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GENETIC STRUCTURE OF PINTADO (*PSEUDOPLATYSTOMA CORRUSCANS*) IN THE INFLUENCE AREA OF ITAIPU BINATIONAL DAM

ESTRUTURA GENÉTICA DO PINTADO (*PSEUDOPLATYSTOMA CORRUSCANS*) NA ÁREA DE INFLUÊNCIA DA BARRAGEM DA ITAIPU BINACIONAL

ESTRUCTURA GENÉTICA DEL PINTADO (*PSEUDOPLATYSTOMA CORRUSCANS*) EN LA ÁREA DE INFLUENCIA DE LA REPRESA DE ITAIPU BINACIONAL

José Alejandro Morales Gómez [1]
Luiz Henrique Garcia Pereira [2]

Resumo: Foram analisados 112 espécimes de *Pseudoplatystoma corruscans*, com marcadores microssatélites, das bacias do Alto e Baixo Paraná, para checar o impacto da fragmentação de habitat causado por usinas hidrelétricas com canais de piracema. Altos níveis de diversidade genética foram encontrados, sendo as amostras estruturadas em dois grupamentos correspondentes as duas bacias.
Palavras-chave: Diversidade genética. Fragmentação. Migração. Ictiofauna.

Abstract: We analyzed 112 samples of *Pseudoplatystoma corruscans*, with microsatellite markers from Upper and Lower Parana River Basins to check the impact of fragmentation by power plant dam with fishes pass. High genetic diversity was found in the sampled places being structured in two clusters belonging to the two basins.
Keywords: Genetic diversity. Fragmentation. Migration. Ichthyofauna.

Resumen: Fueron analizados 112 ejemplares de *Pseudoplatystoma corruscans*, con marcadores de microssatélite en las cuencas hidrográficas del Alto e Bajo Paraná, para chequear el impacto de la fragmentación de hábitat causado por las represas hidroeléctricas con escalas para peces. Fueron encontrados altos niveles de diversidad genética en los lugares muestreados, siendo estructurados en dos grupos que pertenecen a las dos cuencas previamente mencionadas.
Palabras-clave: Diversidad genética. Fragmentación. Migración. Ictiofauna.

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Introduction

Human disturbance of the aquatic environment has led to a critical decline in aquatic diversity (Dudgeon et al., 2006). Among the main causes of disturbance are overexploitation, pollution, habitat loss, introduction of alien species, and habitat fragmentation (Agostinho et al., 2005; Dudgeon et al., 2006; Carpenter et al., 2011; Gouskov et al., 2016). But for fishes, habitat fragmentation has been highlighted because aquatic organisms are restricted to their linear dendritic habitats and cannot avoid anthropogenic barriers (Agostinho et al., 2005; Vörösmarty et al., 2010). One of the main causes of river fragmentation are the dams built to produce electricity, to store water and for agricultural purposes (Agostinho et al., 2005; Nilsson et al., 2005; Lehner et al., 2011). In fact, dams, mainly to produce electricity, have been constructed in all major river systems of the world (Nilsson et al., 2005; Dugan et al., 2010; Vörösmarty et al., 2010; Liermann et al., 2012). Liermann et al. (2012) pointed out that the Neotropical region as one of the ecoregions most impacted by dams. In Brazil there are 4,467 hydroelectric power plants in operation, 203 in construction, and 610 planned, most of them in the south and southeast regions including the Paraná river Basin (Aneel, 2016). As an example, according to Liermann et al. (2012), only 43.19% of the watercourses of the Upper Paraná river basin remain unobstructed.

Habitat fragmentation can subdivide populations, interrupt the natural migratory routes and interfere directly with the levels of diversity and genetic structure of populations and species (Vrijenhoek, 1998; Keyghobadi, 2007; Esguícero; Arcifa, 2010). Low genetic diversity can reduce population viability via genetic mechanisms such as inbreeding depression, the accumulation of deleterious mutations, and the loss of adaptive potential (Frankham et al., 2010; Freeland et al., 2011). The long-term persistence of a species depends on enough genetic diversity to survive in variable or changing environments (Hughes et al., 2008; Freeland et al., 2011).

