Estuarine, Coastal and Shelf Science 149 (2014) 80–86

Contents lists available at ScienceDirect

Estuarine, Coastal and Shelf Science

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

Microsatellite variation and genetic structuring in *Mugil liza* (Teleostei: Mugilidae) populations from Argentina and Brazil

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ARTICLE INFO

Article history:
Received 31 March 2014
Accepted 20 July 2014
Available online 1 August 2014

Keywords:
gene flow
genetic diversity
mullet
SSR markers
fisheries management
migratory fishes

ABSTRACT

The mullet *Mugil liza* is distributed along the Atlantic coast of South America, from Argentina to Venezuela, and it is heavily exploited in Brazil. We assessed patterns of distribution of neutral nuclear genetic variation in 250 samples from the Brazilian states of Rio de Janeiro, São Paulo, Santa Catarina and Rio Grande do Sul (latitudinal range of 23–31°S) and from Buenos Aires Province in Argentina (36°S). Nine microsatellite loci revealed 131 total alleles, 3–23 alleles per locus, *He*: 0.69 and *Ho*: 0.67. Significant genetic differentiation was observed between Rio de Janeiro samples (23°S) and those from all other locations, as indicated by F_{ST}, hierarchical analyses of genetic structure, Bayesian cluster analyses and assignment tests. The presence of two different demographic clusters better explains the allelic diversity observed in mullets from the southernmost portion of the Atlantic coast of Brazil and from Argentina. This may be taken into account when designing fisheries management plans involving Brazilian, Uruguayan and Argentinean *M. liza* populations.

1. Introduction

The mullet *Mugil liza* Valenciennes, 1836 is a pelagic fish distributed along the Atlantic coast of South America, from Venezuela to Argentina (Menezes et al., 2010; Siccha-Ramirez et al., 2014). This mullet is commercially exploited in Brazil and Argentina, where peak catches can surpass 18,000 tons/year (González-Castro et al., 2009; MPA, 2011). Commercial catches in Brazil occur especially between May and August, following reproductive migration (Miranda and Carneiro, 2007; MMA, 2007; Vieira et al., 2008) and fishes are caught mainly during migration (Lemos et al., 2014). This resource, shared with Argentina and Uruguay, was declared overexploited by the Brazilian Ministry of Environment a decade ago (MMA, 2004). Although fishing rules do exist for regulating the exploitation of this species (IBAMA, IN N° 171/2008) there is still a lack of information on the dynamics and structure of mullet populations in Brazil (Garbin et al., 2014; Lemos et al., 2014) and no plan is available for responsible managing of this resource.

Mugil liza is a marine migrant estuarine-dependent fish (sensu Potter et al., 2013) marine single spawner (Albieri and Araújo, 2010; González-Castro et al., 2011; Lemos et al., 2014). The spawning areas for *M. liza* remains uncertain, but in South of Brazil evidences from artisanal and purse seine fisheries appoint the area between the North of Rio Grande do Sul State and North of Santa Catarina State (Brazil) as the main spawning area for the southern

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http://dx.doi.org/10.1016/j.ecss.2014.07.013
0272-7714/

populations (Vieira and Scalabrin, 1991; Garbin et al., 2014; Lemos et al., 2014). After spawning, marine currents carry the juveniles to enter the estuaries (Vieira, 1991) and at 5–6 years old mullets are recruited to the adult population and reproduce once a year (Garbin et al., 2014; Lemos et al., 2014). Thus, given their reproductive and ecological characteristics are expected that mullets will be composed of discrete subpopulations of adults that exchange migrants through the pelagic larval phase (Durand et al., 2012).

Over recent years, there has been considerable debate on the taxonomy of *Mugil liza* (Cousseau et al., 2005; González-Castro et al., 2008, 2009, 2011, 2012; Menezes et al., 2010; Durand et al., 2012; Sicha-Ramirez et al., 2014). This debate is fueled with the increasing accumulation of ecological, genetic and morphological evidence supporting the classification of *M. liza* as a single species (Albieri and Araújo, 2010; Menezes et al., 2010; Sicha-Ramirez et al., 2014). However, as proposed by González-Castro et al. (2012), different populations of *M. liza* would occur along the species distribution range from Cuba to Argentina.

Aspects of population dynamics, evolutionary processes, migration, genetic drift, effective population size and sex-biased dispersal in fishes are described by both mitochondrial DNA (mtDNA) and nuclear markers (e.g. microsatellites) (Avise, 1994; Durand et al., 2013). Both types of markers differ in their mutational rates and inheritance mode; therefore, they could concurrently contribute to describe a complete picture of ongoing evolutionary processes and to better characterize the demographic history of natural fish populations (Durand et al., 2013). Mitochondrial DNA markers picture the more ancient history of populations and evolutionary processes (e.g. speciation), while nuclear markers are used to describe contemporary processes and resolving population structure on a finer scale (Goudet et al., 1996; González-Castro et al., 2012; Durand et al., 2013; but see Karl et al., 2012). To date, however, most genetic studies conducted in populations of *Mugil* spp. inhabiting the Atlantic coast of South America have primarily used mitochondrial markers (Fraga et al., 2007; Heras et al., 2007; Aurelle et al., 2008; Heras et al., 2009; Sicha-Ramirez et al., 2014). In addition, to the extent of our knowledge, and despite their utility, microsatellites have not been employed yet to study South American *Mugil liza*. There is, therefore, an apparent need of more studies using microsatellites to explore patterns of diversity at regional or fine geographic scales that could provide useful information on the dispersal abilities of mullets (Whitfield et al., 2012).

In this study, we investigate the patterns of distribution of nuclear genetic diversity in populations of *Mugil liza* inhabiting an area under strong fishing pressure at the southernmost distribution range of the species in South America. Our results indicate significant differentiation between Niterói (RJ) mullets and other samples from Southern Brazil and one from Argentina. We discuss our findings in the light of the species' dispersal and migratory abilities and of contemporary barriers to gene flow.

2. Material and methods

2.1. Sampling details

Fifty *Mugil liza* were sampled from May to September 2011 at each of four sites on the Brazilian Atlantic coast, Niterói (Rio de Janeiro State), Ubatuba (São Paulo State), Laguna (Santa Catarina State) and Rio Grande (Rio Grande do Sul State), and at one site on the Argentinean Atlantic coast, Lavalle, Bahía Samborombón (Buenos Aires Province) (Table 1; Fig. 1). Fishes were captured by commercial fishermen using gill nets (70–140 mm mesh size, opposing knots) and were transported on ice to the laboratory; a 5 mm^2 tissue sample was taken from near the caudal fin and stored in 100% ethanol at −4 °C until processing.

Table 1
Information on mullets (*Mugil liza*) analyzed in this study: names and abbreviation of sampling sites (four at the Brazilian Southern Atlantic coast and one in Argentina), geographical coordinates, number of specimens sampled (*N*), mean observed heterozygosity (H_O), mean expected heterozygosity (H_E), and average inbreeding coefficient (F_{IS}) overall nine microsatellite loci are shown.

Country	Sample (abbreviation)	Geographical coordinates	*N*	Mean H_O	Mean H_E	F_{IS}
Brazil	Niterói (RJ)	S22°58′23″; W42°49′41″	50	0.716	0.716	0.177
Brazil	Ubatuba (SP)	S23°27′53″; W44°59′12″	50	0.654	0.694	0.180
Brazil	Laguna (SC)	S28°27′14″; W48°49′01″	50	0.655	0.682	0.054
Brazil	Rio Grande (RS)	S31°58′34″; W52°7′26″	50	0.693	0.699	−0.115
Argentina	Lavalle (BsAs)	S36°10′37″; W57°1′36″	50	0.669	0.685	0.161

2.2. DNA isolation and microsatellites' amplification

Total genomic DNA was extracted from tissue samples using a standard phenol-chloroform procedure (Sambrook et al., 1989) and dissolved in 60 μL of TE buffer. A set of nine microsatellite loci previously described for the "*Mugil cephalus*" complex were used to genotype each sample: Muce-9 (Xu et al., 2010), Mcs17FM, Mcs16DM, Mcs2DM (Miggiano et al., 2005) Mce-4, Mce-11, Mce-14, Mce-24 and Mce-27 (Shen et al., 2010). Polymerase Chain Reaction (PCR) amplifications were performed in a final volume of 12.5 μl containing: 10–50 ng of DNA, 1X PCR buffer, 10 mM of each primer, 100 mM $MgCl_2$, 10 mM dNTPs and 0.5 U of *Taq* Platinum® (Invitrogen, São Paulo, Brazil). The PCR reaction profile included an initial denaturizing step at 95 °C for 5 min, 35 cycles of 94 °C for 30 s, annealing at the specific temperature of each primer for 30 s and extension at 72 °C for 30 s, with a final extension at 72 °C for 5 min. Amplified products were separated on 6% denaturing polyacrylamide gels using silver staining (following Creste et al., 2001). Allele sizes were estimated by comparing bands to 10 bp and 50 bp DNA ladder standards (Invitrogen, Brazil).

2.3. Data analyses

Genotypes were inspected for the presence of null alleles, large allele drop-out and/or stuttering using Micro-Checker v2.2.3 (van Oosterhout et al., 2004). GenAlEx v6 (Peakall and Smouse, 2006) was used to estimate the number of alleles per locus, allele frequencies and expected (*He*) and observed (*Ho*) heterozygosities and to conduct assignment tests. The inbreeding coefficient (F_{IS}) (Weir and Cockerham, 1984) and genotypic linkage disequilibrium (*LD*) were computed using default settings in Genepop v4.0 (Rousset, 2008). The same program was used to test for the departure of genotypic proportions from those expected under Hardy–Weinberg equilibrium (HWE), adjusting the alpha level for multiple comparisons with the Bonferroni procedure (Rice, 1989). A possible recent reduction in population size was investigated using Bottleneck v1.2.02 (Piry et al., 1999), with 1000 replications, and a two-phase mutation model (TPM) with a 7:3 ratio of single-step: multi-step mutations and 30% of variance. The significance of results was evaluated with the Wilcoxon sign-ranked test. A Mantel test was carried out in IBDWS v3.23 (Jensen et al., 2005) to examine the correlation between genetic distance and geographic distance (measured in kilometers along the coastline).

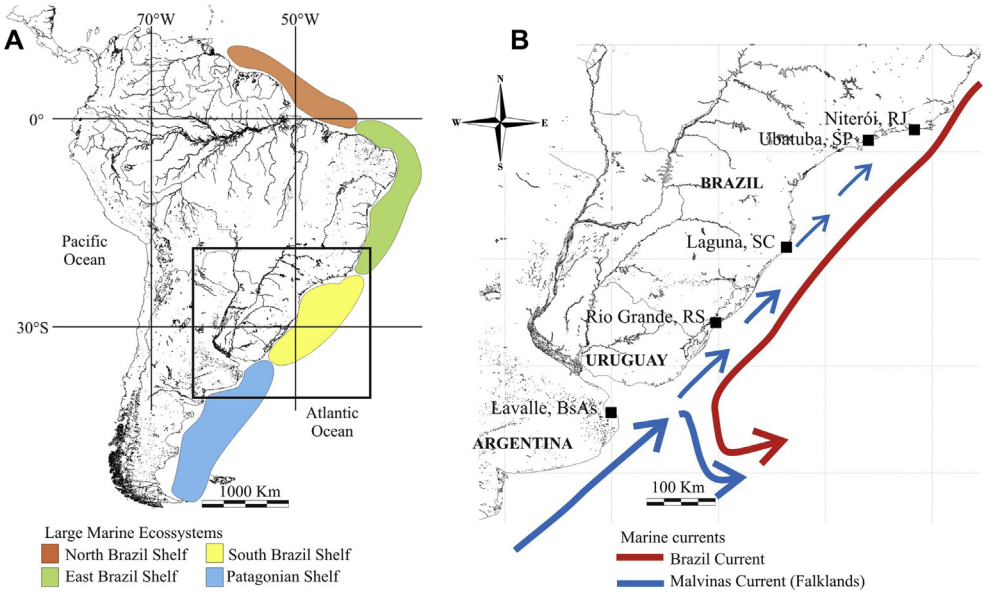

Fig. 1. Maps of the study area. A) schematical representation of the Large Marine Ecosystems (LMEs) along the coast of South America. The black square indicates the area enlarged in B; B) detail of Southern Brazilian and Northern Argentinean Atlantic coast and shelf region showing the approximate location of sampling sites (black squares). Red and blue arrows indicate the direction of predominant marine currents in the winter. Adapted from Sherman and Duda (1999) and Matano et al. (2010). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

To inspect patterns of genetic structuring, an Analysis of Molecular Variance (AMOVA) was conducted in Arlequin v3.5.1.3 (Excoffier et al., 1992). Additionally, the package hierfstat (Goudet, 2005) implemented within the R environment (R Core Team, 2013) was used to compute the F_{ST} index (Weir and Cockerham, 1984) between pairs of samples. Also, to inspect the patterns of genetic differentiation in the absence of apparent barriers to gene flow, a hierarchically nested analysis was carried out in hierfstat. The compared nested levels were: 1) all sampling sites separately; 2) Niterói (RJ) samples vs. all other samples together (from preliminary hierarchical analyses in Arlequin program); and 3) Brazilian samples vs. Argentinean sample. Significance of *F*-statistics for each hierarchical level was assessed by 1000 permutations.

The presence of potentially distinct genetic groups was examined using the Bayesian clustering method implemented in STRUCTURE v2.3.1 (Pritchard et al., 2000), based on the multi-locus genotypes. To estimate the number of population clusters (K) within the complete data set, five independent simulations for $K = 1$–5 were run with 100000 burn-in iterations and 10000 data iterations. Analysis was performed using the admixture model of population structure, and correlated allele frequencies amongst populations. The most likely number of population clusters was estimated applying the Evanno et al. (2005) procedure to Structure results, using the online application Structure Harvester (Earl and Vonholdt, 2012).

The number of effective breeders (*Nb*) was estimated as a proxy of the effective population size (*Ne*) using the sibship assignment approach implemented in the program Colony (Jones and Wang, 2010).

3. Results

A total of 131 alleles with an average of 10.8 alleles per locus were observed across the nine microsatellite loci; none of the microsatellite loci showed evidence of null alleles, stuttering or allelic dropout (Table 2). The number of alleles per locus varied considerably, from 3 at locus *Muce-9* to 23 at locus *Mcs2DM* (Table 2). After adjusting for multiple comparisons, none of the populations deviated from HWE at any loci (Table 2). Mean H_O per population overall loci ranged from 0.654 to 0.716 and H_E ranged from 0.682 to 0.716 (Table 1). There was no significant evidence of homozygotes' excess in any population (F_{IS} indexes did not differ from zero) (Table 1). Nonrandom association of alleles (linkage disequilibrium) was evidenced in six out of 180 comparisons performed and involved different pairs of loci in each case. There was no evidence of a recent reduction in population size that could have affected genetic diversity at nuclear loci (all two-tailed Wilcoxon tests were non-significant, Table S1 in Supplementary Material). There was no evidence of a significant relationship between genetic and geographical distances among the five populations (Mantel test results: $r = 0.2547$, $Z = 185.8269$, $P = 0.7563$).

As indicated by F_{ST} values, a low but significant genetic difference was found at nuclear loci between the Niterói (RJ) samples and all other samples (Table 3). AMOVA results showed that only 0.61% of the variation was distributed among regions, whereas 1.77% was allocated among populations within regions, and the remaining 97.62% was distributed within populations (Table S2). Results of hierarchical analyses conducted in Hierfstat showed a significant effect only for the level that considered the Niterói sample separately from all the others (those, in turn, clustered together) (Table S3). Assignment tests revealed that, when sites were separately used as source populations, Niterói sample had the highest number of positively assigned individuals (42%) (Table S3). But, notably, when regions were used as sources (Niterói (RJ) defining one region and the grouping of SP + SC + RS + BsAs defining the other), the percentages of positive assignments increased almost 70% (Table S3). The application of Evanno et al. (2005) procedure to the results of the Bayesian analysis of the entire dataset conducted in Structure revealed a peak at $K = 2$ in the plot of estimated Delta K versus K (Fig. 2), suggesting that two is the most likely number of different population clusters in *Mugil liza* samples.

Given that a significant differentiation was observed between Niterói (RJ) and all the other samples, and to avoid bias, the number of effective breeders (*Nb*) was computed separately for both population clusters (RJ vs. SP + SC + RS + BsAs). Estimates of *Nb* were 197 breeding individuals (95% CI: 111–747) for RJ population and

Table 2
Summary statistics of microsatellite diversity for mullets sampled at four sites along the Southern Brazilian coast and at one site in Argentina. Number of amplified samples (*N*), number of alleles (*Na*), observed heterozygosity (H_O) and expected heterozygosity (H_E) were estimated using GenAlEx (Peakall and Smouse, 2006). Inbreeding coefficient (F_{IS}) estimates (Weir and Cockerham, 1984) and probability values of Hardy–Weinberg equilibrium (HWE) were computed using Genepop v4.0 (Rousset, 2008). Significant adjusted nominal level was 0.001.

Locus	*Muce-9*	*Mcs17FM*	*Mcs16DM*	*Mcs2DM*	*Mce-11*	*Mce-14*	*Mce-24*	*Mce-27*	*Mce-4*
Niterói (RJ)									
N	50	50	49	48	49	50	49	50	48
Na	3	17	13	23	4	9	16	10	5
H_O	0.440	0.920	0.755	0.896	0.571	0.680	0.837	0.680	0.667
H_E	0.528	0.908	0.805	0.933	0.512	0.683	0.831	0.624	0.615
HWE	0.814	0.460	0.056	0.694	0.127	0.514	0.888	0.814	0.809
F_{IS}	−0.079	−0.003	0.072	0.050	−0.105	0.015	0.003	−0.079	−0.074
Ubatuba (SP)									
N	50	50	50	47	50	48	49	50	50
Na	3	19	12	21	3	8	19	11	5
H_O	0.460	0.940	0.780	0.894	0.480	0.396	0.673	0.600	0.660
H_E	0.554	0.913	0.848	0.920	0.502	0.439	0.842	0.606	0.626
HWE	0.404	0.577	0.298	0.218	0.447	0.086	0.006	0.231	0.656
F_{IS}	0.180	−0.019	0.090	0.039	0.054	0.109	0.210	0.0203	−0.045
Laguna (SC)									
N	47	49	49	47	49	47	49	49	50
Na	3	19	12	18	5	7	18	10	4
H_O	0.532	0.898	0.776	0.872	0.408	0.426	0.776	0.571	0.640
H_E	0.552	0.921	0.845	0.917	0.445	0.478	0.812	0.576	0.591
HWE	0.818	0.391	0.125	0.048	0.525	0.263	0.633	0.059	0.208
F_{IS}	0.0535	0.035	0.092	0.059	0.093	0.120	0.055	0.019	−0.073
Rio Grande (RS)									
N	49	49	47	48	49	49	50	50	49
Na	3	18	12	20	5	9	15	11	4
H_O	0.633	0.959	0.851	0.938	0.673	0.490	0.780	0.400	0.510
H_E	0.562	0.928	0.878	0.927	0.550	0.540	0.815	0.457	0.635
HWE	0.256	0.661	0.617	0.250	0.249	0.054	0.221	0.079	0.199
F_{IS}	−0.115	−0.024	0.041	−0.001	−0.215	0.104	0.053	0.135	0.206
Lavalle (BsAs)									
N	50	49	49	50	50	49	50	50	50
Na	3	17	12	18	4	8	16	11	5
H_O	0.480	0.837	0.918	0.880	0.420	0.388	0.720	0.660	0.720
H_E	0.565	0.899	0.866	0.918	0.474	0.357	0.783	0.640	0.663
HWE	0.028	0.219	0.343	0.032	0.223	0.845	0.297	0.886	0.891
F_{IS}	0.161	0.079	−0.051	0.052	0.124	−0.075	0.090	−0.021	−0.075

284 (95% CI: 222–375) breeding individuals for the southern population cluster.

4. Discussion

4.1. Microsatellites' diversity levels

Microsatellite primers developed for *Mugil cephalus* proved to amplify well in *Mugil liza*, most probably due to the low phylogenetic distance between both taxonomical units (3.0–3.6% Siccha-Ramirez et al., 2014). The levels of microsatellite diversity found in the present study in *M. liza* differed depending on the loci compared, regarding the populations of *M. cephalus* studied so far with neutral nuclear loci. For example, at *Muce-9* we found a lower number of alleles (3) than in 30 *M. cephalus* individuals from East China sea in which the primer was originally described (9 alleles, see Xu et al., 2010). At loci *Mcs17FM*, *Mcs16DM* and *Mcs2DM* the number of alleles found in *M. liza* (mean 18, 12 and 20, respectively; Table 2) was comparable to that found in *M. cephalus* from Italy ($N = 180$) and Australia ($N = 18$) (Miggiano et al., 2005). At loci *Mce-*

Table 3
Pairwise estimates of F_{ST} (below diagonal) (Weir and Cockerham, 1984) and their corresponding *P*-values (above diagonal) for comparisons among five samples of *Mugil liza* based on nine polymorphic microsatellite loci. Asterisks (*) indicate significant comparisons ($P < 0.05$). Abbreviations for sampling sites detailed in Table 1.

Sample	RJ	SP	SC	RS	BsAs
Niterói (RJ)	–	0.010*	0.010*	0.020*	0.010*
Ubatuba (SP)	0.009	–	0.440	0.520	0.420
Laguna (SC)	0.011	0.000	–	0.100	0.110
Rio Grande (RS)	0.009	0.000	0.003	–	0.060
Lavalle (BsAs)	0.018	0.000	0.004	0.004	–

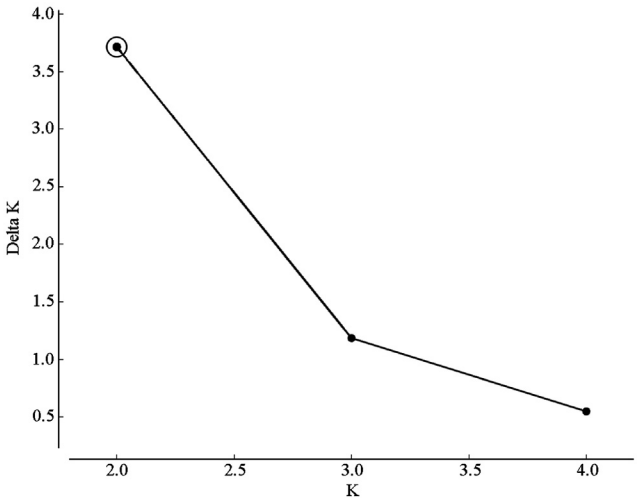

Fig. 2. Results of the Evanno et al. (2005) procedure applied to results of Bayesian clustering analyses performed in Structure (Pritchard et al., 2000) based on data from nine microsatellites for 250 *Mugil liza* samples. The plot of Delta *K* (filled circles, solid line) was calculated as the mean of the second-order rate of change in the likelihood of *K* divided by the standard deviation of the likelihood of *K*, mean($|L''(K)|$)/sd($L(K)$). The maximum Delta *K* was obtained at $K = 2$ (encircled), indicating the presence of two different clusters.

11, Mce-14, Mce-24, Mce-27 and *Mce-4* the number of alleles per locus found in *M. liza* (Table 2) were comparable with those found in ca. 30 *M. cephalus* samples from Taiwan, East Australia, Spain and Peru, in which the primers were described (Shen et al., 2010). On average, levels of observed and expected heterozygosity in *M. liza* from the Southern Atlantic coast of South America were within the range of those found in studies with *M. cephalus* (Miggiano et al., 2005; Shen et al., 2010; Xu et al., 2010; Durand et al., 2013).

4.2. Regional genetic structuring

Based on nuclear markers we found evidence of genetic differentiation between samples of *Mugil liza* from Niterói (RJ) (northern population) and from the Brazilian Southeastern Atlantic coast (southern population), as indicated by F_{ST} (Table 3), hierarchical analyses of population structure and assignment tests (Table S3). In agreement, Bayesian analyses (Fig. 2) indicated that two population clusters could best explain the allelic variation observed in the analyzed samples.

Those results apparently contrast the findings of Siccha-Ramirez et al. (2014) based on analyses of 38 specimens of *Mugil liza*, which suggest that a single mtDNA clade would be present throughout the species' distribution. Although a direct comparison between the present study and that of Siccha-Ramirez et al. (2014) would not be entirely valid, because they refer to different individual samples, it is still useful to regard both studies as complementary. Microsatellite markers picture contemporary processes and can better describe some ecological characteristics and life-history traits of organisms, such as larval transport and dispersal and differences in spawning timing or location, depending on the time-lag. On the other hand, mtDNA are more useful to depict patterns of historical distribution and demographic processes. It is worth noting that, while under most conditions mtDNA yields higher F_{ST} estimates than microsatellites, this is not always true (see Karl et al., 2012 review). A similar finding of significant genetic differentiation at nuclear loci but a single mtDNA group has been recently reported for *Mugil cephalus* samples from the North Eastern Atlantic Ocean, the Mediterranean Sea and the Black Sea (Durand et al., 2013). Those authors proposed that male-biased gene flow could be a possible explanation of such a pattern, but alert to the fact that there is limited ecological evidence to support such a situation in that species (Durand et al., 2013). The scenery is not much different for *M. liza*, although differences in migratory behavior between males and females could account for the pattern observed in this study, there is a lack of ecological evidence in support of this.

Only the Niterói (RJ) *Mugil liza* sample differs significantly from all other samples from the Brazilian Southern Atlantic coast and from Argentina regarding allele frequency distribution at microsatellite loci (Table 3). Similarly, Argentinean samples from Buenos Aires Province do not appear to be significantly differentiated from the studied Brazilian populations – excepting Niterói (RJ) samples (Table 3). The average strait-line geographic distance between RJ and all other studied samples is ca. 1409 km, twice the average documented migratory distance traveled by reproductive mullets of the *Mugil cephalus* species complex (between 240 and 740 km, reviewed in Whitfield et al., 2012). It is, therefore, tempting to suggest that geographic distance could account for the significant genetic structuring found in this study. However, given the lack of significant correlation between genetic and geographic distance (Mantel test), isolation-by-distance alone cannot explain our findings. Thus, other factors might be responsible for the significant differentiation observed in this study. Mullet larvae are passively carried out by oceanic currents, which, in turn, may promote long distance dispersal and gene flow over a wide geographic scale (reviewed in Whitfield et al., 2012). Reproductive migration routes of *M. liza* in South America indicated that Argentinean populations migrate to Brazilian waters during the Southern hemisphere winter (Vieira and Scalabrin, 1991; Lemos et al., 2014).

The existence of specifics oceanographic conditions of spawning for *Mugil liza* between the North of Rio Grande do Sul State and North of Santa Catarina State (Lemos et al., 2014) and marine boundaries that could provide efficient barriers to the dispersal of larvae, and restrict gene flow, has been proposed to explain genetic structuring in other Mugilids (Rocha-Olivares et al., 2000; 2005; Liu et al., 2009; Durand et al., 2013).

The continental shelf and upper slope off southern Brazil is influenced by the seasonal oscillation of the Sub-Tropical Convergence (STC) where Tropical waters of the Brazil Current ($T > 20$ °C) meet cold and low salinity waters of subantartic origin. This originates the Subtropical Water, also called South Atlantic Central Water (Matano et al., 2010). This Water, (10–20 °C) runs northwards under the Brazil Current. The cold water intrusion may surface through seasonal upwelling (Cabo Santa Marta Grande, SC, and Cabo Frio, RJ) and due to mixing processes over the continental shelf region, the temperatures reach approximately 20 °C at the northern boundary south to Rio de Janeiro (23°S) (Haimovici and Perez, 1991; Castello et al., 1997; Matano et al., 2010). Sherman and Duda (1999) recognize three Large Marine Ecosystems (LMEs) in this area. The South Brazil Shelf and the East Brazil Shelf are splitted around 23°S at Cabo Frio (Fig. 1A) and the South Brazil Shelf and Patagonian Shelf divides around 34°S where the coastal and shelf interaction of the STC takes place. In this sense, a possible alternative explanation to the observed genetic differentiation between Rio de Janeiro sample and all other southern samples could be related to the circulation patterns observed in the southern Brazilian Coast and the well-known biogeographical boundary that exists at Cabo Frio. In agreement this hypothesis, several coastal species have congeners or populations with discontinued distribution at Rio de Janeiro and/or Espírito Santo States [e.g. King weakfish *Macrodon atricauda* (Yamaguti, 1979; Santos et al., 2006; Carvalho-Filho et al., 2010); Franciscana dolphin *Pontoporia blainvillei* (Lazarro et al., 2004); white shrimp *Litopenaeus schmitti* (Maggioni et al., 2003); Argentine stiletto shrimp *Artemesia longinaris* (Dumont et al., 2009)].

We cannot rule out the possibility of methodological biases: inappropriate sampling, a low number of loci analyzed or violation of underlying assumptions of analytical models (for example, mutation–drift equilibrium) in our study. Reduced gene flow within the last few generations could be a possible explanation for the significant microsatellite structuring but lack of mtDNA differentiation observed in *Mugil liza* from the Southern Atlantic coast of South America, but this remains to be better elucidated in future studies.

5. Conclusions, implications for management and conservation

In conclusion, this study using microsatellite markers provides the first molecular evidence of the existence of distinct population clusters of *Mugil liza* along the South American Atlantic coast: one represented by Niterói samples (Brazil) and the other including Brazilian (Ubatuba, Laguna e Rio Grande) and Argentinean samples (Lavalle, Buenos Aires Province). The identification of a possible barrier to gene flow in this region provides a base to better understand *M. liza* life-history traits and to interpret the genetic variation inherent to the species complex in adaptive terms (Whitfield et al., 2012).

Mugil liza sampled at Niterói (RJ) harbor different allelic composition than the other Brazilian and the Argentinean samples. Significant divergence of allele frequencies at nuclear or

mitochondrial loci has been used as a criterion to define 'Management Units' (MUs) for conservation purposes, population monitoring and demographic studies (Moritz, 1994). Thus, considering RJ (and northernmost populations) separately from southern populations of *M. liza* could be a good practice in the future, when applying genetic tools to monitor the status of this fish resource and also when designing management plans. Given that more than 95% of *M. liza* catches occur at Rio Grande do Sul and Santa Catarina, especially during the northward reproductive migration (Vieira and Scalabrin, 1991; Lemos et al., 2014), it is essential to consider the information provided in this genetic study when designing a concurrent management plan for Argentina, Uruguay and Brazil.

Despite being an overexploited fisheries resource in South American Waters (MMA, 2004; González-Castro et al., 2009), it appears that the actual volume of catches has not yet reduced the populations of *Mugil liza* studied here to a size that could deplete their genetic diversity (non-significant results of Bottleneck tests). However, the relatively small number of effective breeders estimated based on microsatellite loci (<300 individuals), together with the increased anthropogenic pressures on estuarine regions that are nurseries for species, the species' late sexual maturity (about 5–6 years of age) with annual breeding and the habit of forming migration shoals in the reproductive period (Garbin et al., 2014; Lemos et al., 2014) make *M. liza* very vulnerable to any further increase in fisheries pressure.

Future studies should more fully explore the patterns of nuclear and mtDNA diversity, expanding the sampling sites towards latitudes Northern and Southern of the area surveyed in this study. Expanding the sampling sites towards north of Brazil (Bahia, Pernambuco and Natal States) might help shed light into the number of existing populations throughout the species' distribution in Brazilian coastal waters. Based on our results, we suggest that measures restricting the total volume of mullets captured or protecting the species in its reproductive period are implemented. In addition, we further call for new studies that estimate the effective size of stocks, indirectly assess parameters (growth, reproductive rates, number of recruits, etc.) and monitor genetic diversity. Our findings can effectively contribute to the adequate design of management plans and aid in the conservation of this important and overexploited fish resource in Neotropical Atlantic waters.

Acknowledgments

Authors acknowledge the anonymous reviewer for helpful comments on the manuscript. We thank all the workmates of the Ichthyology and Molecular Biology Laboratories (FURG). This study was supported by REDE SISBIOTA-PELD project (FAPERGS, 11/2262-7; CNPq 563263/2010-5), PELD (Brazilian Long Term Ecological Research Program 403805/2012-0) and CNPq-Universal Proc. 482274/2012-7. CIM acknowledges support from Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET-Argentina). English review was funded by FURG.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ecss.2014.07.013.

References

Albieri, R.J., Araújo, F.G., 2010. Reproductive biology of the mullet *Mugil liza* (Teleostei: Mugilidae) in a tropical Brazilian bay. Zoologia 27 (3), 331–340.
Aurelle, D., Barthelemy, R.-M., Quignard, J.-P., Trabelsi, M., Faure, E., 2008. Molecular phylogeny of Mugilidae (Teleostei: Perciformes). Open. Mar. Biology J. 2, 29–37.
Avise, J.C., 1994. Molecular Markers, Natural History and Distribution. Chapman & Hall, New York, p. 459.
Carvalho-Filho, A., Santos, S., Sampaio, I., 2010. *Macrodon atricauda* (Günther, 1880) (Perciformes: Sciaenidae), a valid species from the southwestern Atlantic, with comments on its conservation. Zootaxa 2519, 45–58.
Castello, J.P., Haimovici, M., Odebrecht, C., Vooren, C., 1997. The continental shelf and slope. In: Seeliger, U., Odebrecht, C., Castello, J.P. (Eds.), Subtropical Convergence Environments. The Coast and Sea in the Southwestern Atlantic. Springer, Berlin, pp. 171–178.
Cousseau, M.B., González Castro, M., Figueroa, D.E., Gosztonyi, A.E., 2005. Does *Mugil liza* Valenciennes 1836 (Teleostei, Mugiliformes) occur in Argentinean waters? Rev. Biol. Mar. Oceanogr. 40 (2), 133–140.
Creste, S., Tulmann-Neto, A., Figueira, A., 2001. Detection of single sequence repeat polymorphisms in denaturing polyacrylamide sequencing gels by silver staining. Plant Mol. Biol. Report. 19, 299–306.
Dumont, L.F.C., Hwang, G., Maclean, N., 2009. The mtDNA control region of the barba-ruça shrimp *Artemesia longinaris* (Decapoda: Penaeidae) and its potential use as a marker for population analysis. Atlântica 31 (2), 199–207.
Durand, J.-D., Shen, K.-N., Chen, W.-J., Jamandre, B.W., Blel, H., Diop, K., Nirchio, M., Garcia de León, F.J., Whitfield, A.K., Chang, C.-W., Borsa, P., 2012. Systematics of the grey mullets (Teleostei: Mugiliformes: Mugilidae): molecular phylogenetic evidence challenges two centuries of morphology-based taxonomy. Mol. Phylogenetics Evol. 64, 73–92.
Durand, J.D., Blel, H., Shen, K.N., Koutrakis, E.T., Guinand, B., 2013. Population genetic structure of *Mugil cephalus* in the Mediterranean and Black Seas: a single mitochondrial clade and many nuclear barriers. Mar. Ecol. Prog. Ser. 474, 243–261. http://dx.doi.org/10.3354/meps10080.
Earl, D.A., Vonholdt, N.M., 2012. STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. Conserv. Genet. Resour. 4, 359–361.
Evanno, G., Regnaut, S., Goudet, J., 2005. Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. Mol. Ecol. 14, 2611–2620.
Excoffier, L., Smouse, P.E., Quattro, J.M., 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. Genetics 131, 479–491.
Fraga, E., Schneider, H., Nirchio, M., Santa-Brigida, E., Rodrigues-Filho, L.F., Sampaio, I., 2007. Molecular phylogenetic analyses of mullets (Mugilidae, Mugiliformes) based on two mitochondrial genes. J. Appl. Ichthyol. 23, 598–604.
Garbin, T., Castello, J.P., Kinas, P.G., 2014. Age, growth and mortality of the mullet *Mugil liza* in Brazil's southern and southeastern coastal regions. Fish. Res. 49, 61–68.
González-Castro, M., Heras, S., Cousseau, M.B., Roldán, M.I., 2008. Assessing species validity of *Mugil platanus* Günther, 1880 in relation to *Mugil cephalus* Linnaeus, 1758 (Actinopterygii). Ital. J. Zool. 75 (3), 319–325.
González-Castro, M., Abachian, V., Perrotta, R.G., 2009. Age and growth of the stripped mullet *Mugil platanus* (Actinopterygii, Mugilidae), in a Southwestern Atlantic coastal lagoon (37°32′S–57°19′W): a proposal for a life- history model. J. Appl. Ichthyol. 25, 61–66.
González-Castro, M., Macchi, G., Cousseau, M.B., 2011. Studies on reproduction of the mullet *Mugil platanus* Günther, 1880 (Actinopterygii, Mugilidae) from the Mar Chiquita coastal lagoon, Argentina: similarities and differences with related species. Ital. J. Zool. 78, 343–353.
González-Castro, M., Ibáñez, A.L., Heras, S., Roldán, M.I., Cousseau, M.B., 2012. Assesment of lineal versus landmarks-based morphometry for discriminating species of Mugilidae (Actinopterygii). Zool. Stud. 51 (8), 1515–1528.
Goudet, J., 2005. Hierfstat, a package for R to compute and test hierarchical F-statistics. Mol. Ecol. Notes 5, 184–186.
Goudet, J., Raymond, M., Meeiis, T., Roussett, F., 1996. Testing differentiation in diploid populations. Genetics 144, 1933–1940.
Haimovici, M., Perez, J.A.A., 1991. Coastal cephalopod fauna of Southern Brazil. Bull. Mar. Sci. 49 (1), 221–230.
Heras, S., Roldán, M.I., González-Castro, M., Cousseau, M.B., 2007. *Mugil cephalus*: cosmopolitan species or species complex? Rapport Commission International Mer Méditerranée 38, 499.
Heras, S., Roldán, M.I., González-Castro, M., 2009. Molecular phylogeny of Mugilidae fishes revised. Rev. Fish. Biol. Fish. 19, 217–231.
IBAMA, 2008. Instrução Normativa N° 171 de 9 de maio de 2008.
Jensen, J.L., Bohonak, A.J., Kelley, S.T., 2005. Isolation by distance, web service. BMC Genet. 6 (13), 1–6.
Jones, O.R., Wang, J., 2010. COLONY: a program for parentage and sibship inference from multilocus genotype data. Mol. Ecol. Resour. 10 (3), 551–555.
Karl, S.A., Toonen, R.J., Grant, W.S., Bowen, B.W., 2012. Common misconceptions in molecular ecology: echoes of the modern synthesis. Mol. Ecol. 21, 4171–4189.
Lazarro, M., Lessa, E.P., Hamilton, H., 2004. Geographic genetic structure in the franciscana dolphin (*Pontoporia blainvillei*). Mar. Mammal Sci. 20 (2), 201–204.
Lemos, V.M., Varela Jr., A.S., Schwingel, P.R., Muelbert, J.H., Vieira, J.P., 2014. Migration and reproductive biology of *Mugil liza* (Teleostei: Mugilidae) in south Brazil. J. Fish Biol. http://dx.doi.org/10.1111/jfb.12452.
Liu, J., Brown, C.L., Yang, T., 2009. Population genetic structure and historical demography of grey mullet, *Mugil cephalus*, along the coast of China, inferred by analysis of the mitochondrial control region. Biochem. Syst. Ecol. 37, 556–566.
Maggioni, R., Rogers, A.D., Maclean, N., 2003. Population structure of *Litopenaeus schmitti* (Decapoda: Penaeidae) from the Brazilian Coast identified using six polymorphic microsatellite loci. Mol. Ecol. 12, 3213–3217.

Matano, R.P., Palma, E.D., Piola, A.R., 2010. The influence of the Brazil and Malvinas Currents on the southwestern Atlantic Shelf circulation. Ocean Sci. 6, 983–995.
Menezes, N.A., Oliveira, C., Nirchio, M., 2010. An old taxonomic dilemma: the identity of the western south Atlantic Lebranche Mullet (Teleostei: Perciformes: Mugilidae). Zootaxa 2519, 59–68.
Miggiano, E., Lyons, R.E., Li, Y., Dierens, L.M., Crosetti, D., Sola, L., 2005. Isolation and characterization of microsatellite loci in the striped mullet, *Mugil cephalus*. Mol. Ecol. Notes 5, 323–326.
Miranda, L.V., Carneiro, M.H., 2007. A pesca da tainha *Mugil platanus* (Perciformes: Mugilidae) desembarcada no Estado de São Paulo: Subsídio ao Ordenamento. Série Relatórios Técnicos do Instituto de Pesca 30, 1–13.
MMA, Ministério do Meio Ambiente, 2004. Instrução normativa IBAMA N° 05, de 21 de maio de 2004. Reconhece como espécies ameaçadas de extinção e espécies sobreexplotadas ou ameaçadas de sobreexplotação, os invertebrados aquáticos e peixes, constantes dos Anexos a esta Instrução Normativa. Diário Oficial da União, Brasília. No. 102.
MMA, Ministério do Meio Ambiente, 2007. Estatistica da pesca 2007 Brasil: grandes regioes e unidades da federação. Instituto Brasileiro do Meio Ambiente e Recursos Naturais Renováveis – IBAMA, Ministério do Meio Ambiente – MMA, Brasília.
Moritz, C., 1994. Defining 'Evolutionarily Significant Units' for conservation. Tree 9 (10), 373–375.
MPA, Ministério do Meio Ambiente, 2011. Boletim estatístico da pesca e aquicultura. MPA, Brasília, p. 60.
Peakall, R., Smouse, P.E., 2006. GenAlEx 6: genetic analysis in Excel. Population genetic software for teaching and research. Mol. Ecol. Notes 6, 288–295.
Piry, S., Luikart, G., Cornuet, J.M., 1999. BOTTLENECK: a computer program for detecting recent reductions in the effective population size using allele frequency data. J. Hered. 90, 502–503.
Potter, I.C., Tweedley, J.R., Elliott, M., Whitfield, A.K., 2013. The ways in which fish use estuaries: a refinement and expansion of the guild approach. Fish Fish. http://dx.doi.org/10.1111/faf.12050.
Pritchard, J.K., Stephens, M., Donnelly, P., 2000. Inference of population structure using multilocus genotype data. Genetic 155, 945–959.
R Core Team, 2013. R: a Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. URL. http://www.R-project.org/.
Rice, W.R., 1989. Analyzing tables of statistical tests. Evolution 43, 223–225.
Rocha-Olivares, A., Garber, N.M., Stuck, K.C., 2000. High genetic diversity, large inter-oceanic divergence and historical demography of the striped mullet. J. Fish Biol. 57, 1134–1149.
Rocha-Olivares, A., Garber, N.M., Garber, A.F., Stuck, K.C., 2005. Structure of the mitochondrial control region and flanking tRNA genes of *Mugil cephalus*. Hidrobiológica 15, 139–149.
Rousset, F., 2008. Genepop'007: a complete re-implementation of the genepop software for Windows and Linux. Mol. Ecol. Resour. 8, 103–106.
Sambrook, J., Fritsch, E.F., Maniatis, T., 1989. Molecular Cloning: a Laboratory Manual. Cold Spring Harbor Laboratory Press, New York, p. 1659.
Santos, S., Hrbek, T., Farias, I.P., Schneider, H., Sampaio, I., 2006. Population genetic structuring of the king weakfish, *Macrodon ancylodon* (Sciaenidae), in Atlantic coastal waters of South America: deep genetic divergence without morphological change. Mol. Ecol. 15, 4361–4373.
Shen, K.N., Chen, C.Y., