might be driving the co-occurrence patterns between native and non-native species at small spatial scales. This may result in spatial segregation between competing native and non-native species and it might limit the native geographic ranges, possibly resulting in alterations in the

taxonomic and functional diversity of native communities. Since geographic barriers are constantly being suppressed by anthropogenic activities and different communities are starting to co-occur, we need to understand what impacts this will cause to the native biodiversity.

Keywords: competition, dam impacts, fish community, functional diversity, invasion, spatial segregation.

2.1 Introduction

A central question in ecology is to identify the factors driving the spatial distribution of organisms and their coexistence (MacArthur & Levins, 1967). Species coexistence is predicted to be driven by the biological characteristics of species and mediated by the availability of resources in the environment (MacArthur & Levins, 1967). For instance, a pair of species sharing limited resources (i.e., similar ecological niches) will compete to limit the exploitation of the resources by the other species (the limiting similarity principle; MacArthur & Levins, 1967; Tilman, 1982). Therefore, competition is a strong driver of coexistence patterns that shapes the observed co-occurrence of species in ecosystems (Diamond, 1975; Novella-Fernandez et al., 2021). When competition reaches a certain level not sustainable for one species, competitive exclusion can occur and is a key driver of community assembly and spatial segregation (the competitive exclusion principle; Hardin, 1960), limiting the co-occurrence between competing species (Reitalu et al., 2008; Rauschert et al., 2012). However, competitive interactions among organisms are strongly affected by human-induced environmental changes modulate the spatial distribution of organisms, the availability of resources and, consequently, the coexistence patterns observed at local and global scales (Vitousek et al., 1997; Blois et al., 2013; O’Brian, 2019).

Biological invasions are a global phenomenon with important implications on biodiversity and ecosystem functioning (Sala et al., 2000). The introduction of non-native species creates novel ecological interactions among organisms, including the coexistence between native and non-native species that did not share a coevolutionary history. Competing species that have coevolved can coexist because they display behaviour to partition space, time, or resources, becoming temporally and/or spatially segregated (i.e., different realised niche; Reitalu et al., 2008; Schuette et al., 2013; Grassel et al., 2015). For example, in carnivore communities, niche partitioning is expected to structure the community as species present different peaks of activity during daytime (Schuette et al., 2013). At the opposite, species that did not coevolve may not be able to achieve this spatial and/or temporal segregation and their coexistence may not be possible (Whitney & Gabler, 2008; Priddis et al., 2009). In cases when non-native species display higher competitive abilities than native species, they may modify the spatial distribution of native populations (Parker et al., 1999). Studies on the co-occurrence patterns between functionally similar native and non-native species suggested that a long time after introduction, native and non-native species can become spatially segregated at fine spatial scales, and this allow coexistence (Richter-Boix et al., 2013; Smith et al., 2019; Pascual-Rico et al., 2020). Therefore, the lack of co-evolutionary history between native and non-native species can help to observe the transient dynamics of species displacement during or after a species invasion (Sax et al., 2007) and biological invasions represent an unique opportunity to investigate how ecological and evolutionary processes may shape local patterns of diversity within communities (Verhoeven et al., 2011).

In this study, we aim to understand the drivers of co-occurrence between native and non-native species. Our first objective was to test the relationship between

functional dissimilarity and co-occurrence for pairs of native and non-native species. Assuming high competition between native and non-native species due to high functional similarity caused by a lack of co-evolutionary history, we hypothesised that functionally similar native and non-native species presented lower co-occurrence than expected by chance. Our second objective was to test if coexistence was affected by species status (native and non-native). Our hypothesis was that native and non-native species co-occur less when compared to pairs of native species. To test these hypotheses, we used as a unique model system, i.e., the massive introduction of > 30 non-native fish species that occurred after a dam construction that eliminated a geological barrier in a Neotropical floodplain (Júlio Júnior et al., 2009). Non-native species that historically occurred downstream the geological barrier colonised the areas located upstream following dam construction (Júlio Júnior et al., 2009), with both native and non-native species originating from the same basin but from two distinct ecoregions (i.e., large areas “encompassing one or more freshwater systems with a distinct assemblage of natural freshwater communities and species”; Abell et al., 2008).

2.2 Methods

2.2.1 Study area

This study was conducted in the Upper Paraná River floodplain, located in the Upper Paraná hydrographic ecoregion (*sensu* Abell et al., 2008) in Southern Brazil.

Historically, the Upper Paraná ecoregion was separated from the lower part of the Paraná River basin (the Lower Paraná ecoregion) by a natural and effective barrier, the Sete Quedas Falls (average water volume of 13,000 m³/s; Fig. 1). However, the construction of the Itaipu Reservoir in 1982, 150 km downstream the Sete Quedas, completely flooded the falls, allowing the colonisation and spread of several species

endemic to the Lower Paraná ecoregion into the upper part of the river (Júlio Júnior et al., 2009). Around 33 new introduced species were registered after the flooding, distributed in 14 families and six orders, and many of them became relatively abundant after the introduction.

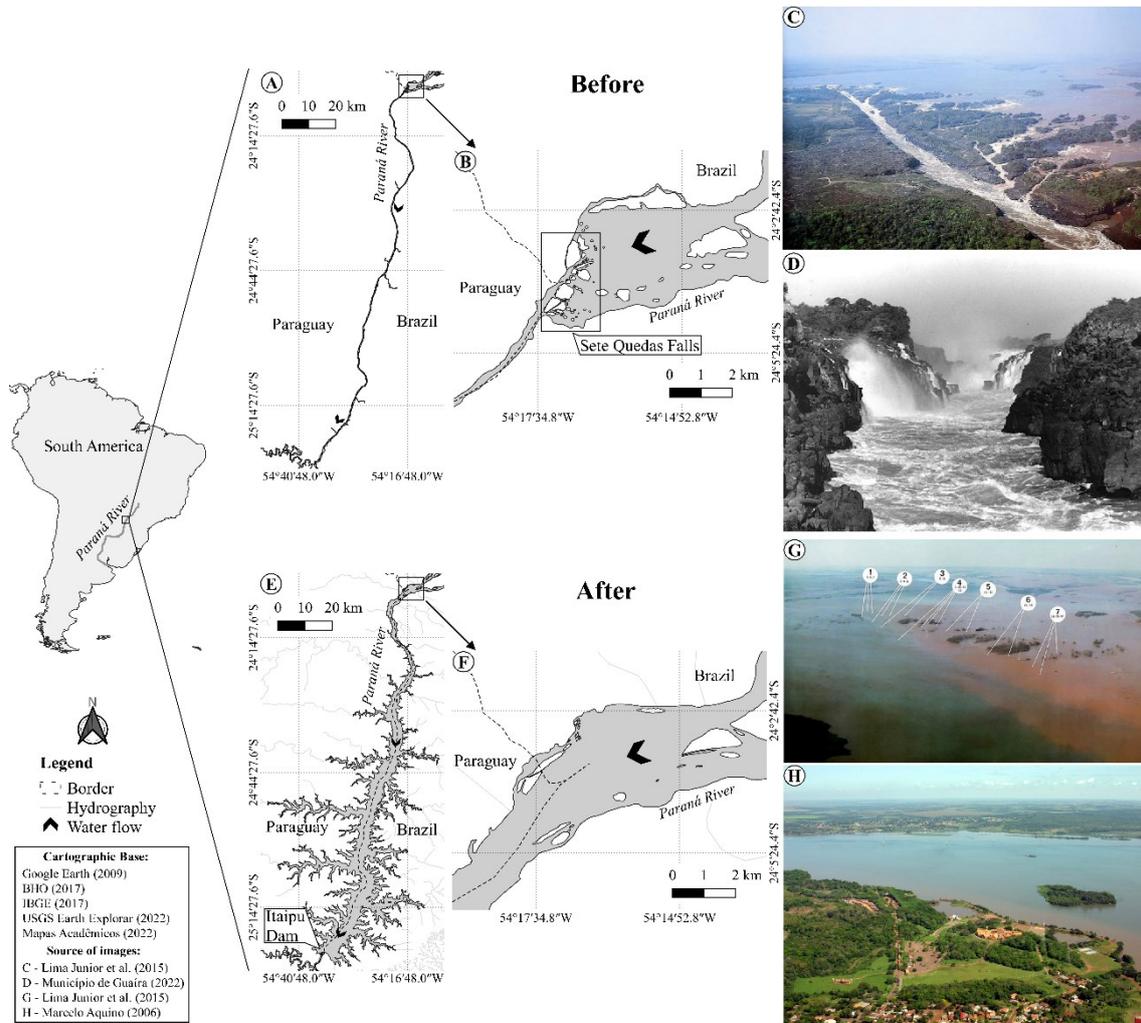


Figure 1. The flooding of the Sete Quedas Falls caused by the construction of the Itaipu Dam. A, B, C, and D show the falls before the construction, and E, F, G, and H show the completely disappearance of the falls after the Itaipu Dam construction.

2.2.2 Fish sampling

Fish distribution data were obtained from a long-term ecological research program developed at the Upper Paraná River floodplain (Angulo-Valencia et al., 2022). Fish were sampled in periods of low water (April to September) in two distinct surveys between 2000 and 2001 and between 2010 and 2011. These surveys were selected because they have the highest number of sites sampled. In the first survey, fish were sampled in May and August in each year. In the second survey, fish were sampled in June and September in each year; overall a total of eight samplings. Sampling was conducted in 20 sites of the floodplain (main channel of the river and hydrologically connected and not connected floodplain lakes; Appendix A) to capture a larger spatial distribution of species. 160 sampling events were performed during this period. To sample fish, sets of gillnets with different mesh sizes (24, 30, 40, 50, 60, 70, 80, 100, 120, 140 e 160 mm between opposite knots) were deployed in each sampling site for 24h and all sampled individuals were identified to the species level (Angulo-Valencia et al., 2022). Native and non-native species were classified considering the hydrographic ecoregion delineation made by Abell et al. (2008) and the filling of the Itaipu Reservoir and the subsequent species introduction. Species from the Lower Paraná ecoregion that spread in the Upper Paraná ecoregion after removal of the Sete Quedas Falls barrier were considered as non-native species.

2.2.3 Co-occurrence

For each possible combination of species, we quantified a species co-occurrence metric using the ‘cooccur’ R package (Griffith et al., 2016). It applies a probabilistic model to find the probability of two species co-occurring given the data (observed co-occurrence; OCo) and the probability that the same pair of species would co-occur at

random (PCo). We consider co-occurrence as both species of a pair occurring at the same time at a given site. However, following Thuiller et al. (2010), we use the term coexistence to infer when both species are frequently interacting on a small spatial scale. Because we were interested in investigating if a pair of species co-occurred more or less than expected by chance, we scaled the observed by the expected co-occurrence using the quotient of OCo and PCo. Therefore, values of scaled co-occurrence lower than one indicate that species co-occur less than expected by chance, values equal one indicate that species co-occur the same as expected by chance, and values higher than one indicate that species co-occur more than expected by chance. We removed pairs of species that showed scaled co-occurrence values equal zero in further analyses since our objective was to evaluate pairs of species with lower and/or higher co-occurrence, and not absence of co-occurrence. Among the 2703 pairs of species, 996 pairs of species never co-occurred. Therefore, the scaled co-occurrence was estimated for 1707 pairs of species, of which 871 are pairs of native species and 836 are pairs of native and non-native species.

2.2.4 Functional diversity

Trait-based approaches are highly recommended to estimate dissimilarity between native and non-native species (Thuiller et al., 2010). To estimate functional dissimilarity, we first obtained the functional traits of the studied species from FISHMORPH (Brosse et al., 2021) that include 10 morphological traits (nine unitless ratios and body size; Appendix B), commonly used in assessments of morphological diversity of freshwater fishes. Five species from the Upper Paraná River floodplain were not available in this database (*Hypostomus strigaticeps*, *Hoplias* sp.2, *Hoplias*

sp.3, *Trachelyopterus* sp. and *Potamotrygon amandae*) and, for these species, we measured functional traits using pictures following Brosse et al. (2021).