Although habitat fragmentation affects the entire aquatic fauna, it is more pronounced in migratory species due to the interruption of their natural reproductive migratory routes. This interruption interacts directly with the metapopulation dynamic, with consequences to gene flow, diversity and genetic structure (Petrere-jr, 2002; Esguícero, Arcifa, 2010; Liermann et al., 2012). In this way, some surveys have demonstrated that in fragmented rivers migratory fish

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species use the non-fragmented tributaries as alternative routes to migration and reproduction (Baumgartner et al., 2004; Sanches et al., 2006; Antonio et al., 2007; Makrakis et al., 2012; da silva et al., 2015).

In the case of Paraná river, until 1982, before the construction of the Itaipu Dam, the fish fauna of Upper Paraná River (UP) was isolated from the fish fauna of remaining portions of Paraná and Paraguay rivers (LP) by the Sete Quedas Falls (Agostinho et al., 1995; Bonetto, 1998). The reservoir of Itaipu Dam inundated an area of about 1350 km2 (Hahn et al., 2007) that included the Sete Quedas Falls (Langeani et al., 2007; Julio-junior et al., 2009). The inundation of Sete Quedas falls moved the barrier between these two basins downriver by 150 km below the Itaipu Dam, allowing the mixing of populations and species from the two regions. At present, at least 33 species initially limited to the LP portion have successfully colonized the UP portion (Julio-junior et al., 2009).

On the other hand, some actions have been taken to mitigate the impact of dams as barriers, such as the construction of fish passes. However, the effectiveness of these systems is contested in many surveys that point to its faulty design which, only allows for the passage of a few species, with low efficiency. Additionally, most fish passages on dams only allow upstream movement (Noonam et al., 2002; Agostinho et al., 2002; 2007a,b,c; Pelicice; Agostinho, 2008).

With the advance of molecular techniques and analytical methods, a set of tools is now available, which allows for comparison between historical and recent events that shape the genetic structure of populations. The employment of these tools allows for the identification of changes during the life history of the species and populations, including recent human disturbances (Lange et al., 2010; Putman; Carbone, 2014; Ishiyama et al., 2015).

In these context, we analysed a large migratory catfish, *Pseudoplatystoma corruscans* Spix & Agassiz, 1829 (Pimelodidae: Siluriformes), previously documented having structured populations (Pereira et al., 2009; Abreu et al., 2011), to assess the consequences of barriers created by dams in the natural migratory fish species populations. Therefore, using microsatellites markers, we assess the diversity and genetic structure of three populations of *P. corruscans* in the Paraná River basin, which are, nowadays, isolated from each other at least by one Dam. We compare historical and recent migration rates among populations to identify

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changes in the genetic history of this species and relate them to human disturbance (Dam barriers) in the natural hydrographic environment.

Materials and Methods

P. corruscans, study area and sample collection.

P. corruscans is known to make long-distance migrations for reproduction (upstream migration – between October to December) and for feeding (downstream migration – between January to September) (Barthem; Goulding, 1997; Agostinho et al., 2003; Carolsfeld et al., 2003; Resende, 2003). Like many other large-sized pimelodid species, *P. corruscans* is important because of their high commercial value and ecological role as voracious predators (Bayley; Petrere, 1989; Barthem; Goulding, 1997; Petrere et al., 2002; Carolsfeld et al., 2003). In a previous study, significant genetic structure was detected among six *P. corruscans* populations in the Paraná river basin was detected that pointed to a possible homing behavior (Pereira et al., 2009).

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For this study, we analyzed 112 *P. corruscans* specimens from three locations in the Paraná River Basin that are separated by hydroelectric power plants. Two sampling location, Paranapanema river (UP-1) (53 specimens) and Ivinhema river (UP-2) (19 specimens) are from the Upper Paraná basin (UP), the other, Paraná river (LP) (40 specimens) is located in the Lower Paraná-Paraguay basin (LP) (Figure. 1). The UP-1 population are isolated from the UP-2 and LP populations by six and seven different hydroelectric power plants, respectively, in which, four of them do not present fish passes (Figure. 1).

