# Anthropogenic river fragmentation reduces long-term viability of the migratory fish *Salminus brasiliensis* (Characiformes: Bryconidae) populations

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Life-history, geographical barriers, and damming can shape the genetic diversity of freshwater migratory fish, which are particularly vulnerable to anthropogenic impacts. We investigated the genetic diversity of *Salminus brasiliensis*, a long-distance migratory species that is recognized as an important provider of ecosystem services. We implemented microsatellite analyses to assess genetic diversity and simulate future scenarios for evaluating the long-term viability of dammed and non-dammed populations from the Uruguay River. High levels of genetic diversity were detected for all sampled populations. However, effective population sizes were lower in the uppermost river stretches, where the landscape is highly fragmented. Population structure analysis indicated two spatial genetic populations. It is suggested that this genetic structure preserves populations partially isolated by an ancient natural barrier, instead of being a result of the presence of dams. The simulated genetic scenarios indicated that genetic variability of *S. brasiliensis* populations from upstream dams could collapse over the years, mainly due to the reduction in the number of alleles. Therefore, besides helping to better understand issues related to the influence of dams on the genetic diversity of migratory fish, our results are especially relevant for driving local fishery policies and management actions for the species conservation.

Keywords: Cascade of reservoirs, Genetic diversity, Genetic population structuring, Natural barrier, River fragmentation.

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História de vida, barreiras geográficas e barramento dos rios podem moldar a diversidade genética de grandes peixes migratórios de água doce, que são particularmente vulneráveis a impactos antrópicos. Nós investigamos a diversidade genética de *Salminus brasiliensis*, uma espécie migratória de longa distância que é reconhecida como um importante provedor de serviços ecossistêmicos. Realizamos análises de microssatélites para avaliar a diversidade genética e simular cenários futuros, possibilitando estimar a viabilidade em longo prazo de populações situadas em regiões com e sem represas do rio Uruguai. Altos níveis de diversidade genética foram detectados para todas as populações amostradas. Contudo, os tamanhos populacionais efetivos foram menores nos trechos superiores do rio, onde a paisagem é altamente fragmentada. A análise da estrutura populacional indicou duas populações genéticas espaciais. Sugere-se que esta estrutura genética preserva populações parcialmente isoladas por uma antiga barreira natural, ao invés de ser resultado da presença de barragens. Os cenários genéticos simulados indicaram que a variabilidade genética das populações de *S. brasiliensis* situadas a montante das barragens entraria em colapso ao longo dos anos, principalmente como resultado da redução do número de alelos. Portanto, além de ajudar a entender melhor questões relacionadas à influência de barragens na diversidade genética de peixes migradores, nossos resultados são especialmente relevantes para a condução de políticas pesqueiras locais e ações de manejo para a conservação das espécies.

Palavras-chave: Barreira natural, Cascata de reservatórios, Diversidade genética, Estruturação genética de populações, Fragmentação de rios.

## INTRODUCTION

Large freshwater migratory fish species commonly require complex habitats to complete their life cycle (Winemiller, 2005). These species have been increasingly affected by anthropogenic impacts, mainly river damming promoted by hydropower plants (Agostinho *et al.*, 2007). Rivers formatted by a cascade of reservoirs are particularly harmful for long-distance migratory fish (Pelicice *et al.*, 2018), once they can reduce or even prevent migration during downstream-upstream movements (Khedkar *et al.*, 2014; Pelicice *et al.*, 2015). Dams can cause substantial disruption to river systems and fish communities (Dudgeon *et al.*, 2006; Vörösmarty *et al.*, 2010) by fragmenting and reducing available habitat for local populations. This scenario favors a reduction of the effective population size, an increase in the genetic drift and inbreeding, and a consequent genetic diversity reduction (Yamamoto *et al.*, 2004; Bessert, Ortí, 2008). Genetic diversity has been recognized as one of the three major levels of biodiversity (McNeely *et al.*, 1990). It constitutes a primordial material for evolutionary change over time, and it is fundamental for species adaptation and conservation (Meffe, 1990; Carroll, Fox, 2008; Allendorf *et al.*, 2013). Therefore, the species persistence depends on the ability of its populations to adapt in response to anthropogenic and habitat disturbances (Oro, 2020).

Natural populations are dynamic systems and the life-history of a given species has a great influence on both the genetic diversity level and its spatial distribution (Selander, Kaufman, 2012). Freshwater migratory fish species, for example, have been frequently associated with a panmictic population model, showing high genetic diversity distributed in a single large population (Sivasundar *et al.*, 2001; Garcez *et al.*, 2011; Aguirre-Pabón *et al.*, 2013; Ferreira *et al.*, 2017). However, this is not a general rule, and in several case studies, the genetic diversity has been shown partitioned in the structured population (*e.g.*, Hatanaka *et al.*, 2006; Sanches *et al.*, 2007). Different mechanisms, such as isolation by space associated to the presence of physical barriers (Sekine *et al.*, 2002) or homing behavior (Batista, Alves-Gomes, 2006; Carvajal-Vallejos *et al.*, 2014), and isolation by time during spawning season (Braga-Silva, Galetti Jr., 2016; Ribolli *et al.*, 2017), can lead to population structuring indeed.

