Fisheries Research xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Fisheries Research

journal homepage: www.elsevier.com/locate/fishres

Research Paper

Genetic differentiation in populations of lane snapper (*Lutjanus synagris* – Lutjanidae) from Western Atlantic as revealed by multilocus analysis

Danillo Silva[a], Kely Martins[a], Joiciane Oliveira[b], Raimundo da Silva[a], Iracilda Sampaio[b], Horacio Schneider[b], Grazielle Gomes[a,*]

[a] *Laboratory of Applied Genetics, Institute of Coastal Studies, Universidade Federal do Pará, Campus Bragança, Alameda Leandro Ribeiro, S/N, Aldeia, Bragança, Pará, Brazil*

[b] *Laboratory of Genetics and Molecular Biology, Institute of Coastal Studies, Universidade Federal do Pará, Campus Bragança, Alameda Leandro Ribeiro, S/N, Aldeia, Bragança, Pará, Brazil*

ARTICLE INFO

Handled by George A. Rose

Keywords:
Gene flow
Brazilian coast
Marine fishes
Nuclear DNA

ABSTRACT

Population genetic structure, genetic diversity and molecular demography of lane snapper *Lutjanus synagris* were assessed using mitochondrial DNA (control region, cytochrome b and NADH Dehydrogenase, subunit 4) and two nuclear introns (intron 2 of the S7 ribosomal protein gene – S7-2, and intron 6 of the L3 ribosomal protein gene – RPL3). Here, we collected specimens from Brazilian and Colombian coasts. In addition, we downloaded available sequences from prior studies conducted in Caribbean and US waters. Population genetic structure analysis did not detect any genetic substructure signal along the Brazilian coast. However, comparisons between Brazilian and Colombian samples provided a significant and high level of genetic subdivision, probably due to the freshwater outflow of the Amazon. Our data also showed a higher genetic diversity index (both mtDNA and nuDNA) in the Colombian populations compared to Brazil. It is should be probably associated with the environmental changes and historical population size differences between these regions. Also, was detected an asymmetric gene flow from Brazil to Colombia, which can explain the high genetic diversity found in the last region, besides being probably, an older and stable population. Bayesian skyline plots clearly indicated that Brazilian population has experienced a historical demographic expansion, which probably began after Last Glacial Maximum (LGM). On the other hand, our analysis did not detect any demographic change signal for the Colombian population in this period. Our analysis based on a combined dataset indicates that the Atlantic *L. synagris* populations are interconnected in different levels through this region. While some comparisons have showed no significant difference over hundreds or thousands of kilometers, there were strong population differentiation signals between other localities considering a similar geographical distance.

1. Introduction

Many populations of marine species display little or no genetic differentiation over large geographic distances (Hauser and Carvalho, 2008). In general, this pattern arises from the absence of physical barriers and current circulation, thus determining high levels of gene flow among populations. Furthermore, several taxa are characterized by long periods of pelagic larval stage that might favor the dispersal throughout their geographic range (Waples, 1998). Indeed, phylogeographic patterns and the genetic structure of many marine species are correlated with oceanic features such as currents (White et al., 2010) and strongly influenced by historical and biological features of each taxon.

Climate changes during Pleistocene period have deeply affect the genetic diversity and the phylogeographic pattern of several vertebrates, including marine fishes (Avise et al., 1998). Nonetheless, the role of paleoclimatic effects in the genetic structure of several coastal fish species remains poorly understood, because of the scarcity of phylogeographic studies, particularly in South Atlantic region (Beheregaray, 2008).