Functional dissimilarity between species was calculated using the functional traits of each species (**F** matrix) and by calculating the Gower's distance (Gower, 1966) on the **F** matrix to obtain the functional dissimilarity matrix. Gower's distance was used because it is the most indicated metric when there are some missing trait values (Marie et al., 2015). We estimated the best functional space following Maire et al. (2015) and here, it presented eight dimensions (mean standardised distance = 0.000475). The functional dissimilarity between species was measured as the standardised distance between each pair of species in the functional space. Therefore, lower values indicate that species are closer in the functional space (more functionally similar) and higher values indicate that species are more distant in the functional space (more functionally dissimilar).

2.2.5 Data analysis

We first performed a Permutational Multivariate Analysis of Variance (PERMANOVA) to test whether native and non-native species overall differ in their functional traits. We then performed a Linear Mixed Model (LMM; Zuur et al., 2009) using the scaled co-occurrence as response variable to test the relationship between functional dissimilarity and co-occurrence for native and non-native pairs of species. As predictor variables, we used the functional distance as fixed term and the identity of native species as random factor with a random intercept. We tested if there was a positive and significant relationship between scaled co-occurrence and functional distance (positive β estimate and $p < 0.05$). For LMM analysis, we used the *lmer* function in 'lme4' package (Bates et al., 2015), the 'lmerTest' package (Kuznetsova et

al., 2017) to obtain model significance and the *r.squaredGLMM* function from the ‘MuMIn’ package (Barton, 2020) to obtain model performance.

To test if coexistence patterns differ with species status, we compared scaled co-occurrence values between pairs of native species (native × native) and pairs of native and non-native species (native × non-native) using a Linear Mixed Model (LMM) with the scaled co-occurrence as response variable. As predictor variables, we used the group as fixed term and the identity of native species as random factor with a random intercept. For LMM analysis, we used the same functions mentioned above for the first hypothesis. We log-transformed the scaled co-occurrence data to test both hypotheses. All analyses were performed in R software version 4.1.2 (R Core Team, 2021).

2.3 Results

A total of 53 native and 25 non-native fish species were registered in the Upper Paraná River floodplain, belonging to six orders (Characiformes, Gymnotiformes, Myliobatiformes, Cichliformes, Pleuronectiformes and Siluriformes), 22 families and 53 genus (Appendix C). Native species were present in all 160 sampling events and non-native species were absent in two sampling events. Among the 10 most common species (occurring in more than 50% of the sampling events), six were native and four were non-native species (Appendix E, Fig. S1). Overall, the functional traits of native and non-native fish species did not differ significantly (PERMANOVA, $F = 1.15$; $p = 0.33$, Fig. 2).

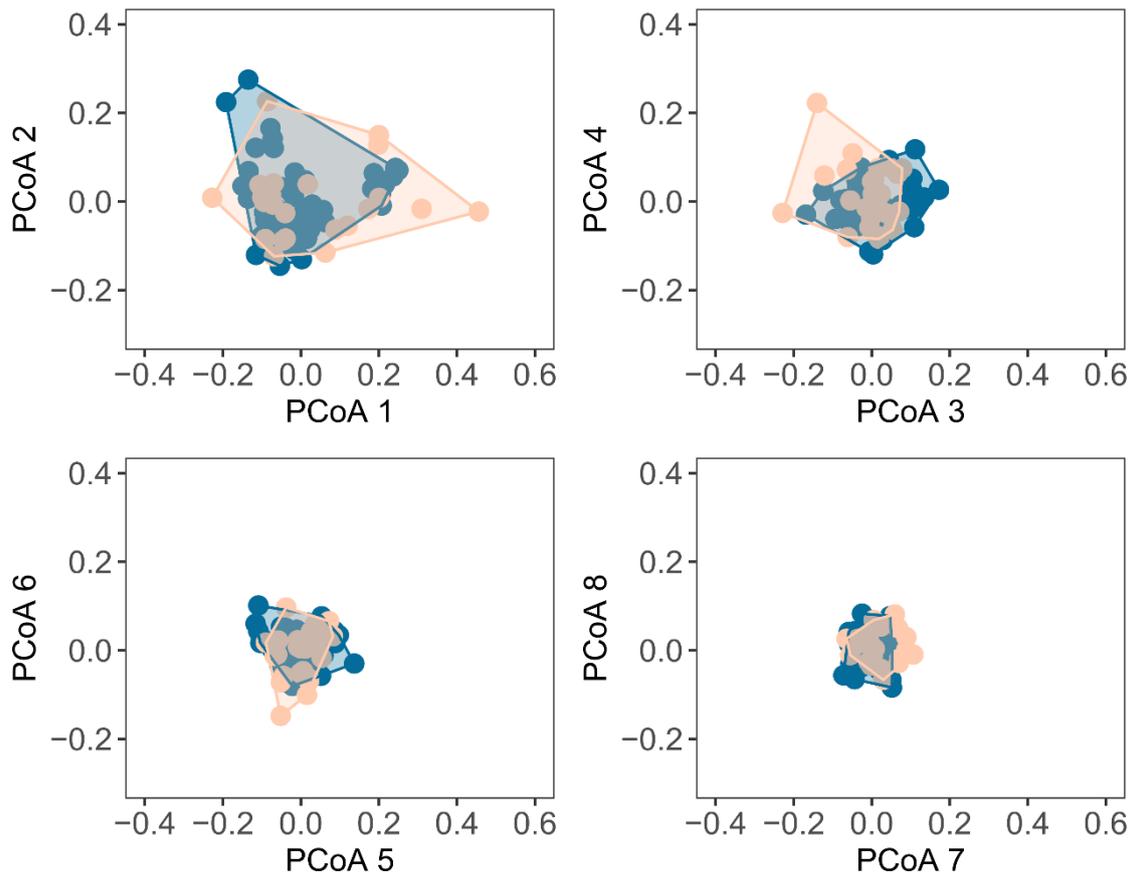


Figure 2. Functional space of native (blue) and non-native (orange) species along the eight functional axes.

There was a large variation in the scaled co-occurrence among the 871 pairs of native species (Fig. 3a): 25.83% of pairs of species co-occurred less than expected by chance (values lower than one; yellow squares in Fig. 3) and 70.15% of pairs of species co-occurred more than expected by chance. For 836 pairs of native and non-native species, 25.48% of pairs of species also co-occurred less than expected by chance and 71.29% of pairs of species co-occurred more than expected by chance, but with a lower degree when compared to native pairs of species (less dark squares in Fig. 3b than in Fig. 3a).

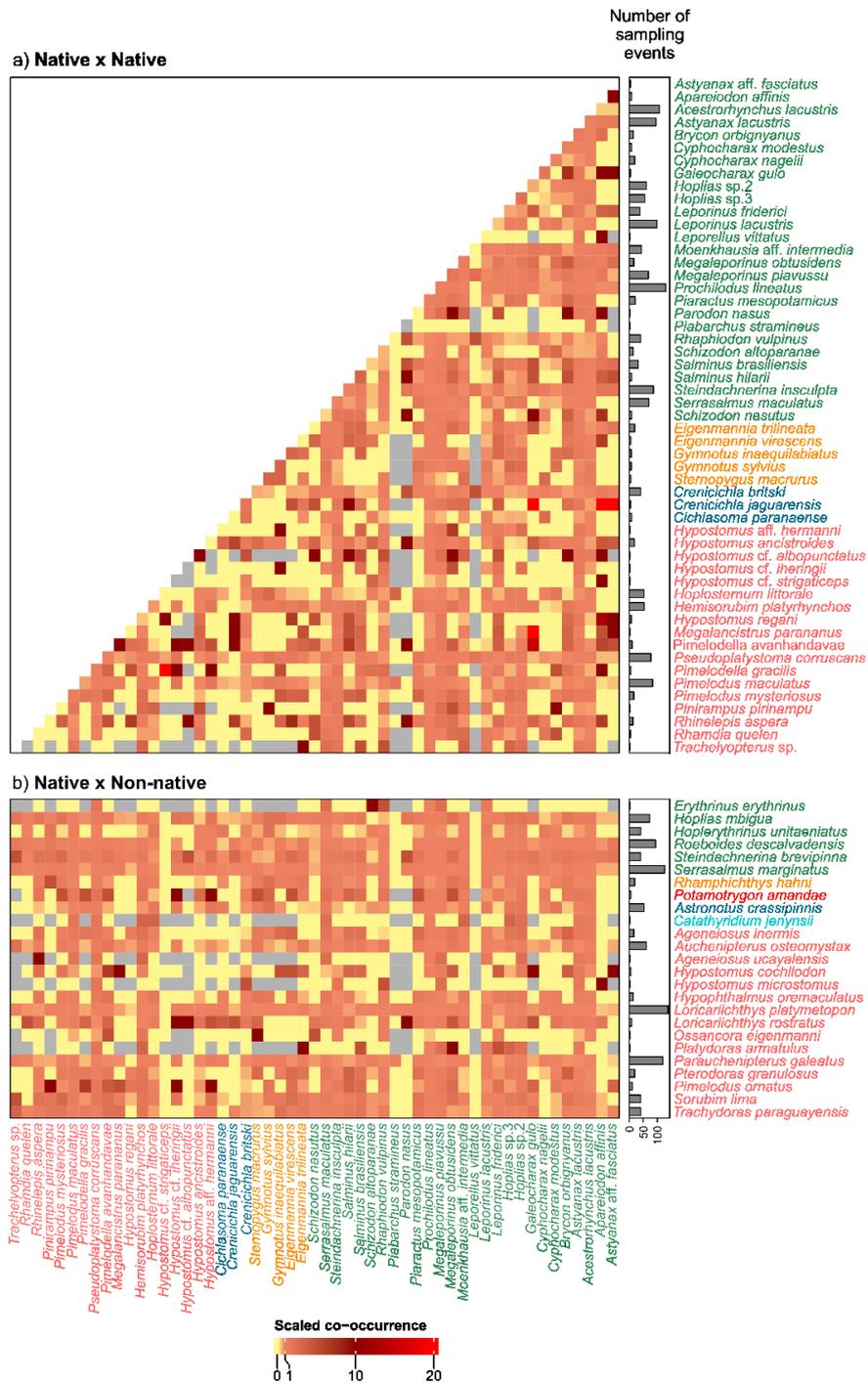


Figure 3. Scaled co-occurrence values between a) native species and b) native and non-native species. Grey squares represent pairs of species that did not co-occur. Right bar plots represent species occurrence. Different colours in species names indicate different taxonomic orders. Green: Characiformes; orange: Gymnotiformes; dark blue: Cichliformes; pink: Siluriformes; red: Myliobatiformes; light blue: Pleuronectiformes.

The functional distance between pairs of native and non-native species in the functional space ranged from 0.048 (more similar species) to 0.742 (less similar species) (Appendix E, Fig. S2). For native and non-native pairs of species, there was a positive and significant relationship between the scaled co-occurrence and the functional distance between species (Linear Mixed Model; r^2 conditional: 0.12, $p < 0.0001$; Fig. 4), indicating that the more similar the native species were from the non-native species, the less they co-occurred. We also found a significant difference in the co-occurrence between pairs of native species and pairs of native and non-native species (Linear Mixed Model; r^2 conditional: 0.18, $p < 0.001$), indicating that native and non-native species co-occur less when compared to the co-occurrence only between native species (Fig. 5).

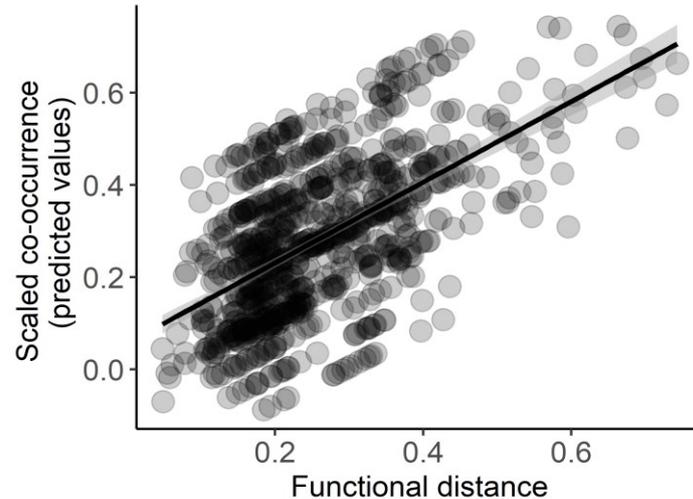


Figure 4. Significant relationship between functional dissimilarity and co-occurrence (scaled values, log-transformed) between native and non-native pairs of species (native \times non-native group).

