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GENETIC CONNECTIVITY OF MULLETS (*Mugil liza*) BETWEEN RODRIGO DE FREITAS LAGOON AND A CONSERVATION UNIT IN STATE OF RIO DE JANEIRO, SOUTHEASTERN BRAZIL

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Abstract: We analyzed the genetic connectivity between mullets (*Mugil liza*) captured around the protected Natural Monument of Cagarras Islands (MoNa Cagarras) and inside Rodrigo de Freitas Lagoon, using microsatellite markers polymorphisms. Our data revealed the occurrence of 31 shared alleles (from 41 sampled), a high similarity in both allelic frequencies and genetic diversity and lack of differentiation between collection points ($F_{ST} = 0.000$, $p > 0.05$, STRUCTURE best estimative K = 1), results which, analyzed together, are strongly indicative of panmixia. We conclude that individuals collected inside the Rodrigo de Freitas Lagoon are genetically similar to those individuals collected around MoNa Cagarras. Given the importance of estuaries for the reproduction and development of individuals of *M. liza*, it is recommended that the Rodrigo de Freitas Lagoon to be managed in order to maintain genetic connectivity and diversity between the two ecosystems.

Keywords: coastal ecosystem; gene flow; microsatellite

The mullet *Mugil liza* (Mugiliformes: Mugilidae) is a widely distributed pelagic species of the Atlantic Coast in South America, occurring from Venezuela to Argentina (Menezes *et al.* 2010, Siccha-Ramirez *et al.* 2014). The species is mainly related to sheltered and coastal waters with a predominant (but not mandatory) migratory behavior to the open sea at the spawning time (Vieira 1991, Fortunato *et al.* 2017). Most of the individuals enter estuarine waters and uses it as a nursery, remaining in this habitat until the recruitment for adult population (Vieira *et al.* 2008, Potter *et al.* 2013, Garbin *et al.* 2014), but some individuals may spend part or whole life exclusively in estuaries or in open sea (Fortunato *et al.* 2017). This is a very important fisheries resource for local fishermen in the municipality of Rio de Janeiro since it is one of the most abundant fisheries inside Rodrigo de Freitas Lagoon (Andreata *et al.* 2002).

Genetic markers have been used as an effective tool to improve the understanding of important

aspects of population dynamics and structure, as well as the evolutionary processes and genetic drift of fish species (Cuéllar-Pinzón *et al.* 2016). Through the analysis of genetic variation, it is possible to analyze migration events, the contribution of each population to the formation of genetic stocks and connectivity between meta-populations (Kasapadis *et al.* 2012). In Brazil, previous genetic analysis indicates the existence of two distinct populations of *M. liza* along the coast: 1) One population restricted to the state of Rio de Janeiro; and 2) another population distributed (at least) from the State of São Paulo (Brazil) through Argentina (Mai *et al.* 2014).

The "Monumento Natural do Arquipélago das Ilhas Cagarras – MoNa Cagarras" (Natural Monument of Cagarras Island Archipelago) is a fully protected conservation unit (UC) that consists of four islands (Cagarras, Palmas, Comprida and Redonda) and the islets Filhote da Cagarras and Filhote da Redonda (Figure 1). The purpose of the UC is to preserve the remnants of the Atlantic Forest domain ecosystem, the refuges and nesting areas of migratory seabirds, and the local scenic beauty (Brasil 2020). Around the islands water depths reach 20 m and are abundant in fish and a closed area for fishing (Bertoncini *et al.* 2013). This closed area is recognized as important in maintaining fisheries abundance near this area (Amorim & Monteiro-Neto 2016).

The Rodrigo de Freitas Lagoon (RFL) is an important ecosystem of Rio de Janeiro (Figure 1) to reproduction, nutrition, and growth of several fish species. It is also used for commercial fishing, tourism, leisure, and water sports activities (Gonzalez *et al.* 2006). Rodrigo de Freitas Lagoon had its origin in the drowning of old fluvial basins generated by transgressive–regressive variations of sea level that occurred in the past 6,000 years (Amador 1997). Currently, the artificial channel called "Jardim de Alah" that was constructed in the 1920s makes the connection between RFL and the open sea. However, for a long time, the lagoon was an enclosed area with sporadic sea connections only at times of rising tide. This long-term isolation between RFL and the Ocean may have led to the emergence of two genetically

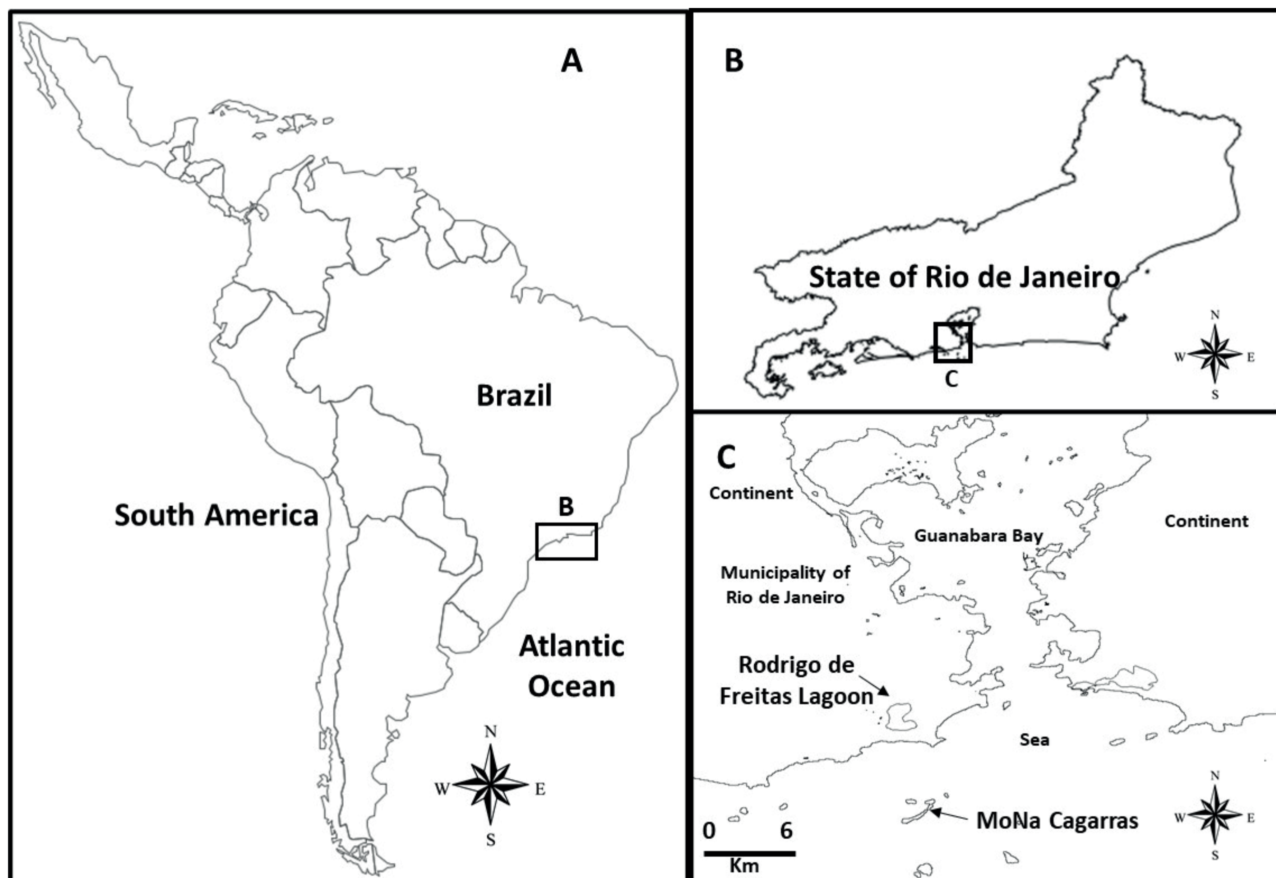

Figure 1. Map of South America (A), Rio de Janeiro State (B) and municipality of Rio de Janeiro (C) showing sampling locations of *M. liza*.

distinct mullet populations. Another possible scenario could be that frequent fluctuations in mortality rate, due to pollution in the lagoon, would lead to a rapid differentiation between two areas. Based on these premises, this study aimed to evaluate the genetic connectivity degree between mullets collected inside LRF and in the near areas of MoNa Cagarras.

Muscle tissue samples were obtained from fish captured between August and September 2018 in RFL (N = 28) and in areas near MoNa Cagarras (N = 34). These samples were stored in 70 % ethanol or frozen immediately after collection for further processing. DNA extraction was performed according to the saline extraction protocol (modified from Miller *et al.* 1988). The extracts were quantified in nanodrop and high-quality DNA was stored at -20 °C. Polymerase Chain Reaction (PCR) assays were performed using heterologous primers developed for a congeneric species. Seven heterologous microsatellite primers (Miggiano *et al.* 2005, Shen *et al.* 2010) were selected and used in PCR amplification. In order to genotype the microsatellites, we employed the method described in Schuelke (2000). PCR reactions were performed using 1U Taq DNA polymerase (Sinapse), 0.2 mM of each dNTPs, 0.5 µM of each primer, 2 mM $MgCl_2$, 1X PCR buffer and 1 µL DNA (30 ng/µL), in a final volume of 15 µL. Amplification conditions were: an initial cycle at 94 °C for 5 minutes (min), followed by 43 cycles (35 amplification cycles followed by 8 cycles for incorporation of fluorescence) from 1 min at 94 ºC, 1 min at 53 ºC and 1 min at 72 ºC. An additional extension cycle was 72 °C for 30 min. Amplified products were detected by 1 % (v/v) agarose gel electrophoresis in 0.5X TBE buffer, stained with ethidium bromide and visualized under UV light. Genotyping was performed on a 3130xl automatic sequencer with GeneScan 500 (Life technologies) as size marker.

The resulting electropherograms were analyzed using the GeneMarker software (SoftGenetics LLC - free trial version) in which the genotypes were manually determined. Descriptive statistics (i.e., gene frequencies, number of alleles, accordance with Hardy-Weinberg Equilibrium, and linkage disequilibrium) were calculated in FSTAT 2.9.3 (Goudet 2001). To assess the occurrence of problems inherent to genotyping was used the Micro-Checker (Van Oosterhout *et al.* 2004). The pairwise F_{ST} fixation index calculation was performed using Arlequin 3.5 program (Excoffier & Lischer 2010). A correspondence factorial analysis (AFC) was made to visualize the distribution of genetic variation among individuals in a multidimensional space in the Genetix 4.05 (Belkhir *et al.* 2002). The genetic structure was also analyzed with a Bayesian analysis as implemented in the STRUCTURE (Pritchard *et al.* 2000). Analyses were performed with one million Monte Carlo Markov Chain (MCMC) steps, discarding the first 20 % of iterations as burn-in. Each analysis was repeated 5 times for each simulated value of K, which ranged from 1 to 2 groups. Structure Harvester was used to infer the most likely number of clusters (K) (Earl & Von Holdt 2012).

Among the seven markers used, three were monomorphic and were excluded from further analysis. None of the sampled loci showed any evidence of amplification artifacts, Hardy-Weinberg Equilibrium deviations, or linkage disequilibrium between loci. The analyzed data revealed the occurrence of 41 alleles of which 31 are shared among individuals sampled from both locations. A high similarity in allelic frequencies (Figure 2) and genetic diversity (Table 1) was observed among the samples.

The F_{ST} structuring index showed genetic differences between individuals from the two locations (F_{ST} = 0.000, $p > 0.05$). High genetic similarity revealed by Bayesian partition (K = 1) (Figure 3) and AFC (Figure 4), is indicative of a consistent panmixia scenario.

The genetic data allow the conclusion that the individuals collected inside the Rodrigo de Freitas Lagoon are genetically very similar to those individuals collected around MoNa Cagarras. Thus, we may conclude that mullets can migrate between MoNa Cagarras and Rodrigo de Freitas Lagoon and, given the importance of estuaries for the reproduction and development of individuals of the species, it is recommended that the Lagoon be managed in order to maintain genetic connectivity and diversity between the two ecosystems, what could be relevant in maintaining the region's fishing stock and in the conservation of the species.

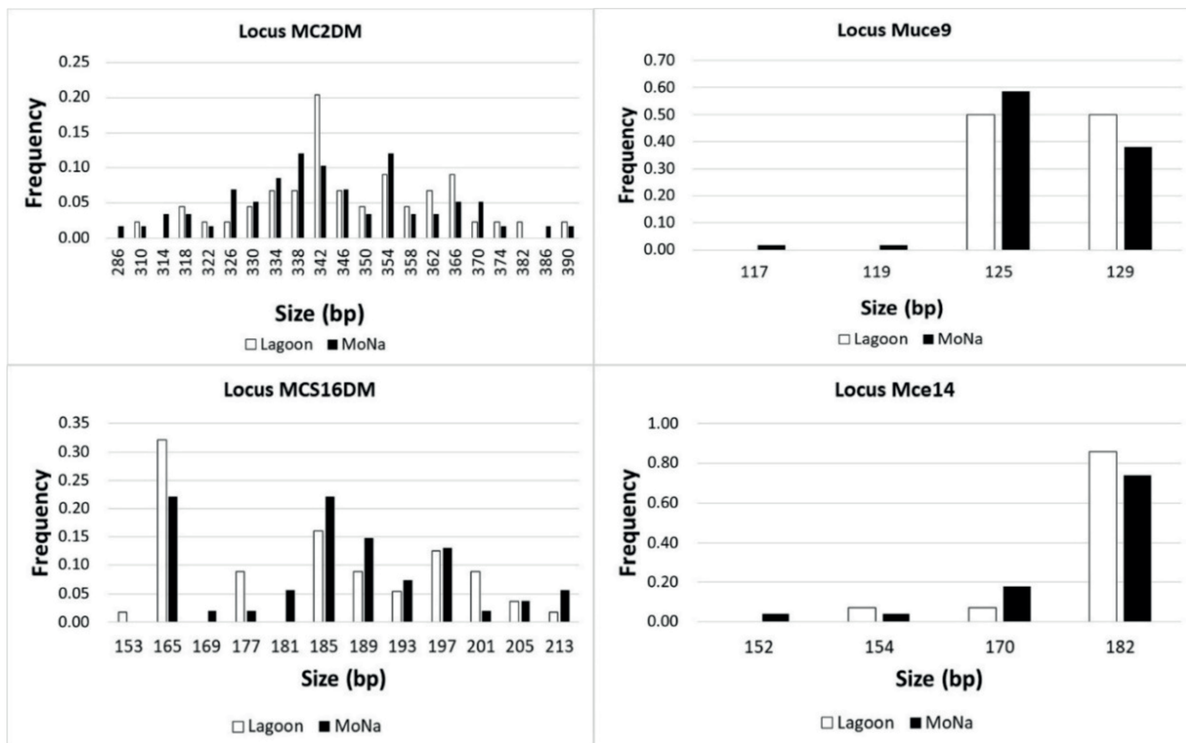

Figure 2. Allele frequencies from four sampled microsatellite *loci*. White bars represent allele frequencies in individuals from the Rodrigo de Freitas Lagoon and Black bars represent allele frequencies in individuals from the MoNa Cagarras. Alleles are labelled according to their size in number of base pairs (bp).

Table 1. Genetic diversity per *locus* and sampling location. N_A: Number of alleles. Number of analyzed individuals: Rodrigo de Freitas Lagoon (N=28); MoNa Cagarras (N=34).

Locus	Rodrigo de Freitas Lagoon		MoNa Cagarras	
	Gene diversity	N_A	Gene diversity	N_A
MC2DM	0.937	18	0.946	20
Muce9	0.505	2	0.524	4
Mcs16d	0.843	10	0.867	11
Mce14	0.369	3	0.428	4

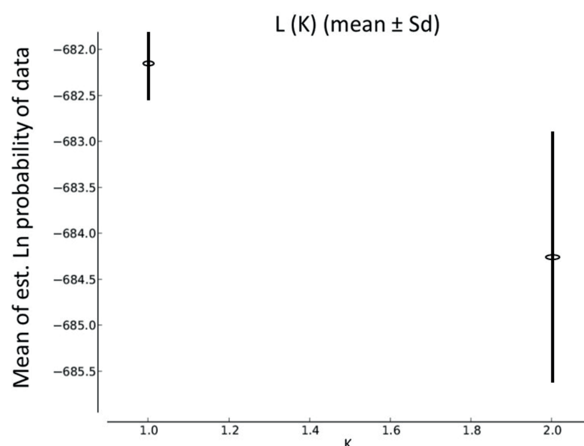

Figure 3. Log-likelihood values of K from K = 1 to K = 2 obtained from Bayesian Clustering analysis implemented in STRUCTURE.

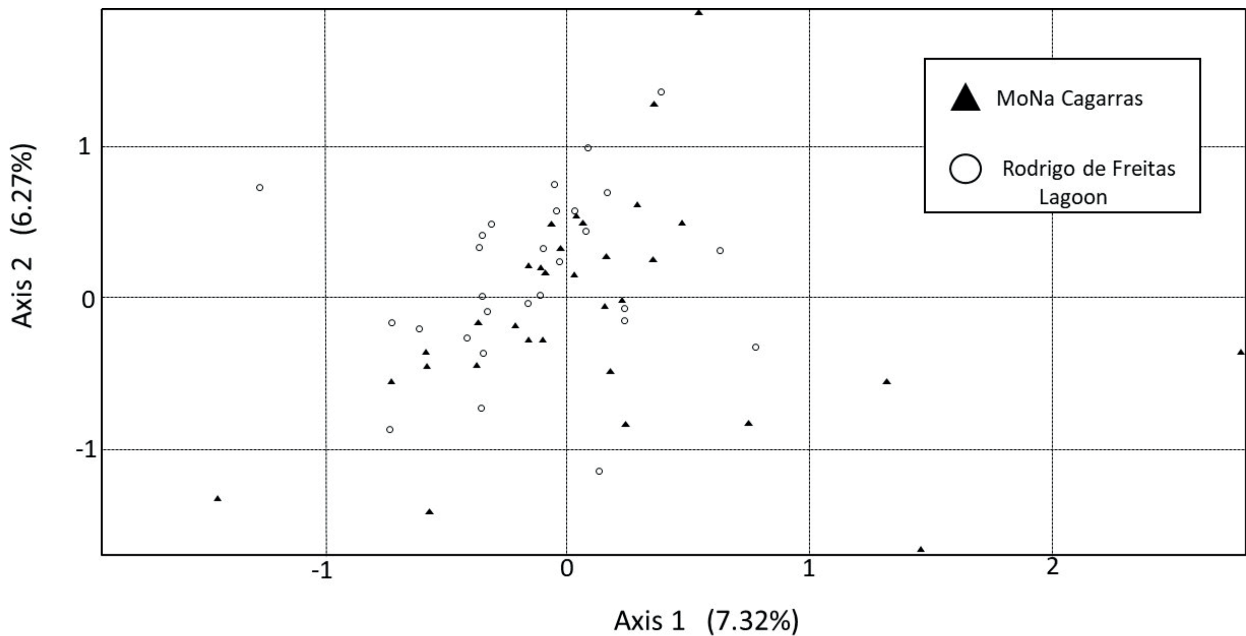

Figure 4. Scatter plot from Factorial Analysis of Correspondence of each individual. Individuals from Rodrigo de Freitas Lagoon are presented as white circles and individuals from MoNa Cagarras are presented as black triangles.

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