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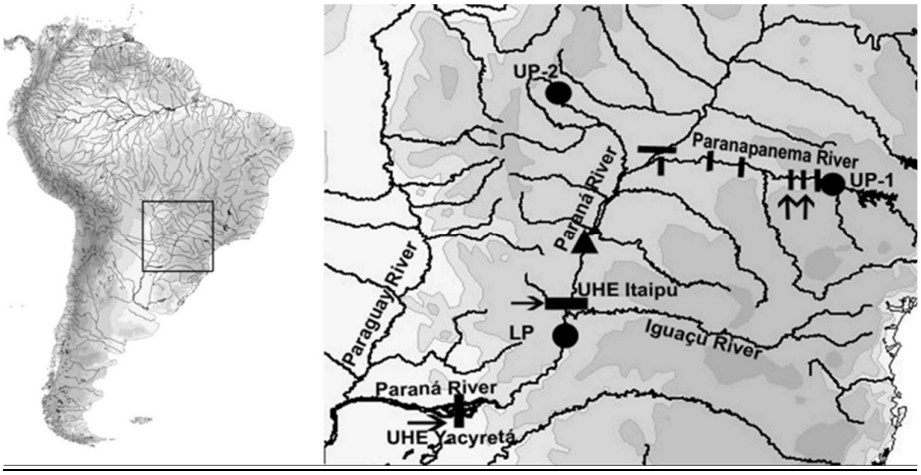
The UP-2 population is isolated from LP population by one hydroelectric power plant that present fish passes (Figure. 1). Two samples (UP-1 and UP-2) were collected in tributaries of the Paraná river during spawning season (between October and January of 2004-2005 years) and one (LP) was collected in the Paraná river in February of 2005, just at the beginning of the feeding season. Fin clips were preserved in ethanol absolute until DNA extraction and in the tissue collection of the Laboratório de Biologia e Genética de Peixes (LBP) at Universidade Estadual Paulista (UNESP), São Paulo State, Brazil.

Figure 1 - Local of samples.



Map showing the points of collection of the three *P. corruscans* populations analyzed from the Paraná River Basin, Brazil. Circles = samples location; lines = power plant dams; arrows = the power plant dams with fish pass. Fonte: Pereira et al., 2009, modificado.

DNA extraction and microsatellite genotyping.

To extract genomic DNA, about 0.1 mg of tissue was incubated in 200 µl of 5% Chelex at 65°C overnight. The samples were screened for variation at each of seven microsatellite loci (Pcor01, Pcor02, Pcor05, Pcor10, Pcor21 (Revaldaves et al., 2005), Pcor23 and Pcor28 (Pereira et al., 2009)). PCR amplification reactions were conducted with a final volume of 12.5 µl with about 10 ng of DNA, 0.25 µM of each primer, 0.2 mM of dNTP, 1.2 mM of $MgCl_2$, 0.2 U of Taq-Pht DNA polymerase, 1X PCR buffer (50 mM KCl, 10 mM Tris–HCl, 0.1% Triton X-100,

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and 1.5 mM MgCl2) and water. We used the following PCR profile for the loci Pcor01, Pcor02, Pcor05, Pcor21, Pcor23 and Pcor28: initial denaturation at 95 ºC for 5 min, followed by 30 cycles of 10 s at 95 °C, 15s at annealing temperature of 55 °C, and 15 s at 72 °C, with a final extension at 72 °C for 10 min. For the locus Pcor10, the PCR profile consisted of an initial denaturation at 95 °C for 5 min, followed by 30 cycles of 30 s at 95 °C, 30 s at the annealing temperature of 48 °C, and 30 s at 72 °C, with a final extension at 72 °C for 10 min. Amplified products were resolved in 6% polyacrylamide gels stained with silver nitrate. To ensure the best resolution on the polyacrylamide gels, the electrophoresis was performed in a 30 cm gel run at 150 volts for 8-10 hours to allow a good separation of the alleles, which differ in two bases. Microsatellite alleles were identified by their size by base pairs numbers. Allele lengths were determined by reference to a 10 bp ladder (10 bp DNA Ladder – Invitrogen, Carlsbad, CA) using the software Kodak digital science 1D. To minimize the screening error of the microsatellites, all electrophoresis runs were carried out with three lanes of ladder, one in the centre of the gel and one on the either sides. Additionally, in each gel we ran at least one sample with known allele size which had been previously sequenced to confirm size.

Genetic variation analysis

Allelic count, privative alleles, expected and observed heterozygosity (HE, HO), and deviation from Hardy-Weinberg equilibrium (HWE) were obtained with GenAlEx 6.502 (Peakall; Smouse, 2006; 2012). The frequency of null alleles was calculated according to Dempster et al. (1977) with the FreeNA software (Chapius; Estoup, 2007).