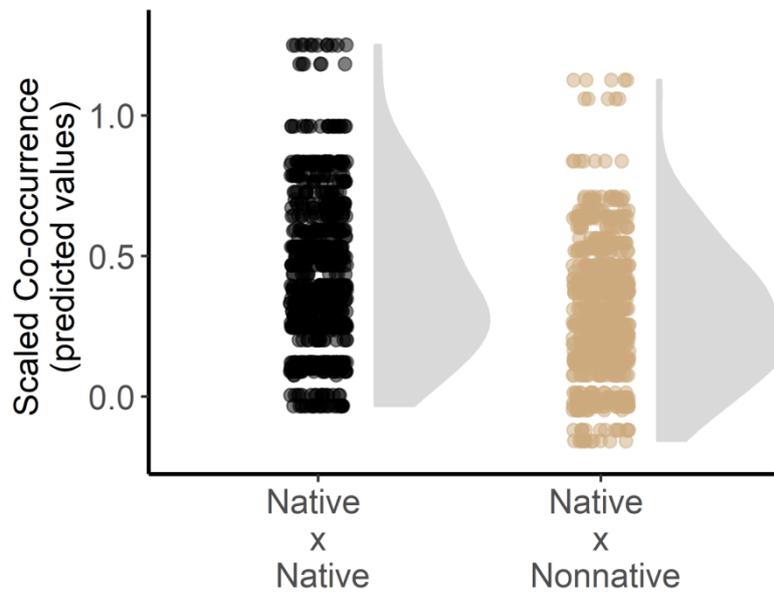


Figure 5. Co-occurrence (scaled values) for pairs of only native species (black points; native \times native group) and for pairs of native and non-native species (gold points; native \times non-native group).

2.4 Discussion

The factors driving species coexistence have been widely studied, with several theories being tested (Mouillot et al., 2007), but most ecosystems in the world are being impacted by species introduction (Bellard et al., 2016). Therefore, studying how non-native species coexist with native species is an opportunity to better understand the ecological mechanisms that shape community assembly and species coexistence (Gallien and Carboni, 2017). Using a unique case that allowed the introduction and establishment of multiple non-native species in a Neotropical river, we provide novel insights into the co-occurrence patterns of native and non-native species. We found that more functionally similar pairs of species (i.e., native and non-native) tended to co-occur less, supporting our first hypothesis, indicating that the co-occurrence patterns of native and non-native fish species, in the Upper Paraná River floodplain, were driven by

their functional dissimilarity. We also found support for our second hypothesis, since the co-occurrence of pairs of native and non-native species was lower when compared to the co-occurrence between pairs of native species, indicating that coexistence is affected by species status. Therefore, biotic interactions (i.e., competition) likely drive co-occurrence patterns, making competing native and non-native species more spatially segregated, and, eventually, limiting their geographic ranges (Wiszniewski et al., 2012; Novella-Fernandez et al., 2021).

During introduction, non-native species usually face multiple ecological filters (Gallien and Carboni, 2017). One of them is the environmental barrier, where local environmental conditions filter species based on their ecological niches and physiological adaptations (e.g., environmental filtering theory; Gallien and Carboni, 2017). This filter may allow non-native species with pre-adaptations to the new environment to co-occur on a regional scale with native species – as postulated by Darwin (1859) in the pre-adaptation hypothesis (Li et al., 2015; Park et al., 2020). Accordingly, we found that the functional characteristics between native and non-native species were not significantly different, suggesting that non-native species could spread and establish in the floodplain as demonstrated in the results. However, the distribution of non-native species can also be affected by biotic filters (Diez et al., 2008; Gallien and Carboni, 2017), because non-native species could interact with native species. Negative biotic interactions such as competition might occur, and it is believed to be strong at local spatial scales where the environment and resources are homogenous (Davies et al., 2005; 2011; Mouillot et al., 2007; Park et al., 2020). In our study system, co-occurrence of species was evaluated at fine spatial scales (river and floodplain lakes), and we found an effect of the functional diversity on species spatial distribution patterns. This effect depended on the level of similarity between species: the more similar a pair of species

is, the less they co-occur. Therefore, despite native and non-native species show increased functional similarity (which allowed non-native species to inhabit and spread throughout the floodplain), their co-occurrence is mediated by the spatial segregation at fine scales, possibly resulted from competing interactions.

However, different mechanisms can also allow non-native species interactions with native species, even for closely related ones (Pereira et al., 2007). Trophic segregation can make species use similar trophic niche but with specialised diets (Alves et al., 2017) or in different periods during the day (Schuette et al., 2013), limiting direct competition. Shifts in resource use can also alleviate or worsen interactions between native and non-native species, which can be caused by fluctuations in water level and food availability (Reinas et al., 2022). In floodplains, the seasonal changes of chemical and physical characteristics, community composition and resource availability caused by the flood pulse have consequences for the coexistence of native and non-native species (Thomaz, 2021). For instance, shifts in the trophic niche during the flood period (e.g., Abujanra et al., 2009; Quirino et al., 2015; 2017) may reduce the niche overlap between species and alleviate competition, enhancing the possibility of coexistence over longer periods. Therefore, environmental changes can modulate coexistence between native and non-native species by influencing species interactions (i.e., competition), which will directly influence invasion success and ecological impacts.

We found that pairs of native species showed increased co-occurrence when compared to co-occurring native and non-native species. Competing native species (i.e., co-evolved) have adapted themselves according to interactions with other species, which is often an evolutionary advantageous strategy to avoid direct competition and to increase biodiversity (Linnell & Strand, 2000). For instance, subordinate species can display different strategies to avoid direct contact with dominant competitors (i.e., niche

partitioning; Schuette et al., 2013). However, native and non-native species may not display mechanisms to avoid competition (Pascual-Rico et al., 2020). In this case, the co-occurrence between similar native and non-native species might increase competition and result in habitat niche shift (Rodrigues et al., 2018; Moquet et al., 2020; Pascual-Rico et al., 2020) and/or even competitive exclusion (Hardin, 1960; Bøhn et al., 2008). Therefore, the lack of both coevolutionary history and differences in functional diversity between native and non-native species resulted in different patterns of co-occurrence when considering the species status.

Integrating information on the functional diversity to understand the spatial distribution of organisms has potential to improve our understanding of co-occurrence patterns between native and non-native species, providing us ways to understand how species coexistence may respond after introductions. The results suggest that biotic interactions modulate the patterns of coexistence between native and non-native species, indicating that negative interactions may affect specific species and functional traits. This may lead to alterations in taxonomic and functional diversity, so we highly recommend more studies that evaluate how communities and ecosystem functions changed after invasions. Besides, studying different spatial scales may also help us to understand which processes are acting in each one, and this is of great importance to make predictions and extrapolations (Gallien and Carboni, 2017). Additionally, environmental factors (filters) are also important drivers of the coexistence between native and non-native species, and it is also necessary to consider environmental changes when evaluating invaded communities. As anthropogenic processes are increasingly present in natural environments and suppressing natural barriers between non-co-evolved communities (Júlio Júnior et al., 2009; Wood et al., 2021), evaluating

the potential impacts of such invasions on native organisms and recipient ecosystems is needed.

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3 NON-NATIVE SPECIES ABUNDANCE DECREASES CO-OCCURRENCE BETWEEN NATIVE AND NON-NATIVE SPECIES THROUGH TIME AT ANY PHYLOGENETIC DISTANCE

ABSTRACT

Monitoring non-native species over time offers different answers regarding their effects on communities. As the non-native species population increases, greater are their impacts on the native community, which may vary to the phylogenetic proximity between species. We aimed to assess co-occurrence time series between native and non-native species in a 30-year time scale. We tested if the co-occurrence between native and non-native species is driven by the abundance of the non-native species and by the time after the introduction. We also tested if the effect of the abundance is mediated by the phylogenetic distance. We sampled fish in the Upper Paraná River floodplain, Southern Brazil and used Linear Mixed Model to test our hypothesis. We found that the effect of time since introduction in driving species co-occurrence was less important than the effect of the non-native abundance. Co-occurrence values decreased with the increase in non-native abundance for any phylogenetic level, but the intensity of the effect was different between different phylogenetic distances. We found that for greater values of non-native abundance, native and non-native species that are phylogenetically distant co-occur less than phylogenetically close species. The low effect of time since introduction can be related to the annual variability in non-native abundance, instead of a long-term tendency as expected. Therefore, co-occurrence patterns may be more sensitive to the variability in non-native population attributes than time since introduction. Non-native species are affecting both phylogenetically close and distant native species, by competition processes between niche differences and between

different competitive abilities. We show that evaluating patterns of several non-native species may provide a broader understating of the entire community after invasions.

3.1 Introduction

The introduction of new species through human intervention has increased exponentially worldwide and represents one of the greatest threats to biodiversity at local and regional scales (Simberloff 2003; Blackburn et al. 2014; Bellard et al. 2016). Species introduced outside of their original distribution often causes dramatic impacts on the population of native species, community dynamics and functional structure of ecosystems (Catford et al. 2009; Simberloff et al. 2013; Toussaint et al. 2018), possibly resulting in the decline of biodiversity and the extinction of native species (Clavero et al. 2009). These impacts have already been well studied (Strayer 2012), but we still face some challenges when studying the processes structuring invasion patterns. An important issue is monitoring non-native species over time, which offers different answers regarding their effects on communities, since several common ecological and/or evolutionary processes should modulate the effect of a non-native species over time (Strayer et al. 2006). Therefore, time since introduction should be explicitly considered to adequately understand the effects of many non-native species (Strayer et al. 2006), since they continue to rise globally with few signs of saturation (Mormul et al. 2022).

The invasion process can be divided into stages: transport, introduction, establishment and spread (Blackburn et al. 2011). In the last stage, the population of the non-native species increases and may cause greater impacts on the native community, since the effects of a non-native species increase with its abundance (Strayer et al. 2006; Muñoz and Cavieres 2008; Jucker et al. 2013). Therefore, we should expect that if a

non-native species increases its abundance through time, then its impacts should increase as well. For instance, some studies have evaluated if the spatial distribution of native and non-native species changes with time after introduction and they found that native species were displaced from their optimal distribution and even excluded from the habitat after invasions (Bohn et al. 2008; Rodrigues et al. 2018; Smith et al. 2019). This is supported by the theory that competing and non-co-evolved species may not co-occur, since they don't display behaviour to partition space, time, or resources (Reitalu et al. 2008; Schuette et al. 2013; Grassel et al. 2015). Competing species usually share similar traits, and this may be assumed by phylogenetic similarity between them ('phylogenetic signal'; Blomberg et al. 2003; Jucker et al. 2013). Therefore, if the non-native species is phylogenetically close to a native species, it might be expected that they will be spatially separated, which avoids competitive interactions (Procheş et al. 2008; Weber and Strauss 2016), decreasing their co-occurrence. Furthermore, the density of competing species is also known to affect the strength of competition (Muñoz and Cavieres 2008; Wood et al. 2021), so relative abundance and similarity between species can interact in determining their co-occurrence.

Considering this, evaluating the co-occurrence in invaded communities through time may provide answers to ecological and evolutionary processes that shape biological invasions. If the non-native species can adapt to the new environmental conditions faster than the native species can evolve mechanisms to reduce the harmful effects of introduction, long-term coexistence between them may not be possible (Priddis et al. 2009). If so, it may result in changes and/or losses in taxonomic, functional and phylogenetic diversity of native communities through time (Jucker et al. 2013; Toussaint et al. 2018; Angulo-Valencia et al. 2022). As many communities are constantly suffering with invasions and they may change before being studied, long-

term studies are important keys to assess possible alterations of native communities. In this study, we aimed to assess co-occurrence time series between native and non-native species of fish in a 30-year time scale. We tested if the co-occurrence changed as the invasion process of the non-native species proceeded, especially at the last stage, when non-native species are expected to increase their abundance. We expected that the co-occurrence between native and non-native species decreases with the increase in abundance of the non-native species and with time after the introduction (Fig. 1; left panel). Besides, we also tested if the effect of the abundance is mediated by the phylogenetic distance (hereafter PD) between the native and the non-native species. We expected that co-occurrence decreases with non-native species abundance for phylogenetically similar species, but not necessarily for phylogenetically distant species, which are assumed to compete less (Fig. 1; right panel). We hope that studying the natural variability of population attributes (e.g. abundance) will allow us to make predictions and extrapolations for other communities, and evaluating this through time series will help us elucidate important patterns within invasion biology.

