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Research Article

Population genetic structure revealed by a school of the freshwater migratory fish, *Brycon hilarii*

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ABSTRACT. It is believed that different genetic populations of migratory fishes can co-exist in a single hydrographic system. Although different populations may occupy and explore the river together, they segregate during the spawning season and consequently the population genetic structuring is maintained. Genetic variation of a *Brycon hilarii* spawning school and samples from different collection sites in the Miranda River basin were surveyed using seven microsatellites. Population structuring was revealed by a significant differentiation of the spawning school related to the supposed local populations. The genetic differentiation detected may be supported by behavior during the reproductive season that promotes the maintenance of the genetic integrity of different populations. These data may contribute toward the understanding of the behavior and biology of these fish as well as fishery management and species conservation programs.

Keywords: Characidae, Bryconinae, neotropical fish, population structuring, microsatellite, Brasil.

INTRODUCTION

During flood seasons, Neotropical freshwater migratory fish form large schools that swim upstream at reproduction time (Lowe-McConnell, 1987; Carolsfeld et al., 2003). Studies on radio-telemetry have shown the ability of these fishes to migrate several hundred kilometers for spawning or feeding (Lowe-McConnell, 1987; Godinho & Kynard, 2006; Godinho et al., 2007). Therefore, a single large panmitic population within a continuous hydrographic system would be an expected situation for these migratory fishes.

However, a number of studies have indicated that even vagile fish species may contain populations genetically different within a single hydrographic basin. It is believed that two or more genetic populations of migratory fish can co-exist within a
Molecular analysis of *Brycon orthotaenia* (= *Brycon lundii*) enable to detect a significant population structuring in the São Francisco River basin, Brazil (Wasko & Galetti, 2002). These authors hypothesized that this migratory fish constitute different coexisting and comigrating populations along the main river channel. Similar results were reported for another migratory fish that also inhabits the São Francisco River – *Prochilodus argenteus* (Hatanaka & Galetti, 2003; Hatanaka et al., 2006).

Although different populations may occupy and explore the river together, they segregate during the spawning season and consequently the population genetic structuring is maintained. Homing behavior (Gerking, 1959; Hatanaka et al., 2006) and spawning waves (Jorgensen et al., 2005) have been hypothesized as mechanisms responsible for maintaining the genetic cohesiveness of a fish population. Homing is a behavior mainly observed in salmonids and carps (Stähl, 1987), in which mature individuals return to the birthplace for reproduction rather than going to other equally viable places (Gerking, 1959). Mark-recapture and radio tracking studies have evidenced that migratory fish species, such as *Prochilodus lineatus* (Godoy, 1975), *P. argenteus* (Godinho & Kynard, 2006) and *Pseudoplatystoma corruscans* (Godinho et al., 2007) show homing behavior. Spawning waves constitute different populations that spawn at a same location but at different times, even if with some overlap, as observed in some herring species (McPherson et al., 2003; Jorgensen et al., 2005).

Fish have diverse reproductive behaviors with a rich natural-history literature on mating systems ranging from pelagic group spawning, cooperative breeding to social monogamy (for a review, see Avise et al., 2002). Several reports have documented the inbreeding avoidance through the kin recognition among fish species (Pusey & Wolf, 1996; Gerlach & Lysiak, 2006; Ala-Honkola et al., 2010). On the other hand, recent studies have demonstrated that a number of fish species are able to recognize relatives or non-relatives individuals, and that they can show preference for schooling or mating with the relative ones (Odling-Smee & Braithwaite, 2003; Ward & Hart, 2003; Fraser et al., 2005). These mechanisms could provide a limited exchange of reproductive individuals between populations, resulting in genetic differences. However, the life history movements and spawning of freshwater Neotropical fish are poorly known (Godinho et al., 2007).

Significant genetic differentiation was recently revealed between populations of *Brycon hilarii* inhabiting small streams of the Miranda River basin in the Bonito region of western Brazil (Sanches & Galetti, 2007). These authors assessed the genetic variation of this species during different times of the year and detected a significant population differentiation. According to the authors, since significant differences were detected between population subunits, spawning schools organize themselves in such way as to maintain the integrity of each subunit residing in the system.