Population differentiation analysis

Initially, we used the Bayesian assignment method as implemented in STRUCTURE 2.3.4 (Pritchard et al., 2000) to find the most probable number of genetic cluster among all samples. The method clusters individuals into populations using multilocus genotype information by minimizing the Hardy–Weinberg and linkage disequilibrium that would result if individuals from different, randomly mating populations, were incorrectly grouped into a common population. To determine the number of genetic clusters (K) in STRUCTURE, we explored different values of K ranging from K = 1 to K = 5. Ten independent runs for each K

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value were performed with a Markov chain Monte Carlo (MCMC) of 106 iterations following a burn-in of 105 iterations, assuming admixture model without any prior information and correlated allele frequencies. We used the ΔK method for inferring the K value that best fitted our genetic data (Evanno et al., 2005) with STRUCTURE HARVESTER (Earl, 2012). STRUCTURE runs were averaged for each value of K using the 'greedy algorithm' in CLUMPP (Jakobsson; Roenerg, 2007), and the resulting Q matrices were graphically visualized using DISTRUCT (Rosenberg, 2004), representing the membership of individuals assigned to each genetic cluster. To check the presence of some substructure in the initial genetic clusters revealed by the first round of the structure analysis, we conducted new runs, with the same parameters, separately on each genetic cluster obtained.

We also obtained the global and the pair-wise FST index using the ENA method implemented in FreeNA (FSTENA) software (Chapius; Estoup, 2007), which corrects for the presence of null alleles as a measure of genetic structure. We applied a Mantel test to verify the correlation of pairwise multilocus FST index and geographical distances using the programme isolde supplied with the Genepop 4.2 package (Raymond; Rousset, 1995; Rousset, 2008). Their significance was tested with 10,000 permutations. The distance among sample locations were obtained using a scaled map measuring the distances by rivers canals.

In addition, we used the GENECLASS2 software (Piry et al., 2004) that uses a Bayesian approach to assign individuals to their population of origin.

Historic and contemporary migration rate estimation

We used the Migrate-n software v.3.6.11 (Beerli, 2008) to assess the historical migration rate for approximately 4Ne generations in the past. MIGRATE-n estimates the mutation-scaled migration rate M (m/μ) and the effective population size θ (4Neμ) based on the maximum-likelihood approach using the coalescent theory and MCMC (Beerli, 2008). Following the directions of the author, the MIGRATE-n was run two times. The first run used an estimate of FST as a starting parameter to calculate θ and M values, which were used in the second run as new starting parameters. Both runs were conducted with 10 short chains of 103 sampled, 100 recorded and three final chains of 105 sampled, 103 recorded.

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We used the program BAYESASS v.3.0.4 (Wilson; Rannala, 2003) to assess the contemporary migration rate over the last few generations (3-5 generations). BAYESASS uses a Bayesian method with MCMC to estimates the contemporary migration rate. Initially, we conducted exploratory runs to adjust the delta values (a (allele frequency), f (inbreeding coefficient) and, m (migration rate)) to ensure that 20–60% of the total changes were accepted.

Next, we ran the software using 107 iterations with a burn-in of 106 iterations and a sampling frequency of 100. To compare historical and contemporary migration rates, we followed the protocol proposed by Gibbs, Chiucchi (2010), which used the values of m directly generated by BAYESASS and estimated m from values of M (m/μ) generated by MIGRATE-n by multiplying all M values by a conservatively estimated microsatellite mutation rate (μ) of 5.10-4, which is considered to be the average mutation rate in many species (Yue et al., 2007; Ishiyama, 2015).

Results

Genetic variation

A total of 78 alleles were observed with numbers of alleles per locus ranging from five (Pcor01 and Pcor28) to 19 (Pcor10) with an average of 11.14 alleles per locus (as shown in the table 1, available only in the online version). The mean observed and expected heterozygosities were 0.54 ± 0.05 and 0.66 ± 0.04, respectively. Null allele frequencies calculated by FreeNA ranged from 0.0 to 32.6%. The highest values were observed for the loci Pcor10 in all populations. Thus, we ran subsequent analyses with and without Pcor10, but no substantial differences were observed (data not shown). Besides this, the global FST remained unchanged after applying the ENA correction method with all seven loci; hence, all loci were kept for subsequent analyses. Significant departures from HWE ($P<0.0071$, adjusted according to Bonferroni correction, $K=7$) were detected at the population level for 11 comparisons. However, no loci showed consistent deviations across all populations, except for locus Pcor10, probably due to the high frequency of null alleles observed (as shown in the table 1).

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Table 1 - Summary of microsatellite data for each population of *P. corruscans* analyzed.

Pop		Loc01	Loc02	Loc05	Loc10	Loc21	Loc23	Loc28
UP-1	N	53	51	52	44	51	47	50
	Na	4	10	9	8	11	10	6
	Ho	0,377	0,686	0,673	0,295	0,922	0,702	0,380
	He	0,489	0,833	0,768	0,455	0,814	0,761	0,330
	HWE	0,537	0,501	0,000*	0,000*	0,000*	0,000*	1,000
	R	0.0629	0.0674	0.0560	0.1381	0.0000	0.0375	0.0000
UP-2	N	19	19	19	19	19	19	19
	Na	3	7	6	7	5	6	4
	Ho	0,579	0,789	0,632	0,526	0,632	0,526	0,421
	He	0,525	0,749	0,615	0,758	0,748	0,741	0,586
	HWE	0,745	0,588	0,978	0,000*	0,007	0,023	0,001*
	R	0.0000	0.0139	0.0000	0.1255	0.0632	0.1085	0.1291
LP-1	N	40	38	38	27	40	40	40
	Na	4	9	9	17	7	9	4
	Ho	0,075	0,711	0,605	0,259	0,650	0,750	0,250
	He	0,287	0,803	0,823	0,867	0,820	0,836	0,313
	HWE	0,000*	0,012	0,002*	0,000*	0,000*	0,001*	0,328
	R	0.2063	0.0605	0.1241	0.3259	0.0841	0.0400	0.0501

N, number of individuals; A, number of alleles; Ho, observed heterozygosity; He, expected heterozygosity; HWE, result of Hardy Weinberg probability test for deviation from expected Hardy-Weinberg proportions with P-value = 0.05 (adjustment Bonferroni correction P = 0.0071; K = 7), *,significant; and R, null allele frequency per loci

Population differentiation

The STRUCTURE software assigned the individuals from the three sample locations to two genetic clusters (K = 2, in a run of K = 1 to 5) corresponding to the two basins (Upper Paraná river basin and Lower Paraná-Paraguay river basin) (Figure. 2). In addition, we ran the samples of UP basin separately to examine some substructure, that result was negative (data not shown). Beside the STRUCTURE software not revealed genetic structuration between UP-1 and UP-2 sample localities, the subsequent analyses were carried out considering the two clusters revealed by STRUCTURE software (UP and LP) and considering the three sample localities separately.

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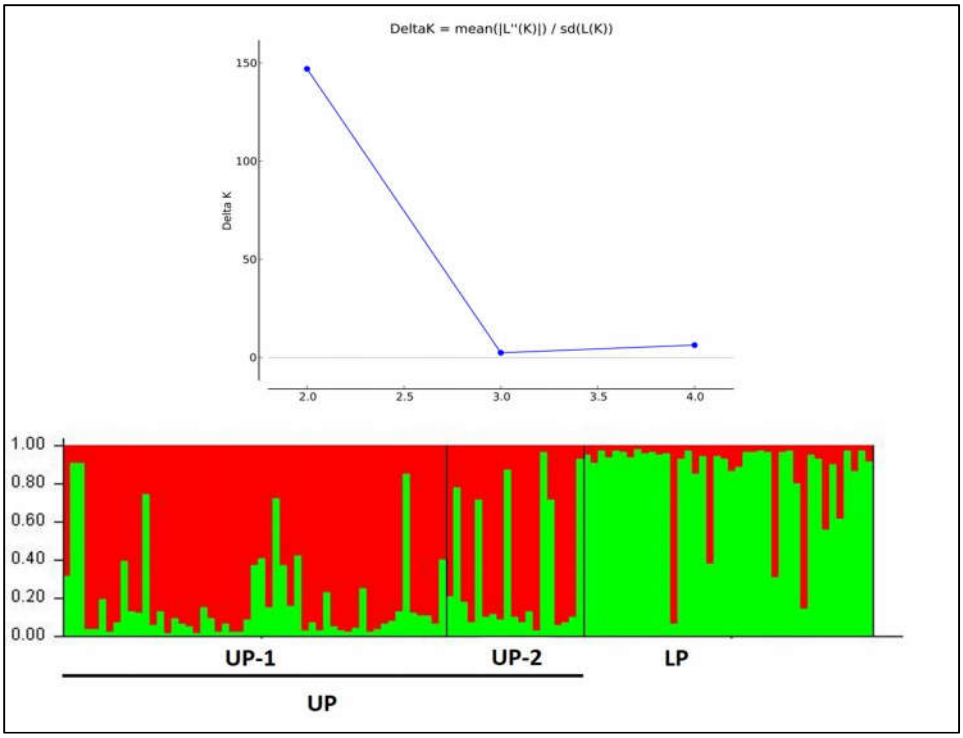
Figure 2 - Inference of the number of clusters and STRUCTURE-like plot.



STRUCTURE analyses. Above = ΔK analysis showing the best K that fitted our genetic data; below = bar plots showing the association among genetic cluster and sample locations.

The analyses were conducted in this way to try to identify the possible impacts of the river fragmentation between UP-1 and UP-2 sample localities. The pair-wise FST index of differentiation, corrected by ENA, showed moderate genetic structure among the two basins analysed (0.0867) (Table. 2A) and among the three sample locations analysed (ranging from 0.0856 between UP-2 and LP to 0.1153 between UP-1-LP) (Table. 2B) with a global FST = 0.0867 (0.0329-0.1709) and 0.1048 (0.0448-0.1745, with 95% confidence interval estimates), respectively. Individual multilocus genotypes were used to assign individuals to their population of origin using GeneClass2 software.

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Table 2 - Pair-wise FST differentiation index.

A			
	UP	LP	
UP	-	0.1023 (0.0348-0.2021)	
LP	0.0867 (0.0329-0.1709)	-	
B			
	UP-1	UP-2	LP
UP-1	-	0.1070 (0.0144-0.2184)	0.1359 (0.0366-0.2580)
UP-2	0.0971 (0.0150-0.1912)	-	0.0866 (0.0464-0.1450)
LP	0.1153 (0.0346-0.2207)	0.0856 (0.0485-0.1354)	-

(A) is considering STRUCTURE clusters and (B) is considering the three sample localities. Above diagonal = without ENA correction; below diagonal = with ENA correction. (95% confidence interval between parentheses). All values are highly significant $p<0.001$.

Considering the UP and LP basins, 104 out of 112 individuals (92.9%) were assigned correctly to it basin of origin (Table. 3A). Considering the three sample localities, 99 out of 112 individuals (88.4%) were assigned correctly to the location from which they were sampled (Table 3B). We found 16 alleles privative to UP basin, all of them at UP-1 sample (one with frequency ≥5%) and 14 alleles privative to LP basin (three with frequency ≥5%)The Mantel test showed no association between genetic divergence (FST) and geographical distances ($R2 = 0.83$; p-value = 0.46).

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Table 3 - Assignment test.

A				
Pop	UP	LP		Individuals
UP	69 (95.8%)	5 (12. 5%)		72
LP	3 (4.2%)	35 (87.5%)		40

B				
Pop	UP-1	UP-2	LP	Individuals
UP-1	49 (92.4%)	2 (10.5%)	3 (7.5%)	53
UP-2	1 (1.9%)	15 (79.0%)	2 (5.0%)	19
LP	3 (5.7%)	2 (10.5%)	35 (87.5%)	40

(A) Considering STRUCTURE clusters and (B) Considering the three samples localities.

Historic and contemporary migration rates

The historical migration rates M (m/μ) estimated by MIGRATE-n considering the two basins (UP and LP) showed values of 8.22 (7.70-8.56) from LP to UP and 13.28 (12.39-13.87) from UP to LP. The migration rate between UP-LP was asymmetrical (no overlapping 95% confidence intervals for pairwise migration) (Figure. 3a). The analysis comparing UP-1 and UP-2 samples (UP basin), the values of M ranged from 2.10 (1.91-2.31) to 3.44 (3.17-3.73) from UP-1 to UP-2 and from UP-2 to UP-1, respectively. The migration rate was asymmetrical between pairs of sample locations (Figure. 3a).

To compare historical and contemporary migration rates, we converted the migration mutation scaled values (M=m/μ) to migration rate per generation (mh) using a mean microsatellite mutation rate of μ = 5x10-4 following the protocol proposed by Gibbs, Chiucchi (2010). All comparisons showed low values for historic migration rates <1.0% (ranged from 0.1% to 0.7%) (Figure. 3a).

The θ value (4Neμ) ranged from 1.39 to 7.92 (Figure. 3a) with effective population size (Ne) ranging from 694.90 to 3960.05.The contemporary migration rate (mc) estimated at the basin scale showed values of 1.71% (1.50-1.90%) from UP to LP and of 2.54% (2.20-2.80%) from LP to UP (Figure. 3b). The comparisons between UP-1 and UP-2 sample localities showed values ranged from 1.66% (1.33-1.99%) to 5.92% (5.20-6.64%) from UP-2 to UP-1

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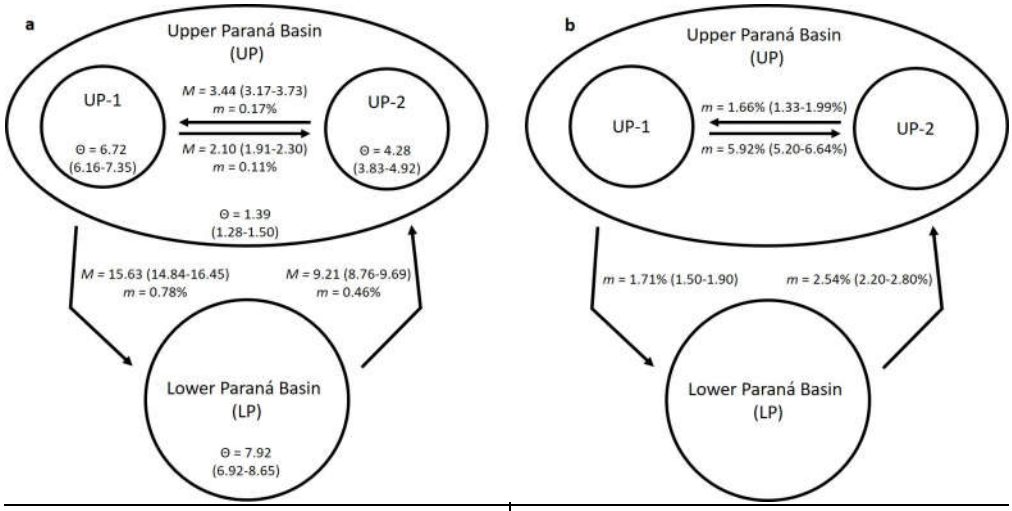
and from UP-1 to UP-2, respectively (Figure. 3b). To all comparisons, the contemporary migration rate was asymmetrical.

Figure 3 - Historical and contemporary migration among *P. corruscans* populations.



Graphics showing the historic (a) and recent (b) migration rates among samples analyzed. Arrows indicate the migration direction. M (m/μ) = mutation-scaled-migration rate; m = migration rate; θ (4Neμ) = effective population size; between parenthesis = 95% confidence interval.

Discussion

Genetic variation and population differentiation

Our results showed that, besides the current fragmentation condition of the habitat, the *P. corruscans* specimens display high levels of genetic diversity. The values of mean allele number and HE (as shown in the table 1) are consistent with the mean number observed in other fish species (Dewoody; Avise, 2000; Matsumoto; Hilsdorf, 2009; Sanches et al., 2012; Berdugo; Barandica, 2014), including other species of catfishes (SO et al., 2006; Batista et al., 2010; Telles et al., 2014).

The STRUCTURE software analysis pointed to the existence of two genetic clusters coinciding with the Upper Paraná Basin (UP) and Lower Paraná-Paraguay Basin (LP) (Figure. 2) corroborating the hypothesis that the Salto de Sete Quedas Falls was an effective barrier to migration of *P. corruscans* specimens. This hypothesis is reinforced by Godinho et al. (1991),

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which observed that siluriform species such as *P. corruscans* cannot migrate upriver thought large waterfalls. The FST differentiation index showed significant values of moderate structure between UP and LP basins, such as, among the three sample sites analyzed (Table. 2). These structure were also confirmed by the assignment test performed (Table. 3) and by the presence of a high number of private alleles. Beside the STRUCTURE software not indicate genetic structure between UP-1 and UP-2 sample localities, the FST differentiation index and the assignment test showed that there is significant structuration between them.

In a previous study, Pereira et al. (2009) found significant genetic structure among six *P. corruscans* populations in the Paraná river Basin and suggested a possible homing behavior to justify the population structure in this migratory species. Homing behavior has been reported for females of *P. corruscans* from São Francisco River Basin (Godinho et al., 2006) and, for others Neotropical migratory catfish species such as *P. reticulatum* in the Paraguay river Basin (Abreu et al. 2009) and *Brachyplatystoma rousseauxii* in the Amazonian basin (Batista; Alves-Gomes, 2006).