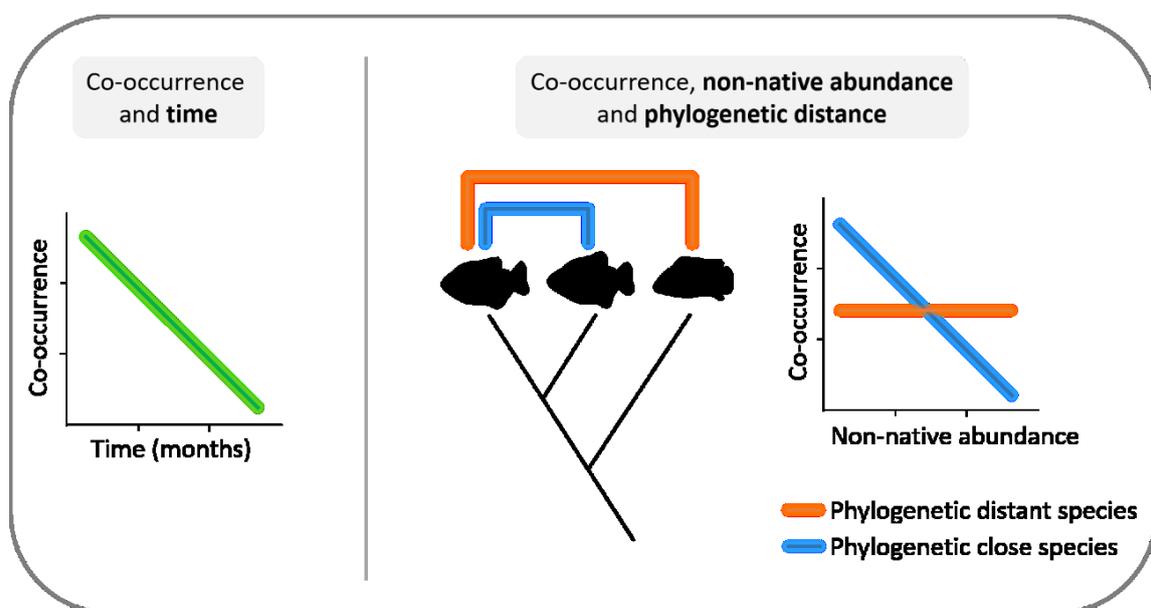


Figure 1. Co-occurrence between native and non-native species is expected to be driven by the time (left panel), abundance and phylogenetic distance (right panel). The co-occurrence is expected to decrease through time. The effect of the non-native abundance is mediated by phylogenetic distance: the co-occurrence decreases with abundance for phylogenetically similar species, but not necessarily for phylogenetically distant species.

3.2 Methods

3.2.1 Study area

This study was conducted in the Upper Paraná River floodplain, located in Southern Brazil (Fig. 2). The main river of the floodplain is the Paraná River, the tenth largest river in the world in terms of water discharge and fourth in terms of the drainage area (5×10^8 m³/year; 2.8×10^6 km², respectively; Agostinho and Zalewski 1996). The floodplain is the last dam-free stretch of the Paraná River in Brazil, comprising a 230 km stretch between two large reservoirs: the Porto Primavera Dam upstream and the Itaipu Reservoir downstream (22°40'S to 22°52'S and 53°12'W to 53°38'W). In this stretch, there is an intricate anastomosis involving secondary channels, tributary rivers and some floodplain lakes permanently connected to the river and some isolated and connected indirectly to the river by groundwater or only during flooding events (Granzotti et al. 2018; Ruaro et al. 2020). It is estimated that the region harbours approximately 4500 large animal and plant species (Agostinho et al. 2013), due to its high environmental heterogeneity provided by several habitats and seasonal flooding (Agostinho et al. 2004). Therefore, the area is considered a priority target for conservation, mostly because of its high socioeconomic relevance and refuge for biodiversity in the Southern part of South America (Agostinho et al. 2004).

Despite its high biodiversity, the Upper Paraná River floodplain is one of the most threatened South American wetlands (Ruaro et al. 2020), suffering with several impoundments constructed upstream and downstream the floodplain. These impoundments have changed several conditions in the floodplain, including its hydrology by reducing the variability in the flood pulse (Agostinho et al. 2009) and the limnologic conditions such as the increase in water transparency and reduction of both sediment and nutrient transport (Roberto et al. 2009; Stevaux et al. 2009). All these impacts contribute to the invasion by non-native species and its establishment, causing an increase in the number of non-native fish species. According to Gubiani et al. (2018), the Upper Paraná River ecoregion hosts the largest number of non-native fish species in the Neotropics (105 species). One of the most severe impact occurred with the filling of the Itaipu Reservoir in 1982, which flooded a geographic barrier located downstream the floodplain and allowed the introduction of at least 33 fish species in habitats where they had not previously occurred (Júlio Júnior et al. 2009). Additionally, activities such as aquarium trade, stocking, aquaculture and sport fishing also contributed to the invasion of non-native fish species in the floodplain (Ruaro et al. 2020).

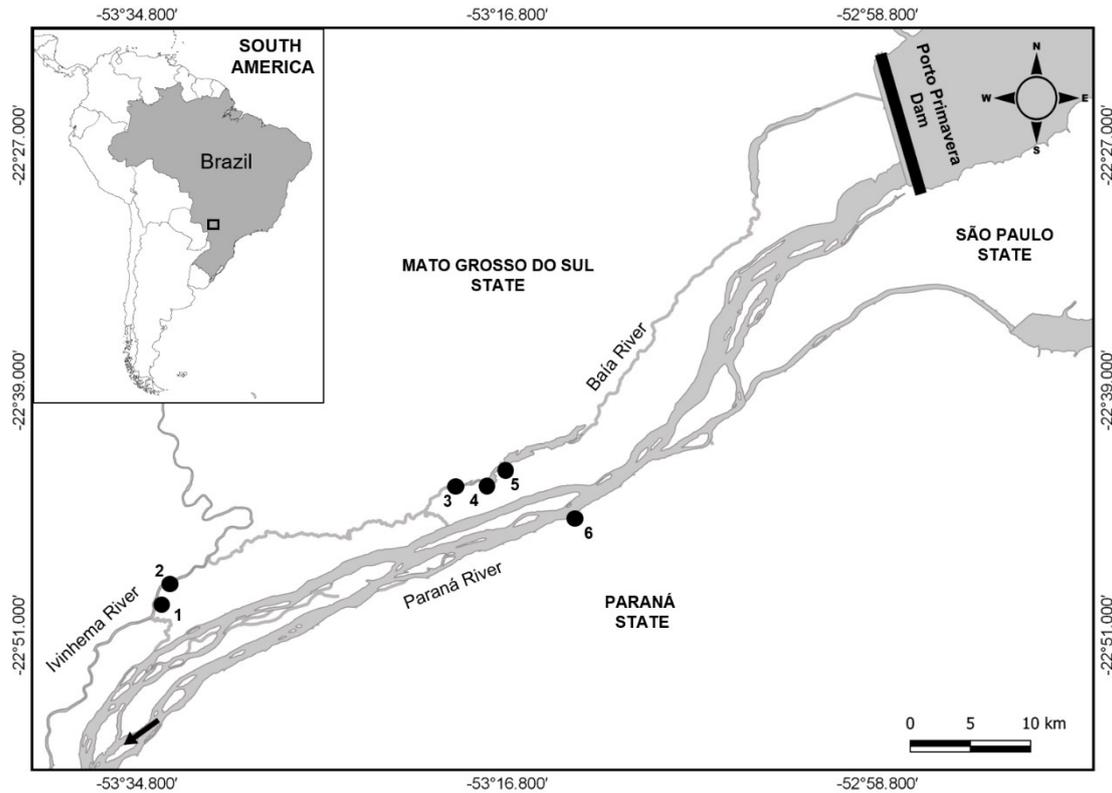


Figure 2. Sampling sites in the Upper Paraná River floodplain. 1 – Patos Lake; 2 – Ivinhema River; 3 – Guaraná Lake; 4 – Baía River; 5 – Fechada Lake; 6 – Paraná River. The black arrow indicates flow direction.

3.2.2 Fish sampling

We obtained fish community data from different projects conducted by the Nucleus of Research in Limnology, Ichthyology and Aquaculture (“*Núcleo de Pesquisas em Limnologia, Ictiologia e Aquicultura*” - Nupélia) at the State University of Maringá, Southern Brazil. These projects encompassed a 30-year time scale between 1987 and 2017 with a sampling periodicity almost continuous despite two short unsampled periods (1989-91 and 1995-99). Sampling was made quarterly in all surveys, generally carried out in March, June, September and December, with the exception of 1988 (three sampling events), 2003 and 2017 (semiannual sampling). Six sites were

sampled in almost the entire time series (five sites in 1992, 1993 and 1995) including lotic - main channel of the river - and lentic environments - floodplain lakes. Sampling is distributed in 87 months, totalizing 510 sampling events performed during this period. To sample the fish community, set of gillnets with different mesh sizes (24, 30, 40, 50, 60, 70, 80, 100, 120, 140, and 160 mm between opposite knots; 24 mm mesh used only from 2000 on) were deployed in each sampling site for 24h and all sampled individuals were identified to the species level. The abundance of non-native species in each sample was expressed in capture per unit effort (CPUE – number of individuals per area of gillnet per 24h). Native and non-native species were sampled, euthanized and classified according to Ota et al. (2018).

3.2.3 Co-occurrence

To estimate the co-occurrence between pairs of native and non-native species we applied the metric developed by Griffith et al. (2016), available in the ‘cooccur’ package from the R software (R Core Team 2021). This metric applies a probabilistic model to find the probability of two species co-occurring given the data (observed co-occurrence; OCo) and the probability that the same pair of species would co-occur at random (PCo). Because we were interested in investigating if a pair of species co-occurred more or less than expected by chance, we scaled the observed by the expected co-occurrence using the quotient of OCo and PCo. Therefore, values of scaled co-occurrence lower than 1 indicate that species co-occur less than expected by chance, values equal 1 indicate that species co-occur the same as expected by chance, and values higher than 1 indicate that species co-occur more than expected by chance. In order to obtain a temporal series for each pair of species, we estimated the co-occurrence in each month. We chose to estimate the co-occurrence by month because

the spatial distribution and abundance of species change according to the hydrological cycle, and to group this in one single value by year might cause confusion between different explaining factors. Finally, we removed pairs of species that showed scaled co-occurrence values equal to zero in further analyses since our objective was to evaluate pairs of species with lower and/or higher co-occurrence, and not absence of co-occurrence.

3.2.4 Phylogenetic distance

To obtain the PD between pairs of species, we used a set of 100 phylogenetic hypotheses compiled from the bony fish phylogeny of Rabosky et al. (2018). We used the *cophenetic* function of the ‘ape’ package (Paradis and Schliep 2019) from R software version 4.1.2 (R Core Team 2021). This provided us 100 phylogenetic distance matrices. However, these distance matrices were very similar to each other (pairwise correlations ranging from 0.98 to 0.99). Therefore, we randomly chose one of the distance matrices to use in the following analysis using the *sample* function of the ‘base’ package (R Core Team 2021).

3.2.5 Data analysis

For each month and each pair of native – non-native species, we estimated the co-occurrence value, the abundance of the non-native species and time since introduction. We estimated the abundance (CPUE) for all non-native species as the mean abundance across all sites in each month where they were sampled. Time since introduction was considered as the time elapsed in months after the first registered occurrence of the species in the floodplain. To minimize the effect of a low number of samples for pairs of species that occurred sporadically in the time series, we removed

pairs of species that occurred in less than 25% of the complete time series. Therefore, we only evaluated pairs of species that occurred in at least 21 months, which resulted in 623 pairs of species, 37 native and 28 non-native species.