*B. hilarii* is a medium-sized migratory species, widely distributed throughout the Paraguay River basin (Lima, 2003). It is appreciated for meat quality and sport fishing, as it is very voracious at the time of capture. Due to the beauty of its color, it is also the main tourist attraction for underwater observation activities in the clear river waters of the Bonito region (Sabino & Andrade, 2003). Although this species is not vulnerable, the impact of visitors on the ichthyofauna has increased in the Bonito region, leading to a reduction in species richness (Sabino & Andrade, 2003).

Brazil has about 21% of all freshwater fish species in the world with around two thousand catalogued species (Agostinho et al., 2005; Buckup & Menezes, 2003). However, according to a Brazilian environmental agency (MMA, 2004), 134 of these species were considered endangered, including members of the genus *Brycon*. This loss of biodiversity is directly related to the impact on aquatic continental ecosystems, including pollution, eutrophication, silting, the construction of dams, fisheries and the introduction of exotic species (Agostinho et al., 2005).

According to the previous study (Sanches & Galetti, 2007), in which significant differences were detected between population subunits of *B. hilarii*, and assuming that this structuring reflects the reproductive moment of these fish, the present study surveyed the genetic variation of a *B. hilarii* spawning school and local populations from different collection sites in the Miranda River basin using microsatellites. The goals of this study were to identify the genetic composition of a spawning school and local populations and examine the genetic differentiation between these samples, providing useful genetic data for knowledge on biology, ecology and conservation of this fish.

**MATERIALS AND METHODS**

**Sample collection**

The collection was performed between 2000 and 2004. A total of 128 individuals of *B. hilarii* were obtained at three small streams of the Miranda River...
basin pertaining to the larger Paraguay River basin (MS, Brazil): Formoso River (Fo; n = 30), Peixe River (Pe; n = 19) and the main channel of the Miranda River (Mi; n = 19) (Fig. 1). A spawning school (Sch) was sampled (n = 62) in the main channel of the Miranda River. Fo and Pe are independent tributaries and both flow into the Miranda River.

Blood samples were collected from the caudal peduncle of the fish using hypodermic syringes containing 0.5 M EDTA (0.5 mL blood: 0.1 mL EDTA). Before releasing the fish back into the capture sites, all specimens were marked with nylon strings tied to the dorsal fin base in order to prevent recapture.

Microsatellite analysis

Total genomic DNA was extracted from blood samples using a saline buffer method and precipitation with ethanol (Lahiri & Nurnberger, 1991). Genetic variability was analyzed at seven microsatellite loci, Bh5, Bh6, Bh8, Bh13, Bh15, Bh16, Bh17, previously reported (Sanches & Galetti, 2006). PCRs were carried out using the method described by Schuelke (2000) in a final volume of 15 µL, containing 50 ng of DNA, 0.2 mM dNTPs, 1 x PCR buffer (20 mM Tris-HCl, pH 8.4 and 50 mM KCl; LGC Biotecnologia), 8 pmol of each reverse primer and 6-FAM, HEX or NED M13 (-21) fluorescent labeled primer as well as 2 pmol of the forward primer, 1.5 mM MgCl2 and 0.5 U of Taq DNA Polymerase (LGC Biotecnologia). PCR conditions for all loci were as follows: 1 cycle at 95°C (5 min), 35 cycles at 94°C (30 s), 56°C (45 s) and 72°C (45 s), followed by 12 cycles at 94°C (30 s), 53°C (45 s), 72°C (45 s) and a final extension at 72°C for 10 min. The microsatellite loci were analyzed on a MegaBACE 1000 automated sequencer and the alleles were scored with the Genetic Profiler (Amersham Biosciences).

Data analysis

Within population genetic variability, as measured by the number of alleles (NA), inbreeding coefficient (Weir & Cockerham, 1984) (FIS), observed heterozygosity (HO) and expected (HE) heterozygosity were obtained using the GENEPOP software (Raymond & Rousset, 1995). Allelic richness (Petit et al., 1998) was calculated using the FSTAT software (Goudet, 1995). Significant differences in heterozygosity and allelic richness values were evaluated between sites using the non-parametric Kruskal-Wallis test through BIOESTAT software (Ayres et al., 2003).