Dam impacts

To verify the impact of the habitat fragmentation due to a hydroelectric power plant on the connectivity and genetic structure of *P. corruscans*, we compared the historical and contemporary migration rates. MIGRATE-n and BAYESASS estimate migration rates on a historical (approx. 4Ne generations in the past) and contemporary (the past two-five generations) timescale, respectively. The historical migration rates considering both basin and inside of UP basin were <1%, revealing a restricted gene flow among all sample locations (Figure. 3a). These results agree with the genetic structure revealed by STRUCTURE and the FST index analysis showing that, even if a minimal gene flow exists, it is not enough to homogenize the populations. The absent or low migration rate between the two basins (UP and LP) was expected due to the effectiveness of the physical barrier, the Sete Quedas Falls, between these two basins (Agostinho et al., 1995; Bonetto, 1998). On the other hand, the absent or low gene flow found between sample locaties of UP basin can be a result by the possible existence of homing behavior pointed to this species (Godinho et al., 2006; Pereira et al., 2009).

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The contemporary migration rate considering the basin scale was asymmetrical with values of 1.71% (1.50-1.90%) from UP to LP and of 2.54% (2.20-2.80%) from LP to UP (Figure. 3b). In general, the gene flow between both basins remains low (<2.6%) but has increased to about 3.8-4.2 times higher than the historical migration rate. Itaipu hydroelectric power plant was constructed in 1982, and it reservoir inundated an area of about 1350 km2 (Hahn et al., 2007) that included the Sete Quedas Falls (Langeani et al., 2007; Julio-Junior et al., 2009). The inundation of Sete Quedas falls moved the barrier between these two basins to 150 km below the Itaipu Dam, allowing for mixing of the populations and species from the two previously isolated basins. Thus, this mixing could be the cause of the increase in the contemporary migration rate found between the two basins.

On the other hand, the Itaipu Dam has a fish pass, which started to work in 2002, with an aim to allow the upriver movement of the migratory fishes (Makrakis et al., 2007). Considering that our samples were collected between 2003-2005, we could have obtained the first migrants that passed through the fish pass. If that is the case, the contemporary migration rates could be reflecting these first migrants. However, there are several criticisms to the effectiveness of the fish passes present in the Neotropical rivers and, in general, these systems were constructed to only allow upriver movement (Agostinho et al., 2002; 2007a; 2007b; 2007c; Makrakis et al., 2007; Pelicice; Agostinho, 2008). Based on these facts, we believe that the first hypothesis is the most plausible to our observed results.

At the regional scale (UP-1 – UP-2), the comparisons between historical e recent migration rates showed interesting results. The recent migration rate showed an increase from 9.6 to 56 times to historical migration rate, indicating genetic changes in these samples sites (Figure. 3). The recent migration rates were 1.66% from UP-2 to UP-1 and 5.92% from UP-1 to UP-2 (Figure. 3).

Nowadays, the Ivinhema river (UP-2), a tributary of Paraná river which is free of barriers (power plants), is isolated from Paranapanema river sample location (UP-1) by six Dam barriers of which, only for two of which fish passes are present (Figure. 1). In conclusion, presently there is an effective barrier between UP-1 and UP-2. This species uses to migrate downriver to feed in the main river canals which works as feeding area where they remain between January to September until to the next reproduction season (Resende, 2003). Some of

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the feedings areas are represented by the lower portions of Paranapanema river, and mainly, by the Itaipu reservoir. Then, many individuals from UP-1 locality could be retained in the feeding areas after the hydroelectric power plant construction in the Paranapenema river. In the same way, some individuals from LP-2 locality could be retained at higher portions of Paranapanema river.

Thus, the increase of the recent migration rate between these two localities could be a reflect, such as in basin level analysis, of the mixing of previous structured populations.

Our results show that the *P. corruscans* populations are under the effects of river fragmentation, mainly by mixing previous genetic structure populations, which are altering their original genetic structure. The mixing of these previous structured populations could lead a homogenization of its populations, in the next few generations, and a possible loss of local diversity.

In conclusion, predicting the consequences of rivers habitat fragmentation on fish species is essential to their conservation and management. In this context, our results are relevant, giving a first insight of the potential consequences of fragmented river system due Dam constructions to migratory fishes, especially among those that present genetic structured populations, such as *P. corruscans*. These findings showing the urgency of more studies with this focus in this and others fish species to understanding the population genetics dynamic and to support monitoring, conservation and management actions to preserve the ichthyofauna.

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