In order to test if the co-occurrence between native and non-native species was driven by non-native species abundance, time and phylogenetic distance, we performed a Linear Mixed Effect Model (LME; Zuur et al. 2009) using the log-transformed scaled co-occurrence as response variable. We included the log-transformed non-native species abundance, time and PD as predictors, and the seasonality (dry or wet season) and identity of the species pair as random factors. The random factors were chosen for the following reasons: 1) seasonality (i.e., periods of low and high water levels in each year according to the hydrological cycle of the floodplain) may affect the co-occurrence between species, such that co-occurrence could be similar within seasons but different between seasons; adding the seasonality as random factor implies that we assume the presence of its effect but we are not interested in it; 2) each pair of species shows a specific trend of co-occurrence over time due to the idiosyncrasies of each one (e.g., non-native species were introduced at different times), but we are also not interested in this. We included the random factors in a nested structure (seasonality nested within each species pair, which represented a time series). To minimize the effect of temporal autocorrelation, we added a correlation structure in the model (corAR1, corARMA and corCAR1). To assess the best correlation structure, we ranked competitive models with different correlation structures according to AIC. To perform the model, we used the *lme* function in the 'nlme' package (Pinheiro et al. 2021) in the R software version 4.1.2 (R Core Team 2021).

3.3 Results

Our data presented occurrence information through time for 37 native and 28 non-native species from the Upper Paraná River floodplain (Appendix D). Overall, non-native species are very common in the floodplain: among the 10 most common species in occurrence, four are native (*Prochilodus lineatus*, *Astyanax lacustris*, *Leporinus friderici* and *Leporinus lacustris*) and six are non-native (*Loricariichthys platymetopon*, *Serrasalmus marginatus*, *Schizodon borellii*, *Parauchenipterus galeatus*, *Pterygoplichthys ambrosetti*, *Auchenipterus osteomystax*). Among the non-native species, the lowest abundance was 0.36 (number of individuals/area*24h) for *Trachydoras paraguayensis*, and the highest was 418.48 (number of individuals/area*24h) for *Serrasalmus marginatus*. The time since introduction ranged from 36 to 87 months. As for the PD, a congeneric pair of native and non-native species showed the lowest distance value (2.69), and the most distant pairs of species had a PD of 354.95 (several pairs; Appendix E, Fig. S3). Overall, most pairs of species showed intermediary values of PD (Appendix E, Fig. S4).

We gathered co-occurrence time series for 623 pairs of species. The shortest time series presented 21 co-occurrences, and the longest presented 86 co-occurrences. We created heatmaps for the two most common non-native species (*L. platymetopon* and *S. marginatus*), in order to illustrate the variation in the co-occurrence through time for each species pair (Fig. 3).

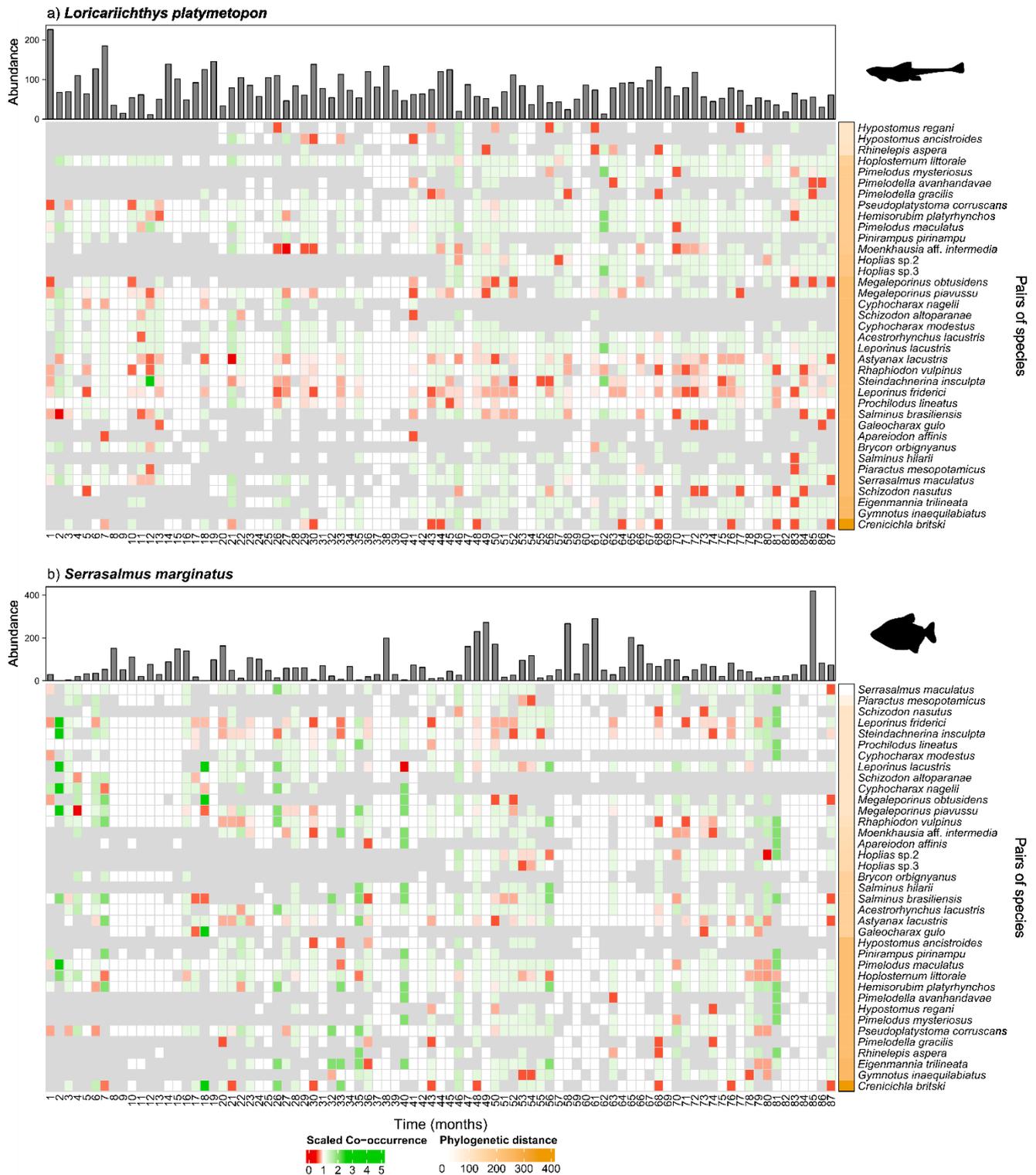


Figure 3. Heatmap of the scaled co-occurrence through time for pairs of species with a) *Loricariichthys platymetopon* and b) *Serrasalmus marginatus*. Grey squares represent lack of co-occurrence. Top bar plots represent non-native species abundance through time.

We found that non-native abundance, phylogenetic distance and time since introduction affected the co-occurrence between native and non-native species. Scaled co-occurrence values decreased with increasing non-native abundance (Linear Mixed Model; Table 1). However, we found an interaction between abundance and phylogenetic distance, so that, in higher values of non-native abundance, phylogenetically distant native and non-native species pairs co-occur less than phylogenetically close species (Fig. 4). As for time since introduction, we found that co-occurrence increased through time, but the effect was low when compared to the non-native abundance (see T-value in Table 1).

Table 1. Linear Mixed Model results between scaled co-occurrence and time since introduction, log-abundance and phylogenetic distance. Bold values are significant variables. The standard deviations for the random effects were 0.10782 for wet season and 0.33581 for residuals.

Parameter	Estimate	Confidence intervals		T-value
		Lower-95	Upper-95	
Intercept	0.41117	0.36549	0.45684	17.84794
Time since introduction	0.00073	0.00052	0.00094	6.91371
log Abundance	-0.08849	-0.10015	-0.07683	-15.31874
Phylogenetic distance	0.00003	-0.00015	0.00021	0.00639
log Abundance * phylogenetic distance	-0.00007	-0.00012	-0.00002	-2.52737

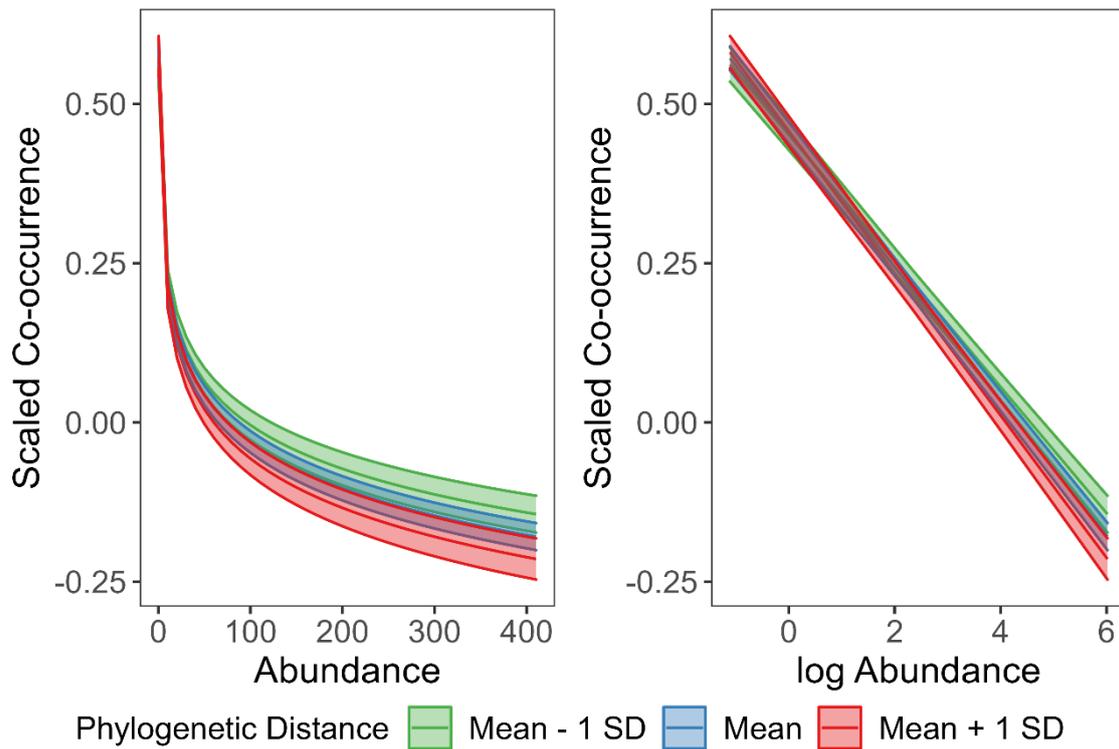


Figure 4. The phylogenetic distance between species mediates the relationship between abundance and scaled co-occurrence. Different colours represent different levels of phylogenetic distance: green – smaller distance; blue – medium distance; red – higher distance.

3.4 Discussion

Evaluating the ecological processes related to the effects of non-native species' is extremely important to understand and mitigate the impacts of biological invasions. Many questions have been raised until now (see Gallien and Carboni 2017 for examples), but few are related to how the effects of invasion change through time. Most studies report the acute effects of invasions (i.e. immediately after a new species arrives), which are also important, but chronic effects represent the eventual outcomes of a species invasion and have great ecological and economic interest (i.e. after various ecological and evolutionary processes have come into play; Strayer et al. 2006). Here,

assessing the co-occurrence time series of pairs of native and non-native species, we provide information about how co-occurrence between them may be modelled through time. First, the results showed that the effect of time since introduction in driving species co-occurrence was less important than the effect of the non-native abundance. This suggests that population attributes that are variable through time are more important in determining non-native species impacts than the time after introduction. Second, as expected, we provide evidence that greater non-native abundance decreases the co-occurrence between native and non-native species. However, the intensity of this effect is mediated by the phylogenetic distance between the pair of species. Contrary to our predictions, we found that the direction of the effect of the non-native abundance is the same independently of the phylogenetic distance (a negative effect), but phylogenetically distant species are more spatially segregated when the abundance is high.