Since hydropower dams can have a huge influence on long-distance migratory fish, we hypothesized that a reduction in the genetic diversity across a longitudinal down to upstream gradient along a dammed river would occur, as well as higher inbreeding rates in populations from fragmented and isolated river stretches. To test both hypotheses, we adopted as a study model the long-migratory fish *Salminus brasiliensis* (Cuvier, 1816), inhabiting the Middle and Upper Uruguay River. This rheophilic fish, known as Dourado or "river king", is an important fishery resource for the local communities of the Upper Uruguay River, where the reduction in fishing catches, probably due to human impacts, such as fragmentation, habitat reduction and overfishing, have already been reported (Schork, Zaniboni-Filho, 2017).

Overall, we used microsatellite markers to assess contemporary genetic diversity and population structure for *S. brasiliensis* populations from dammed and non-dammed river stretches. Microsatellites have been widely spread used for genetic studies, and they have already been successfully used to evaluate the impact of fragmentation on connectivity among fish populations (Valenzuela-Aguayo *et al.*, 2020). We also simulated future scenarios to evaluate the long-term viability of the species. We expect that populations located in a river stretch free of anthropic fragmentation will present higher contemporary genetic diversity and greater long-term viability in comparison with the populations from the dammed upstream river stretches.

## MATERIAL AND METHODS

**Study area.** The Uruguay River is the youngest watershed of the major La Plata basin and is mainly covered with Precambrian and Paleozoic rocks of the Brazilian Shield (Albert, Reis, 2011). This geological formation distinguishes the Upper Uruguay from the other rivers of the La Plata basin, running in a valley embedded without marginal lagoons, with a series of pools and rapids, standing out two important geographical features. The ancient Augusto César Gorge canyon (popularly known as "step ant"; Fig. 1A), situated in the upper section, was flooded by the reservoir of the Itá hydroelectric dam in 1999; and the Yucumã (or Moconá) falls, located in the upper boundary of the middle section of the Uruguay River, dropping of 12 m and forming waterfalls with approximately 1800 m long, the widest in South America (Fig. 1B) (Zaniboni-Filho, Schulz, 2003).

The geological formation of the Upper Uruguay River has high hydroelectric potential, and currently, the main river course is fragmented by four large hydroelectric dams: Barra Grande (completed in 2005), Machadinho (completed in 2002), Itá (completed in 2000), and Foz do Chapecó (completed in 2010), while Middle Uruguay still presents dam-free stretches of the river (Fig. 1).

**Sampling.** We collected 108 adult individuals of *S. brasiliensis* at five distinct areas (Fig. 1): downstream Barra Grande dam and upstream Machadinho dam (Pop1 – sampled between 2003–2012, n = 12); upstream Itá dam and downstream Machadinho dam (Pop2 - 2006–2011, n = 29); downstream Itá and upstream Foz do Chapecó dam (Pop3 - 2010–2011, n = 23); downstream Foz do Chapecó and upstream Yucumã falls (Pop4 - 2010–2011, n = 25); and downstream Yucumã falls (Pop5 - 2010–2011, n = 19). In each sampling year, fish samples were collected throughout the year (not considering reproductive season or floods and droughts periods). Sampling was mostly conducted using hooks and long lines. The fish were not anesthetized for collection in the field because after removing the fin fragment, the individuals were immediately released into the river. Few individuals were caught using gill nets and were discarded when dead or kept as voucher. Voucher of *S. brasiliensis* from the Upper Uruguay River was deposited in the fish collection of Museu de Zoologia da Universidade Estadual de Londrina, Brazil (MZUEL 15570). Fragments of the caudal fin were removed, identified, and preserved in 95% ethanol.
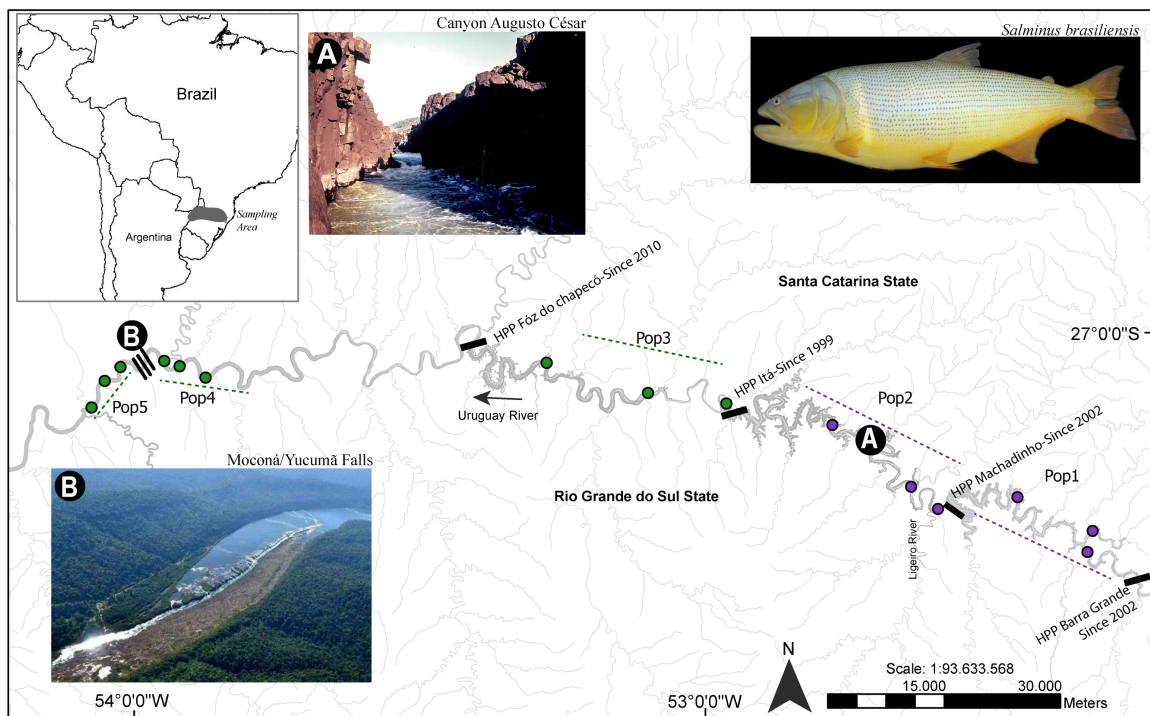


**FIGURE 1** | Study area of *Salminus brasiliensis* populations in the Uruguay River basin, southern Brazil. **A**. Picture of Canyon Agusto César Gorge, Upper Uruguay River (acquired rights); **B**. Picture of Yucumã our Moconá Falls (google font: https://7mar.com.ar/mocona). *Salminus brasiliensis* (personal picture).

**DNA isolation and microsatellite amplification.** Total DNA was obtained from caudal fin clips following a salt extraction method (Aljanabi, Martinez, 1997). Polymerase chain reactions (PCR) were performed using eleven polymorphic microsatellite loci [Sfra02, Sfra10, Sfra13, Sfra03, Sfra04, Sfra18, Sfra14, Sfra05, Sfra15 (Rossini *et al.,* 2011), Sh05 (Silva, Hilsdorf, 2011), and BoM2 (Barroso *et al.*, 2003)]. PCRs were carried out following the M13-labelled primer protocol (Schuelke, 2000), in 10 μL mixes containing 30 ng of template DNA, 1X of *GoTaq® Colorless Master Mix 2X (*Promega); 8 pmol of M13 primer with FAM, TET or HEX label; 8 pmol of forward primer and 2 pmol of reverse primer (this latter with an M13 sequence tail). PCR conditions used 5 min at 94°C, followed by 35 cycles of 45 sec at 95°C, 45 sec at the original primer annealing temperature (Barroso *et al.*, 2003; Rossini *et al.*, 2011; Silva, Hilsdorf, 2011), and 45 sec at 72°C; 10 cycles of 30 sec at 94°C, 45 sec at 53°C; and a final cycle of 10 min at 72°C. Genotyping was carried out in a MegaBace 1000 automatic sequencer (GE Healthcare Life Science), and alleles were scored using Fragment Profiler Software Suite v1.2 (GE Healthcare Life Science) with ET-ROX 550-R (size standard between 50 and 550 bp).

**Genetic diversity analysis.** All genetic diversity analyses were carried out within and between the five sampled populations (Pop1, Pop2, Pop3, Pop4, and Pop5), representing the scenario of river stretches as population groups. By creating pooled samples by river stretch, our aim was to assess the genetic diversity of populations from dammed riverscape without connections, which would allow gene flow events. Null alleles, allelic dropout, and stuttering were investigated using Micro-Checker v2.3 (Van Oosterhout *et al.*, 2004). The number of alleles per locus (*A*), expected (*He*), and observed (*Ho*) heterozygosity, effective number of alleles (*Ae*), and deviations from Hardy-Weinberg equilibrium (*HWE*) were calculated in GenAlex v6.5 (Peakall, Smouse, 2012), adjusting for significance with sequential Bonferroni corrections (Rice, 1989). Linkage disequilibrium (*LD*) between pairs of loci was computed in Genepop v1.2 (Raymond, Rousset, 1995). Inbreeding coefficient ($F_{IS}$) (Weir, Cockerham, 1984) and allelic richness (*Ar*; Leberg, 2002) were performed in FSTAT v2.9 (Goudet, 2001). The linkage disequilibrium method and parametric confidence intervals based on the chi-square approximation (Waples, 2006) were implemented in the analysis with a minimum allele frequency of 0.05 and without frequency restriction (data not shown).

The contemporary effective population size (*Ne*) for each population was estimated from data on Linkage Disequilibrium (*LD*) using the NeEstimator 1.31 software (Do *et al.*, 2014). Considering that *Ne* can be underestimated when sample size is small and/or uneven (Waples, 2006; Peel *et al.*, 2013), we standardized the sample size up to 30 individuals for all populations, using the Hybridlab software (Nielsen *et al.*, 2006). This software creates new individuals (genotypes) randomly based on the allele frequencies within each sampled population, and it has been used to simulate new individuals for conservation management (Castilho *et al.*, 2012; Yokogawa *et al.*, 2013).

In addition, we simulated the effects of hypothetical population bottleneck scenarios on the genetic diversity in the future using the BOTTLESIM 2.6 software (Kuo, Janzen, 2003). Selection, migration, and mutation are not included in the simulation model (Kuo, Janzen, 2003). The only evolutionary force considered is genetic drift, an appropriate assumption for populations located between dams or natural barriers. Despite this limitation, the method has been used as a conservation approach for the

most diverse taxa (Lippé *et al.*, 2006; Moraes *et al.*, 2017; Reid-Anderson *et al.*, 2019; Dai *et al.*, 2020). We estimated the genetic diversity through the estimated effective number of alleles and estimated expected heterozygosity of *S. brasiliensis* for the next 100 years. Using both the allelic frequency data and the empirical population size (*Ne*), we ran simulations with 100%, 75% and 50% of the empirical population size retained.

The scenarios used the following parameters: completely overlapping generations (100%); reproductive system (dioecious); expected longevity of the organism (11 years; Winemiller, Rose, 1992); age of reproductive maturation (two years; Barbieri *et al.*, 2000); empirical population size (using a standardized sample size N = 30 by simulating new individuals for each population; Pop1 = 160, Pop2 = 108, Pop3 = 740, Pop4 = 260, Pop5 = 526; Tab. 1); time frame to simulate (100 years); and the number of iterations (1,000).

**Population structure analysis.** Spatial population genetic structure of *S. brasiliensis* was investigated using the Bayesian clustering STRUCTURE v.2.3.3 software (Pritchard *et al.*, 2000; Falush *et al.*, 2003). The admixture model with correlated allele frequencies was used without specifying sampling locations. The model was run with the likely number of clusters (K) ranging from 1 to 9, in a total of ten repetitions, using a burn-in period of 200,000 iterations followed by 600,000 Markov Chain Monte Carlo (MCMC) iterations. The optimal value of K was selected following Evanno *et al.* (2005), and the STRUCTURE bar plot was visualized by using the webapp (http://pophelper.com) POPHELPER.

Genetic differentiation was assessed by the $F_{ST}$ index (Weir, Cockerham, 1984) between pairs of sampled populations using Arlequin v3.11 (Excoffier *et al.*, 2005) and sequential Bonferroni corrections (Rice, 1989). We calculated the $F_{ST}$ index between populations sampled upstream and downstream the Augusto César Gorge canyon, as well between upstream and downstream the Yucumã waterfall, for investigating the influence of these geographical barriers on the genetic structure. Analysis of Molecular Variance (AMOVA), also implemented in Arlequin v3.11, was conducted to check the existence of the partition of variance among groups and populations.

Population structuring between sampled populations was also assessed by Discriminant Analysis of Principal Components (DAPC), a multivariate method designed to identify and describe clusters of genetically related individuals. This tool allows the identification of genetic clusters and gives us a graphical representation of between-group structures to disentangle complex population structures, which may not be evident in other methods (Jombart *et al.*, 2010). The DAPC analysis was performed using the adegenet package (Jombart *et al.*, 2008), implemented in the R software (R Development Core Team, 2017).

## RESULTS

**Genetic diversity analysis.** All 11 microsatellite loci were highly polymorphic with a mean number of alleles varying from 9.3 (Pop1) to 14.4 (Pop2 and Pop3) (Tab. 1). Mean *Ho* ranged from 0.754 (Pop1) to 0.896 (Pop3), while *He* ranged from 0.817 (Pop1) to 0.848 (Pop2), effective number of alleles from 6.1 (Pop1) to 9.5 (Pop2), and

allele richness ranged from 8.1 (Pop 1 and Pop4) to 8.3 (Pop2, Pop3 and Pop5). No linkage disequilibrium among loci within populations was detected (data not shown). Pop1 and Pop2 showed positive values of inbreeding coefficient ($F_{IS}$, 0.078 and 0.198, respectively), indicating a homozygous excess in these populations located upstream of the stretch studied; distinctly to the populations situated downstream (Pop3, Pop4, and Pop5), that exhibited negative $F_{IS}$ values (–0.075 to –0.030). Initially Pop1 showed negative $Ne$ values due to the small sample size (data not shown); however, after standardizing all sample size (N = 30) using the Hybridlab software, the $Ne$ ranged from 108.1 to 740.6 (Tab. 1). The infinite values were observed in the upper interval in Pop3, Pop4 and Pop 5, probably due to very large $Ne$ (Waples, Do, 2010).

The simulated scenarios of population bottleneck (75 and 50% of the current population size; Fig. 2) showed loss of genetic diversity ($Ae$) in the population located in the uppermost portion of the dam cascade system (Pop1 and Pop2). Of note, the $He$ retained did not reach values below 80% of the current genetic diversity (represented by 100%). The $Ae$ reduction was faster than $He$ and, in the worst scenario (Pop2 with 50% of $Ne$ reduction), the $Ae$ retained reached in 100 years values smaller than 60% of the current genetic diversity. We observed an intense decline of genetic diversity in the sampled populations located in the uppermost portion of the cascade systems (Pop1 and Pop2), and Pop4.

**Population structure analysis.** Bayesian clustering analysis without prior information separated the group of individuals in two genetic clusters (*K* = 2; **S1**); one formed by individuals from Pop1 and Pop2, and the other composed by Pop3, Pop4 and Pop5 (Fig. 3A). This same population structuring pattern with these two principal genetic clusters was also detected by the DAPC analysis (Figs. 3B,C). AMOVA analysis revealed that 97.2 % of the total genetic diversity occurred within individuals, while 1.90 % of the total variation occurred among sampled populations. We detected an identical divergence among sampled populations, with significance $F_{ST}$ values between Pop1 *vs*. Pop3, Pop4 and Pop5, and Pop2 *vs*. Pop3, Pop4 and Pop5 (Tab. 2). The pairwise divergence between individuals from Pop1+Pop2 *vs*. Pop3+Pop4+Pop5 showed significant genetic differentiation ($F_{ST}$ = 0.030, P < 0.05).

**TABLE 1 |** Summary of genetic diversity estimates in *Salminus brasiliensis* based on eleven microsatellite loci. *N*: samples size; *A*: mean number of alleles; *Ae*: No. of effective alleles; *Ar*: allelic richness; *Ho*: observed heterozygosity; *He*: expected heterozygosity; $F_{IS}$: population inbreeding coefficient; *Ne*: effective population size (using a standardized sample size (N = 30) by simulating new individuals for each specific population); Inf: infinite.

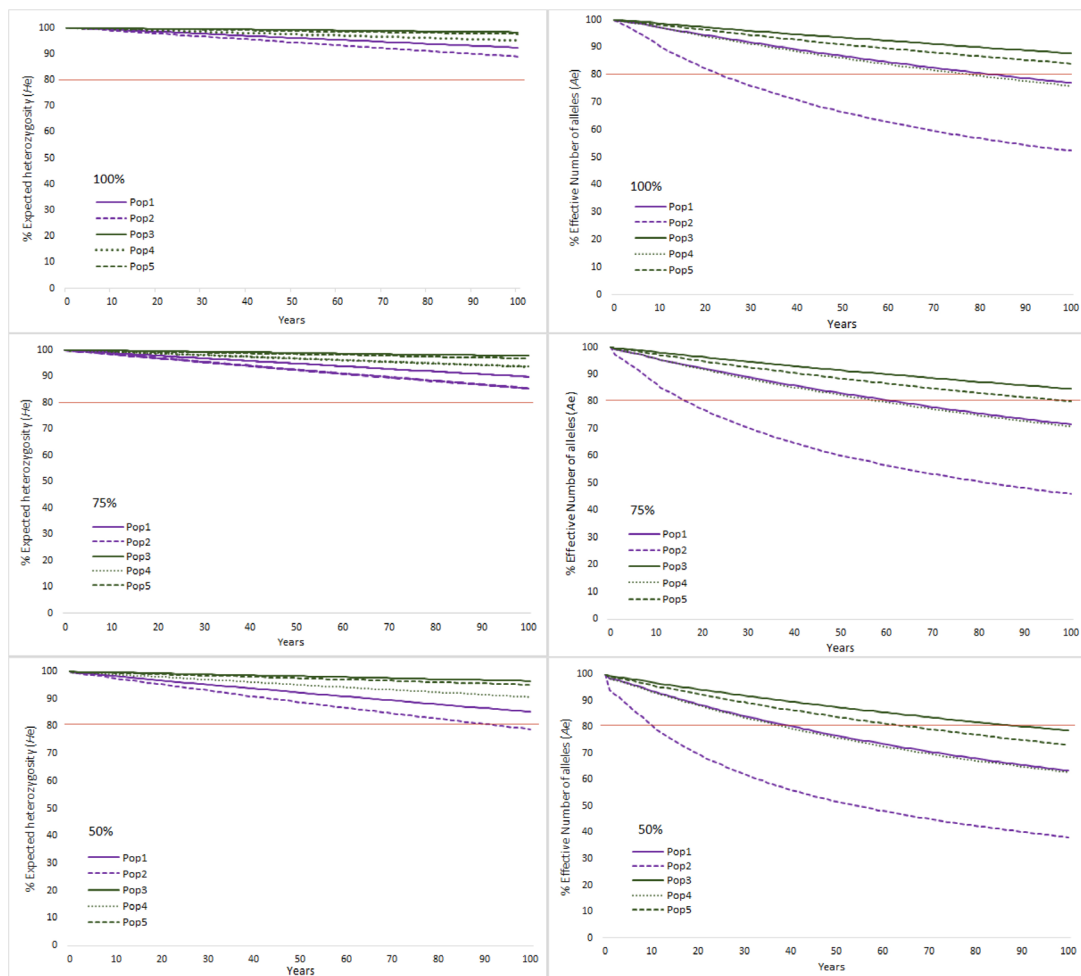| Sampled population | *N* | *A* | *Ae* | *Ar* | *Ho* | *He* | $F_{IS}$ | *Ne* (range; 95% CIs) |
|---|---|---|---|---|---|---|---|---|
| Pop1 | 12 | 9.3 | 6.1 | 8.1 | 0.754 | 0.817 | 0.078 | 160.5 (68.3-789.1) |
| Pop2 | 29 | 14.4 | 9.5 | 8.3 | 0.799 | 0.848 | 0.052 | 108.1 (54.5-847.1) |
| Pop3 | 25 | 14.4 | 8.6 | 8.3 | 0.896 | 0.832 | -0.075 | 740.6 (322-inf.) |
| Pop4 | 25 | 13.5 | 7.8 | 8.1 | 0.863 | 0.834 | -0.030 | 260.9 (81.8-inf.) |
| Pop5 | 19 | 12.8 | 8.3 | 8.3 | 0.888 | 0.830 | -0.075 | 526.8 (275.6-inf.) |

FIGURE 2 | Predicted genetic diversity in *Salminus brasiliensis* populations with distinct fragmentation levels over the next 100 years. Using BOTTLESIM 2.6, we estimated the retained percentage of effective number of alleles (*Ae*) and expected heterozygosity (*He*) under 100%, 75% and 50% of retain bottleneck scenarios. In all populations, the current population size is unable to maintain 80% of current genetic diveristy, which it is indicated by a red line.

TABLE 2 | **Pairwise genetic distance**, $F_{ST}$ in populations of *Salminus brasiliensis* based on eleven microsatellites. Values in bold indicate significant differences.

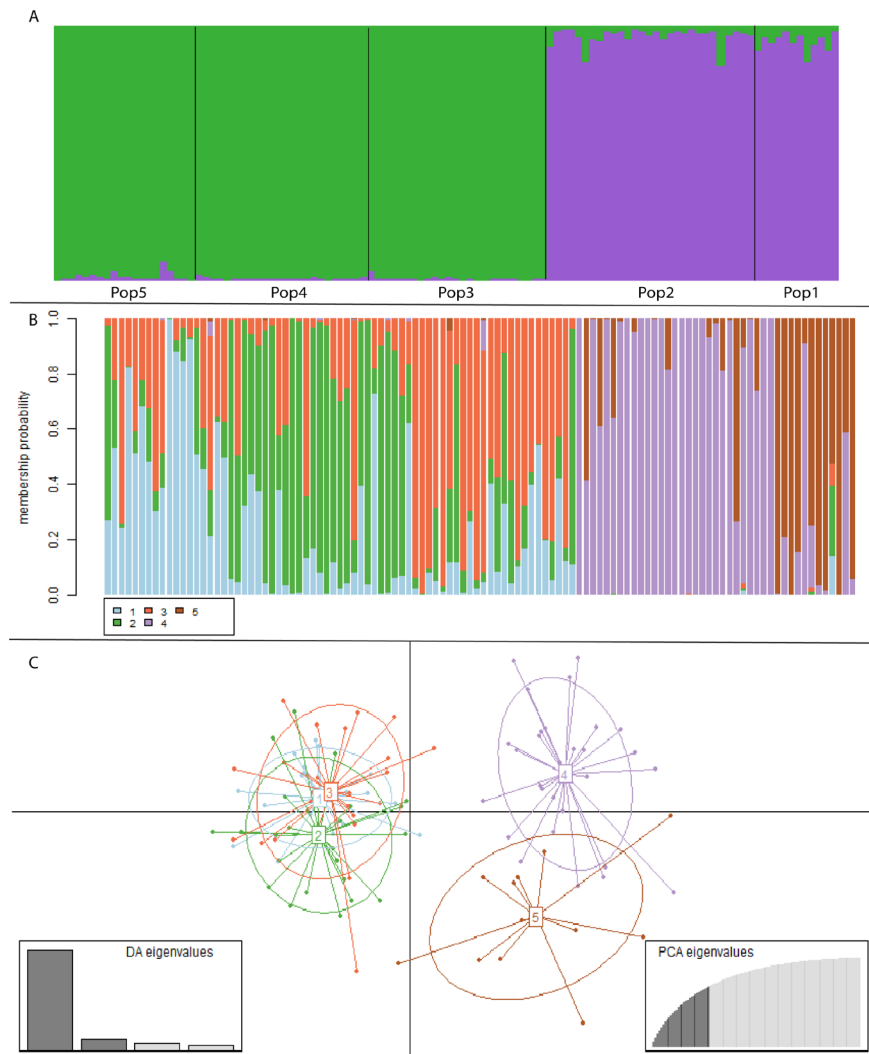| Sampled population | Sampled population | $F_{ST}$ | *p*-value |
|---|---|---|---|
| Pop1 | Pop2 | 0.000 | 0.410 |
| | Pop3 | **0.028** | 0.005 |
| | Pop4 | **0.028** | 0.005 |
| | Pop5 | **0.028** | 0.005 |
| Pop2 | Pop3 | **0.030** | 0.005 |
| | Pop4 | **0.036** | 0.005 |
| | Pop5 | **0.029** | 0.005 |
| Pop3 | Pop4 | 0.002 | 0.060 |
| | Pop5 | 0.001 | 0.225 |
| Pop4 | Pop5 | 0.002 | 0.225 |
| (Pop1 + Pop2) | (Pop3 + Pop4 + Pop5) | **0.030** | 0.005 |

FIGURE 3 | **A.** *Salminus brasiliensis* population structure from the Bayesian cluster analysis for *K* = 2 (see also **S1**). Black lines separate the different sampled populations based on location (Pop1, Pop2, Pop3, Pop4 and Pop5; Fig. 1). **B.** DAPC scatterplots and membership probabilities show the first two principal components of the DAPC. Populations are represented in different colors: 1 - Lilac (Pop5); 2 - Green (Pop4), 3 - Orange (Pop3), 4 - Lilac (Pop2) and 5 - Brown (Pop1). **C.** Membership probabilities (in bar plots), represent individuals in different clusters.

## DISCUSSION

Our results suggest that genetic diversity loss is quite inevitable in *S. brasiliensis* inhabiting the Uruguay River, and that it may compromise the viability of the fragmented populations isolated by dams in the uppermost portion of this hydrographic system in 100 years. It is believed that when conservation of endangered wild population is focused, the goal is retaining at least 80–90% of the initial genetic diversity over 100 years (Grueber, Jamieson, 2008; Corti *et al*., 2011; Shivaprakash *et al*., 2014). Our simulated scenarios of population bottleneck showed that lesser values of genetic diversity (*Ae*) will be reached in the populations located in the uppermost portion of the dam cascade system (Pop1 and Pop2). Although the *He* retained in 100 years was always above 80%

of the current genetic diversity (represented by 100%), these results do not represent a signal of population persistence in future scenario, since after a population bottleneck *He* decays slower than *Ae* (Frankel, Soulé, 1981; Allendorf *et al.*, 2013).

It is already recognized that anthropic fragmentation limits gene flow between populations and decreases population size. As a result of this population bottleneck, genetic diversity decreases, compromising the population viability (Frankham *et al.*, 2010). While the river fragmentation by damming constitutes a major threat to global freshwater species diversity in the world (Dudgeon *et al.*, 2006; Arthington *et al.*, 2010; Vörösmarty *et al.*, 2010), little is known on its long-term genetic consequences within and between populations of migratory fish (Baggio *et al.*, 2018). The approach presented here allows us to infer on the genetic diversity changes over 100 years, corresponding to approximately 50 generations of *S. brasiliensis*.

This observed reduction on the genetic diversity seems to be directly connected with river fragmentation and its known consequences, *e.g.*, reducing migration routes, habitat change, reduction of living area, and reduction of effective population size and gene flow (Esguícero, Arcifa, 2010). Our results suggested that *S. brasiliensis* populations possibly will experience different levels of impacts due to the river fragmentation. Both populations (Pop1, Pop2) sampled in the upper part of the Uruguay River will possibly suffer a higher impact over time, showing lower genetic diversity and high inbreeding than the populations located more downstream. This inference is especially valid for Pop1 and Pop2, and it can be directly associated to their living area. Inhabiting the uppermost river stretch, limited by two adjacent dams and lacking large tributaries (this stretch is enclosed in a valley, and the tributaries are short and have many waterfalls; Zaniboni-Filho, Schulz, 2003; Silva *et al.*, 2012; Fig. 1), appears as the worst conditions for spawning and recruiting of new individuals. It is quite probable that our reduced sampling of Pop1 is already a consequence of this river fragmentation. This whole picture may explain the lower contemporary genetic diversity (*A* = 6.1) observed and the most unfavorable scenarios obtained in the future simulations for this population (Fig. 2).

On the other hand, the remaining populations (Pop3, Pop4, Pop5) located downstream Itá dam presented the best scenario for maintaining genetic viability in the long term, representing the healthiest populations in this study. However, the future scenario for Pop3 may be overestimated, since the presence of Foz do Chapecó dam, which started operation in 2010 (the same year of our Pop3 sampling), can restrict gene flow among downstream populations, and reduce genetic diversity of Pop3. Located in the most downstream river portion sampled, Pop5 inhabits a dam-free stretch of the Middle Uruguay River, within the Turvo State Park, and it is a stretch that still has the original characteristics of the river (Zaniboni-Filho, Schulz, 2003), with large spawning and recruiting areas for fish (Ziober *et al.*, 2015; Reynalte-Tataje *et al.*, 2020). It is the sampling area with the least anthropic impact (*e.g.*, land use, such as cities, agriculture, and livestock) of all rivers stretches studied. Populations with a larger number of individuals have higher genetic diversity and, consequently, better chances of surviving anthropic actions and environmental changes (Vrijenhoek *et al.*, 1985). The other sampled populations showed an intermediate reduction in genetic diversity in the long-term, particularly evaluated by the alleles number. Although long-term viability was simulated using the *Ne* with infinite values and in some populations that could indicate low precision and accuracy (*e.g.*, sampling errors or a low number of markers; Waples,

Do, 2010), these values can also be associated with large populations (Hare *et al.*, 2011; Waples *et al.*, 2018), and many of the theoretical models in population genetics assume an infinite population size (Allendorf, Luikart, 2009). Large populations are observed in freshwater fish (Garcez *et al.*, 2011; Souza-Shibatta *et al.*, 2018), as may be the case with these *Salminus brasiliensis* populations.

It appears that the long-term survival of *S. brasiliensis* populations in the Upper Uruguay River will largely depend on the effectiveness of protected areas in supporting viable populations, as represented by the downstream populations, which can serve as genetic stocks to help replenish upstream populations, that will have their genetic diversity reduced over the years. Previous ichthyoplankton studies within the site inhabited by Pop2 identified only three spawning events of *S. brasiliensis*, along nine years of monitoring, associated with a higher frequency of rain during spring and summer (Reynalte-Tataje *et al.*, 2012). The presence of dam-free tributaries, as the Ligeiro River, offers lotic environments that allow the spawning of migratory fish, contributing to the viability of its population (Reynalte-Tataje *et al.*, 2012).

The population genetic structure analyses revealed the occurrence of spatially structured genetic populations of *S. brasiliensis* in the upper portion of the Uruguay River basin. Individuals inhabiting upstream Itá dam (Pop1 and Pop2) were genetically differentiated ($F_{ST}$ = 0.030, P < 0.05) from those sampled downstream (Pop3, Pop4 and Pop5). Higher levels of genetic differentiation are not expected among populations of migratory fish, often showing lower values ($F_{ST}$ < 0.05; Wright, 1978) of genetic differentiation (Lopes *et al.*, 2007; Braga-Silva, Galetti Jr., 2016; Ribolli *et al.*, 2017). However, despite being the oldest hydroelectric plant along the Upper Uruguay River, Itá HPP had its gates closed in 1999, and our fish sampling occurred only seven to 12 years after the damming. It is not expected that a short period would be enough for promoting a fish population structuring due to the dam presence (Bessert, Orti, 2008). However, it is well known that the Itá dam replaced the Augusto César Gorge canyon (Fig. 1), completely flooded by the Itá reservoir. We suggest that this canyon could have represented a semi-permeable physical barrier, mainly during the dry seasons, facilitating the genetic differentiation between up and downstream populations related to the canyon. Nowadays, this semi-permeable canyon was replaced by the dam, an impassable barrier. In contrast, the Yucumã waterfall, believed to represent a temporary obstacle for fish migrating (Zaniboni-Filho, Schulz, 2003), seems not to lessen gene flow between downstream (Pop5) and upstream (Pop4) *S. brasiliensis* populations. Thus, the spatial population structuring of *S. brasiliensis* between the middle and upper portion of the Uruguay River appears to be only due to the ancient canyon's presence. A fish community study conducted in this area, before the Itá dam construction, also revealed differences in the fish communities inhabiting up and downstream this canyon (Meurer, 2010). It reinforces the idea that the ancient canyon represented a last natural barrier for the free movement of fish. Therefore, this differentiation is relevant and could be amplified over the years due to presence of both Itá and Machadinho dams, which disrupted the connectivity between downstream (Pop3, Pop4, Pop5) and upstream (Pop1, Pop2) populations, and between Pop1 and Pop2. It reinforces the use of each sampled population in simulated future scenarios.

In summary, our results show that river fragmentation by damming can have a significant impact on the population viability of the migratory fish *S. brasiliensis*. The

maintenance of viable populations of this large emblematic fish will at least partially depend on the populations living in non-anthropized areas, serving as stocks of genetic diversity that can be used for mitigation measures, such as restocking in fragmented and isolated populations. Considering the life history of *S. brasiliensis* and the fragmentation of habitat caused by the Upper Uruguay River's cascade system, the future scenario is of collapse mainly for the uppermost populations. Additionally, we highlight the importance of maintaining river stretches without dams, as observed in the Middle Uruguay River, which allows the long-term maintenance of migratory fish populations. This approach, applied for the first time for Neotropical migratory fish, can be used for rheophilic species that inhabit the large and fragmented rivers of South America, and contribute for conservation of these fishes.

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**Josiane Ribolli:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Supervision, Validation, Writing-original draft, Writing-review and editing.

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## ETHICAL STATEMENT

## COMPETING INTERESTS

The authors declare no competing interests.

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