Exact-significance testing methods (Guo & Thompson, 1992), implemented in GENEPOP, were used to analyze deviations from the Hardy-Weinberg equilibrium (10,000 batches/10,000 iterations). Evidence for linkage disequilibrium for all pairs of loci was investigated through the Markov Chain Monte Carlo method in GENEPOP. The presence of null allele was investigated for each locus using MicroChecker (Van Oosterhout et al., 2004).

A hierarchical partitioning of the genetic diversity was carried out using an analysis of variance framework as implemented in software Arlequin 2.0 (Schneider et al., 2000). Population differentiation also was estimated using FST (Weir & Cockerham, 1984) obtained with the Arlequin. Pairwise population differentiation in genotype and gene frequencies were assessed using the exact test implemented in
GENEPOP. The critical values for multiple comparisons were adjusted using the Benjamini and Yekutieli correction (Benjamini & Yekutieli, 2001). An analysis of isolation by distance was performed with the Mantel test using GENEPOP considering only the local populations.

Population structure was assessed using a model-based Bayesian procedure implemented on the Structure 2.3.3 program (Pritchard et al., 2000, 2007). This analysis was carried out assuming the admixture model and non correlated allele frequencies. Three individual repetitions of each K estimate (1-8) were run (500,000 iterations, burn-in of 200,000 iterations).

Among the relatedness estimators there is not one estimator that overpowers the others (Van de Casteele et al., 2001; Csillery et al., 2006). We used the most widely used relatedness index (RQG), (Queller & Goodnight 1989; Van Horn et al., 2008) obtained for all possible pair of individuals of each population by means of GeneAlEx 6 (Peatll & Smouse, 2006). This relatedness index measures the extent to which two individuals share alleles that are identical by descent (Queller & Goodnight, 1989). We used Kruskal-Wallis test implemented in SPSS 13.0 to compare average relatedness obtained for each identified genetic population.

RESULTS

A total of 57 alleles (mean of 40.75 per population) were identified over all loci, ranging from 4 (Bh16) to 11 (Bh8 and Bh17) alleles per locus (Table 1). No evidence of linkage disequilibrium (P > 0.05) was detected for any pair of loci. Sixteen alleles were exclusive to a single population, four of which were found in Fo, one in Pe, two in Mi and nine in the Sch population. It is important to state that one of the Sch-exclusive alleles was the second most frequent allele among these fish (Bh8/ allele 227 bp/ frequency = 0.200).

Two populations (Fo and Sch) exhibited a departure from Hardy-Weinberg expectations with a deficit of heterozygosity (Fis significantly higher than zero) when the data was analyzed across all loci (Table 2). The analysis on a locus-by-locus basis demonstrated that the loci Bh6 and Bh13 exhibited departures from HWE in two populations (Fo and Mi, Fo and Sch, respectively) (Appendix). According to the Micro-Checker analysis, both these loci (Bh6 and Bh13) demonstrated evidence for null alleles.

The populations studied showed similar genetic variation, as measured by heterozygosity, gene diversity and allelic richness values (Table 2), and no significant differences were found among them (Kruskal-Wallis, P = 1). The allele richness ranged from 5.20 to 5.66 and the gene diversity from 0.66 to 0.68. The observed and expected heterozygosities ranged of 0.59-0.61 and 0.66-0.67, respectively.

The analysis of molecular variance (AMOVA) revealed a significant genetic differentiation between populations (FST = 0.0184; P = 0.00032). Of the total genetic variation, 1.84% was attributed to interpopulation divergence and 98.16% to the individual differences within populations (Table 3). Pair-wise analysis identified significant differentiation as revealed by the distribution of gene and genotypes frequencies (Table 4). FST values were low, but demonstrated significant divergence between populations (Table 4). These analyses pointed to a same result, in which the spawning school exhibited significant difference when compared to any supposed local population. Considering the local populations, Fo and Pe presented significant genetic differentiation. On the other hand, when the 128 genotypes were submitted to Bayesian clustering analysis, one cluster were identified with a higher probability (K = 1; P(K/X) = 0.99). The Mantel test identified no correlation between the genetic and geographic distance of the local populations.