The results showed that there was an increase in the co-occurrence between native and non-native species through time. We expected that the co-occurrence would decrease through time, assuming that the non-native abundance would continuously increase through the years studied. However, in Fig. 3 it is possible to see that abundance for the two non-native species showed a high variability between months. This indicates that an annual variability of non-native abundance is more important than a long-term tendency. This could justify the lower effect of time since introduction when compared to the higher effect of non-native abundance in driving the co-occurrence between native and non-native species. We believe we found this result due to the specificity of our study system. Floodplains systems are mostly controlled by the hydrological regime, characterized by seasonal floodings that drive physical, chemical and biological changes on floodplain habitats (Thomaz et al. 2007). These changes

affect fish species distributions at a local scale, as well as species abundance via resources limitation and competitive exclusion (Hitt and Chambers 2014; Thomaz 2021). Therefore, including the variability of abundance through a time scale that encompasses extreme events (i.e. such as large floods for floodplain fish) was extremely important to capture the real effect of this variable (Strayer et al. 2006).

Regarding the effect of the non-native species abundance, we found that abundance affected the co-occurrence patterns between native and non-native species. The results showed that when non-native abundance reached high values, the co-occurrence with native species decreased. After an introduction, it is expected that non-native species abundance increases or decreases through time (Strayer et al. 2006). When it increases, it can cause the displacement from optimal habitats of native species, leading to alterations in spatial distribution, decreasing abundance or even excluding the native species. This impact has already been reported by some authors (Muñoz and Cavieres 2008; Jucker et al. 2013; Pascual-Rico et al. 2020; Clavero et al. 2022), and some of them relate the increase and spread of the non-native population to the displacement of native species. The increase in abundance intensifies the competition imposed by non-native species, generally because non-native species are superior competitors (Melbourne et al. 2007). Therefore, in order to avoid competition, native species might be spatially displaced and/or excluded from the habitat (Parker et al. 1999; Cheng et al. 2009; Jessen et al. 2018). By altering native populations, non-native species may cause marked shifts in taxonomic, functional and phylogenetic diversity of native communities (Jucker et al. 2013; Toussaint et al. 2018). However, when non-native abundance is low, co-occurrence with native species is higher, and the chances of greater impacts on native biodiversity are lower. As mentioned above, the seasonal flooding in floodplains systems provides the variability in species abundance (Thomaz

et al. 2021). Therefore, preserving the seasonal variability of the floodplain is extremely important for the maintenance of native biodiversity by allowing the coexistence of native and non-native species.

We expected that phylogenetically close species should co-occur less when the non-native abundance was higher, and that phylogenetically distant species would be less affected by high non-native abundance. This prediction suggests that competition would spatially segregate similar native and non-native species (Gois et al. 2015), but the results only partially supported this. The association between non-native abundance and phylogenetic distance indicated that non-native abundance decreases co-occurrence in any level of phylogenetic distance, but the intensity of this effect is lower for phylogenetically distant species, which co-occur less when abundance is high.

According to the “Darwin’s naturalization hypothesis” (Daehler 2001; Thuiller et al. 2010), non-native species that are not phylogenetically related to the native species (i.e. distant species) should co-occur more, because they would not share similar characteristics and would compete less for resources. However, Darwin (1859) also postulated that non-native species that are phylogenetically related to the native species in the new environment should obtain success in the invasion, because they would share similar pre-adaptations to local conditions (“pre-adaptation hypothesis”; Thuiller et al. 2010; Li et al. 2015). The first hypothesis is aligned with the limiting similarity principle (MacArthur & Levins 1967; Tilman 1982), which predicts that ecologically similar species will exclude one another due to strong niche overlap. The second hypothesis is aligned with the assumption that environmental filtering will structure communities and filter similar species to inhabit determined environmental conditions (Melbourne et al. 2007). Therefore, the results suggest that non-native species are impacting both phylogenetically close and distant native species, by competition

processes between niche differences (i.e. between phylogenetically close species) and between different competitive abilities (i.e. between phylogenetically distant species), when competitively weaker species might be displaced. HilleRisLambers et al. (2012) suggested this outcome of competition among species, and Jucker et al. (2013) supported it for invaded communities. We believe the obtained results follow the same expectations, and that both the limiting similarity principle and the environmental filtering hypothesis can explain community co-occurrence after invasions.

The results show that evaluating information on co-occurrence between native and non-native species through time might improve the study of ecological and evolutionary processes of biological invasions. Here, we integrated co-occurrence time series with abundance and phylogenetic distance of an invaded community and we showed that evaluate patterns of several non-native species may provide a broader understating of the community after invasions. Many studies are focused on analysing the impacts of non-native species on similar native species, but we show here that phylogenetically distant species may suffer with negative impacts as well. Therefore, we highlight the need of studying the non-native effects on communities through time, so we can capture the impacts as a whole. For that, we should consider population and community attributes when evaluating invasion impacts. Besides, analysing data sets through time also allow us to considerate the variability in the community after changes in environmental characteristics (Strayer et al. 2006), which affects the population dynamics of species and can be helpful to the maintenance of native biodiversity. We also highlight the preservation of the natural temporal dynamics of habitats (e.g. the seasonal flooding in floodplain systems), which is extremely important to prevent greater impacts on native communities. We hope these findings will inspire the

development of future research on community effects through time and support science-based management decisions regarding non-native species.

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4 CONCLUDING REMARKS

Based on the results obtained, it was possible to identify two factors that model the co-occurrence between native and non-native species. The first one is the relationship between species, both functional and phylogenetic relationship. At any level of relationship between native and non-native species, there may always be a modification in the spatial distribution of species when the environment is invaded. The second one is the non-native species abundance. The high abundance of non-native species is a great threat for the spatial distribution of native species. This is concerning for the native biodiversity, once species abundance is a population attribute that can vary depending on the environment, which means that the non-native effect may be unpredictable, instead of occurring with time. Finally, using different approaches is also important to evaluate non-native species. The co-occurrence was evaluated under different scales and historical context, and in all of them, it was always affected. Therefore, it is extremely important that future researchers consider different approaches and scales, so one can estimate the different processes and impacts that may exist. To summarize, the whole native community will be affected by non-native species, and mitigation actions to preserve natural habitats and to control the spread and establishment of non-native species are essentials.

APPENDIX LIST

APPENDIX A – SAMPLING SITES IN THE UPPER PARANÁ RIVER

FLOODPLAIN, BRAZIL

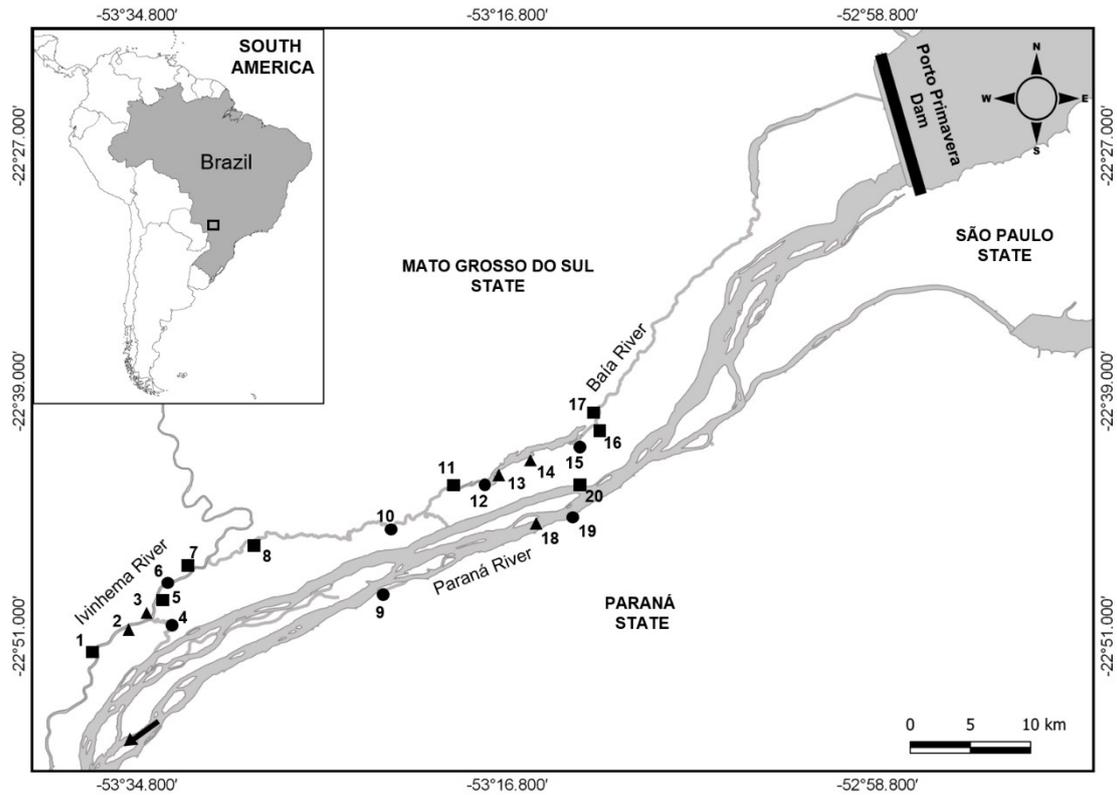


Figure S1. Sampling sites in the Upper Paraná River floodplain, Brazil. Different shapes represent different environments (circles – lotic environments; squares – connected floodplain lakes; triangles – not connected floodplain lakes). The black arrow indicates flow direction.

**APPENDIX B – MORPHOLOGICAL TRAITS USED IN THE ANALYSIS,
THEIR FORMULA AND POTENTIAL LINK WITH FISH FUNCTIONS *SENSU*
BROSSE ET AL. (2021).**

Table 1. Morphological traits used in the analysis, their formula and potential link with fish functions *sensu* Brosse et al. (2021). Bl = body length; Bd = body depth; Eh = eye position; Ed = eye diameter; Hd = head depth; Mo = mouth height; Jl = maxillary jaw length; PFi = pectoral fin position; PFl = pectoral fin length; CFd = caudal fin depth; CPd = caudal peduncle depth.

Morphological traits	Formula	Potential link with fish functions
Maximum body length (MBI)	MBI	Metabolism, trophic impacts, locomotion ability, nutrient cycling
Body elongation (BEI)	$\frac{Bl}{Bd}$	Hydrodynamism
Vertical eye position (VEp)	$\frac{Eh}{Bd}$	Position of fish and/or of its prey in the water column
Relative eye size (REs)	$\frac{Ed}{Hd}$	Visual acuity
Oral gape position (OGp)	$\frac{Mo}{Bd}$	Feeding position in the water column
Relative maxillary length (RMI)	$\frac{Jl}{Hd}$	Size of mouth and strength of jaw
Body lateral shape (BLs)	$\frac{Hd}{Bd}$	Hydrodynamism and head size
Pectoral fin vertical position (PFv)	$\frac{PFl}{PFi}$	Pectoral fin use for swimming
Pectoral fin size (PFs)	$\frac{Bl}{PFl}$	Pectoral fin use for swimming
Caudal peduncle throttling (CPT)	$\frac{Bl}{CPd}$	Caudal propulsion efficiency through reduction of drag

**APPENDIX C – LIST OF FISH SPECIES FROM THE UPPER PARANÁ RIVER
FLOODPLAIN SAMPLED IN 2000, 2001, 2010 AND 2011 AND THEIR STATUS.**

