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ORIGINAL PAPER

Invasion dynamics of the white piranha (*Serrasalmus brandtii*) in a Neotropical river basin

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Abstract Clarifying the intricate history of unrecorded fish invasions represents an important step in understanding the invasion process. Here, we elucidate the invasion of a Neotropical river basin in Southeastern Brazil by a very efficient predator, the white piranha (*Serrasalmus brandtii*). We used a temporal series of population dynamics data (2008–2016) and analyses of mitochondrial DNA sequences (COI, 16S, and control region) of specimens from the entire native (São Francisco River Basin, Southeastern Brazil) and invaded (Jequitinhonha River Basin, JRB) ranges. We detected low genetic diversity ($h = 0.5835$) and strong genetic structure in the invasive range (Fst = 0.9141), despite high genetic diversity ($h = 0.8900$) and low genetic structure (Fst from 0.0740 to 0.1348) in the native range. High genetic structure in the invaded range was associated with a hydroelectric dam that prevented the admixture of two independent introductory acts into the JRB (downstream and upstream of the Irapé Dam). The rapid invasion capability of *Serrasalmus brandtii*, with few propagules, indicates that the species should be included in ecological risk assessments for restocking efforts of other commercially or ecologically important fish species and dam construction in Brazil. The combination of genetic and population dynamic datasets enabled the reconstruction of a top predator fish invasion in the Neotropics and shed light on ecological factors that influenced its invasion success.

Keywords Biological invasion · DNA · Invasive success · Reservoirs · Dam · Jequitinhonha River Basin

Introduction

Biological invasions are considered the main driver of recent extinctions (Blackburn et al. 2019) and a

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primary threat to freshwater ecosystems (Reid et al. 2019), but also offer a great opportunity to investigate the role of top predators in regulating biodiversity (Pringle et al. 2019). Invasive species can also cause negative economic impacts (Bajer et al. 2016; Gallardo et al. 2016) due to habitat degradation and massive loss of ecosystem services (D'Antonio et al. 2001; Walsh et al. 2016). The economic losses caused by the introduction of non-native fish alone was estimated to be more than $1 billion per year in the United States (Pimentel 2007).

Clarifying the intricate history of unrecorded fish invasions is an important step in understanding and managing the invasion process (Colautti and Lau 2006). Notably, when the introduction is unintentional, little information is often available regarding the origin of the introduced species, date of introduction, invasion route, vector types, number of independent introductions, and secondary spread pathways. Moreover, intentional introductory acts, such as restocking programs, may lead to the accidental introduction of undesired species because other non-native species may be accidentally carried along without being detected. In Europe, at least 18% of non-native fish species were accidentally introduced (Holčík 1991).

The lack of data regarding biological invasions such as the initial propagule size, proper taxonomic identification, and population dynamics, hampers management and mitigation of ecological impacts. Comparing early and late invasion stages allows the understating of ecological changes caused by invasive species and factors that might influence the invasive success. For example, Carol et al. (2009) collected data on growth and diet for the European catfish (*Silurus glanis*) in the Iberian Peninsula and compared three populations corresponding to the earliest introductions (> 30 years ago) with two recently introduced populations in reservoirs. Their results indicated that the total length and age of the catfish belonging to recent introductions consisted of smaller and younger catfish with significantly higher condition and size-specific growth rates compared to older (as well as native) populations. Feiner et al. (2013) evaluated the role of life history shifts such as age, length at maturity, gonadosomatic index, and growth for the successful invasion of white perch (*Morone americana*) across three reservoirs, with introduction time ranging from 1 to 21 years since initial detection. They detected an increased growth and higher mean reproductive investment within fish from the newly introduced population.

Molecular tools can be valuable to reconstruct the invasion history (Kalinowski et al. 2010; Kinziger et al. 2014; Cristescu 2015) and its outcomes, such as assessment of propagule pressure and hybridization with native species. In the Neotropics, the use of mitochondrial and microsatellite DNA markers made possible the genetic characterization of the invasion of a fish predator, the Amazonian peacock bass (*Cichla* spp.), into multiple watersheds in Southeastern of Brazil (Oliveira et al. 2006; de Carvalho et al. 2009, Carvalho et al. 2014). A low propagule pressure, leading to reduced genetic diversity in the invaded range, did not hinder the peacock bass from successfully invading this region (Carvalho et al. 2014). In the Jequitinhonha River Basin (JRB) in eastern Brazil, molecular studies detected a previously overlooked hybrid swarm between the native fish *Prochilodus hartii* and introduced *Prochilodus* spp. from neighboring river basins (Sales et al. 2018). The Irapé Dam (a hydroelectric dam in the Middle JRB) seems to work as a barrier to the hybrid spread because populations upstream of the dam had higher proportions of pure native species when compared to downstream sites (Sales et al. 2018).

The invasion process may be facilitated when it takes place in severely modified habitats that possess lower biotic resistance, altered native population assemblages, and increased niche availability (Shea and Chesson 2002; Herbold and Moyle 1986), such as found in river impoundments (Havel et al. 2005; Johnson et al. 2008). Vacant niches can be particularly well explored by introduced species with broad diet requirements (Korsu et al. 2012). Moreover, the establishment probability of non-native freshwater fish might be improved when an introduced species is native to a neighboring basin, as the introduced range might have similar ecological conditions (Kinziger et al. 2014). Biological features such as plasticity in resource use, prolonged reproductive period, and parental care can also enhance invasive potential (Carvalho et al. 2014; Estoup et al. 2016).

The fish *Serrasalmus brandtii* (commonly known as white piranha or pirambeba) was first registered in the JRB in 2006, downstream from Irapé Dam, a hydroelectric reservoir built between 2002 and 2006 (Andrade et al. 2018). The development of a DNA barcode library for the JRB fish fauna enabled Pugedo et al. (2016) to identify the invasive white piranha as *S. brandtii*, a species native to the neighboring São Francisco River Basin (SFRB). White piranhas have a large diet breadth (Pompeu 1999; de Trindade and Jucá-Chagas 2008), but they feed especially on fish fins (i.e. pterygophagous), and an increase in their number has been associated to higher proportions of mutilated netted fish in the JRB (Andrade et al. 2018). Species of the *Serrasalmus* genus are renowned predators that alter trophic dynamics (Alves et al. 2017). In addition, they may attack fishermen, swimmers, and bathers, thus posing a threat to human health (Mol 2006; Haddad and Sazima 2010). Considering their potential harm to humans, low commercial value, and predatory feeding behavior, the white piranha was probably introduced accidentally into the JRB; this introduction was not documented.

In this study we carried out an eight-year population monitoring of the white piranha upstream (lentic environment) and downstream (lotic environment) from Irapé Dam reservoir in the Middle JRB. We were able to monitor the piranha's invasion into the Irapé reservoir since its first detection in 2009, only 3 years after the river's closure. Thus, the early detection of piranha introduction allowed us to assess the invasive population dynamics and to test the hypothesis that reservoirs facilitate biological invasions. We predicted that the signal of recent introduction into the reservoir would result in rapid population growth composed of smaller (younger) specimens. This population growth would be the result of higher investment in reproduction as the species occupy vacant niches in a severely modified lentic reservoir habitat. On the other hand, within the downstream site, which represents an older invasive stage, we expect less investment in reproduction and lower population growth due to a less impacted lotic environment.

Using three mitochondrial genes, we assessed the population structure from both the native (SFRB) and invaded (JRB) ranges aiming at the molecular identification of introduced piranhas, identification of the number of introductory acts, assessment of the propagule pressure and detection of source population(s). We would reject the single introductory act hypothesis of piranhas into the JRB if a strong genetic structure between the downstream and upstream Irapé Dam sample sites was detected. In this scenario, the dam may not be allowing gene flow, and the genetic admixture of distinct genetic pools resulting from the different native source propagules introduced upstream and downstream of the dam. Moreover, by comparing the genetic diversity and population structure of the entire native and invasive ranges as proxies for propagule pressure and introductory routes, respectively, we were able to reconstruct the invasion dynamics of the piranha into the entire Jequitinhonha River Basin.

Materials and methods

Sampling for molecular analyses

We collected 130 *S. brandtii* specimens between January 2006 and July 2016 from three sites in the Upper São Francisco River (USF; n = 41), four in the Middle São Francisco River (MSF; n = 54), and two in the Lower São Francisco River (LSF; n = 35), representing the entire native range of the species (Fig. 1a; Table S1—Supplementary material). For the JRB, we sampled 71 individuals from three sites upstream of Irapé Dam (UID; n = 30) and nine downstream from Irapé Dam (DID; n = 41) in the main Jequitinhonha River and its tributaries. The invasive specimens were collected between May 2012 and January 2016 across the recorded occurrence area (Fig. 1a; Table S1—Supplementary material).

DNA extraction, amplification, and sequencing

We extracted DNA from fin clips and muscle tissue, previously fixed in ethanol 100% and stored at − 4 °C, using a saline extraction method adapted from Aljanabi and Martinez (1997). We amplified a fragment of the COI (cytochrome c oxidase subunit 1) gene using the FishF1 and FishR1 primers (Ward et al. 2005) and the 16S rDNA using the H-2609 and L-1987 primers (Palumbi et al. 1991) from 10 *S. brandtii* specimens (eight native and two non-native). A fragment of the mitochondrial control region (CR) was amplified using the SbrandtiiCR_F (5′ CCATCCAATTCACTTTCACAAG 3′) and SbrandtiiCR_R (5′ AAAACCACTCGTTGATTACGC 3′) primers designed in this study, from 201 specimens (130 native and 71 invasive).

Polymerase Chain Reactions (PCRs) were performed in a thermal cycler (Veriti® 96—Well Thermal

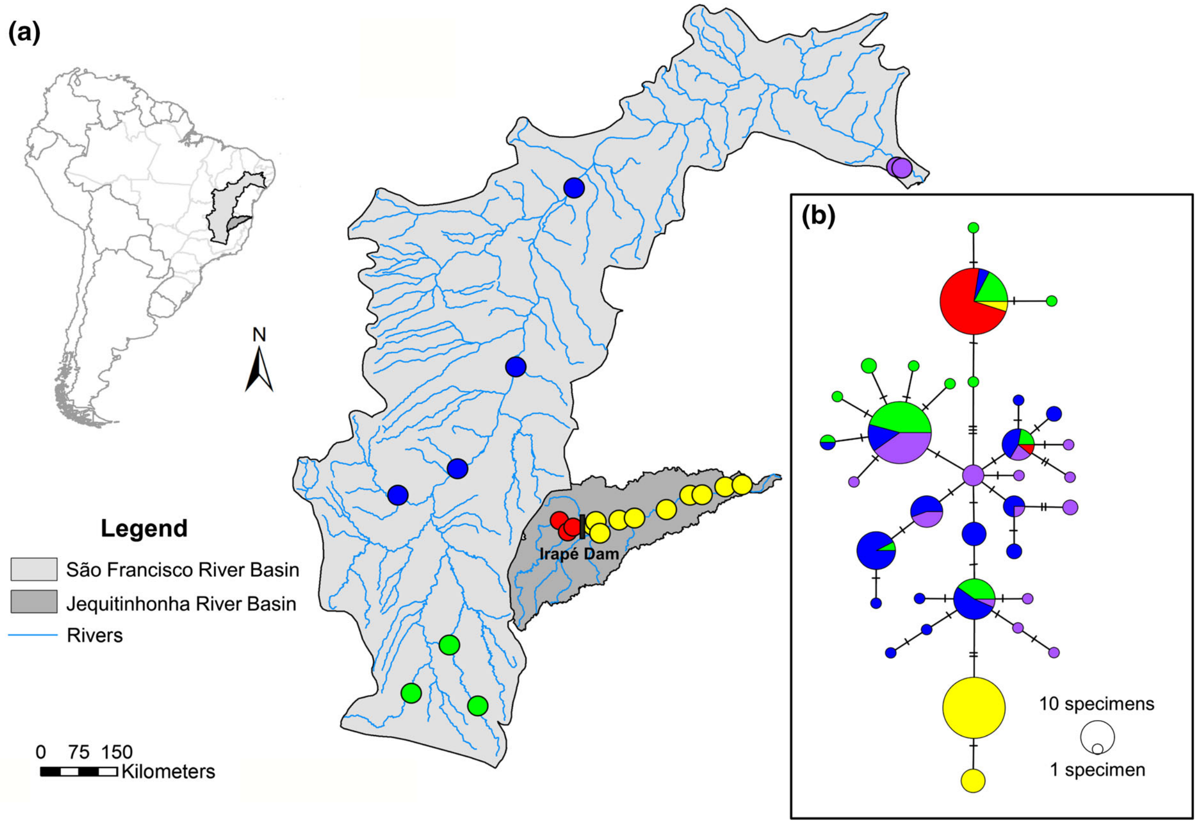


Fig. 1 Study area map (**a**) and control region haplotype network (**b**) for *S. brandtii*. Light blue lines indicate rivers. The area in light grey represents the São Francisco River Basin (SFRB) and the dark grey shows the Jequitinhonha River Basin (JRB). In the native range: green circles = Upper SFRB, blue circles = Medium SFRB, and purple circles = Lower SFRB. In the invaded range: red circles = Upstream of Irapé Dam (UID) and yellow circles = Downstream from Irapé Dam (DID)

Cycler, Applied Biosystems) using 10.0 µl of a solution composed of 7.0 µl of ultrapure water, 0.3 µl of dNTP (10 mM), 1.0 µl of a 10× Buffer containing $MgCL_2$, 0.25 µl of each primer (10 µM), 0.2 µl of Taq DNA polymerase (5 U/µl) and 1.0 µl of template DNA. PCR conditions consisted of an initial denaturation for 5 min at 94 °C followed by 35 cycles of denaturation for 1 min at 94 °C, primers annealing for 1 min at 54 °C (COI), 55 °C (16S) or 59 °C (CR), extension for 1 min at 72 °C, and one final extension step for 7 min at 72 °C.

The resulting amplicons were sequenced bidirectionally using a commercial BigDye® Terminator v3.1 Cycle Sequencing Kit in an automated DNA sequencer ABI 3500 (Applied Biosystems). Each DNA sequence was deposited in GenBank under accession numbers MK728330-MK728530 (CR), MK728531-MK728540 (16S) and MK738113-MK738122 (COI).

COI and 16S data analysis

White piranhas introduced into the JRB were initially identified at the genus level as *Serrasalmus* sp. and later as *S. brandtii* (Pugedo et al. 2016). Considering the complex taxonomy and systematics of piranhas, the difficulty of species identification within the group (Freeman et al. 2007), we decide to perform a molecular identification of introduced specimens. This was done using the COI and 16S markers by including samples from the putative native basin and other Serrasalminae species. We included COI sequences of *S. brandtii* (Accession numbers [AN]: HM405230 and HM405231), *S. maculatus* (AN: JN989229 and JN989230), *S. marginatus* (AN: JN989232 and JN989233), *Pygocentrus nattereri* (AN: AP012000 and NC_015840), and *P. piraya* (AN: HM405211 and HQ600848). We also included 16S sequences of *S. brandtii* (AN: DQ384720 and

DQ384721), *S. maculatus* (AN: DQ384738 and HQ171285), *S. marginatus* (AN: DQ384742 and DQ384743), *P. nattereri* (AN: AY788074 and NC_015840), and *P. piraya* (AN: DQ384718 and DQ384719).

We assembled the consensus haplotype sequences (contigs) using DNA Baser 4.16 software (Heracle BioSoft) and aligned the contigs using the ClustalW method (Thompson et al. 1994) implemented in MEGA 6.0 (Tamura et al. 2013), followed by manual conference and edition. The best-fit model of evolution was identified based on Bayesian Information Criterion (BIC) scores in MEGA 6.0. For each marker, we built a Neighbor-joining tree with 1000 replications and estimated the genetic distance between species using MEGA 6.0.

Control region data analysis

We used the CR to assess genetic diversity, as a proxy of the propagule pressure, and the genetic structure of the native and invaded range. We compared haplotypes between the native and introduced populations to identify the most likely source population of the white piranha introduction into the JRB.

The nucleotide and haplotype diversities were estimated using the DnaSP 5.10.1 (Librado and Rozas 2009). Genetic structure was estimated by comparing the USF, MSF, and LSF sections in the native range to the UID and DID from the invaded range (JRB). Genetic structure was inferred using the pairwise Fixation Index (Fst) estimated in Arlequin 3.11 software (Excoffier et al. 2005). We also conducted a Bayesian analysis of population structure in the BAPS 6.0 program (Corander et al. 2008), without prior geographical information, to estimate the most probable number of genetic clusters. We used a maximum population number (k) of 11, which considered the nine native and the two invaded (upstream and downstream from Irapé dam) sample sites. A median-joining (Bandelt et al. 1999) haplotype network of the CR haplotypes was built using PopART 1.7 (Leigh and Bryant 2015).

Fish monitoring data

We assessed white piranha abundance from fish assemblage data from the Irapé Dam fish monitoring program. We used data from two sites in the UID segment, located in the reservoir (IR2 and IR3) and one site from DID (IR4). Fish samplings were done quarterly (February, May, August, and November) from 2008 to 2013 and in 2016. The remaining years, 2014 and 2015, had two and three fish samplings, respectively. Fish were captured with gillnets placed randomly overnight (stretched size meshes = 2.6, 3, 4, 5, 6, 7, 8, 10, 12, 14, and 16 cm). In the UID sites, we sampled the littoral zone of the Irapé Dam reservoir (IR2: 16° 43′ 47.79″ S, 42° 34′ 38.87″ W; IR3: 16° 42′ 49.43″ S, 42° 38′ 19.95″ W), while in the DID site the gillnets were settled in the tailrace and spillway plunge area (IR4: 16° 44′ 26.11″ S, 42° 34′ 9.35″ W). For each site we calculated the white piranha mean annual capture per unit effort (CPUE) as the mean number of individuals captured in 100 m^2 nets using the R-Studio version 1.1.442 (R Development Core Team 2016).

Fish that had not been mutilated by other white piranhas had their standard length measured and were compared to the size at first maturity in their native range (Honorato-Sampaio et al. 2009). We calculated the proportion of white piranhas matching immature (standard length $\leq$ 131 mm) and adult (standard length $\geq$ 145 mm) sizes from each site. We did not consider individuals whose standard length was between 132 and 144 mm as they might be adult males or immature females.

Results

Molecular taxonomy

After trimming the COI sequences, an alignment of 652 base pairs (bp) was obtained. Five haplotypes were detected when considering both the native and invaded ranges. The trimmed 16S sequence alignment consisted of 525 bp. As expected, all detected 16S and COI haplotypes from the native and invasive ranges clustered with the *S. brandtii* GenBank sequences (Fig. 2b).

The COI and 16S markers clearly differentiated all five Serrasalminae species (Fig. 2; Tables 1, 2). The mean genetic distance between specimens collected in the invaded (JRB) and native (SFRB) ranges was low (0.24/0.02%, COI and 16S, respectively).

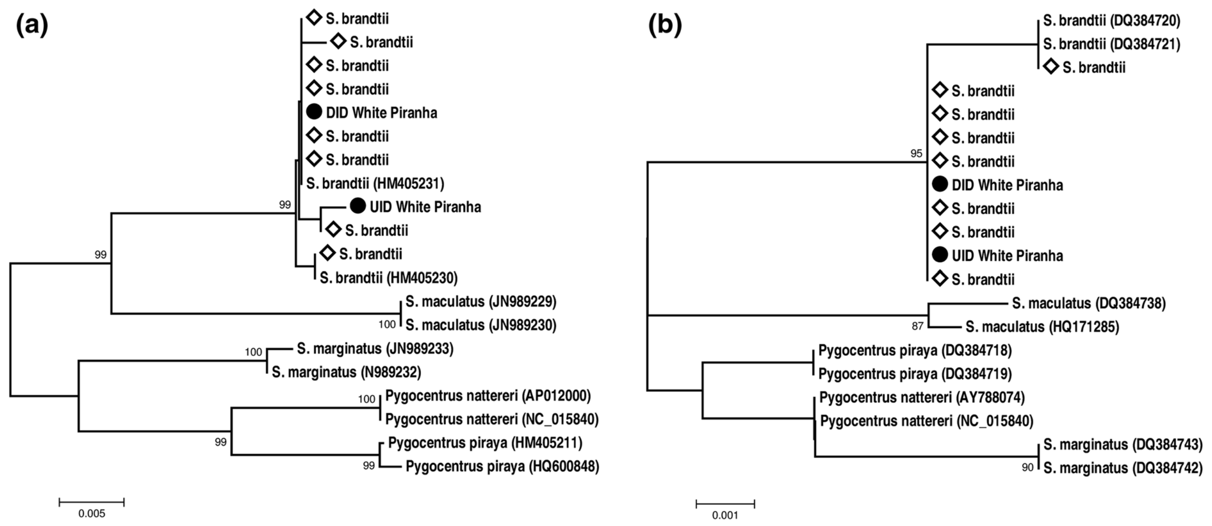


Fig. 2 COI (**a**) and 16S (**b**) Neighbor-joining trees. The sequences obtained in this study from invasive specimens are tagged with a circle, while the sequences from native specimens are tagged with a diamond. Sequences downloaded from the GenBank database are shown with their respective accession number in parentheses

Table 1 COI pairwise mean genetic distance (%) between Serrasalminae species and native (São Francisco River Basin) and invasive (Jequitinhonha River Basin) populations

Groups	1	2	3	4	5	6	7
1. Native	**0.14**						
2. Invasive	0.24	**0.38**					
3. *S. brandtii*	0.14	0.28	**0.19**				
4. *S. maculatus*	3.77	3.89	3.79	**0.00**			
5. *S. marginatus*	4.39	4.39	4.39	5.21	**0.19**		
6. *Pygocentrus nattereri*	5.16	5.31	5.10	5.72	3.99	**0.00**	
7. *Pygocentrus piraya*	5.26	5.42	5.21	5.82	3.89	2.40	**0.19**

The mean genetic divergence within each group is highlighted in bold

Table 2 16S pairwise mean genetic distance (%) between Serrasalminae species and native (São Francisco River Basin) and invasive (Jequitinhonha River Basin) populations

Groups	1	2	3	4	5	6	7
1. Native	**0.05**						
2. Invasive	0.02	**0.00**					
3. *S. brandtii*	0.17	0.19	**0.00**				
4. *S. maculatus*	1.08	1.06	1.25	**0.19**			
5. *S. marginatus*	1.18	1.15	1.35	1.25	**0.00**		
6. *Pygocentrus nattereri*	0.79	0.77	0.96	0.86	0.38	**0.00**	
7. *Pygocentrus piraya*	0.79	0.77	0.96	0.86	0.77	0.38	**0.00**

The mean genetic divergence within each group is highlighted in bold

Genetic diversity and structure

After manual inspection and trimming ambiguous ends, an alignment of 640 bp was obtained for the CR for 201 white piranhas. We detected a total of 34 haplotypes with 28 variable sites including both native and invaded ranges (Table S2—Supplementary Material).

Within the native range, we recovered 32 haplotypes, with haplotype diversity (h) of 0.890. When comparing the three sections of the SFRB (upper, middle, and lower), the genetic diversity remained high, ranging from $h = 0.8073$ in the USF (13 haplotypes) to $h = 0.9050$ in the MSF (16 haplotypes) (Fig. 1b; Table 3). Values of nucleotide diversity (π) were very similar across the native range. Within the invaded range, we recovered a h of 0.5835 and π of 0.0057, with a total of four haplotypes. Both the UID and DID segments in the JRB presented low genetic diversity: $h = 0.0667$ and $\pi = 0.0005$ with two haplotypes, and $h = 0.3020$ and $\pi = 0.0014$ with three haplotypes, respectively (Fig. 1b; Table 3).

Pairwise Fst ranged from 0.07 to 0.13 in the native range (Table 4). In the invaded range, high genetic differentiation was recovered when comparing upstream and downstream Irapé segments (Fst = 0.91). The only haplotype shared between both invaded segments, which is also the most abundant haplotype in UID, was only detected in the DID during the last sampling campaign (January 2016) at a site immediately downstream from Irapé dam. High genetic differentiation was detected between invaded and native segments (Fst ranged between 0.55 and 0.76; Table 4).

Table 4 Pairwise genetic differentiation indices (Fst) between invaded and native basin segments

	USF	MSF	LSF	UID	DID
USF	–	0.0000	0.0270	0.0000	0.0000
MSF	0.1348	–	0.0000	0.0000	0.0000
LSF	0.0740	0.0745	–	0.0000	0.0000
UID	0.5862	0.7019	0.7582	–	0.0000
DID	0.5501	0.5913	0.6229	0.9141	–

Pairwise Fst values are placed below the diagonal, and their respective significance p values above diagonal

USF upper São Francisco River Basin (SFRB), *MSF* middle SFRB, *LSF* lower SFRB, *UID* upstream of Irapé Dam, *DID* downstream from Irapé Dam

The BAPS output resulted in six genetic clusters across SFRB and JRB (Fig. 3). Each invaded segment presented two genetic clusters. The dominant cluster in the DID segment was exclusive to this region, while the other was shared with UID, USF, and MSF. The shared cluster between both invaded regions was the dominant in UID, while the second less abundant cluster was found in specimens from all native sections (Fig. 3).

Fish monitoring data

Fish monitoring was carried out between 2008 and 2016, before and after the first detection of the white piranha in the hydroelectric reservoir and in one sample site downstream of the dam where piranhas had been previously detected. We collected 309 *S. brandtii* from site IR2, 257 from IR3 (both in the reservoir), and 40 from IR4 (downstream from Irapé dam). The first capture in the reservoir happened in

Table 3 Number of samples, haplotypes, haplotype diversity (h), and nucleotide diversity (π) by basin segment and haplotype diversity by population of *S. brandtii* recovered from the invaded (JRB = Jequitinhonha River Basin) and native (SFRB = São Francisco River Basin) ranges based on the control region

River Basin	Segment	No. Samples	No. Haplotypes	Hd	π	Hd	π
SFRB	USF	41	13	0.8073	0.0044	**0.8900**	**0.0041**
	MSF	54	16	0.9050	0.0038		
	LSF	35	14	0.8235	0.0031		
JRB	UID	30	2	0.0667	0.0005	**0.5835**	**0.0057**
	DID	41	3	0.3020	0.0014		

USF upper SFRB, *MSF* middle SFRB, *LSF* lower SFRB, *UID* upstream of Irapé Dam, *DID* downstream from Irapé Dam

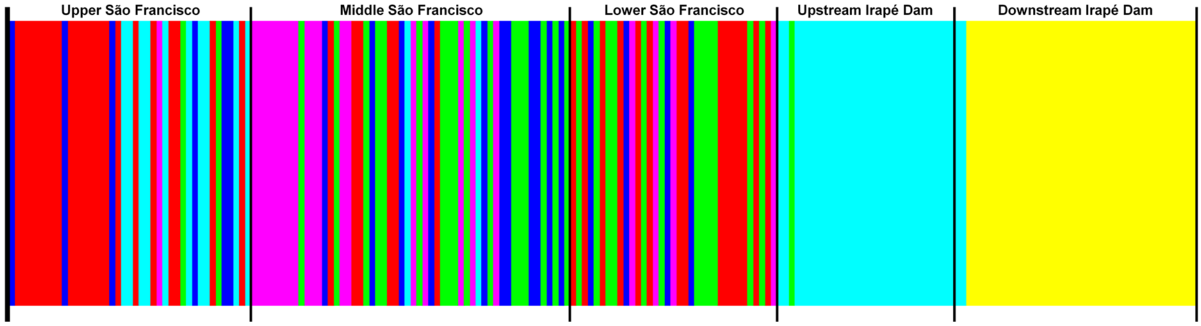


Fig. 3 BAPS output without geographical information for k = 11. Each specimen is represented by a vertical line and the colors represent the distinct genetic clusters

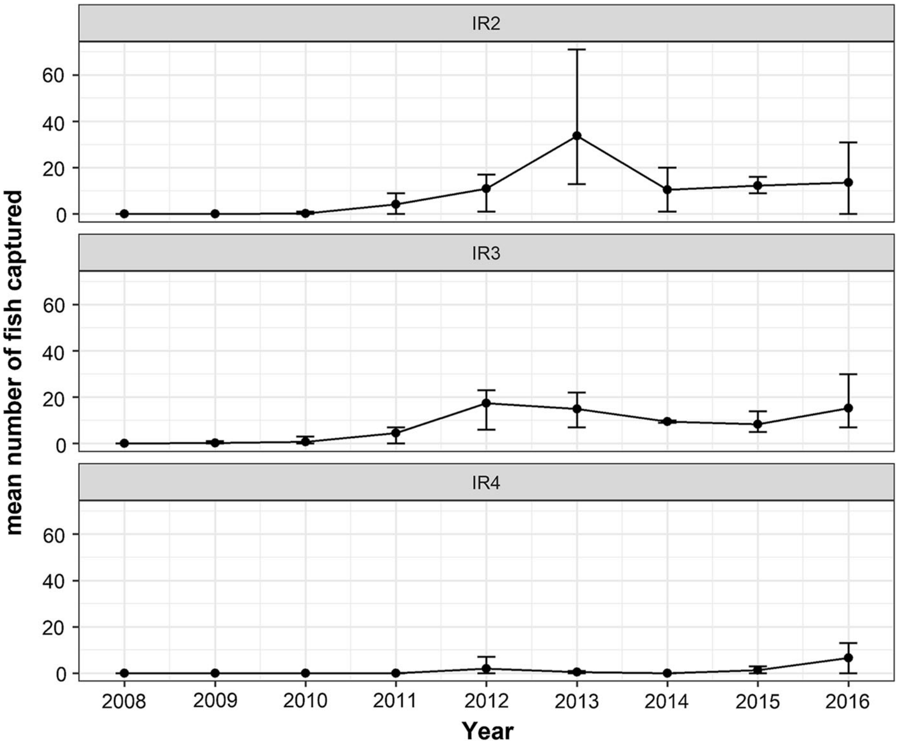
November 2009 for IR3 and November 2010 for IR2. An increase in the mean annual capture as well as a variation between the smallest to the largest captures was observed in subsequent years (Figs. 4, 5).

Although the very first record for this species in the JRB dates back to 2006 downstream from Irapé Dam (i.e. IR4), it was only captured again at this site in 2012. Captures remained frequent after that, but with the mean number of fish captured at site IR4 lower than at sites IR2 and IR3 (Fig. 4). Higher proportions of fish matching immature sizes were recorded in both UID sites when compared to the DID site (IR2: immature = 0.53, adult = 0.45; IR3: immature = 0.49, adult = 0.43; and IR4: immature = 0.22, adult = 0.75) (Fig. 5).

Discussion

We tracked the white piranha invasion in the JRB via a long-term population monitoring in the invaded range and genetically characterized the invasion process by thoroughly sampling the native (SFRB) and invaded (JRB) basins. A DNA barcode gap was observed

Fig. 4 *S. brandtii* mean annual capture in three sites in the invaded range: lentic environment sites IR2 and IR3 from the Irapé Dam reservoir and one lotic environment, site IR4, downstream from Irapé Dam. Error bar whisker plots limit the capture range for that particular year (upper = maximum, lower = minimum)



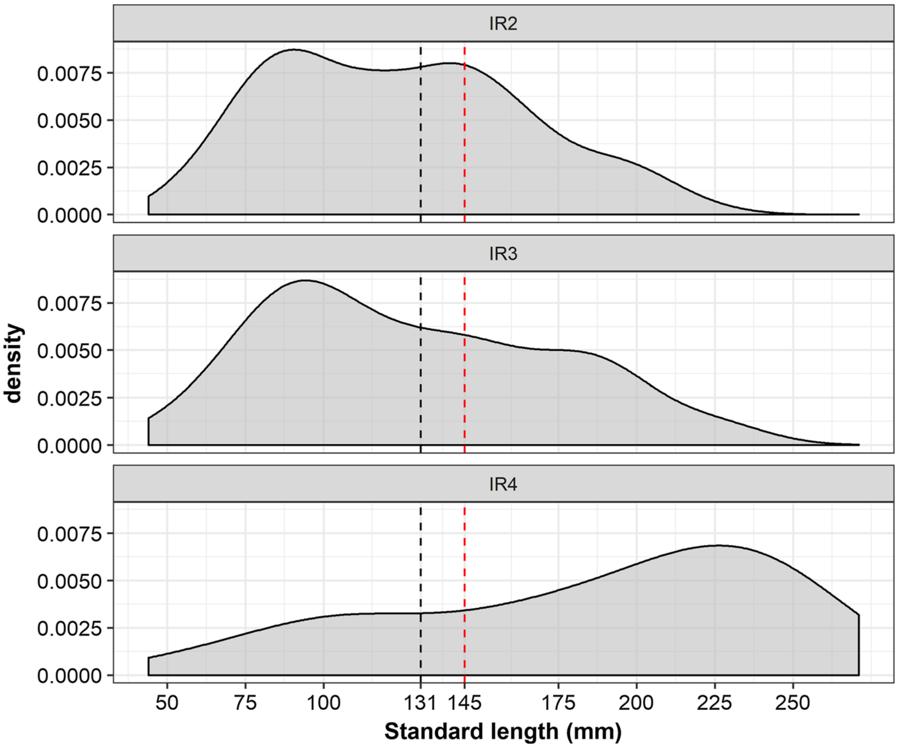


Fig. 5 Density plots for the size structure of *S. brandtii* populations in each site. IR2 and IR3 denote the sampling sites located in the reservoir, and IR4, immediately downstream from Irapé Dam. Dotted vertical lines match the size at first maturity for males (black) and females (red)

within the Serrassalminae subfamily, which enabled the identification of introduced piranhas as *S. brandtii* in the two JRB invasive populations using data from two mitochondrial regions (COI and 16S). For the JRB, we detected a low propagule pressure using the hypervariable mitochondrial control region that suggests a great invasive potential of the white piranha assuming a small number of founders.

The introduction of the white piranha into the JRB was first recorded in 2006, downstream from Irapé Dam (Andrade et al. 2018). We detected this species in the Irapé Reservoir during monitoring in 2009, only 3 years after the construction of the Irapé hydroelectric dam (Andrade et al. 2018). Our long-term monitoring before and during the invasion provides valuable information regarding population dynamics. The population monitoring at the two sites within the reservoir (UID) followed similar population trends, whether in number of individuals or in size structure. Downstream from Irapé Dam (DID), the presence of *S. brandtii* took longer to be detected since the monitoring started in 2008, and fewer individuals, mainly adults, were captured. Differences in captured numbers between DID and UID sites may be due to *S. brandtii* fast colonization and pre-adaptation to lentic environments, since native populations are reported to be more abundant in floodplain lakes when compared to riverine habitats (Pompeu 1999; Honorato-Sampaio et al. 2009). Pre-adaptation in its natural area (Silva et al. 2006) may explain fast population growth in manmade reservoirs. Additionally, *S. brandtii* has a broad dietary plasticity and ontogenetic variation in its feeding habits (Pompeu 1999; Oliveira et al. 2004; de Trindade and Jucá-Chagas 2008) which enables it to explore multiple trophic niches while reducing intraspecific competition. Moreover, piranhas explore a unique niche since they are the only pterygophagous fish species in the JRB, which exhibit a mutilating feeding behaviour (Andrade et al. 2018).

The greater abundance of smaller (younger) fish in the reservoir may be related to the more recent introduction of the white piranha at this site and to the hydrologic alterations promoted by damming (Agostinho et al. 2009). This is consistent with the strong association between invasive species success and newly built impoundments that results in increased niche availability and a high disturbance regime typical of most reservoirs (Johnson et al. 2008). Therefore, at an early invasive stage the white piranha invasive population may be reproducing faster to colonize and occupy vacant niches in the reservoir environment. On the other hand, individuals from the older invasion downstream from the Irapé Dam (Fig. 5—IR4) presented a lower number of young specimens reflecting an older and stable invasion stage. Similar population trends were reported for the invasive populations of the European catfish (*Silurus glanis*) (Carol et al. 2009) and white perch (*Morone americana*) (Feiner et al. 2013), showing that life history plasticity confers an important advantage to invasive species and allow them to adapt for successful transitions throughout the invasion process.

A strong genetic structure was detected between the invaded range sites UID and DID (Fst = 0.9141) shown by the lack of shared haplotypes until the last fish sampling, when the dominant haplotype in UID was first detected in the site located downstream from the dam (DID). This supports the hypothesis that the Irapé Reservoir was colonized by white piranhas belonging to a secondary introductory act from its native range, not piranhas previously introduced downstream from Irapé Dam. Artificial dams have been reported as barriers to gene flow (de Almeida et al. 2003; Pamponet et al. 2008; Khedkar et al. 2014) and isolation caused by the Irapé Dam allowed the detection of multiple introductory acts. However, in January 2016 the dominant haplotype in the upstream population was detected for the first time downstream from Irapé Dam. It is unclear if the species was able to ultimately overcome the barrier or if human-mediated translocation of individuals promoted this admixture. We predict that the genetic signature of two distinct introductory acts in the JRB should decrease due to the recently detected upstream to downstream gene flow.

A moderate genetic differentiation (Fst ranged from 7.4 to 13.48%) was observed for *S. brandtii* among the native range (SFRB) segments. Considering the sedentary habit of this species and the broad sampled range, the widespread distribution of some haplotypes was not expected for *S. brandtii*. Non-migratory fishes often exhibit an isolation by distance (IBD) pattern of genetic differentiation (Leuzzi et al. 2004; Sofia et al. 2006; Pamponet et al. 2008; but see Brauer et al. 2018 for a comparison of IBD versus dentrically-driven differentiation). A recent study suggested that mtDNA datasets are often not suitable to test for IBD, and that multilocus markers such as microsatellites and SNPs are more appropriate to test hypotheses of spatial population genetic structure (Teske et al. 2018). However, Hubert et al. (2007) found evidence for genetic structure even at a small geographical scale for the black piranha *S. rhombeus* in the Upper Amazon Basin using intron length polymorphism and mitochondrial DNA sequences. On the other hand, Frantine-Silva et al. (2015) detected relatively low levels of genetic structure in four sedentary species in the Upper Paraná River Basin and argued that periodic floods enabled the transit of individuals between localities. The periodic floods may have enabled the connectivity of *S. brandtii* populations from geographically distant localities, such as the lower and upper SFRB with shared haplotypes separated by more than 1400 km. The white piranha is prone to colonize floodplain lakes (Pompeu 1999) and could have used them as stepping stones to disperse across long distances.

The lack of strong population structure in the native range did not allow a precise identification of the propagule source. The two haplotypes detected at the invaded range site UID were also detected from the native range sites USF and MSF, while the other two haplotypes detected at invaded range site DID could not be found in the native range (Fig. 1). The BAPS clustering analysis detected similar clusters between the site UID and both the USF and MSF native sites, but the most representative cluster recovered for the DID site did not match any other cluster from the native range. Therefore, after a thorough analysis of the entire native range (Fig. 1), we could trace the introductory route of the secondary introduction act in the UID as belonging to the MSF or USF, but the propagule source of the primary and older introduction of white piranha could not be traced.

The great genetic diversity reduction in JRB when compared to SFRB, allied to our first recording of few piranha specimens before the rapid population establishment, strongly suggests low propagule pressure and a short lagging period of invasion. Recurrent gene flow due to repeated introductions will prevent loss of genetic diversity in invasive populations (Dlugosch and Parker 2008), but low propagule pressure results in reduced genetic diversity as observed for the piranha introduction into the JRB. Reduced genetic diversity has been reported extensively for invasive species (Tsutsui et al. 2000; Hagenblad et al. 2015) and is usually related to low propagule pressure. For example, Carvalho et al. (2014) detected reduced genetic diversity for invasive populations of peacock bass (*Cichla kelberi* and *C. piquiti*) from Southeastern

Brazilian river systems compared to native populations from the Amazon Basin, indicating low propagule pressure. On the other hand, high propagule pressure was considered the reason for no reduction in the genetic diversity of an introduced population of European smelt (*Osmerus eperlanus*) in a Norwegian lake (Hagenlund et al. 2015).

Although we suggest that at least two independent introductory acts were responsible for the piranha introduction into the JRB, we speculate that its introduction may have occurred accidentally or possibly accompanied restocking actions with fingerlings of other species. For instance, the restocking of the commercially important fish *Prochilodus hartti* is likely to have been responsible for the hybrid swarm effect observed in the Jequitinhonha after the introduction of several other *Prochilodus* species native to neighboring basins, such as the São Francisco Basin (Sales et al. 2018). Given that restocking actions are conducted by hydroelectric companies due to Brazilian environmental law obligations, we recommend stronger regulations and enforcement for restocking to avoid the accidental introduction of highly invasive species. We suggest that every restocking should be preceded by the molecular identification of breeders to avoid the introduction of non-native or hybrid species, and the use of eDNA metabarcoding (Sales et al. 2019) to check for the presence of hitchhiking species during large scale captive breeding efforts for restocking, and removing the obligation to restock from Brazilian environmental law. We also recommend the inclusion of *S. brandtii* in the ecological risk assessment for dam construction and restocking of other commercially or ecologically important fish species in Brazil.

The invasive success of piranhas into the JRB was not influenced by the lack of genetic diversity, but population dynamics confirms theoretical predictions that reservoirs facilitate biological invasions. This is consistent with a scenario where the white piranha rapidly colonized the reservoir, after a short lag phase, reaching a plateau in only 4 years following the first detection. The hydroelectric dam not only facilitated the invasion of the piranha by strongly altering the upstream environment, but also enabled the characterization of a population's invasive dynamics due to a secondary introductory act in a Neotropical river basin. Our combination of genetic and population dynamics tools enabled the reconstruction of a top predator fish invasion in the Neotropics and shed light on to the ecological factors that influenced its invasion success.

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References

Agostinho A, Pelicice F, Gomes L (2009) Dams and the fish fauna of the Neotropical region: impacts and management related to diversity and fisheries. Braz J Biol 68:1119–1132. https://doi.org/10.1590/s1519-69842008000500019

Aljanabi SM, Martinez I (1997) Universal and rapid salt-extraction of high quality genomic DNA for PCR-based techniques. Nucleic Acids Res 25:4692–4693. https://doi.org/10.1093/nar/25.22.4692

Alves GHZ, Figueiredo BRS, Manetta GI et al (2017) Trophic segregation underlies the coexistence of two piranha species after the removal of a geographic barrier. Hydrobiologia 797:57–68. https://doi.org/10.1007/s10750-017-3159-6

Andrade FR, Silva LD, Guedes I, et al (2018) Non-native white piranhas graze preferentially on caudal fins from large netted fishes. Mar Freshw Res 70:585–593. https://doi.org/10.1071/MF18202

Bajer PG, Beck MW, Cross TK et al (2016) Biological invasion by a benthivorous fish reduced the cover and species richness of aquatic plants in most lakes of a large North American ecoregion. Glob Change Biol 22:3937–3947. https://doi.org/10.1111/gcb.13377

Bandelt HJ, Forster P, Röhl A (1999) Median-joining networks for inferring intraspecific phylogenies. Mol Biol Evol 16:37–48. https://doi.org/10.1093/oxfordjournals.molbev.a026036

Blackburn TM, Bellard C, Ricciardi A (2019) Alien versus native species as drivers of recent extinctions. Front Ecol Environ. https://doi.org/10.1002/fee.2020

Brauer CJ, Unmack PJ, Smith S et al (2018) On the roles of landscape heterogeneity and environmental variation in determining population genomic structure in a dendritic system. Mol Ecol. https://doi.org/10.1111/mec.14808

Carol J, Benejam L, Benito J, García-Berthou E (2009) Growth and diet of European catfish (*Silurus glanis*) in early and late invasion stages. Fundam Appl Limnol 174:317–328. https://doi.org/10.1127/1863-9135/2009/0174-0317

Carvalho DC, Oliveira DAA, Sampaio I, Beheregaray LB (2014) Analysis of propagule pressure and genetic diversity in the invasibility of a freshwater apex predator: the peacock bass (genus *Cichla*). Neotrop Ichthyol 12:105–116. https://doi.org/10.1590/S1679-62252014000100011

Colautti RI, Lau JA (2006) Contemporary evolution during invasion: evidence for differentiation, natural selection, and local adaptation. Mol Ecol 24:1999–2017. https://doi.org/10.1111/mec.13162

Corander J, Marttinen P, Sirén J, Tang J (2008) Enhanced Bayesian modelling in BAPS software for learning genetic structures of populations. BMC Bioinform 9:539. https://doi.org/10.1186/1471-2105-9-539

Cristescu ME (2015) Genetic reconstructions of invasion history. Mol Ecol 24:2212–2225. https://doi.org/10.1111/mec.13117

D'Antonio C, Meyerson LA, Denslow J (2001) Exotic species and conservation. In: Soulé ME, Orians GH (eds) Conservation biology: research priorities for the next decade. Island Press, Washington, DC, pp 59–80

de Almeida FS, Sodré LMK, Contel EPB (2003) Population structure analysis of *Pimelodus maculatus* (Pisces, Siluriformes) from the Tietê and Paranapanema Rivers (Brazil). Genet Mol Biol 26:301–305. https://doi.org/10.1590/S1415-47572003000300014

Carvalho DC, de Oliveira DAA, dos Santos JE et al (2009) Genetic characterization of native and introduced populations of the neotropical cichlid genus *Cichla* in Brazil. Genet Mol Biol 32:601–607. https://doi.org/10.1590/S1415-47572009005000060

de Trindade MEJ, Jucá-Chagas R (2008) Diet of two serrasalmin species, *Pygocentrus piraya* and *Serrasalmus brandtii* (Teleostei: Characidae), along a stretch of the rio de Contas, Bahia, Brazil. Neotrop Ichthyol 6:645–650

Dlugosch KM, Parker IM (2008) Founding events in species invasions: genetic variation, adaptive evolution, and the role of multiple introductions. Mol Ecol 17:431–449. https://doi.org/10.1111/j.1365-294X.2007.03538.x

Estoup A, Ravigné V, Hufbauer R et al (2016) Is there a genetic paradox of biological invasion? Annu Rev Ecol Evol Syst 47:51–72. https://doi.org/10.1146/annurev-ecolsys-121415-032116

Excoffier L, Laval G, Schneider S (2005) Arlequin (version 3.0): an integrated software package for population genetics data analysis. Evol Bioinforma 1:117693430500100. https://doi.org/10.1177/117693430500100003

Feiner ZS, Rice JA, Aday DD (2013) Trophic niche of invasive white perch and potential interactions with representative reservoir species. Trans Am Fish Soc 142:628–641. https://doi.org/10.1080/00028487.2013.763854

Frantine-Silva W, Ferreira DG, Nascimento RHC et al (2015) Genetic analysis of five sedentary fish species in middle Laranjinha River (upper Paraná River basin): a case study. Genet Mol Res 14:18637–18649. https://doi.org/10.4238/2015.December.28.13

Freeman B, Nico LEO, Osentoski M et al (2007) Molecular systematics of Serrasalmidae: deciphering the identities of piranha species and unraveling their evolutionary histories. Zootaxa 1484:1–38

Gallardo B, Clavero M, Sánchez MI, Vilà M (2016) Global ecological impacts of invasive species in aquatic ecosystems. Glob Change Biol 22:151–163. https://doi.org/10.1111/gcb.13004

Haddad V, Sazima I (2010) Piranha attacks in dammed streams used for human recreation in the State of São Paulo, Brazil. Rev Soc Bras Med Trop 43:596–598

Hagenblad J, Hülskötter J, Acharya KP et al (2015) Low genetic diversity despite multiple introductions of the invasive plant species *Impatiens glandulifera* in Europe. BMC Genet 16:103. https://doi.org/10.1186/s12863-015-0242-8

Hagenlund M, Østbye K, Langdal K et al (2015) Fauna crime: elucidating the potential source and introduction history of European smelt (*Osmerus eperlanus* L.) into Lake Storsjøen, Norway. Conserv Genet 16:1085–1098. https://doi.org/10.1007/s10592-015-0724-2

Havel JE, Lee CE, Vander Zanden MJ (2005) Do reservoirs facilitate invasions into landscapes? Bioscience 55:518–525. https://doi.org/10.1641/0006-3568(2005)055%5b0518:drfiil%5d2.0.co;2

Herbold B, Moyle PB (1986) Introduced species and vacant niches. Am Nat 128:751–760. https://doi.org/10.1086/284600

Holčík J (1991) Fish introductions in Europe with particular reference to its central and eastern part. Can J Fish Aquat Sci 48:13–23. https://doi.org/10.1139/f91-300

Honorato-Sampaio K, Santos GB, Bazzoli N, Rizzo E (2009) Observations on the seasonal breeding biology and fine structure of the egg surface in the white piranha *Serrasalmus brandtii* from the São Francisco River basin, Brazil. J Fish Biol 75:1874–1882. https://doi.org/10.1111/j.1095-8649.2009.02422.x

Hubert N, Duponchelle F, Nuñez J et al (2007) Isolation by distance and Pleistocene expansion of the lowland populations of the white piranha *Serrasalmus rhombeus*. Mol Ecol 16:2488–2503. https://doi.org/10.1111/j.1365-294X.2007.03338.x

Johnson PTJ, Olden JD, Vander Zanden MJ (2008) Dam invaders: impoundments facilitate biological invasions into freshwaters. Front Ecol Environ 6:357–363. https://doi.org/10.1890/070156

Kalinowski ST, Muhlfeld CC, Guy CS, Cox B (2010) Founding population size of an aquatic invasive species. Conserv Genet 11:2049–2053. https://doi.org/10.1007/s10592-009-0041-8

Khedkar GD, Jamdade R, Kalyankar A et al (2014) Genetic fragmentation in India's third longest river system, the Narmada. Springerplus 3:1–12. https://doi.org/10.1186/2193-1801-3-385

Kinziger AP, Nakamoto RJ, Harvey BC (2014) Local-scale invasion pathways and small founder numbers in introduced Sacramento pikeminnow (*Ptychocheilus grandis*). Conserv Genet 15:1–9. https://doi.org/10.1007/s10592-013-0516-5

Korsu K, Heino J, Huusko A, Muotka T (2012) Specific niche characteristics facilitate the invasion of an alien fish invader in boreal streams. Int J Ecol. https://doi.org/10.1155/2012/813016

Leigh JW, Bryant D (2015) POPART: full-feature software for haplotype network construction. Methods Ecol Evol 6:1110–1116. https://doi.org/10.1111/2041-210X.12410

Leuzzi MSP, de Almeida FS, Orsi ML, Sodré LMK (2004) Analysis by RAPD of the genetic structure of *Astyanax altiparanae* (Pisces, Characiformes) in reservoirs on the

Paranapanema River, Brazil. Genet Mol Biol 27:355–362. https://doi.org/10.1590/S1415-47572004000300009

Librado P, Rozas J (2009) DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. Bioinformatics 25:1451–1452. https://doi.org/10.1093/bioinformatics/btp187

Mol JH (2006) Attacks on humans by the piranha *Serrasalmus rhombeus* in Suriname. Stud Neotrop Fauna Environ 41:189–195. https://doi.org/10.1080/01650520600630683

Oliveira AK, Alvim MC, Peret AC, Alves CBM (2004) Diet shifts related to body size of the pirambeba *Serrasalmus brandtii* Lütken, 1875 (Osteichthyes, Serrasalminae) in the Cajuru Reservoir, São Francisco River Basin, Brazil. Braz J Biol 64:117–124. https://doi.org/10.1590/S1519-69842004000100013

Oliveira AV, Prioli AJ, Prioli SMAP et al (2006) Genetic diversity of invasive and native *Cichla* (Pisces: Perciformes) populations in Brazil with evidence of interspecific hybridization. J Fish Biol 69:260–277. https://doi.org/10.1111/j.1095-8649.2006.01291.x

Palumbi S, Martin A, Romano S et al (1991) The Simple Fool's Guide do PCR, v. 2.0. University of Hawaii, Honolulu

Pamponet VCC, Carneiro PLS, Affonso PRAM et al (2008) A multi-approach analysis of the genetic diversity in populations of *Astyanax* aff. *bimaculatus* Linnaeus, 1758 (Teleostei: Characidae) from Northeastern Brazil. Neotrop Ichthyol 6:621–630. https://doi.org/10.1590/S1679-62252008000400010

Pimentel D (2007) Environmental and economic costs of vertebrate species invasions into the United States. In: Witmer GW, Pitt WC, Fagerstone KA (eds) Manag Vertebr Invasive Species Proc an Int Symp USDA/APHIS/WS, Natl Wildl Res Center, Fort Collins, CO 1–8. https://doi.org/10.1093/nar/gkq443

Pompeu PS (1999) Dieta da pirambeba *Serrasalmus brandtii* Reinhardt (Teleostei, Characidae) em quatro lagoas marginais do rio São Francisco, Brasil. Revta Bras Zool 16:19–26. https://doi.org/10.1590/S0101-81751999000600003

Pringle RM, Kartzinel TR, Palmer TM et al (2019) Predator-induced collapse of niche structure and species coexistence. Nature 570:58–64. https://doi.org/10.1038/s41586-019-1264-6

Pugedo ML, de Andrade Neto FR, Pessali TC et al (2016) Integrative taxonomy supports new candidate fish species in a poorly studied neotropical region: the Jequitinhonha River Basin. Genetica 144:341–349. https://doi.org/10.1007/s10709-016-9903-4

R Development Core Team (2016) RStudio| Open source and enterprise-ready professional software for R

Reid AJ, Carlson AK, Creed IF et al (2019) Emerging threats and persistent conservation challenges for freshwater biodiversity. Biol Rev 94:849–873. https://doi.org/10.1111/brv.12480

Sales NG, Pessali TC, Andrade Neto FR, Carvalho DC (2018) Introgression from non-native species unveils a hidden threat to the migratory Neotropical fish *Prochilodus hartii*. Biol Invasions 20:555–566. https://doi.org/10.1007/s10530-017-1556-4

Shea K, Chesson P (2002) Community ecology theory as a framework for biological invasions. Ecol Evol 17:170–176. https://doi.org/10.1016/S0169-5347(02)02495-3

Silva ARM, Santos GB, Ratton T (2006) Fish community structure of Juramento reservoir, São Francisco River basin, Minas Gerais, Brazil. Rev Bras Zool 23:832–840. https://doi.org/10.1590/S0101-81752006000300031

Sofia SH, Silva CRM, Galindo BA et al (2006) Population genetic structure of *Astyanax scabripinnis* (Teleostei, Characidae) from an urban stream. Hydrobiologia 553:245–254. https://doi.org/10.1007/s10750-005-1106-4

Tamura K, Stecher G, Peterson D et al (2013) MEGA6: molecular evolutionary genetics analysis version 6.0. Mol Biol Evol 30:2725–2729. https://doi.org/10.1093/molbev/mst197

Teske PR, Golla TR, Sandoval-Castillo J, Emami-Khoyi A, van der Lingen C, von der Heyden S, Chiazzari B, van Vuuren BJ, Beheregaray LB (2018) Mitochondrial DNA is unsuitable to test for isolation by distance. Sci Rep 8:8448. https://doi.org/10.1038/s41598-018-25138-9

Thompson JD, Higgins DG, Gibson TJ (1994) CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res 22:4673–4680

Tsutsui ND, Suarez AV, Holway DA, Case TJ (2000) Reduced genetic variation and the success of an invasive species. Proc Natl Acad Sci 97:5948–5953. https://doi.org/10.1073/pnas.100110397

Walsh JR, Carpenter SR, Vander Zanden MJ (2016) Invasive species triggers a massive loss of ecosystem services through a trophic cascade. Proc Natl Acad Sci 113:4081–4085. https://doi.org/10.1073/pnas.1600366113

Ward RD, Zemlak TS, Innes BH et al (2005) DNA barcoding Australia's fish species. Philos Trans R Soc Lond B Biol Sci 360:1847–1857. https://doi.org/10.1098/rstb.2005.1716