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Population Structure of a Neotropical Migratory Fish: Contrasting Perspectives from Genetics and Otolith Microchemistry

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ARTICLE

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Abstract

Developing conservation strategies for migratory fishes requires an understanding of connectivity among populations. Neotropical rivers contain diverse and economically important assemblages of migratory fishes, but little is known about the population biology of most species. We examined the population structure of *Prochilodus mariae*, an abundant migratory fish species found in Venezuelan rivers that plays essential roles in both regional fisheries and ecosystem dynamics. By coupling otolith microchemistry and microsatellite genetic analyses, we were able to evaluate both natal origins of individual fish and genetic structure on a regional level. The chemistry of otolith cores inferred separate breeding grounds for four of six populations, with 75–85% of individuals from each river sharing

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a natal signature that is distinct from the other populations. In contrast, we detected no genetic structure, indicating that gene flow among these rivers prevents population differentiation. These disparate inferences underscore the complexity of conserving migratory species; otolith data suggest that ensuring fishery sustainability requires recognizing distinct breeding stocks, while gene flow reflects the importance of connectivity across the broader river network on an evolutionary time scale. We conclude that multiple methodological approaches may often be necessary to fully understand the spatial ecology and management needs of migratory fishes and, therefore, also influence local management practices.

Understanding the population structure of migratory fishes can provide important information for determining the appropriate spatial scale for their management and conservation (Shaklee and Bentzen 1998). In ecosystems with spatially structured populations and high levels of site fidelity, heavy exploitation or habitat degradation in one location can sometimes have strong effects on that local population without affecting adjacent populations (Heath et al. 2008). Alternatively, if fish migrate widely and interbreed on a regional scale, effects on a single population could be manifested in other areas (Kell et al. 2009). Complexity, including differences among populations and their life history characteristics, can be key to maintaining ecosystem function and services (Hilborn et al. 2003; Schindler et al. 2010).

Migratory fishes display high interspecific variation in their population biology, so it is important to identify whether management and conservation efforts should treat the panoply of local populations as discrete units or instead as a single regional population. Previous studies spanning a wide range of North American taxa have identified many species with high levels of site fidelity and genetic population structure, including such diverse groups as salmonids (McDowall 2001), Swordfish *Xiphias gladius* (Alvarado Bremer et al. 2005), and Atlantic Herring *Clupea harengus* (Ruzzante et al. 2006). Other species disperse and interbreed widely and have little population structure, including Striped Bass *Morone saxatilis* (Mather et al. 2010) and Sea Lamprey *Petromyzon marinus* (Waldman et al. 2008). Population structure can also vary within species; for example, Palkovacs et al. (2008) found high levels of genetic structure for landlocked populations of Alewife *Alosa pseudoharengus*, but low levels in anadromous populations.

Using multiple methods of analyzing movement and population structure is often essential for migratory animals (Rubenstein and Hobson 2004). For fishes, chemical differences between different layers of their ear stones (otoliths) can be used to reconstruct the migration history of individual fish because otolith microchemistry can reflect local stream water chemistry and thus location at the time of accretion (Campana and Thorrold 2001; Wells et al. 2003; Feyrer et al. 2007). While otolith chemistry does reflect ambient chemistry to some extent, other factors can also influence otolith core chemistry and results must be interpreted carefully. For example, physiology, maternal effects, and crystallization can all influence core chemistry (Brophy et al. 2004), especially in species that have yolk sacks like Pacific salmon *Oncorhynchus* spp. (Bacon et al. 2004; Miller and Kent 2009).

More commonly, researchers have assessed fish population structure using genetic markers, such as microsatellites. A smaller number of studies have applied otolith and genetic approaches in parallel. In those studies, the two approaches have provided complementary information about habitat use, movement patterns, and population structure of North American and Australian fishes (Ashford et al. 2006; Bradbury et al. 2008; Barnett-Johnson et al. 2010; Woods et al. 2010; Leis et al. 2011). Chemical tracers can be used to determine whether populations are connected on an ecological time scale, and genetic analyses can be used to determine whether they are connected on an evolutionary time scale. Hence, using multiple types of markers can allow investigators to determine whether populations are connected on multiple time scales (Rubenstein and Hobson 2004; Leis et al. 2011).