Species	Status
<i>Acestrorhynchus lacustris</i> (Lütken, 1875)	Native
<i>Ageneiosus inermis</i> (Linnaeus, 1766)	Non-native
<i>Ageneiosus ucayalensis</i> Castelnau, 1855	Non-native
<i>Apareiodon affinis</i> (Steindachner, 1879)	Native
<i>Astronotus crassipinnis</i> (Heckel, 1840)	Non-native
<i>Astyanax</i> aff. <i>fasciatus</i> (Cuvier, 1819)	Native
<i>Astyanax lacustris</i> (Lütken, 1875)	Native
<i>Auchenipterus osteomystax</i> (Miranda Ribeiro, 1918)	Non-native
<i>Brycon orbignyianus</i> (Valenciennes, 1850)	Native
<i>Catathyridium jenynsii</i> (Günther, 1862)	Non-native
<i>Cichlasoma paranaense</i> Kullander, 1983	Native
<i>Crenicichla britskii</i> Kullander, 1982	Native
<i>Crenicichla jaguarensis</i> Haseman, 1911	Native
<i>Cyphocharax modestus</i> (Fernández-Yépez, 1948)	Native
<i>Cyphocharax nagelii</i> (Steindachner, 1881)	Native
<i>Eigenmannia trilineata</i> López, Castello, 1966	Native
<i>Eigenmannia virescens</i> (Valenciennes, 1836)	Native
<i>Erythrinus erythrinus</i> (Bloch, Schneider, 1801)	Non-native
<i>Galeocharax gulo</i> (Cope, 1870)	Native
<i>Gymnotus inaequilabiatus</i> (Valenciennes, 1839)	Native
<i>Gymnotus sylvius</i> Albert, Fernandes-Matioli, 1999	Native
<i>Hemisorubim platyrhynchus</i> (Valenciennes, 1840)	Native
<i>Hoplerythrinus unitaeniatus</i> (Agassiz, 1829)	Non-native
<i>Hoplias mbigua</i> Azpelicueta, Benítez, Aichino, Mendez, 2015	Non-native
<i>Hoplias</i> sp.2	Native
<i>Hoplias</i> sp.3	Native
<i>Hoplosternum littorale</i> (Hancock, 1828)	Native
<i>Hypophthalmus oremaculatus</i> Nani, Fuster, 1947	Non-native
<i>Hypostomus</i> cf. <i>albopunctatus</i> (Regan, 1908)	Native

<i>Hypostomus ancistroides</i> (Ihering, 1911)	Native
<i>Hypostomus cochliodon</i> Kner, 1854	Non-native
<i>Hypostomus</i> aff. <i>hermanni</i> (Ihering, 1905)	Native
<i>Hypostomus</i> cf. <i>iheringii</i> (Regan, 1908)	Native
<i>Hypostomus microstomus</i> Weber, 1987	Non-native
<i>Hypostomus regani</i> (Ihering, 1905)	Native
<i>Hypostomus</i> cf. <i>strigaticeps</i> (Regan, 1908)	Native
<i>Leporellus vittatus</i> (Valenciennes, 1850)	Native
<i>Leporinus friderici</i> (Block, 1794)	Native
<i>Leporinus lacustris</i> Campos, 1945	Native
<i>Loricariichthys platymetopon</i> Isbrücker, Nijssen, 1979	Non-native
<i>Loricariichthys rostratus</i> Reis, Pereira, 2000	Non-native
<i>Megalancistrus parananus</i> (Peters, 1881)	Native
<i>Megaleporinus obtusidens</i> (Valenciennes, 1836)	Native
<i>Megaleporinus piavussu</i> (Britski, Birindelli, Garavello, 2012)	Native
<i>Moenkhausia</i> aff. <i>intermedia</i> Eigenmann, 1908	Native
<i>Ossancora eigenmanni</i> (Boulenger, 1895)	Non-native
<i>Parauchenipterus galeatus</i> (Linnaeus, 1766)	Non-native
<i>Parodon nasus</i> Kner, 1859	Native
<i>Piabarchus stramineus</i> (Eigenmann, 1908)	Native
<i>Piaractus mesopotamicus</i> (Holmberg, 1900)	Native
<i>Pimelodella avanhandavae</i> Eigenmann, 1917	Native
<i>Pimelodella gracilis</i> (Valenciennes, 1835)	Native
<i>Pimelodus maculatus</i> Lacépède, 1803	Native
<i>Pimelodus mysteriosus</i> Azpelicueta, 1998	Native
<i>Pimelodus ornatus</i> Kner, 1858	Non-native
<i>Pinirampus pirinampu</i> (Agassiz, 1829)	Native
<i>Platydoras armatulus</i> (Valenciennes, 1840)	Non-native
<i>Potamotrygon amandae</i> Loboda, Carvalho, 2013	Non-native
<i>Prochilodus lineatus</i> (Valenciennes, 1836)	Native
<i>Pseudoplatystoma corruscans</i> (Spix, Agassiz, 1829)	Native
<i>Pterodoras granulosus</i> (Valenciennes, 1821)	Non-native
<i>Rhamdia quelen</i> (Quoy, Gaimard, 1824)	Native

<i>Rhamphichthys hahni</i> (Meinken, 1937)	Non-native
<i>Rhaphiodon vulpinus</i> Spix, Agassiz, 1829	Native
<i>Rhinelepis aspera</i> Spix, Agassiz, 1829	Native
<i>Roeboides descalvadensis</i> Fowler, 1932	Non-native
<i>Salminus brasiliensis</i> (Cuvier, 1816)	Native
<i>Salminus hilarii</i> Valenciennes, 1850	Native
<i>Schizodon altoparanae</i> Garavello, Britski, 1990	Native
<i>Schizodon nasutus</i> Kner, 1858	Native
<i>Serrasalmus maculatus</i> Kner, 1858	Native
<i>Serrasalmus marginatus</i> Valenciennes, 1837	Non-native
<i>Sorubim lima</i> (Block, Schneider, 1801)	Non-native
<i>Steindachnerina brevipinna</i> (Eigenmann, Eigenmann, 1889)	Non-native
<i>Steindachnerina insculpta</i> (Fernández-Yépez, 1948)	Native
<i>Sternopygus macrurus</i> (Bloch, Schneider, 1801)	Native
<i>Trachelyopterus</i> sp.	Native
<i>Trachydoras paraguayensis</i> (Eigenmann, Ward, 1907)	Non-native

APPENDIX D – LIST OF FISH SPECIES FROM THE UPPER PARANÁ RIVER FLOODPLAIN SAMPLED BETWEEN 1987 AND 2017

Species	Status	Only for non-native species	
		First occurrence	Vector of introduction
<i>Acestrorhynchus lacustris</i> (Lütken, 1875)	Native		
<i>Ageneiosus inermis</i> (Linnaeus, 1766)	Non-native	December 1993	Construction of impoundments
<i>Apareiodon affinis</i> (Steindachner, 1879)	Native		
<i>Astronotus crassipinnis</i> (Heckel, 1840)	Non-native	February 2000	Aquarium trade
<i>Astyanax lacustris</i> (Lütken, 1875)	Native		
<i>Auchenipterus osteomystax</i> (Miranda Ribeiro, 1918)	Non-native	March 1987	Construction of impoundments
<i>Brycon orbignyanus</i> (Valenciennes, 1850)	Native		
<i>Cichla kelberi</i> Kullander, Ferreira, 2006	Non-native	June 1992	Escapes from recreational angling ponds
<i>Crenicichla britski</i> Kullander, 1982	Native		
<i>Cyphocharax modestus</i> (Fernández-Yépez, 1948)	Native		
<i>Cyphocharax nagelii</i> (Steindachner, 1881)	Native		
<i>Eigenmannia trilineata</i> López, Castello, 1966	Native		
<i>Galeocharax gulo</i> (Cope, 1870)	Native		
<i>Geophagus sveni</i> Lucinda, Lucena, Assis, 2010	Non-native	March 2006	Aquarium trade
<i>Gymnotus inaequilabiatus</i> (Valenciennes, 1839)	Native		
<i>Hemiodus orthonops</i> Eigenmann, Kennedy, 1903	Non-native	June 2008	Functioning of the Canal da Piracema (a fish ladder that connects two distinct regions)
<i>Hemisorubim platyrhynchos</i> (Valenciennes, 1840)	Native		
<i>Hoplerythrinus unitaeniatus</i> (Agassiz, 1829)	Non-native	June 1992	Live bait by anglers or construction of impoundments
<i>Hoplias mbigua</i> Azpelicueta, Benítez, Aichino, Mendez, 2015	Non-native	December 2006	Construction of impoundments
<i>Hoplias</i> sp.2	Native		
<i>Hoplias</i> sp.3	Native		

<i>Hoplosternum littorale</i> (Hancock, 1828)	Native		
<i>Hypophthalmus oremaculatus</i> Nani, Fuster, 1947	Non-native	March 1987	Construction of impoundments
<i>Hypostomus ancistroides</i> (Ihering, 1911)	Native		
<i>Hypostomus cochliodon</i> Kner, 1854	Non-native	August 2002	Construction of impoundments
<i>Hypostomus regain</i> (Ihering, 1905)	Native		
<i>Iheringichthys labrosus</i> (Lütken, 1874)	Non-native	March 1987	Construction of impoundments
<i>Leporinus friderici</i> (Block, 1794)	Native		
<i>Leporinus lacustris</i> Campos, 1945	Native		
<i>Loricariichthys platymetopon</i> Isbrücker, Nijssen, 1979	Non-native	March 1987	Construction of impoundments
<i>Loricariichthys rostratus</i> Reis, Pereira, 2000	Non-native	June 1992	Construction of impoundments
<i>Megaleporinus microcephalus</i> (Garavello, Britski, 1988)	Non-native	May 2002	Fish-farming and escapes from recreational angling ponds
<i>Megaleporinus obtusidens</i> (Valenciennes, 1836)	Native		
<i>Megaleporinus piavussu</i> (Britski, Birindelli, Garavello, 2012)	Native		
<i>Metynnis lippincotianus</i> (Cope, 1870)	Non-native	February 2000	Restocking or aquarium trade
<i>Moenkhausia</i> aff. <i>intermedia</i> Eigenmann, 1908	Native		
<i>Parauchenipterus galeatus</i> (Linnaeus, 1766)	Non-native	March 1987	Construction of impoundments
<i>Piaractus mesopotamicus</i> (Holmberg, 1900)	Native		
<i>Pimelodella avanhandavae</i> Eigenmann, 1917	Native		
<i>Pimelodella gracilis</i> (Valenciennes, 1835)	Native		
<i>Pimelodus maculatus</i> Lacépède, 1803	Native		
<i>Pimelodus misteriosus</i> Azpelicueta, 1998	Native		
<i>Pimelodus ornatus</i> Kner, 1858	Non-native	March 1988	Construction of impoundments
<i>Pinirampus pirinampu</i> (Agassiz, 1829)	Native		
<i>Plagioscion squamosissimus</i> (Heckel, 1840)	Non-native	March 1987	Commercial importance
<i>Prochilodus lineatus</i> (Valenciennes, 1836)	Native		
<i>Pseudoplatystoma corruscans</i> (Spix, Agassiz, 1829)	Native		
<i>Pterodoras granulosus</i> (Valenciennes, 1821)	Non-native	March 1987	Construction of impoundments
<i>Pterygoplichthys ambrosettii</i> (Holmberg, 1893)	Non-native	February 2000	Construction of impoundments or aquarium trade
<i>Rhamphichthys hahni</i> (Meinken, 1937)	Non-native	March 1987	Live bait by anglers or construction of impoundments

<i>Rhaphiodon vulpinus</i> Spix, Agassiz, 1829	Native		
<i>Rhinelepis aspera</i> Spix, Agassiz, 1829	Native		
<i>Roeboides descalvadensis</i> Fowler, 1932	Non-native	March 1987	Construction of impoundments
<i>Salminus brasiliensis</i> (Cuvier, 1816)	Native		
<i>Salminus hilarii</i> Valenciennes, 1850	Native		
<i>Satanoperca</i> sp.	Non-native	March 1987	Construction of impoundments
<i>Schizodon altoparanae</i> Garavello, Britski, 1990	Native		
<i>Schizodon borellii</i> (Boulenger, 1900)	Non-native	March 1987	Restocking
<i>Schizodon nasutus</i> Kner, 1858	Native		
<i>Serrasalmus maculatus</i> Kner, 1858	Native		
<i>Serrasalmus marginatus</i> Valenciennes, 1837	Non-native	March 1987	Construction of impoundments
<i>Sorubim lima</i> (Bloch, Schneider, 1801)	Non-native	September 1987	Construction of impoundments
<i>Steindachnerina brevipinna</i> (Eigenmann, Eigenmann, 1889)	Non-native	August 1994	Construction of impoundments
<i>Steindachnerina insculpta</i> (Fernández-Yépez, 1948)	Native		
<i>Trachydoras paraguayensis</i> (Eigenmann, Ward, 1907)	Non-native	March 1987	Construction of impoundments