The lane snapper *Lutjanus synagris* is a widespread Lutjanidae fish (Perciformes) from the Western Atlantic. It represents a major fishery resource (Menezes and Figueiredo, 1980; Pinto et al., 2013) along its geographic range from North Carolina (USA) to southeastern Brazil (Allen, 1985). Similarly to other snappers, this species usually forms spawning aggregations off the coast, releasing egg batches and larvae with pelagic duration (PLD) of about 30 days (Claro and Lindeman, 2008; Lindeman et al., 2000). Besides the reproductive migration,

* Corresponding author.
E-mail address: graziellefeg@gmail.com (G. Gomes).

http://dx.doi.org/10.1016/j.fishres.2017.10.005
Received 15 March 2017; Received in revised form 27 September 2017; Accepted 10 October 2017

mature individuals are found in reef sites and nearby waters on the continental shelf, while the juveniles inhabit coastal and shallow regions such as estuaries and bays (Riveira-Arriaga et al., 1996). Some life history traits of lane snapper suggest that genetic connectivity among its populations can be expected (e.g. PLD of 30 days, large population size and broadcast spawning). However, few studies about population genetics in this species are restricted to North Atlantic. Using microsatellites and a 590-bp fragment of the mitochondrial NADH dehydrogenase subunit 4 (ND4) gene, (Karlsson et al., 2009) showed two genetic stocks of *L. synagris* from the Gulf of Mexico (western group) and Florida coast (eastern group). Based on the same molecular markers, (Gold et al., 2011) revealed significant genetic divergence between populations of lane snapper from Florida and Caribbean islands. Both studies reported historic declines in effective population size, characterized by low levels of genetic diversity.

Even though *L. synagris* is highly exploited for fisheries, the genetic structure of populations from Western South Atlantic remains unknown, restraining the development of efficient management policies of local or regional stocks. Therefore, the present study aimed for the first time the genetic structure and variation among populations of *L. synagris* from South Atlantic in order to infer their genetic connectivity as well as aspects of demographic history. To provide a reliable phylogeographic scenario, we carried out a multilocus approach based on three mitochondrial (cytochrome b – Cyt b, NADH Dehydrogenase, subunit 4 – ND4, and control region – CR) and two nuclear (intron 2 of the S7 ribosomal protein gene – S7-2, and intron 6 of the L3 ribosomal protein gene – RPL3) markers.

2. Material and methods

2.1. Ethics statement

All the tissues were obtained from dead individuals on commercial landings in the localities mentioned above. During the sampling, *L. synagris* was not endangered or protected. Therefore, there was no need to apply for a license for collection or approval by the Animal Ethics Committee.

2.2. Sampling

A total of 280 specimens of *L. synagris* were collected from fish docks and commercial local markets in eight sites along the coast of Brazil and Colombia from 2001 to 2014 (Fig. 1). Only individuals with reliable data about geographic origin were included in the present study. The morphological identification was based on the available literature (Allen, 1985; Cervigón, 1993; Menezes and Figueiredo, 1980).

The biological samples (fragments of tongue, muscle or fins) were stored in 95% ethanol at −4 °C.

2.3. Laboratory procedures

The total genomic DNA was isolated using the Wizard Genomic DNA Purification kit (Promega) or the phenol-chloroform protocol (Sambrook and Russell, 2001). The DNA quality was verified by electrophoresis in 1% agarose gel. The mitochondrial (mtDNA) and nuclear DNA (nuDNA) fragments were amplified via Polymerase Chain Reaction (PCR) using the primers Dloop-A and Dloop-G (Lee et al., 1995) for CR, FishCytB-F and TruckCytB-R (Sevilla et al., 2007) for Cyt-b; NAP-2 (Arevalo et al., 1994) and ND4LB (Bielawski and Gold, 2002) for ND4, S7RPEX2F and S7RPEX3R (Chow and Hazama, 1998) for S7-2, and RPL35F and RPL36R (Pinho et al., 2010) for RPL3.

