

# The genetic characteristics of invasive Largemouth Bass in southern Brazil

Fabício de A. Frehse<sup>1,2</sup>  | John S. Hargrove<sup>3,4</sup>  | Olaf L. F. Weyl<sup>4,5,6</sup> | Jean R. S. Vitule<sup>2</sup>

<sup>1</sup>Programa de Pós-Graduação em Ecologia e Conservação, Universidade Federal do Paraná, Curitiba, Brazil

<sup>2</sup>Laboratório de Ecologia e Conservação, Departamento de Engenharia Ambiental, Universidade Federal do Paraná, Curitiba, Brazil

<sup>3</sup>Pacific States Marine Fisheries Commission, Eagle, ID, USA

<sup>4</sup>South African Institute for Aquatic Biodiversity (SAIAB), Grahamstown, South Africa

<sup>5</sup>DST/NRF Research Chair in Inland Fisheries and Freshwater Ecology, South African Institute for Aquatic Biodiversity (SAIAB), Grahamstown, South Africa

<sup>6</sup>Center for Invasion Biology, SAIAB, Grahamstown, South Africa

## Correspondence

Fabício de A. Frehse, Universidade Federal do Paraná, Programa de Pós-Graduação em Ecologia e Conservação, Campus Centro Politécnico, Av. Coronel Francisco Heráclito dos Santos, 100, 81530-000, Curitiba, PR, Brazil.

Email: fafrebio@gmail.com

## Funding information

Conselho Nacional de Desenvolvimento Científico e Tecnológico, Grant/Award Number: 457122/2014-5; National Research Foundation, Grant/Award Number: 110507

## Abstract

Largemouth Bass (*Micropterus salmoides*) have been introduced on a global scale for sport fishing but represent a conservation concern given their documented negative impacts on native faunal diversity and abundance. Recent research using molecular data to characterize invasive Largemouth Bass populations elsewhere has demonstrated that populations are typically characterized by limited genetic diversity, or represent a combination of Largemouth Bass and Florida Bass (*Micropterus floridanus*). To test whether these traits were consistent with invasive populations in Brazil, we generated mitochondrial sequence data from four established populations of Largemouth Bass collected in southern Brazil as well as a local aquaculture facility to confirm species identity and quantify levels of genetic diversity. We identified the exclusive presence of Largemouth Bass in the region and observed limited levels of haplotype (haplotype diversity = 0.0684, SE = 0.038) and nucleotide diversity (0.0003, SE = 0.0002) which suggested the presence of a founder effect associated with introduction. Each of the four populations were dominated by a single haplotype that was identical to one recovered from a nearby aquaculture facility, which identified this facility as a potential introduction source.

## KEYWORDS

DNA, fish, freshwater reservoir, genetic diversity, non-native species

## 1 | INTRODUCTION

The introduction and establishment of invasive species are promoting a pattern of global biotic homogenization via the replacement and extinction of endemic species (Bezerra et al., 2019; García-Berthou, 2007; Olden, LeRoy Poff, Douglas, Douglas, & Fausch, 2004). Freshwater ecosystems are particularly susceptible to biological invasions (Marchetti, Moyle, & Levine, 2004) and fishes are

among the most introduced group of aquatic organisms globally (Gozlan, Britton, Cowx, & Copp, 2010). A primary vector for fish introductions is the establishment of populations for recreational fisheries (Ellender & Weyl, 2014; Ribeiro, Collares-Pereira, & Moyle, 2009). As a result, many introduced sport fishes are apex predators in their introduced systems which is a conservation concern given their deleterious impacts on native fauna (Baxter, Fausch, Murakami, & Chapman, 2004; Pereira & Vitule, 2019; Weyl, Moor, Hill, & Weyl, 2010).

The Largemouth Bass (*Micropterus salmoides*) is an apex predator endemic to North America (Froese & Pauly, 2019) that has been introduced into more than 70 countries globally (Hargrove, Weyl, Allen, & Deacon, 2015; Robbins & MacCrimmon, 1974) where it has become the focus of a recreationally and economically important sport fishery (Hargrove, Allen, Weyl, Crandall, & Austin, 2018; Taylor, Weyl, Cowley, & Allen, 2015). The first introduction of Largemouth Bass into Brazil occurred in the 1920s (Godoy, 1954) and, as is the case elsewhere, they are now popular in recreational fisheries (Dairiki, Dias, & Cyrino, 2007). The intentional transfer of these fish between waterbodies has expanded their distribution to ponds and reservoirs in the southern and southeastern regions of Brazil from Rio de Janeiro to Rio Grande do Sul (Schulz & Leal, 2005). To date, research on Largemouth Bass in Brazil has focused on describing their life-history and ecology but little effort has been directed towards understanding the details surrounding their introduction and subsequent spread.