The average intrapopulation relatedness (Table 2) were significantly different among the populations (Kruskal-Wallis test H = 19.6404; P = 0.002). Fish from the school were more closely related as demonstrated by the higher average relatedness value (Dunn post-test, P < 0.001).

DISCUSSION

A number of studies have reported genetic structuring of Neotropical freshwater migratory fish (Wasko & Galetti, 2002; Hatanaka et al., 2006), but the factors that facilitate this structuring are poorly known. The microsatellites used here revealed a significant genetic differentiation between the spawning school and the supposed local populations sampled in the Miranda River Basin. Moreover the two local populations Fo and Pe also presented significant genetic divergence. This same result was not achieved with the Bayesian analysis, but in cases where genetic differentiation is more subtle, testing for allelic frequency differences between groups (FST) can be more powerful than applying Structure (Pritchard et al., 2007).

In the previous study with B. hilarii, RAPD markers also revealed genetic differences between the same sites of the present study for fish collected during the feeding season (Sanches & Galetti, 2007),
Table 1. Analysis on a locus-by-locus basis of four populations of *B. hilarii* using seven microsatellite loci. Number of alleles per locus ($N_A$), allelic richness ($R_A$), observed heterozygosity ($H_O$), expected heterozygosity ($H_E$), inbreeding coefficient ($F_{IS}$) and $P$-value for departures from Hardy-Weinberg expectations. (*) Significant $P$-value <0.05.