Each PCR reaction comprised 2.5 μL of dNTP (1.25 mM), 1.5 μL of buffer (10x), 0.6 μL of $MgCl_2$ (25 mM), 0.6 μL of each primer (50 ng/μL), 0.6 μL of template DNA, 0.1 μL of Taq DNA polymerase (5U/μL) and ultrapure water to complete a volume of 15 μL. The PCR amplification conditions was performed by a first denaturation step at 94 °C for 4 min, followed by 35 cycles at 94 °C for 30 s, 55 °C (mtDNA) or 60 °C (nuDNA) for 35 s, and 72 °C for 1 min plus a final extension at 72 °C for 3 min.

At the following steps, each positive PCR was purified in 20% polyethylenoglicol according to (Paithankar and Prasad, 1991). Then, the purified fragments were submitted to sequencing reaction based on the dideoxynucleotide method (Sanger et al., 1977) using the BigDye Terminator v3.1 Cycle Sequencing Ready Reaction kit (Applied Biosystems). After precipitation, the samples were sequenced automatically using ABI 3500XL Genetic Analyzer sequencer (Applied Biosystems). To nuclear markers, the DNA fragments were sequenced bidirectionally to verify the presence of heterozygous.

2.4. Dataset and analysis of genetic variation

The sequences were checked and aligned using the software BioEdit (Hall, 1999), then, organized in datasets according to each marker. The reconstruction of nuclear haplotypes was performed using the algorithm PHASE (Stephens et al., 2001), available in DnaSP software (Librado and Rozas, 2009). The runs comprised 1000 burn-in iterations and 1000 main iterations. Only haplotypes with a posteriori probability values higher than 0.7 were used a posteriori.

The DnaSP was performed to identify and estimate the haplotype (or alleles, for nuclear data) frequencies and the number of polymorphic sites. The levels of polymorphism for each marker were calculated according to the haplotype (h) and nucleotide (π) diversity indexes by (Nei, 1987), available in the software ARLEQUIN v. 3.5 (Excoffier and Lischer, 2010).

Moreover, to provide a wide analysis of genetic structure and diversity in *L. synagris*, we combined ours generated ND4 sequences with those reported by Karlsson et al. (2009) and Gold et al. (2011) and stored in the GenBank (codes EU025755-EU025734, EU676011-EU676018, and HM369112-HM369131). Thus, the ND4 database comprised 503 sequences, encompassing samples of *L. synagris* from most of their range along Western Atlantic.

2.5. Genetic structure and phylogeography

The analysis of molecular variance (AMOVA) was assessed to estimate the components of genetic variance within and among populations of *L. synagris*, knowing that it allows to test the population genetic structure based on frequency and numbers of mutations among haplotypes (Excoffier et al., 1992). A first one parameter of AMOVA was carried out independently for each marker (five runs). To this analysis, it was considered only samples from Brazil and Colombia and all collection sites along that region into as a single group. A second one parameter of AMOVA joined all datasets available in Atlantic Ocean for ND4 gene (i.e. Brazil, Colombia, Florida, Gulf of Mexico and Puerto Rico). Here, we also regarded all samples as four groups. The significance of fixation indexes (Φ_{ST}) was obtained after 10,000 non-parametric permutations for both cases.

Levels of genetic differentiation among the collection sites were estimated by two pairwise F_{ST} parameters according to (Weir and Hill, 2002), using 10,000 bootstrap permutations and a significance level of 0.05. First, comparisons of pairwise (F_{ST}) were made for all samples from South America (Brazil and Colombia) and independently markers. Second, comparisons of pairwise (F_{ST}) for all Atlantic Ocean (Brazil, Colombia, Puerto Rico, Florida and Gulf of Mexico) samples using a ND4 gene dataset (or data plus data from GenBank). Here, we pooled some collection sites based on results from previous studies Karlsson et al. (2009) and Gold et al. (2011). That way, five populations were considered: Brazil, Colombia, Florida, Gulf of Mexico and Puerto Rico (that included the islands Saint Thomas and Saint Croix). Both analysis above described were inferred using ARLEQUIN v. 3.5 (Excoffier and Lischer, 2010).