Ongoing developments in genetic techniques and statistical analyses (Cristescu, 2015; Estoup & Guillemaud, 2010) have resulted in the ability to reconstruct introduction pathways, quantify demographic changes associated with establishment, and identify sources of introduction (Hargrove, Weyl, & Austin, 2017). In the context of Largemouth Bass, work from southern Africa using nuclear microsatellites has demonstrated that although multiple source populations were initially used for establishment, levels of genetic diversity across introduced populations were low and consistent with a founder effect (Hargrove et al., 2017). Furthermore, mitochondrial (mtDNA) sequence data was used to successfully identify a putative source population from within the native range. A follow up study by Weyl, Schirrmann, Hargrove, Bodill, and Swartz (2017) identified many populations of Largemouth Bass in South Africa were a combination of Largemouth Bass and its closely related sister species the Florida Bass (*Micropterus floridanus*). The dynamics of hybridization among Largemouth Bass populations in southern Africa was further described by Hargrove, Weyl, Zhao, Peatman, and Austin (2019), which revealed a wider distribution of Florida Bass alleles than previously thought, and the presence of populations established with Largemouth Bass that have through time become dominated by Florida Bass alleles. Combined, the above research on non-native populations of Largemouth Bass identified several important trends associated with their introduction. First, many populations have become successfully established from small number of propagules (Bai, Lutz-Carrillo, Quan, & Liang, 2008; Hargrove et al., 2017) which implies that limited genetic diversity alone may not prevent establishment. Second, many populations of what were thought to be Largemouth Bass were actually a combination of Florida Bass, Largemouth Bass, and their hybrids (Hargrove, Weyl, et al., 2019; Weyl et al., 2017). From the context of invasive species management, identifying which species of Black Bass (*Micropterus* spp.) are present is critically important, as Florida Bass and Largemouth Bass may be differentially impactful on native fishes and insects (Weyl et al., 2017).

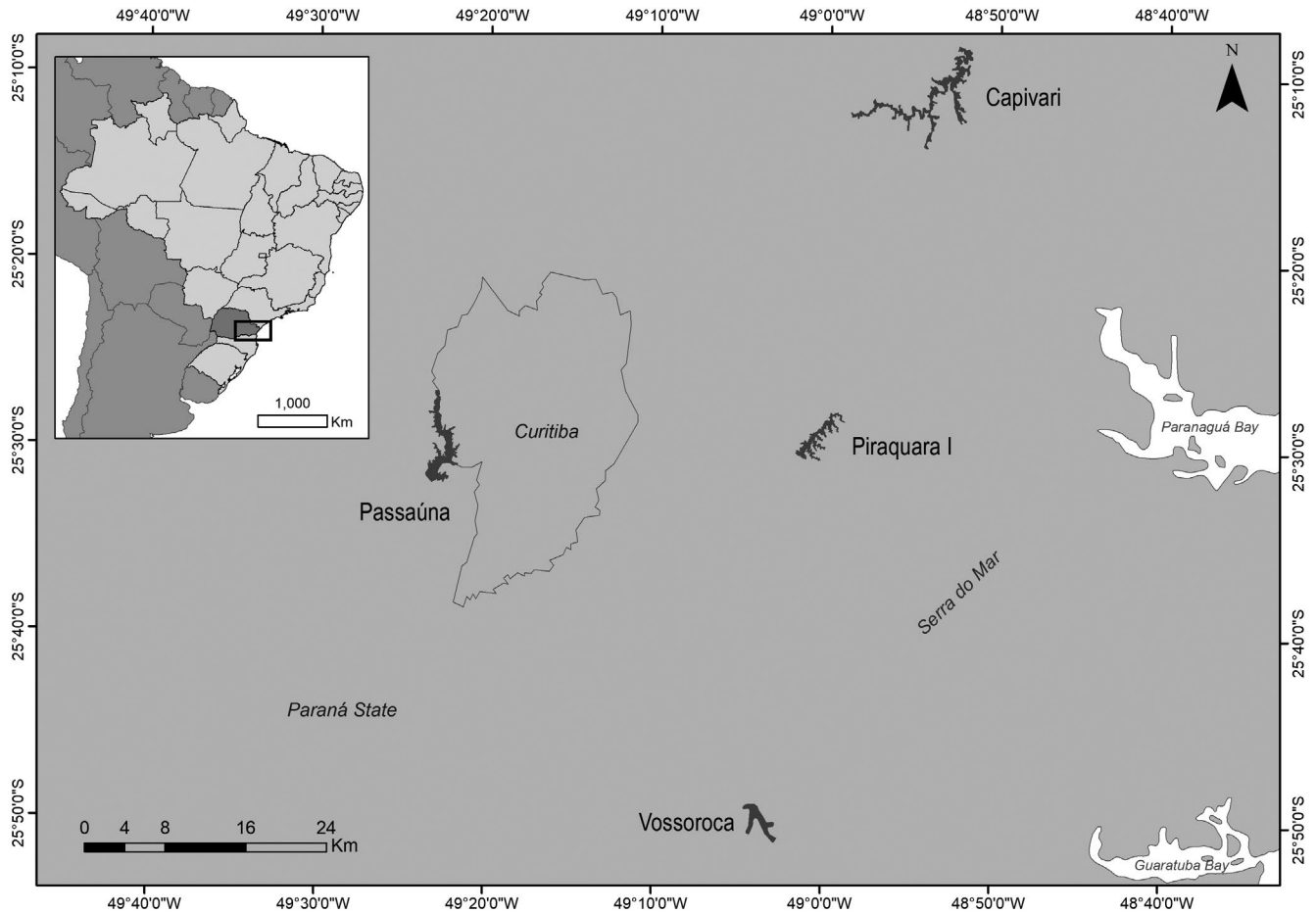
To assess whether genetic trends in Largemouth Bass populations from southern Africa were also displayed by Largemouth Bass populations in Brazil, we utilized mitochondrial DNA sequence data to characterize four Largemouth Bass populations sampled from southern Brazil. Our objectives were to identify which species were present (i.e., Largemouth Bass, Florida Bass, or both) and to quantify genetic diversity within populations to characterize the details associated with introduction (e.g., presence of founder effects). In addition, we compared the haplotypes recovered from four reservoir populations with samples from an aquaculture facility to determine whether this was a likely a source of introduction.

## 2 | METHODS

### 2.1 | Study sites and sample collections

Samples were collected from four impoundments near the city of Curitiba in the state of Paraná, southern Brazil (Figure 1). Two water bodies represented public water supply reservoirs (Passauna and Piraquara I) and two were constructed for power generation purposes (Cativari and Vossoroca). The Passauna and Piraquara I reservoirs are administered by the Sanitation Company of Paraná (SANEPAR) and human activities such as swimming, boating, and fishing were not allowed at the time of the study. In contrast, the Cativari and Vossoroca reservoirs are administered by the Energy Company of Paraná (COPEL) and no restrictions on recreational use were in place at the time of the study (for reservoir details see Table 1). In addition, samples also were obtained at an aquaculture facility near the city of Toledo, in the west of Paraná state, which represents one of the main aquaculture sites in Brazil. Samples were collected by angling during spring (September-December) in 2015 and a 5 mm<sup>2</sup> portion of fin clip was removed from each individual and stored in 95% ethanol.

In the laboratory, a 1 mm<sup>2</sup> portion of fin clip was extracted using the Invitrogen PureLink<sup>®</sup> Genomic DNA Kit following the manufacturer's protocol. Extracted DNA was quantified by spectrophotometer and diluted to a standardized concentration (20 ng/μl) prior to PCR amplification. We selected to amplify an 860 base pair region of the mitochondrial D-loop (control region) gene because of its elevated rate of evolution relative to other mitochondrial genes (Cui, Liu, & Chu, 2010; Jacobsen, Fonseca, Bernatchez, & Hansen, 2015), suitability for discriminating among species of the genus *Micropterus* (Ray, Husemann, King, & Danley, 2012), and established use to evaluate population levels of genetic diversity in Black Bass (Smallmouth Bass: Borden & Stepien, 2006; Spotted Bass: Coughlin, Echelle, Bussche, Cofer, & Fisher, 2003; Ray, Husemann, Lutz-Carrillo, King, & Danley, 2015; Largemouth Bass and Florida Bass: Ray et al., 2012). Up to fifteen individuals per population were amplified using the primer pairs CR-F (5'-GGATTTTAACCCYACCMCT-3') and CR-R (5'-TTCTAGGGCTCATCCTAACATCTTC-3'; Husemann, Ray, King, Hooser, & Danley, 2012). Polymerase chain reactions were performed using the following chemistry: 2.5 μl 10× PCR buffer, 3 mM



**FIGURE 1** A map showing the sampling locations for Largemouth Bass (*Micropterus salmoides*) sampled from four reservoirs in southern

**TABLE 1** Characteristics of reservoirs in the metropolitan region of Curitiba, Paraná, Brazil that were sampled for Largemouth Bass (*Micropterus salmoides*)

Reservoir	Latitude (S)	Longitude (W)	Purpose	Fishing	Opening year	Size (km <sup>2</sup> )
Capivari	25°8'40.50''	48°52'1.03''	Power generation	Allowed	1970	13.5
Passauna	25°31'40.40''	49°23'15.44''	Public supply	Not-allowed	1990	14
Piraquara I	25°30'16.82''	49°1'30.48''	Public supply	Not-allowed	1979	3.3
Vossoroca	25°49'18.73''	49°4'3.79''	Power generation	Allowed	1949	36

## Brazil

MgCl<sub>2</sub>, 0.4 mM dNTP's, 0.25 mM of each primer, 1.5 units Taq DNA polymerase, 50 ng/μl of DNA template, and ddH<sub>2</sub>O to reach a final reaction volume of 25 μl. Thermal cycling conditions were: 94°C for 3 min, followed by, 30 cycles of 60 s at 94°C, 60 s at 58°C, and 120 s at 72°C, followed by a final extension of 72°C for 10 min. PCR products were visualized using a 1.5% SYBR safe (ThermoFisher Scientific) dye stained gel to confirm appropriate amplicon length and reaction success. Successfully amplified DNA samples (i.e., PCR produced an amplified fragment of appropriate length) were further processed (i.e., purified and bi-directionally sequenced) at the Molecular Biology Laboratory, Paraná Federal University and WEMSeq Biotecnologia, Brazil, using their standard protocols.

Chromatograms were edited and assembled using ChromasPro v 1.7.7 (Technelysium).

## 2.2 | Genetic analyses

Following processing, consensus sequences were inputted into the National Center for Biotechnology Information (NCBI) search algorithm BLAST (Altschul, Gish, Miller, Myers, & Lipman, 1990) to retrieve the closest match for species identification. Previous research has validated the use of the control region for species identification in Largemouth and Florida Bass based on fixed nucleotide differences between the

two species (Ray et al., 2012). An important caveat is that identification of hybrids (e.g., offspring born to a Florida Bass × Largemouth Bass) is not possible using mitochondrial DNA sequence data due its matrilineal nature of inheritance in vertebrates (Ballard & Whitlock, 2004). As a result, our primary focus was to detect the presence or absence of species and not the detection of hybrid individuals.

Estimates of haplotype diversity ( $H_D$ ; Nei, 1987), nucleotide diversity ( $\pi$ ; Nei & Tajima, 1981), and number of haplotypes ( $N_H$ ) were generated for the overall and population-specific dataset using DnaSP (Rozas, Sánchez-DelBarrio, Messeguer, & Rozas, 2003). Largemouth Bass DNA sequences from Brazil were aligned with *M. salmoides* and *M. floridanus* D-loop sequences retrieved from GenBank (Table S1). This alignment was constructed using the Clustal x algorithm using default parameters as performed in MEGA7 (Kumar, Stecher, & Tamura, 2016; Thompson, Gibson, Plewniak, Jeanmougin, & Higgins, 1997). The combined sequence set was collapsed into unique haplotypes and DNA sequences of variable length were trimmed to the shortest sequence using FaBox v 1.41 (Villesen, 2007). A minimum spanning haplotype network was generated in PopART (Leigh & Bryant, 2015) with unique haplotypes recovered from Brazil ( $n = 3$ ) and sequences that contained sequence data for the control region ( $n = 37$ ) retrieved from GenBank (Table S1). All sequences generated as part of this study were deposited in GenBank under accession numbers (MN717182–MN717239). In addition to the minimum spanning haplotype network, we generated a neighbor-joining tree in MEGA 7 using unique haplotypes recovered from Brazil and sequences retrieved from GenBank for Largemouth Bass, Florida Bass (*Micropterus floridanus*), and Spotted Bass (*Micropterus punctulatus*; used as an outgroup). The purpose of our neighbor-joining tree was two-fold, to confirm species identity and to identify the most closely related samples from within the native range. The tree was constructed using 2,500 bootstrap iterations and the Tamura-Nei substitution model. The final tree was condensed to show only bootstrap support values >50%.

**TABLE 2** Summary statistics by population for mitochondrial D-loop gene sequences generated for Largemouth Bass (*Micropterus salmoides*) collected from four southeastern Brazil populations

Population	N	$n_H$	$n_{PH}$	$H_F$	$h (\pm SD)$	$\pi (\pm SD)$
Capivari	12	1	0	A (1.00)	0.0000	0.0000
Piraquana I	14	2	1	A (0.93) B (0.07)	0.1429	0.0005
Passauna	16	1	0	A (1.00)	0.0000	0.0000
Vossorooca	12	2	1	A (0.92) C (0.08)	0.1667	0.0008
Hatchery	4	1	0	A (1.00)	0.0000	0.0000
Total	58	3	–	–	0.0684	0.0003

Note: Listed are the population name, sample size (N), number of haplotypes ( $n_H$ ), number of private haplotypes ( $n_{PH}$ ), haplotypes and their frequencies within populations ( $H_F$ ), haplotype diversity ( $h$ ), and nucleotide diversity ( $\pi$ ). Haplotype IDs presented in Figures 2 and 3 (e.g., A, B, and C) are the same as those in column  $H_F$ .

### 3 | RESULTS

Bidirectional sequences were generated for a total of 58 individuals representing 4–16 fish sampled per population (Table 2). The final length of control region DNA sequences from Brazilian samples was 809 base pairs. A total of three unique haplotypes were recovered with a single haplotype being common to all populations. The most frequent haplotype was shared by 56 individuals (97% of all samples). Two additional haplotypes were recovered, one being private to the Vossorooca reservoir population and the other to the Piraquana I reservoir population (Table 2).

We conducted a BLAST (Basic Local Alignment Search Tool) of the DNA sequences we generated and all three haplotypes were most closely related to Largemouth Bass. The percent identity (i.e., the percent of submitted sequence that was identical to a GenBank sequence) for each haplotype was a 99% match (range 97%–99%) to Largemouth Bass records. Sequence similarity between Brazil samples and the second highest match (Florida Bass; *M. floridanus*) was 94%–95% percent identity.

Levels of genetic diversity were low for all sampled populations; the average haplotype diversity across populations was 0.0684 ( $SE = 0.038$ , range = 0.0000–0.1667) and the average nucleotide diversity was 0.0003 ( $SE = 0.0002$ , range = 0.000–0.0008; Table 2). A total of seven variable positions (segregating sites) were detected. When placed into a haplotype network, the most commonly observed haplotype from Brazil (Haplotype A; Table 2) was identical to two separate sequences from North America, one generated from Lake Erie and a second from New York (Figure 2). A DNA sequence from a Largemouth Bass sampled in Pennsylvania, USA was separated by a single base pair difference from the Brazil samples.

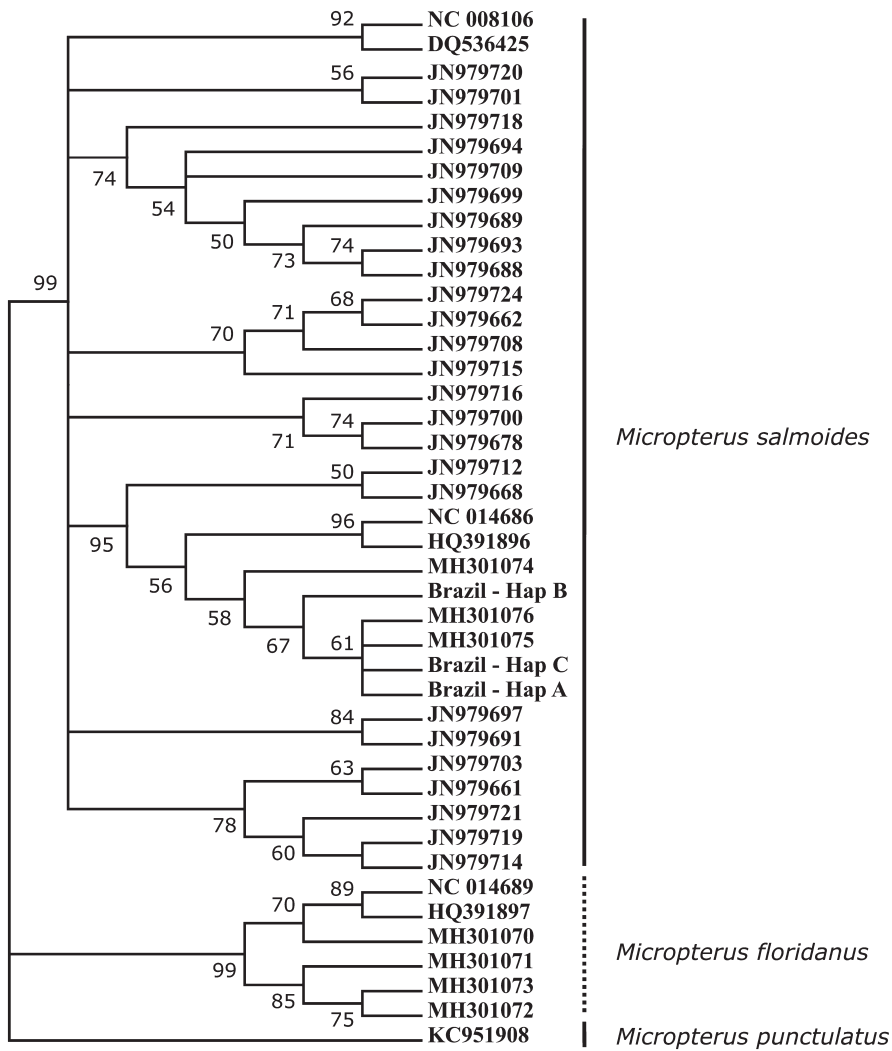
A neighbor-joining tree displaying evolutionary relationships among Largemouth Bass, Florida Bass, and Spotted Bass (used as an outgroup) showed samples collected from Brazil nested within a clade of Largemouth Bass with high bootstrap support (Figure 3). Brazilian haplotypes A and C were most closely related to two haplotypes (MH301075 and MH301076) which were sampled in New York and Lake Erie, respectively. Haplotype B displayed the smallest genetic distance to a sequence sampled in Pennsylvania (MH301074). While bootstrap support among subtrees containing Largemouth Bass was moderate, the division among species was stronger. Polytomies were observed among 9 of the 49 reference sequences (those downloaded from GenBank) used in analysis and were removed from the final tree as they provided no additional phylogenetic information (GenBank accession no. JN979681, JN979677, JN979717, JN979686, JN979680, JN979692, JN979670, JN979669, and JN979723).

### 4 | DISCUSSION

Using a dataset of mitochondrial DNA (mtDNA) sequences, we identified the exclusive presence of Largemouth Bass in a region of Brazil with extensive aquaculture operations. Furthermore, we







**FIGURE 3** A neighbor-joining tree displaying phylogenetic relationships among mitochondrial D loop sequences generated for Largemouth Bass (*Micropterus salmoides*) sampled from introduced populations in Brazil and those retrieved from Largemouth Bass, Florida Bass (*Micropterus floridanus*), and Spotted Bass (*Micropterus punctulatus*) retrieved from GenBank. The evolutionary distances were computed using the Tamura-Nei method with units equal to the number of base substitutions per site. Spotted Bass was used as an outgroup

Lake Erie and New York, USA. While this observation highlights this region within the United States as a potential source population, we advise caution in drawing such a conclusion because the distribution of control region haplotypes across the native range is poorly documented. For example, of the 37 control regions downloaded from GenBank for use in our haplotype network, 34 of them were from a single study (Ray et al., 2012). Future studies examining the source of origin of introduced populations of Largemouth Bass should consider using genes with greater coverage from the native range (i.e., cytochrome *b*, Hargrove et al., 2017). DNA sequences from a local Largemouth Bass aquaculture facility were identical to those recovered in each of the four sampled populations suggesting these operations as a potential source for introductions. Although the breeding, transport, and trade of the invasive Largemouth Bass are forbidden in the state of Paraná (IAP Ordinance 59, 2015), such activities are common due to lack of enforcement. As in other parts of the world (Cambray, 2003; Ellender & Weyl, 2014), sport fishery has been the main vector of introduction of this invasive fish in Brazil (Frehse, Braga, Nocera, & Vitule, 2016), where anglers can buy fish from aquaculture facilities for release at sites where they want to promote sport fishing activities (Vitule, Bornatowski, Freire, & Abilhoa, 2014).

An important consideration for studies that seek to identify species distributions using mitochondrial sequence data (Hargrove et al., 2017; Weyl et al., 2017) involves the nature of inheritance of the selected marker. Both a BLAST search and neighbor-joining tree identified all individuals from Brazil as Largemouth Bass; but hybridization among members of the genus *Micropterus* is common, and the current study analyzed sequence data from the mitochondrial genome which is uniparentally inherited and therefore inappropriate for identifying individuals that are of hybrid origin (e.g., an  $F_1$  or  $F_2$  hybrid or late stage backcross). However, we argue a population that included hybrids would contain haplotypes from both maternal species except in rare cases of asymmetrical hybridization (e.g., where male of species 1 only mates with female of species 2 and not vice versa; Avise et al., 1997). The probability of failing to detect one of two species present can be predicted mathematically as a function of the admixture level in the population ( $A$ ), the number of markers assayed per individual ( $m$ ), and the number of individuals analyzed ( $n$ ),  $P_{n,m} = (1 - A)^{2nm}$ ; Della Croce, Poole, Payn, & Gresswell, 2017). Applying this equation to our system, the probability that we failed to detect Florida Bass by random chance alone was very small ( $7.5 \times 10^{-10}$ ) at moderate rates of admixture ( $A = 0.5$ ) given our sample scheme ( $n = 13.5$  fish per population,

$m = 1$  – which is a conservative value given sequence data revealed multiple diagnostic sites between Largemouth Bass and Florida Bass). At modest levels of admixture ( $A = 0.25$ ) the probability of failing to detect a species is still very low ( $4.2 \times 10^{-4}$ ), and only under low levels of admixture ( $A = 0.10$ ) does the probability of failing to detect Florida Bass begin to rise (0.06). Thus, given our sampling scheme we are confident that the failure to detect Florida Bass was not an artifact of random chance but instead reflected the absence of Florida Bass within our focal populations. Recent developments in sequencing technology have produced larger numbers of species-diagnostic markers distributed across the genome to assess hybridization among Bass species (e.g., single nucleotide polymorphisms SNPs; Li et al., 2015; Zhao et al., 2018), which affords greater resolution and accuracy in mapping species distributions (Hargrove, Rogers, Kacmar, & Black, 2019; Hargrove, Weyl, et al., 2019).

In conclusion, our study demonstrated the exclusive presence of Largemouth Bass in reservoirs around Curitiba. Although these populations have been established for at least 25 years (J. R. S. Vitule, unpublished data), limited levels of haplotypes and nucleotide diversity were found, indicating a founder effect associated with introductions. We also indicate the role of aquaculture facilities associated with sport fishing as the main vectors for this species introduction, reinforcing the need for extensive control over such activities.

#### ACKNOWLEDGEMENTS

We thank the Sanitation Company of Paraná (SANEPAR) and the Energy Company of Paraná (COPEL) for providing sampling permits for the reservoirs. We are grateful to the Prof. Dr. Marcio Pie and his lab crew, Dra. Paula Borges Bassi and Dr. Ricardo Belmonte Lopes, of Federal University of Paraná, for all the support during lab procedures. We are grateful to Prof. Dr. Éder Gubiane for obtaining samples from Toledo, and Lais Carneiro for the help in the elaboration of the study area figure. We also thank the CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior) for the scholarship provided to FAF, and CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico) for research grants provided to JRSV (Process Numbers: 310850/2012-6; 303776/2015-3). This study was financed by the CNPq Universal Announcement (457122/2014-5). OLFW acknowledges support by the National Research Foundation – South African Research Chairs Initiative of the Department of Science and Technology (Grant No. 110507).

#### ORCID

Fabrizio de A. Frehse  <https://orcid.org/0000-0001-7501-0578>

John S. Hargrove  <https://orcid.org/0000-0002-8643-7822>

#### REFERENCES

- Altschul, S. F., Gish, W., Miller, W., Myers, E. W., & Lipman, D. J. (1990). Basic local alignment search tool. *Journal of Molecular Biology*, 215, 403–410.
- Awise, J., Pierce, P., Van Den Avyle, M., Smith, M. H., Nelson, W. S., & Asmussen, M. A. (1997). Cytonuclear introgressive swamping and species turnover of bass after an introduction. *Journal of Heredity*, 88, 14–20.
- Bai, J., Lutz-Carrillo, D. J., Quan, Y., & Liang, S. (2008). Taxonomic status and genetic diversity of cultured Largemouth bass *Micropterus salmoides* in China. *Aquaculture*, 278, 27–30.
- Bailey, R. M., & Hubbs, C. L. (1949). *The black basses (Micropterus) of Florida, with description of a new species* (p. 516). Ann Arbor, MI: Occasional Papers of the Museum of Zoology University of Michigan.
- Ballard, J. W. O., & Whitlock, M. C. (2004). The incomplete natural history of mitochondria. *Molecular Ecology*, 13, 729–744.
- Baxter, C. V., Fausch, K. D., Murakami, M., & Chapman, P. L. (2004). Fish invasion restructures stream and forest food webs by interrupting reciprocal prey subsidies. *Ecology*, 85, 2656–2663.
- Bezerra, L. A. V., Ribeiro, V. M., Freitas, M. O., Kaufman, L., Padial, A. A., & Vitule, J. R. S. (2019). Benthification, biotic homogenization behind the trophic downgrading in altered ecosystems. *Ecosphere*, 10(6), e02757.
- Borden, W. C., & Stepien, C. A. (2006). Discordant population genetic structuring of Smallmouth Bass, *Micropterus dolomieu* Lacepède, in Lake Erie based on mitochondrial DNA sequences and nuclear DNA microsatellites. *Journal of Great Lakes Research*, 32, 242–257.
- Cambrey, J. A. (2003). Impact on indigenous species biodiversity caused by the globalization of alien recreational freshwater fisheries. *Aquatic Biodiversity Developments in Hydrobiology*, 171, 217–230. [https://doi.org/10.1007/978-94-007-1084-9\\_16](https://doi.org/10.1007/978-94-007-1084-9_16)
- Coughlin, W. D., Echelle, A. A., Van Den Bussche, R. A., Cofer, L. M., & Fisher, W. L. (2003). Genetic structure of Spotted Bass (*Micropterus punctulatus*) in the Red and Arkansas River basins: Microsatellite and mitochondrial DNA variation. *The Southwestern Naturalist*, 48, 526–533.
- Cristescu, M. E. (2015). Genetic reconstructions of invasion history. *Molecular Ecology*, 24, 2212–2225.
- Cui, Z., Liu, Y., & Chu, K. H. (2010). Broader pattern of tandem repeats in the mitochondrial control region of Perciformes. *Chinese Journal of Oceanology and Limnology*, 28, 785–794.
- Dairiki, J. K., Dias, C. T. S., & Cyrino, J. E. P. (2007). Lysine requirements of Largemouth Bass, *Micropterus salmoides*: A comparison of methods of analysis of dose-response trials data. *Journal of Applied Aquaculture*, 19, 1–27.
- Della Croce, P., Poole, G. C., Payn, R. A., & Gresswell, R. E. (2017). Early detection of nonnative alleles in fish populations: When sample size actually matters. *Fisheries*, 42, 44–56.
- Ellender, B. R., & Weyl, O. L. F. (2014). A review of current knowledge, risk and ecological impacts associated with non-native freshwater fish introductions in South Africa. *Aquatic Invasions*, 9, 117–132.
- Estoup, A., & Guillemaud, T. (2010). Reconstructing routes of invasion using genetic data: Why, how and so what? *Molecular Ecology*, 19, 4113–4130.
- Frehse, F. A., Braga, R. R., Nocera, G. A., & Vitule, J. R. S. (2016). Non-native species and invasion biology in a megadiverse country: Scientometric analysis and ecological interactions in Brazil. *Biological Invasions*, 18, 3713–3725.
- Froese, R., & Pauly, D. (2019). *FishBase: World Wide Web electronic publication*. Retrieved from <http://www.fishbase.org/version>
- García-Berthou, E. (2007). The characteristics of invasive fishes: What has been learned so far? *Journal of Fish Biology*, 71, 33–55.
- Godoy, M. (1954). Observações sobre a adaptação do Black Bass em Pirassununga, Estado de São Paulo. *Revista Brasileira De Biologia*, 14, 32–38.
- Gozlan, R. E., Britton, J. R., Cowx, I., & Copp, G. H. (2010). Current knowledge on non-native freshwater fish introductions. *Journal of Fish Biology*, 76, 751–786.

- Hargrove, J. S., Allen, M. S., Weyl, O. L. F., Crandall, C. A. C., & Austin, J. D. (2018). Global patterns in the motivations and behaviors of tournament anglers targeting bedding bass. *North American Journal of Fisheries Management*, 38, 334–345. <https://doi.org/10.1002/nafm.10027>
- Hargrove, J. S., Rogers, M. W., Kacmar, P. T., & Black, P. (2019). A state-wide evaluation of Florida Bass genetic introgression in Tennessee. *North American Journal of Fisheries Management*, 39, 637–651. <https://doi.org/10.1002/nafm.10295>
- Hargrove, J. S., Weyl, O. L. F., Allen, M. S., & Deacon, N. R. (2015). Using tournament angler data to rapidly assess the invasion status of alien sport fishes (*Micropterus* spp.) in southern Africa. *PLoS ONE*, 10, e0130056.
- Hargrove, J. S., Weyl, O. L. F., & Austin, J. D. (2017). Reconstructing the introduction history of an invasive fish predator in South Africa. *Biological Invasions*, 19, 2261–2276.
- Hargrove, J. S., Weyl, O. L. F., Zhao, H., Peatman, E., & Austin, J. D. (2019). Using species-diagnostic SNPs to detail the distribution and dynamics of hybridized black bass populations in southern Africa. *Biological Invasions*, 21, 1499–1509. <https://doi.org/10.1007/s10530-018-01912-8>
- Horton, R. A., & Gilliland, E. R. (1993). Monitoring trophy largemouth bass in Oklahoma using a taxidermist network. *Proceedings of the Annual Conference Southeastern Association of Fish and Wildlife Agencies*, 47, 679–685.
- Husemann, M., Ray, J. W., King, R. S., Hooser, E. A., & Danley, P. D. (2012). Comparative biogeography reveals differences in population genetic structure of five species of stream fishes. *Biological Journal of the Linnean Society*, 107(4), 867–885.
- IAP Ordinance 59 (2015). *Portaria do Instituto Ambiental do Paraná - Reconhece a Lista Oficial de Espécies Exóticas Invasoras para o Estado do Paraná, estabelece normas de controle e dá outras providências*. Retrieved from [http://www.iap.pr.gov.br/arquivos/File/Lista\\_invasoras\\_PR\\_corrigeida\\_set\\_2015.pdf](http://www.iap.pr.gov.br/arquivos/File/Lista_invasoras_PR_corrigeida_set_2015.pdf)
- Jacobsen, M. W., da Fonseca, R. R., Bernatchez, L., & Hansen, M. H. (2015). Comparative analysis of complete mitochondrial genomes suggests that relaxed purifying selection is driving high nonsynonymous evolutionary rate of the NADH2 gene in whitefish (*Coregonus* spp.). *Molecular Phylogenetics and Evolution*, 95, 161–170.
- Kinziger, A. P., Nakamoto, R. J., Anderson, E. C., & Harvey, B. C. (2011). Small founding number and low genetic diversity in an introduced species exhibiting limited invasion success (speckled dace, *Rhinichthys osculus*). *Ecology and Evolution*, 1, 73–84.
- Kumar, S., Stecher, G., & Tamura, K. (2016). MEGA7: Molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Molecular Biology and Evolution*, 33, 1870–1874.
- Leigh, J. W., & Bryant, D. (2015). popart: Full-feature software for haplotype network construction. *Methods in Ecology and Evolution*, 6(9), 1110–1116.
- Li, C., Gowan, S., Anil, A., Beck, B. H., Thongda, W., Kucuktas, H., ... Peatman, E. (2015). Discovery and validation of gene-linked diagnostic SNP markers for assessing hybridization between Largemouth bass (*Micropterus salmoides*) and Florida bass (*M. floridanus*). *Molecular Ecology Resources*, 15, 395–404.
- Mabuchi, K. H. S., & Nishida, M. (2008). Mitochondrial DNA analysis reveals cryptic large-scale invasion of non-native genotypes of common carp (*Cyprinus carpio*) in Japan. *Molecular Ecology*, 17, 796–809.
- Marchetti, M. P., Moyle, P. B., & Levine, R. (2004). Alien fishes in California watersheds: Characteristics of successful and failed invaders. *Ecological Applications*, 14, 587–596.
- Neal, J. W., & Noble, R. L. (2002). Growth, survival, and site fidelity of Florida and intergrade Largemouth Bass stocked in a tropical reservoir. *North American Journal of Fisheries Management*, 22, 528–536.
- Nei, M. (1987). *Molecular evolutionary genetics*. New York, NY: Columbia University Press.
- Nei, M., & Tajima, F. (1981). DNA polymorphism detectable by restriction endonucleases. *Genetics*, 97(1), 145–163.
- Olden, J. D., LeRoy Poff, N., Douglas, M. R., Douglas, M. E., & Fausch, K. D. (2004). Ecological and evolutionary consequences of biotic homogenization. *Trends in Ecology & Evolution*, 19, 18–24.
- Pereira, F. W., & Vitule, J. R. S. (2019). The largemouth bass *Micropterus salmoides* (Lacepède, 1802): Impacts of a powerful freshwater fish predator outside of its native range. *Reviews in Fish Biology and Fisheries*, 29(3), 639–652. <https://doi.org/10.1007/s11160-019-09570-2>
- Ray, J. W., Husemann, M., King, R. S., & Danley, P. D. (2012). Genetic analysis reveals dispersal of Florida bass haplotypes from reservoirs to rivers in central Texas. *Transactions of the American Fisheries Society*, 141, 1269–1273.
- Ray, J. W., Husemann, M., Lutz-Carillo, D. J., King, R. S., & Danley, P. D. (2015). Life at the leading edge: Genetic impoverishment of the spotted bass, *Micropterus punctulatus*, at its Western edge. *Environmental Biology of Fishes*, 98, 1823–1832.
- Ribeiro, F., Collares-Pereira, M. J., & Moyle, P. B. (2009). Non-native fish in the fresh waters of Portugal, Azores and Madeira Islands: A growing threat to aquatic biodiversity. *Fisheries Management and Ecology*, 16, 255–264.
- Robbins, W. H., & MacCrimmon, H. R. (1974). *The black bass in America and overseas*. Sault Sainte Marie, Canada: Biomanagement and Research Enterprises.
- Rogers, M. W., Allen, M. S., & Porak, W. F. (2006). Separating genetic and environmental influences on temporal spawning distributions of largemouth bass (*Micropterus salmoides*). *Canadian Journal of Fisheries and Aquatic Sciences*, 63, 2391–2399.
- Rozas, J., Sánchez-DelBarrio, J. C., Messeguer, X., & Rozas, R. (2003). DnaSP, DNA polymorphism analyses by the coalescent and other methods. *Bioinformatics*, 19, 2496–2497.
- Schulz, U. H., & Leal, M. E. (2005). Growth and mortality of black bass, *Micropterus salmoides* (Pisces, Centrarchidae; Lacapède, 1802) in a reservoir in southern Brazil. *Brazilian Journal of Biology*, 65, 363–369.
- Taylor, G. C., Weyl, O. L. F., Cowley, P. D., & Allen, M. S. (2015). Dispersal and population-level mortality of *Micropterus salmoides* associated with catch and release tournament angling in a South African reservoir. *Fisheries Research*, 162, 37–42.
- Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin, F., & Higgins, D. G. (1997). The CLUSTAL X Windows interface: Flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Research*, 25, 4876–4882.
- Villesen, P. (2007). FaBox: An online toolbox for fasta sequences. *Molecular Ecology Notes*, 7, 965–968.
- Vitule, J. R. S., Bornatowski, H., Freire, C. A., & Abilhoa, V. (2014). Extralimital introductions of *Salminus brasiliensis* (Cuvier, 1816) (Teleostei, Characidae) for sport fishing purposes: A growing challenge for the conservation of biodiversity in neotropical aquatic ecosystems. *BioInvasions Records*, 3, 291–296. <https://doi.org/10.3391/bir.2014.3.4.11>
- Weyl, O. L. F., Schirrmann, M. K., Hargrove, J. S., Bodill, T., & Swartz, E. R. (2017). Invasion status of Florida bass *Micropterus floridanus* (Lesueur, 1822) in South Africa. *African Journal of Aquatic Science*, 42, 359–365.
- Weyl, P. S. R., De Moor, F. C., Hill, M. P., & Weyl, O. L. F. (2010). The effect of largemouth bass *Micropterus salmoides* on aquatic macro-invertebrate communities in the Wit River, Eastern Cape, South Africa. *African Journal of Aquatic Science*, 35, 273–281.
- Zhao, H., Li, C., Hargrove, J. S., Bowen, B. R., Thongda, W., Zhang, D., ... Peatman, E. (2018). SNP marker panels for parentage assignment and traceability in the Florida bass (*Micropterus floridanus*). *Aquaculture*, 485, 30–38.



## SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

**How to cite this article:** Frehse FdA, Hargrove JS, Weyl OLF, Vitule JRS. The genetic characteristics of invasive Largemouth Bass in southern Brazil. *J Appl Ichthyol.* 2019;00:1-9. <https://doi.org/10.1111/jai.13987>