APPENDIX E– ADDITIONAL RESULTS

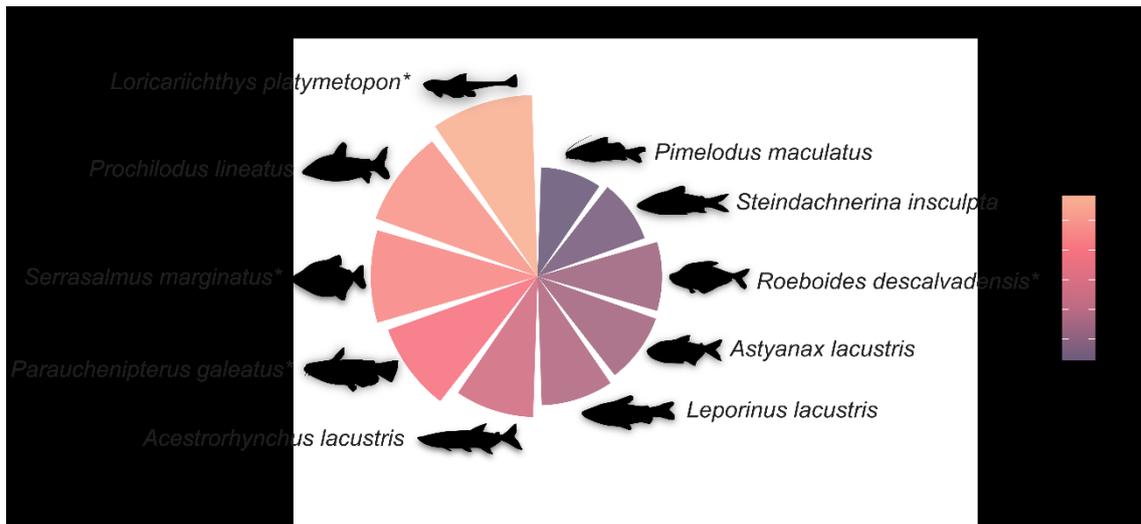


Figure S1. Ten most common species from the Upper Paraná River floodplain. One asterisk after species name represents non-native species.

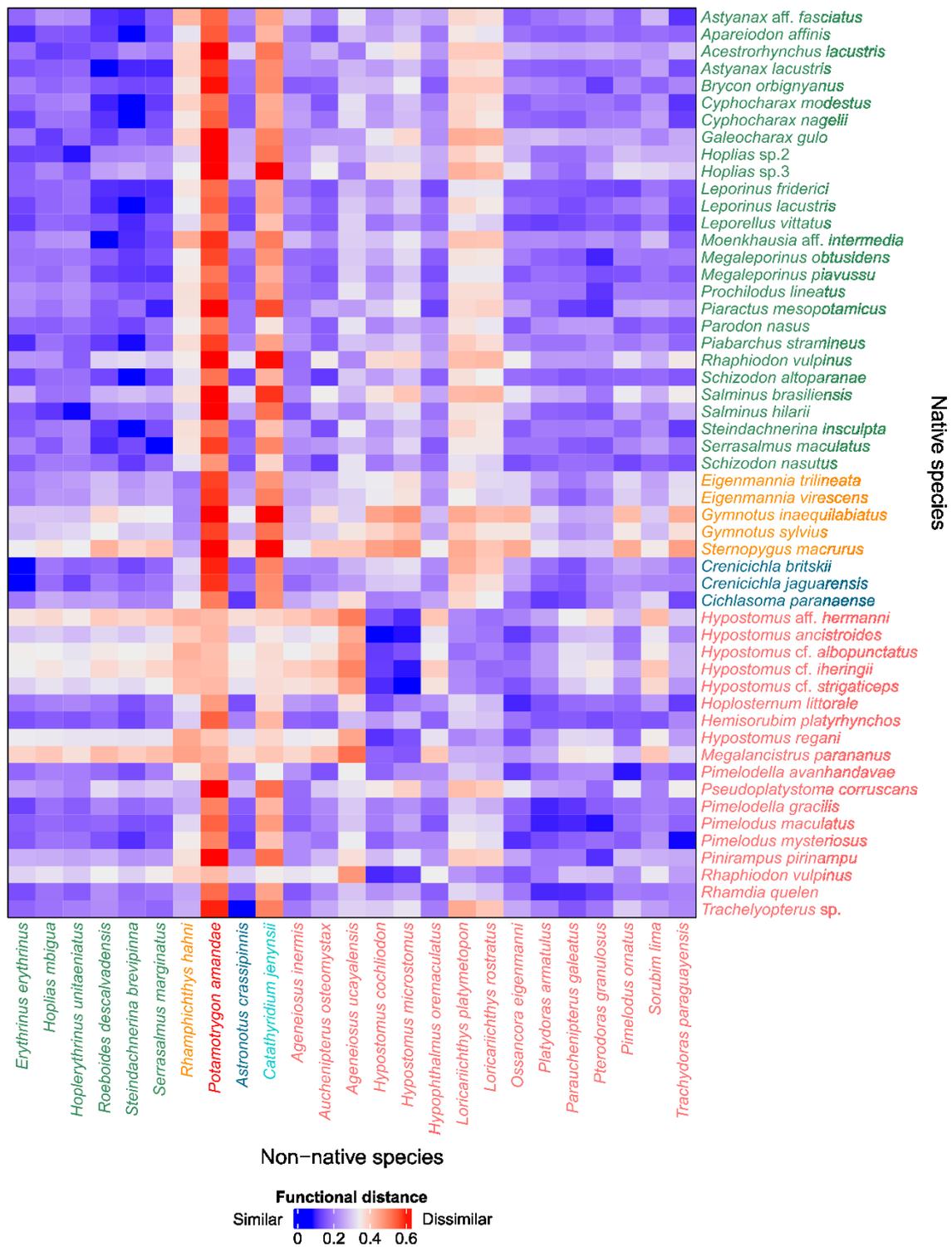


Figure S2. Functional distance between pairs of native and non-native species. Different colours in species name indicate different taxonomic orders. Green: Characiformes; orange: Gymnotiformes; dark blue: Cichliformes; pink: Siluriformes; red: Myliobatiformes; light blue: Pleuronectiformes.

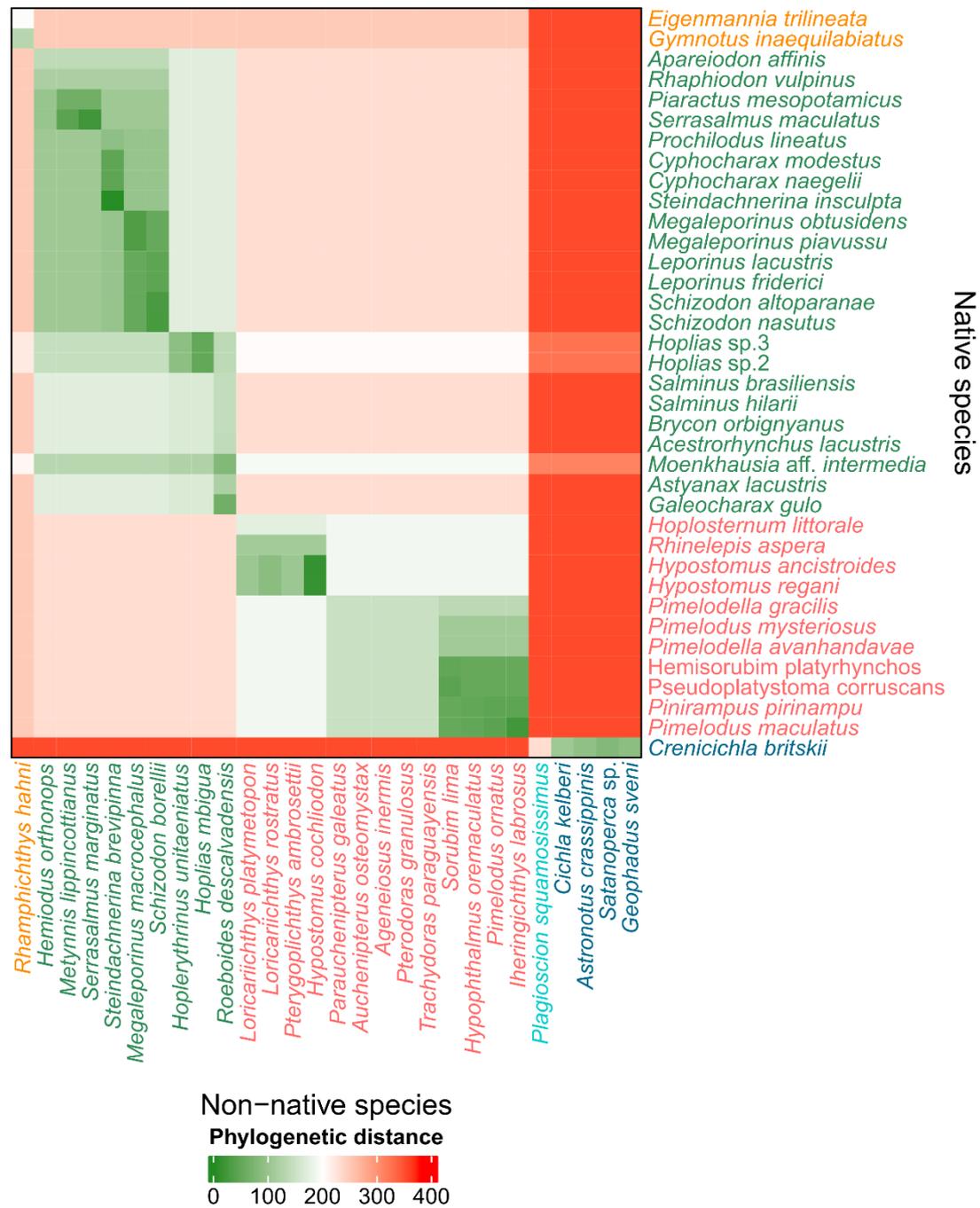


Figure S3. Phylogenetic distance between pairs of native and non-native species. Different colours in species name indicate different taxonomic orders. Orange: Gymnotiformes; green: Characiformes; dark blue: Cichliformes; pink: Siluriformes; light blue: *incertae sedis*.

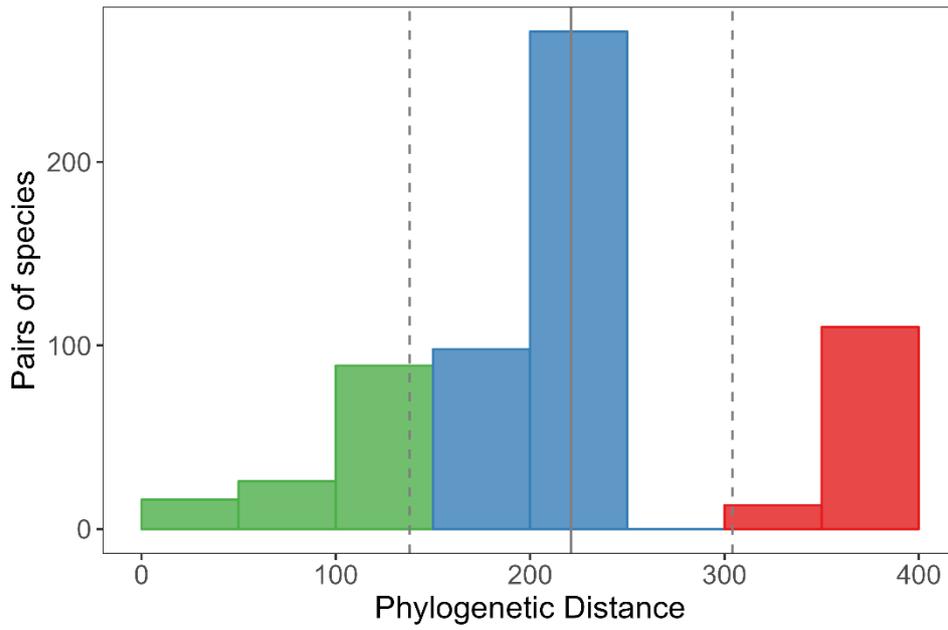


Figure S4. Distribution of phylogenetic distance values. Dashed lines represent ‘mean – standard deviation’ and ‘mean + standard deviation’, respectively, and continuous line represents the mean. Different colours represent different levels of phylogenetic distance: green – smaller distance; blue – medium distance; red – higher distance.