<table>
<thead>
<tr>
<th></th>
<th>$N_A$</th>
<th>$R_A$</th>
<th>$H_O$</th>
<th>$H_E$</th>
<th>$F_{IS}$</th>
<th>$P_{HW}$</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Fo</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bh5</td>
<td>8</td>
<td>7.32</td>
<td>0.83</td>
<td>0.77</td>
<td>0.069</td>
<td>0.211</td>
</tr>
<tr>
<td>Bh6</td>
<td>6</td>
<td>5.48</td>
<td>0.43</td>
<td>0.66</td>
<td>0.353</td>
<td>0.022*</td>
</tr>
<tr>
<td>Bh8</td>
<td>8</td>
<td>6.87</td>
<td>0.86</td>
<td>0.73</td>
<td>0.178</td>
<td>0.271</td>
</tr>
<tr>
<td>Bh13</td>
<td>6</td>
<td>5.97</td>
<td>0.57</td>
<td>0.81</td>
<td>0.296</td>
<td>0.0002*</td>
</tr>
<tr>
<td>Bh15</td>
<td>3</td>
<td>2.65</td>
<td>0.58</td>
<td>0.52</td>
<td>-0.113</td>
<td>0.824</td>
</tr>
<tr>
<td>Bh16</td>
<td>3</td>
<td>2.92</td>
<td>0.25</td>
<td>0.29</td>
<td>0.153</td>
<td>0.502</td>
</tr>
<tr>
<td>Bh17</td>
<td>7</td>
<td>6.90</td>
<td>0.68</td>
<td>0.85</td>
<td>0.203</td>
<td>0.114</td>
</tr>
<tr>
<td><strong>Pe</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bh5</td>
<td>6</td>
<td>5.89</td>
<td>0.58</td>
<td>0.77</td>
<td>0.256</td>
<td>0.246</td>
</tr>
<tr>
<td>Bh6</td>
<td>5</td>
<td>4.99</td>
<td>0.47</td>
<td>0.63</td>
<td>0.250</td>
<td>0.107</td>
</tr>
<tr>
<td>Bh8</td>
<td>6</td>
<td>5.77</td>
<td>0.67</td>
<td>0.59</td>
<td>0.012</td>
<td>0.542</td>
</tr>
<tr>
<td>Bh13</td>
<td>5</td>
<td>5.00</td>
<td>0.74</td>
<td>0.76</td>
<td>0.031</td>
<td>0.116</td>
</tr>
<tr>
<td>Bh15</td>
<td>5</td>
<td>4.89</td>
<td>0.74</td>
<td>0.63</td>
<td>-0.178</td>
<td>0.195</td>
</tr>
<tr>
<td>Bh16</td>
<td>3</td>
<td>3.00</td>
<td>0.32</td>
<td>0.48</td>
<td>0.351</td>
<td>0.087</td>
</tr>
<tr>
<td>Bh17</td>
<td>7</td>
<td>6.89</td>
<td>0.74</td>
<td>0.80</td>
<td>0.077</td>
<td>0.609</td>
</tr>
<tr>
<td><strong>Mi</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bh5</td>
<td>8</td>
<td>8.00</td>
<td>0.82</td>
<td>0.81</td>
<td>-0.016</td>
<td>0.895</td>
</tr>
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<td>Bh6</td>
<td>5</td>
<td>4.89</td>
<td>0.26</td>
<td>0.65</td>
<td>0.600</td>
<td>0.0001*</td>
</tr>
<tr>
<td>Bh8</td>
<td>8</td>
<td>7.83</td>
<td>0.72</td>
<td>0.76</td>
<td>-0.054</td>
<td>0.627</td>
</tr>
<tr>
<td>Bh13</td>
<td>5</td>
<td>5.00</td>
<td>0.72</td>
<td>0.81</td>
<td>0.116</td>
<td>0.279</td>
</tr>
<tr>
<td>Bh15</td>
<td>3</td>
<td>3.00</td>
<td>0.65</td>
<td>0.55</td>
<td>-0.173</td>
<td>0.528</td>
</tr>
<tr>
<td>Bh16</td>
<td>3</td>
<td>3.00</td>
<td>0.33</td>
<td>0.34</td>
<td>0.014</td>
<td>0.513</td>
</tr>
<tr>
<td>Bh17</td>
<td>8</td>
<td>7.89</td>
<td>0.78</td>
<td>0.80</td>
<td>0.033</td>
<td>0.466</td>
</tr>
<tr>
<td><strong>Sch</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bh5</td>
<td>9</td>
<td>6.95</td>
<td>0.69</td>
<td>0.80</td>
<td>0.142</td>
<td>0.129</td>
</tr>
<tr>
<td>Bh6</td>
<td>7</td>
<td>5.47</td>
<td>0.42</td>
<td>0.55</td>
<td>0.237</td>
<td>0.029</td>
</tr>
<tr>
<td>Bh8</td>
<td>7</td>
<td>5.94</td>
<td>0.81</td>
<td>0.76</td>
<td>-0.075</td>
<td>0.746</td>
</tr>
<tr>
<td>Bh13</td>
<td>5</td>
<td>4.95</td>
<td>0.56</td>
<td>0.75</td>
<td>0.259</td>
<td>0.0028*</td>
</tr>
<tr>
<td>Bh15</td>
<td>4</td>
<td>2.92</td>
<td>0.53</td>
<td>0.53</td>
<td>-0.007</td>
<td>0.0312*</td>
</tr>
<tr>
<td>Bh16</td>
<td>4</td>
<td>3.23</td>
<td>0.31</td>
<td>0.39</td>
<td>0.221</td>
<td>0.069</td>
</tr>
<tr>
<td>Bh17</td>
<td>9</td>
<td>7.46</td>
<td>0.80</td>
<td>0.84</td>
<td>0.041</td>
<td>0.589</td>
</tr>
</tbody>
</table>

Fo: Formoso River, Pe: Peixe River, Mi: Miranda River, Sch: Reproductive school.

corroborating with the differentiation detected between Fo and Pe. These populations can comprise resident fish group into these two independent tributaries of the Miranda River Basin. Miranda River with 43.787 km² of drainage area is one of the main rivers of the Pantanal, where there are numerous marginal and nutrient-rich lagoons that are ideal for feeding (SEMA/MS, 1994). In this way, its large size and the availability of food resources may facilitate the accumulation of several populations minimizing the chance of detecting genetic structuring through F statistic.
Table 2. Number of alleles ($N_A$), allelic richness ($R_A$), observed heterozygosity ($H_O$), expected heterozygosity ($H_E$), inbreeding coefficient ($F_{IS}$), $P$-values for departures from Hardy-Weinberg expectations ($P_{HW}$) and average relatedness ($R_{QG}$) found in four populations of *B. hilarii* using seven microsatellite loci.