The objective of our study was to examine the population structure of a major migratory Neotropical fish, *Prochilodus mariae,* on a regional scale using multiple analytical approaches in parallel. Very little is known about the population structure of migratory fishes in tropical rivers, where many species migrate annually between breeding and feeding areas. South American rivers contain diverse assemblages of migratory fishes, including many long-distance migrants, that support key regional fisheries and are threatened by anthropogenic environmental changes (Barletta et al. 2010). The few existing studies on population structure of migratory fishes in Neotropical rivers suggest wide variation among species, ranging from migratory catfish (Spotted Sorubim *Pseudoplatystoma corruscans*) with high levels of population structure (Pereira et al. 2009) to migratory characins (*Prochilodus* spp.) with no detectable population structure (Hatanaka et al. 2006; Carvalho-Costa et al. 2008). Effective conservation and management of Neotropical fish faunas require a better understanding of their movement patterns and population structure, yet this information is lacking even for most heavily exploited species.

Our focal species, *P. mariae*, and its close relatives are a dominant component of fish biomass and fishery yields in rivers across South America (Barbarino Duque et al. 1998; Sivasundar et al. 2001; Hoeinghaus et al. 2004; Taylor et al. 2006). Prochilodontids typically make extensive annual migrations. *Prochilodus mariae* is iteroparous and migrates yearly from the foothill streams of the eastern slope of the Andes to seasonally inundated floodplains, where they spawn. Field studies have documented the strong influence of *P. mariae* migrations on the structure and functioning of river ecosystems in Venezuela

(Flecker 1996; Taylor et al. 2006; McIntyre et al. 2007), but the implications of these migrations on population structure have not been evaluated. Genetic studies on related species indicate low levels of genetic structure among populations (Turner et al. 2004; Carvalho-Costa et al. 2008). A better understanding of the population biology of *P. mariae* and other fishery species is especially important because these migrants face overfishing (Allan et al. 2005) and increasing degradation of both floodplain and upland habitats caused by deforestation and other human activities, as well as installation of major dams in the Orinoco and Amazon basins (Nilsson et al. 2005).

We coupled otolith chemistry and population genetic methods to address two objectives: (1) infer movement patterns of individual *P. mariae* using otolith microchemical analysis, and determine whether adults using different upland rivers share a common lowland breeding area and (2) determine whether populations of *P. mariae* from different rivers are genetically isolated, or instead disperse and interbreed frequently enough to be managed as a panmictic population.

METHODS

Site Descriptions and Sample Collections

During the 2006 dry season (January–March), we collected *P. mariae* from six river sites in the Apure drainage of the Orinoco basin in Venezuela (Table 1; Figure 1). Some sites were only a few kilometers apart (e.g., Rio Las Marias and Rio Morador), while others were connected to the remainder of the sites only through the Apure River (e.g., Rio Paguey). Two of the sites were on the Rio Portuguesa, which we refer to as Upper Portuguesa, an Andean foothill site, and Lower Portuguesa for a larger downstream site located in the floodplain. All six populations were included in otolith microchemical analysis, but the two downstream sites were excluded from genetic analysis due to sampling constraints (Figure 1; Table 1). Approximately 20 fish were collected from each site by local fishermen using cast nets. Whole fish heads were frozen for later otolith extraction, and muscle samples were preserved in 95% ethanol for DNA analysis.

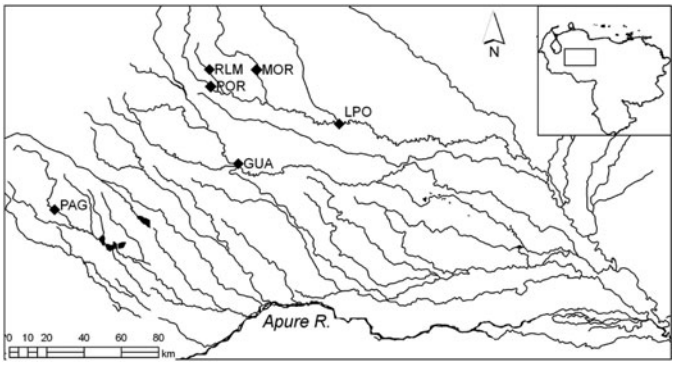

FIGURE 1. Map of the study area showing six collection sites. Sites are abbreviated as follows in all figures and tables: RLM = Rio Las Marias, MOR = Rio Morador, POR = Upper Rio Portuguesa, PAG = Rio Paguey, LPO = Lower Rio Portuguesa Gammareno, GUA = Rio Guanare.

Otolith Microchemical Analysis

We analyzed chemical signatures of the core and edge of the otolith from each individual fish. The edge of the otolith is the most recently accreted layer and reflects the chemistry of the environment inhabited just before collection. We examined otolith edge chemistry to determine whether there is likely to be sufficient chemical variation within the system to separate discrete spawning grounds. Otoliths were analyzed for strontium (Sr), barium (Ba), and magnesium (Mg) and reported as ratios to calcium (Ca). The ratio of stable isotopes of strontium (^{87}Sr:^{86}Sr) were also quantified from raw count data.

We extracted the sagittal otolith of each individual using standard techniques (Campana 1999; Bickford and Hannigan 2005) and stored them in plastic tubes until processing. Otoliths were thin-sectioned with a low-speed saw and then polished with fine-grit and velvet polishing pads until the core was distinctly visible. Otoliths were washed with Milli-Q water and sonicated, then analyzed with dry laser ablation (New Wave 213 nm Nd:YAG) dynamic reaction cell-inductively coupled plasma–mass spectrometry (7500c Agilent) at the University of Alaska–Fairbanks. We used a spot size of 20 μm for both edge and core chemistry analyses, which was the smallest spot size with good resolution and was estimated to represent approximately 10 days of otolith accretion. The core region was approximately 45 μm in diameter. Laser energy was set at 100% with

TABLE 1. Study site characteristics. Number of individuals sampled per river, mean SL, and condition indices (SD in parentheses) are described. Otolith and Genetic columns indicate whether those types of analyses were conducted on fish from each site.

Site	Site type	Number of individuals	SL	Condition index	Otolith	Genetic
Rio Guanare (GUA)	Floodplain	21	27.4 (3.5)	1.48 (0.40)	yes	no
Rio Morador (MOR)	Foothill	16	24.8 (1.8)	1.29 (0.15)	yes	yes
Rio Paguey (PAG)	Foothill	20	29.8 (2.1)	1.27 (0.08)	yes	yes
Upper Portuguesa (POR)	Foothill	20			yes	yes
Lower Portuguesa (LPO)	Floodplain	20	25.3 (2.6)	1.45 (0.14)	yes	no
Rio Las Marias (RLM)	Foothill	25	22.0 (2.3)	1.38 (0.12)	yes	yes

the frequency at 20 Hz and the number of bursts at 200. Flow of the carrier gas, Ar, was set at 1.05 mL/min. Each sample was ablated using a line width size of 25 μm and data were averaged.

Results were calibrated against a silicate glass certified reference material (NIST 610) and corrected for gas blanks before every sample. Samples were run in a randomized order and each sample measurement was preceded by a gas blank measurement with recalibration (gas blank and NIST SRM 610) every 10 samples. Background counts were monitored for 20 s before and after each sample. Concentrations of all elements (Ca, Sr, Ba, Mg) were calculated relative to NIST SRM 610 after proper correction for gas blank, matrix, and drift effects. This allowed us to compare elemental abundances relative to Ca content across otolith samples (Campana 1999). All measurements were greater than the limit of detection and external precision (i.e., relative standard deviations) was better than 3% for all elements and for each isotope of Sr.

Microsatellite DNA Genotyping

We developed a microsatellite DNA library for *P. mariae* (Barker et al. 2011) and genotyped approximately 20 individuals from each site using 11 microsatellite loci (Table 2). We used a universal tag method to generate fluorescently labeled PCR products for genotyping (Schuelke 2000). We analyzed labeled PCR products on a 3100 Genetic Analyzer (Applied Biosystems) and determined allele sizes using GENEMAPPER version 3.0 (Applied Biosystems).

Statistical Analyses

Otolith microchemistry.—We used quadratic discriminant function analysis (QDFA) and a jackknife procedure to cross validate chemical signatures found at each site based on edge and core data. First, we used QDFA to classify fish into groups based on edge chemistry. This classification was expected to match the known collection sites. Since many freshwater systems are geochemically similar, edge chemical classification allowed us to evaluate whether there was sufficient background variation in chemistry among rivers to distinguish sites. We used multivariate analysis of variance (MANOVA) to determine whether multivariate chemical signatures were significantly different among sites and analysis of variance (ANOVA) to identify differences for individual elemental signatures differed among sites for both core and edge chemistry.

We performed an identical QDFA analysis using the core chemical data to determine whether individuals from each site shared a chemically similar natal environment. Since *P. mariae*

TABLE 2. Characteristics of 11 *P. mariae* microsatellite loci, including locus name, GenBank accession number, repeat motif, fluorescent dye color, primer sequence (F: forward primer, R: reverse primer), annealing temperature (T_a), $MgCl_2$ concentration, allele size range (bp: base pairs), number of individuals genotyped (n), number of alleles (N_A), number of private alleles, observed heterozygosity (H_o) and expected heterozygosity (H_e).

Locus and GenBank accession Number	Repeat motif and fluorescent dye color	Primer sequence (5′-3′)	T_a (°C)	$MgCl_2$ (mM)	Allele size range (bp)	n	N_A	Number private alleles	H_o	H_e
Pmar01	(AGAT)21	F: CCACAAGAAGATAGACCCAACGAT	62	1.6	280–413	74	33	10	0.95	0.96
JF832396	VIC	R: TTTCATATACCGGTCTGCCTTTCT								
Pmar06	(TATC)16	F: CATGTCCTTGAATTTCCCTCTGTC	62	1.6	118–269	72	34	15	0.58	0.96
JF832398	FAM	R: CTGGTTGATCTGGCTCTTGCTGGTA								
Pmar07	(AGAT)14	F: GCCCTCCCTTAGTCACCCTGTTA	62	2.0	321–405	73	24	10	0.52	0.93
JF832400	FAM	R: AATACCAAATTTATAAGTTGCGTCCACA								
Pmar11	(GTT)11	F: ATAATAAATCCAGGTGCAGCTTCAGTCA	62	2.0	190–226	75	11	1	0.87	0.84
JF832395	PET	R: CTTCAGAGTCCACATCACCATCAGTAAC								
Pmar17	(TATC)20	F: TAGGCAATGTGACTGAGTGTGAGT	62	1.6	196–302	75	19	7	0.93	0.91
JF832405	NED	R: GAGCCGTTTAATAACAGCCTACAG								
Pmar19	(TTG)10	F: CAGGCATGTACCAACCCAAACC	62	1.6	270–325	75	17	6	0.76	0.88
JF832404	FAM	R: ATGAGTCAAATAACGGCACAGG								
Pmar20	(CAA)13	F: TTCCAGCACCAGATAAACCA	62	1.6	191–237	75	17	3	0.68	0.90
JF832402	NED	R: GGCTAAACCTGCTCGTCCTCT								
Pmar22	(TTG)15	F: AGCCTGACGCTAGTCCTTTTCTT	62	1.6	187–238	75	33	8	0.96	0.95
JF832397	VIC	R: GTATAATTCCGGCTGTTTTAGTTCAATA								
Pmar27	(AAC)18	F: GGTCAAAAATTCCCATAAACACAT	60	1.6	292–378	73	26	9	0.58	0.89
JF832399	FAM	R: TATAGTAAACAAGAACAATCAAAGAGA								
Pmar28	(GTT)19	F: ATATTTGTTTTTGTCCATTCATCA	60	1.6	198–239	68	15	2	0.39	0.90
JF832401	NED	R: CACTGGGTATTTACTTTGTTATTGT								
Pmar29	(TC)18	F: ACTTGAGTGGCGACTGGTCCTG	60	1.6	143–172	75	14	3	0.87	0.87
JF832403	VIC	R: CTCTGAGTCAGCTGACGGTGCTC								

reproduce while migrating downstream (Barbarino Duque et al. 1998), it is unlikely that the natal environments are the same as the collection environments. Hence, we were only able to explore whether natal sites were distinct and shared among individuals from a population. We conducted all otolith chemistry statistical analyses using R (R Development Core Team 2010).

Microsatellite DNA.—Tests for Hardy–Weinberg equilibrium and linkage disequilibrium were performed using GENEPOP version 3.4 (Raymond and Rousset 1995). We used F_{ST} as a measure of population differentiation and calculated pairwise F_{ST} estimates using Arlequin version 3.1 (Excoffier et al. 2005). Genetic structure was also analyzed using Bayesian clustering methods in two programs to ensure the consistency and hence reliability of the results: STRUCTURE version 2.2 (Pritchard et al. 2000) and GENELAND version 2.0.12 (Guillot et al. 2005). Both STRUCTURE and GENELAND use a Markov Chain Monte Carlo (MCMC) approach to infer the most likely number of genetic groups (K). The cluster analysis in GENELAND uses GPS coordinates of collection sites to account for isolation by distance, which can increase detection of low levels of population structure.

In STRUCTURE, we performed MCMC runs with values of K set to 1 to 10 to determine the most likely number of genetic groups. We chose that range for K because we wanted to include all likely numbers of genetic populations for our four geographic populations. For each value of K, we conducted 10 independent runs using the admixture model, the correlated allele frequency model, and alphapropsd set to 0.001 (higher values led to substantial variations of alpha along the runs). For each run, a burn-in period of 50,000 steps followed by one million steps of data collection was sufficient to ensure MCMC convergence. To infer the most likely number of genetic groups in the data set, we looked for the value of K that gave the highest lnP(D) (the most appropriate method with the type of plot of lnP(D) as a function of K we obtained [see Results, Pritchard et al. 2000; Evanno et al. 2005]).

In GENELAND, we performed 10 runs of 1,000,000 MCMC iterations of the spatial model, where K was allowed to vary between 1 and 10 (number initialized to 3) with the following set of parameters that were chosen after conducting preliminary runs to adjust their values: maximum rate of the Poisson process used to generate the Voronoi cells = 500, maximum number of nuclei in the Poisson–Voronoi tessellation = 500, allele frequency model = Dirichlet. The uncertainty associated with the spatial coordinates of specimen collection was 100 m. The most likely K was inferred as the modal number of genetic groups estimated among 1,000,000 iterations of the best of the 10 runs. To select the best run, we used the posterior density of the runs (after a burn-in of 200,000 iterations) as an estimator of their quality: the posterior density is estimated for each state of the parameters, explored along the Markov chain, and represents the posterior probability of that current state of parameters.

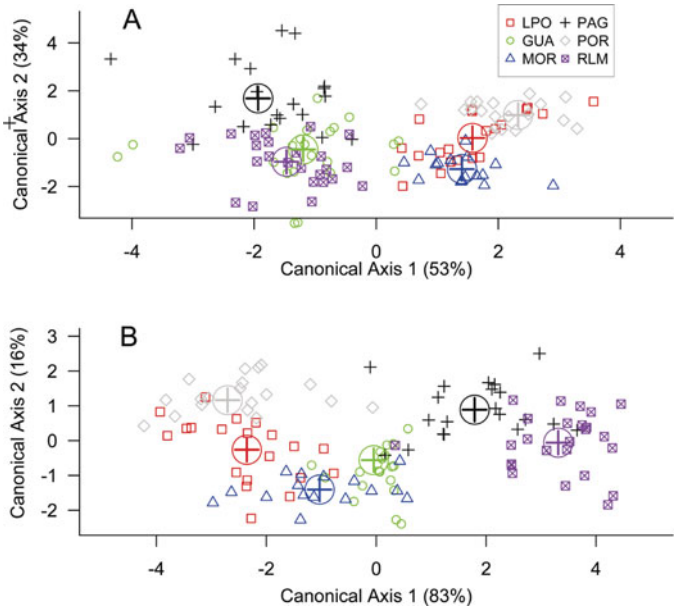

FIGURE 2. Plots demonstrating spatial variation in multivariate otolith chemistry (canonical axes are a compilation of Sr:Ca, Ba:Ca, Mg:Ca, and ^{87}Sr:^{86}Sr chemical signatures). Panel **(A)** shows variation in otolith core chemistry and Panel **(B)** shows variation in otolith edge chemistry. Each small point represents an individual fish and the large points represent the centroid for each site group. [Figure available online in color.]

RESULTS

Otolith Microchemistry

Cross validation of otolith edge chemistry resulted in moderate to good classification of individuals to each collection site; 65–80% of individuals were correctly classified (Table 3). The

TABLE 3. Classification results from QDFA for otolith edges (top) and cores (bottom). Table shows the percentage of individuals from each collection site that are classified into each site. Total numbers of individuals collected are shown in parentheses next to the collection site label. Numbers in bold italics indicate that the collection and classification sites are the same.

	Classification site					
Collection site	PAG (%)	RLM (%)	GUA (%)	MOR (%)	LPO (%)	POR (%)
			Edge			
PAG (20)	***65.0***	25.0	5.0	0.0	0.0	5.0
RLM (26)	16.0	***80.0***	4.0	0.0	0.0	0.0
GUA (21)	4.8	0.0	***76.2***	14.3	4.8	0.0
MOR (16)	0.0	0.0	25.0	***68.8***	6.3	0.0
LPO (20)	0.0	0.0	5.0	20.0	***60.0***	15.0
POR (20)	0.0	0.0	10.0	0.0	15.0	***75.0***
			Core			
PAG (20)	***70.0***	5.0	25.0	0.0	0.0	0.0
RLM (26)	0.0	***80.0***	20.0	0.0	0.0	0.0
GUA (21)	19.0	33.3	***38.1***	4.8	4.8	0.0
MOR (16)	0.0	0.0	0.0	***81.3***	18.8	0.0
LPO (20)	0.0	0.0	5.0	35.0	***20.0***	40.0
POR (20)	0.0	0.0	0.0	0.0	25.0	***75.0***

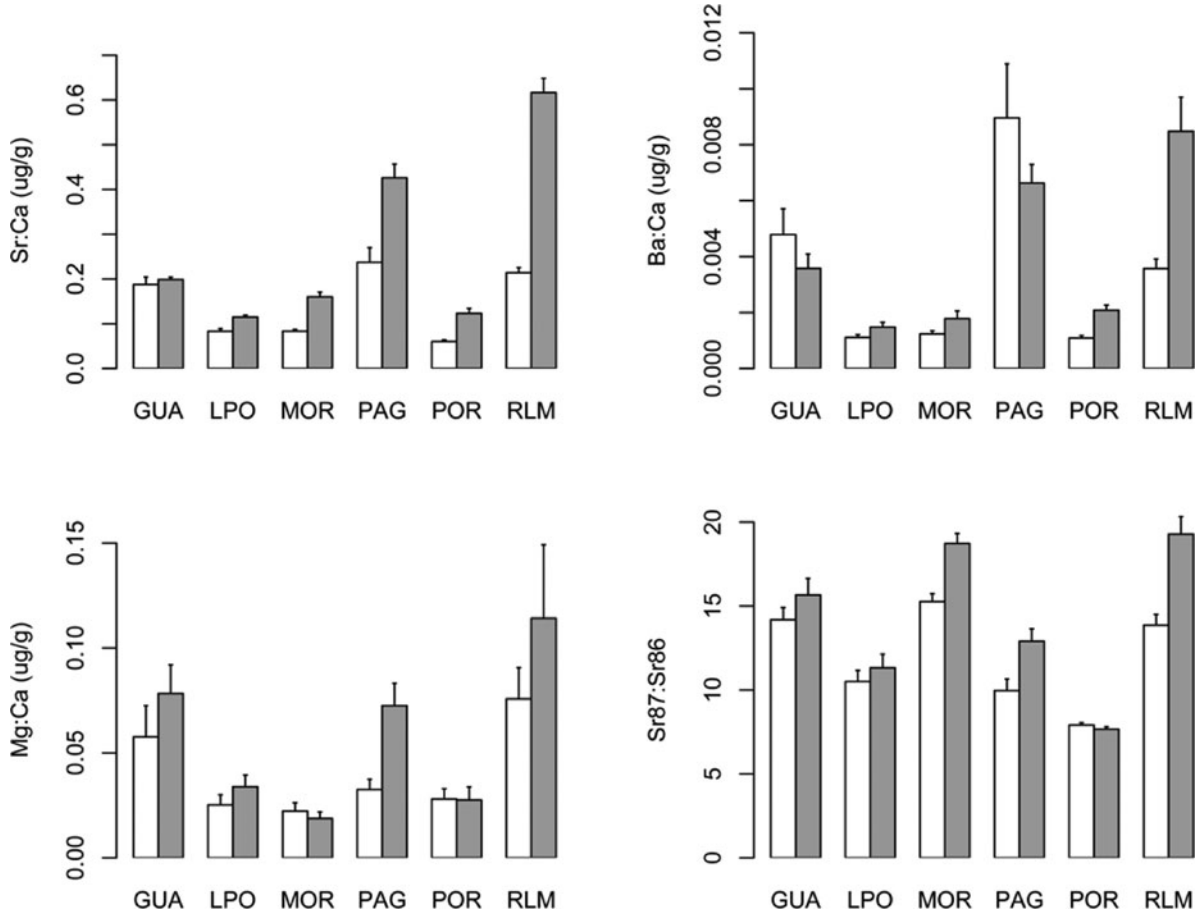

FIGURE 3. Elemental ratios from the core and edge of otoliths collected at each river. Site codes are specified in Figure 1. Error bars show the confidence interval. White bars represent core chemistry and grey bars represent edge chemistry.

MANOVA results indicate that otolith chemical signatures differed significantly among the six sites for both edge and core chemistry (Figure 2; Core Chemistry: Pillai's trace statistic = 1.6093, $F = 15.617$, df = 20, $P < 0.001$; Edge Chemistry: Pillai's trace statistic = 1.4683, $F = 13.456$, df = 20, $P < 0.001$). All individual element signatures were also significantly different among sites (Figure 3; Table 4). The most frequent misclassifications were of fish collected from two sites along the same river (Upper Portuguesa and Lower Portuguesa), where edge chemical signatures were similar. Based on chemical variation among otolith cores, a high proportion of individuals from each of the smaller, foothill rivers (Paguey, Las Marias, Upper Portuguesa, Morador) were classified by cross validation as sharing a natal site distinct from those of other populations. In contrast, fish from the larger, downstream rivers (Guanare, Lower Portuguesa) showed wide variation in natal site classifications (Figure 2; Table 3).

Microsatellite DNA

Our microsatellite markers showed high levels of genetic diversity (11–34 alleles per locus). Genotype data showed no significant evidence of gametic disequilibrium or deviation from Hardy–Weinberg proportions. In addition, there was no significant difference between observed and expected heterozygosity for microsatellite loci. Pairwise *F*st values indicate extremely low levels of population differentiation (Table 5).

Both Bayesian clustering analyses indicate that all populations comprise a single genetic group; there was no detectable population structure at the spatial scale of our study

TABLE 4. Results of ANOVA indicate differences among sites for each elemental signature in the otolith. Otolith core and edge results for the four elemental signatures are presented.

Elemental ratio	Otolith region	*F*-value	*P*-value
Sr:Ca	Edge	104.36	<0.001
Sr:Ca	Core	60.837	<0.001
Mg:Ca	Edge	13.668	<0.001
Mg:Ca	Core	7.085	<0.001
Ba:Ca	Edge	45.583	<0.001
Ba:Ca	Core	51.448	<0.001
Sr 87:86	Edge	26.179	<0.001
Sr 87:86	Core	24.44	<0.001

TABLE 5. Pairwise F_{ST} values for the four sites used in genetic analyses.

	Site			
Site	RLM	PAG	POR	MOR
RLM	0.00000			
PAG	0.00326	0.00000		
POR	0.00822	0.00946	0.00000	
MOR	0.00246	0.00696	0.00856	0.00000

(Figure 4). Analysis in STRUCTURE showed that all individuals are most likely drawn from a single genetic population ($K = 1$). GENELAND also indicated a single population ($K = 1$). These results indicated that gene flow in this species was sufficient to prevent genetic differentiation at the spatial scale of our collections.

DISCUSSION

Otolith chemical analyses suggest that most individuals from each collection site share a distinct, common natal site, but microsatellite genetic analyses did not detect any genetic differentiation or structure among populations of *P. mariae*. Based on otolith edge chemical data, we were able to correctly classify 68–80% of individuals to the river where they were collected, which is relatively high classification success compared to similar studies in other freshwater ecosystems (Feyrer et al. 2007). Differences in otolith edge chemistries among sites implied environmental heterogeneity within the system and gave confidence that otolith core chemistry might differ should different river populations exploit geographically different spawning sites. Our results show that a high proportion of individuals (70–81%) from the four upstream collection sites exhibit distinctive natal signatures (Table 3), implying that breeding areas for these stocks are largely distinct.

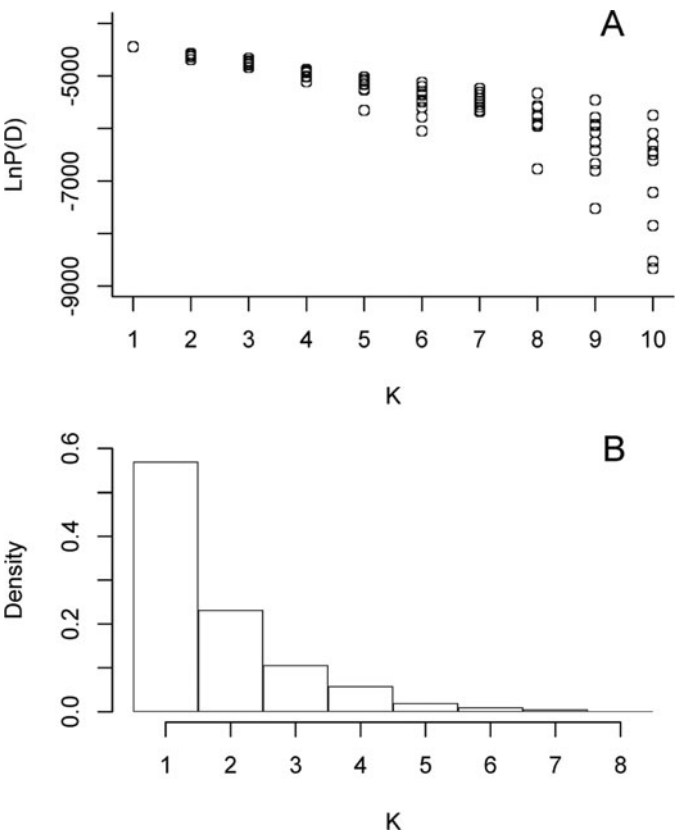

FIGURE 4. Results from genetic analysis in STRUCTURE and GENELAND. Panel **(A)** shows STRUCTURE results. Values on the *y*-axis are the estimated log of the probability of the data and show a relative estimate of the fit of the clustering solutions to the data for each number of genetic groups (K). Panel **(B)** shows results from genetic analysis in GENELAND. Each bar represents the proportion of MCMC iterations that inferred that number of genetic groups (K).

Despite the otolith chemistry evidence for distinct breeding areas, there was sufficient genetic mixture among populations to prevent population genetic differentiation. The simplest explanation for this pattern is that the level of mixing among populations overrides the genetic structure arising from spatial segregation of breeding. Our assignments based on QDFA indicate up to 30% mixing of individuals, i.e., up to 30% individuals were assigned to a different natal site than the site in which they were collected. If this level of mixing carries through to the breeding season, then it would be no surprise that *P. mariae* are effectively panmictic within the study region. In addition, since this species breeds following the downstream migration, interbreeding among populations may easily occur during passage downriver. *Prochilodus mariae* are iteroparous migrants, so individual fish also have multiple opportunities to spawn during the course of their lifetime, including the possibility that fish that return to a river that has different chemistry than their natal site may continue to associate with a nonnatal population and contribute to genetic mixing over many breeding seasons.

The data we collected for this study do not allow us to differentiate between these mechanisms for genetic homogenization, but future studies of *P. mariae* or other migratory fishes could collect data along a full ontogenetic transect of otoliths to investigate whether individual fish migrate to breed and feed in the same locations throughout their lives (Limburg et al. 2011). Additional sampling during the breeding season may also provide key information about whether population structure exists. If individuals return to breed in the same location every year, populations may have stronger genetic structure if they were sampled during the breeding season and a comparison of population structure before and after breeding could be very informative.

Both the spatial patterns inferred from otolith microchemistry and the lack of genetic structure should be important considerations for the management of the heavily exploited *P. mariae* fishery. Because at least some interbreeding occurs among populations, migrants from nearby populations could help to maintain genetic diversity and breeding populations in the face of heavy exploitation. However, the dominance of spatial segregation of breeding sites evident from otolith chemistry indicates that while there is enough mixing to prevent genetic

differentiation, it is probably too little to support a heavily harvested population by immigration alone. Thus, severe overfishing or habitat destruction in either breeding or feeding areas used by each stock is likely to strongly diminish its viability.

Our results suggest that tracer methods, such as otolith microchemical analyses, are especially valuable in systems where there is little or no population structure. For *P. mariae*, the molecular markers we used did not allow us to detect low levels of population structure. The limited geographic scale of our study did not allow a complete assessment of genetic structure for the species, but other studies also found low levels of population structure in prochilodontids. For instance, a case study of *Prochilodus costatus* (Carvalho-Costa et al. 2008) and a broad review of migratory fish biogeography in South America (Turner et al. 2004) both indicated that individual prochilodontid movements are very widespread and populations are genetically homogenous. In addition, our sampling included one river (Rio Paguey) that is disconnected from the remainder of the sites by the Rio Apure (a large tributary to the Orinoco River). Nevertheless, fish from the Rio Paguey were part of the same genetic group as the other sites. Otolith chemical data allowed us to detect population structure on the spatial scale of our study sites even though no genetic differences existed.

Relatively few studies have used both otolith and genetic markers in concert to study migratory fish population biology, but some have gleaned additional insight from using both methods (Miller et al. 2005; Baerwald et al. 2007; Feyrer et al. 2007; Bradbury et al. 2008; Barnett-Johnson et al. 2010; Woods et al. 2010). In other systems, coupled otolith and genetic approaches have mostly produced complementary results; specifically, genetic and otolith approaches both indicate that population boundaries occur in the same location, with few exceptions (Thorrold et al. 2001).

Our analysis of *P. mariae* suggests that different markers may indicate different levels of connectivity: we did not detect genetic structure despite identifying geographic structure using otolith chemical markers. The lack of genetic structure in our system does not necessarily contradict otolith chemical results but does indicate that it may be necessary to consider both ecological connectivity (e.g., otolith chemistry) and evolutionary connectivity (e.g., microsatellites) to understand systems with extremely low levels of population structure. Other coupled methodological combinations besides otolith and genetic markers also show promise for understanding animal movements; previous studies have used genetic and physiological markers to provide insight into fish migrations (Cooke et al. 2008), and others have coupled stable isotope markers with genetics to understand bird and butterfly migrations (Rubenstein and Hobson 2004).

Our results provide basic information on the population biology of one important migratory Neotropical species, but additional study is needed to effectively manage and conserve *P. mariae* and other migratory Neotropical fishes in the face of habitat destruction and overharvest (Barbarino Duque et al. 1998; Mateus et al. 2004). Population structure has been strong and easy to detect for some South American species (Pereira et al. 2009), but most genetic analyses have identified high levels of genetic diversity and very low levels of genetic structure (Hatanaka et al. 2006; Carvalho-Costa et al. 2008; Calcagnotto and DeSalle 2009; Iervolino et al. 2010). Despite the ubiquity and utility of variable genetic markers to analyze population structure (Hudson 2008), our results suggest that genetic data should not be treated as definitive when no population structure is detected. Coupling direct methods of tracking individual animals (e.g., telemetry) with indirect methods of assessing population connectivity (e.g., genetics) is likely to produce robust information about population structure that will be appropriate for the limited temporal and spatial scales of many management efforts. In the case of *P. mariae*, populations are managed by local communities on a small scale, so understanding whether populations are connected may have important implications for whether local stewardship and resource management will actually result in a more sustainable fishery. Our case study of *P. mariae* demonstrates the complex nature of understanding population structure.

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