<table>
<thead>
<tr>
<th></th>
<th>$N_A$</th>
<th>$R_A$</th>
<th>$H_O$</th>
<th>$H_E$</th>
<th>$F_{IS}$</th>
<th>$P_{HW}$</th>
<th>$R_{QG}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fo</td>
<td>41</td>
<td>5.44</td>
<td>0.60</td>
<td>0.66</td>
<td>0.097</td>
<td>0.0007*</td>
<td>-0.035</td>
</tr>
<tr>
<td>Pe</td>
<td>37</td>
<td>5.20</td>
<td>0.61</td>
<td>0.67</td>
<td>0.109</td>
<td>0.079</td>
<td>-0.073</td>
</tr>
<tr>
<td>Mi</td>
<td>40</td>
<td>5.66</td>
<td>0.61</td>
<td>0.67</td>
<td>0.096</td>
<td>0.022</td>
<td>-0.055</td>
</tr>
<tr>
<td>Sch</td>
<td>45</td>
<td>5.27</td>
<td>0.59</td>
<td>0.66</td>
<td>0.108</td>
<td>0.0007*</td>
<td>-0.012</td>
</tr>
</tbody>
</table>

*Significant $P$-value $<0.00714$, following Benjamini & Yekutiele correction; (Fo) Formoso River; (Pe) Peixe River; (Mi) Miranda River; (Sch) Reproductive school.

Table 3. Analysis of molecular variance (AMOVA) between four populations of *Brycon hilarii*. The data present the degrees of freedom (df), sum of squared deviation (SSD), variance component estimates and partitioning of the total genetic diversity.

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>df</th>
<th>SSD</th>
<th>Variance component</th>
<th>Percentage of variation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Among populations</td>
<td>3</td>
<td>8.465</td>
<td>0.025</td>
<td>1.84%</td>
</tr>
<tr>
<td>Within populations</td>
<td>254</td>
<td>342.99</td>
<td>1.350</td>
<td>98.16%</td>
</tr>
<tr>
<td>Total</td>
<td>257</td>
<td>351.45</td>
<td>1.375</td>
<td></td>
</tr>
</tbody>
</table>

Table 4. Genetic differentiation between four populations of *Brycon hilarii*. $P$-values from exact test of genotype and gene differentiation; $F_{ST}$ and respective $P$-values.

<table>
<thead>
<tr>
<th></th>
<th>Gene differentiation ($P$)</th>
<th>Genotype differentiation ($P$)</th>
<th>$F_{ST}$ ($P$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fo x Pe</td>
<td>0.0015*</td>
<td>0.010</td>
<td>0.0284 (0.000*)</td>
</tr>
<tr>
<td>Fo x Mi</td>
<td>0.331</td>
<td>0.584</td>
<td>-0.0032 (0.442)</td>
</tr>
<tr>
<td>Fo x Sch</td>
<td>0.000*</td>
<td>0.000*</td>
<td>0.0254 (0.000*)</td>
</tr>
<tr>
<td>Pe x Mi</td>
<td>0.258</td>
<td>0.572</td>
<td>-0.0074 (0.198)</td>
</tr>
<tr>
<td>Pe x Sch</td>
<td>0.000*</td>
<td>0.000*</td>
<td>0.0317 (0.000*)</td>
</tr>
<tr>
<td>Mi x Sch</td>
<td>0.000*</td>
<td>0.000*</td>
<td>0.0273 (0.006*)</td>
</tr>
</tbody>
</table>

*Significant $P$-value $<0.00833$, following Benjamini & Yekutiele correction. Fo: Formoso River, Pe: Peixe River; Mi: Miranda River; Sch: Reproductive school.

The genetic divergence found between the spawning school and any other population may indicate that the school is composed by individuals from different localities than the examined local populations. This result may explain the presence of exclusive alleles in high frequency, reinforcing the idea of population
structuring within a same hydrographic system. Spawning schools may use different sites for the reproduction and for feeding.

The genetic divergence found in the spawning school may be the result of behavior at reproduction time that reflects in the genetic structure of the fish populations (Laikre et al., 2005). A number of studies have demonstrated the relationship between reproductive behavior, social organization and genetic structure of fish populations, such as the importance of homing behavior in the genetic structuring of salmon populations that spawn in different sites (Stähl, 1987). Similarly, genetic distinctiveness has been found between resident and anadromous *Oncorhynchus mykiss* fish (Narum et al., 2004) as well as temporal differentiations in a herring species (*Clupea harengus*), which were attributed to sampling in different spawning waves (Jorgensen et al., 2005). Recent studies have demonstrated familiarity and kin association in fish (Ward & Hart, 2003; Fraser et al., 2005). It has been suggested that schooling with relatives could promote benefits (Hamilton, 1964; Quinn & Busack, 1985) by performing co-operative behaviours such as predator inspection (Wisenden & Smith, 1998), reduced levels of intraspecific aggression (Brown & Brown, 1993), enhancement of social learning in groups (Swaney et al., 2001), improvement of natal homing to breeding areas in migratory species (Olsen, 1989). The high relatedness value found for fish from school may be evidence of kin association or reproductive behaviors, such as homing and spawning waves.

However, life history and spawning are insufficiently known in Neotropical migratory freshwater species. In the São Francisco Basin, recent studies using telemetry were carried out with two commercially important species, *P. argenteus* and *P. corruscans*, in which migrations were investigated. Both species exhibited a dualistic migration pattern (Godinho & Kynard, 2006; Godinho et al., 2007). In *P. argenteus*, some fish were resident presenting a small total linear home range (≤1 km) and used the same grounds for spawning and non-spawning activities. Though, most fish were migratory, with a large total linear home range (≥53 km) and different reaches for spawning and foraging (Godinho & Kynard, 2006). Moreover, radio-tagged fish exhibited spawning-site homing behavior over two successive seasons and some fish also demonstrated very precise homing to non-spawning sites. In *P. corruscans*, the spatial distribution of the fish was greater during the non-spawning season than the spawning season (Godinho et al., 2007). These studies corroborate the genetic data when homing was addressed to explain the maintenance of population structuring observed in *P. argenteus* (Hatanaka & Galetti, 2003).

Studies on fish populations commonly encounter significant genetic differentiations between populations, but with low FST values (Wirth & Bernatchez, 2001; Hatanaka et al., 2006). Weak differentiation has been especially detected in vagile species of great abundance, with wide distribution and no visible barriers to gene flow (Knutsen et al., 2003; Jorgensen et al., 2005). The degree and pattern of differentiation is related to the amount of gene flow between population units, and even when genetic structuring is identified, different populations present some exchange of individuals (Pettersson et al., 2001; Narum et al., 2004; Jorgensen et al., 2005), as was observed here in the spawning school.

Although the magnitude of FST is still an issue of intense debate (Jorgensen et al., 2005), certainly it is advisable to consider the existence of subdivisions within a population than treating it as a single population, which could result in the depletion of genetic variation (Laikre et al., 2005).

The maintenance of genetic diversity depends on the preservation of gene flow among the populations of a system (Narum et al., 2004; Laikre et al., 2005). Therefore, the population structuring could maintain the existence of distinct genetic populations and avoid panmixia. On the other hand, gene flow ensures the introduction of variation into the genetic populations, avoiding inbreeding and the loss of genetic variation in the species as a whole.

In the present study, the population structuring of *B. hilarii* was demonstrated by the genetic differentiation of a spawning school in relation to the local populations and between Fo and Pe. This population structuring may be supported by behavior that promotes the maintenance of the genetic integrity of different subpopulations during the reproductive season. Before and after reproductive seasons, fish from different population units may be resident (as Fo and Pe) or mixed and spread throughout the hydrographic system (as Mi). But, the spawning school also may consist of a population unit from a different locality than the examined sites of the present study.

The understanding of the genetic structure of natural populations as well as the identification of population units and the knowledge of how these units are distributed throughout the environment are essential to management decisions and the conservation of the genetic resources (Moritz, 1994; Paetkau, 1999). Thus, data on the genetic structure of *B. hilarii* constitute tools of great importance contributing to the
understanding of the behavior and biology of these fish as well as fishery management and species conservation programs.

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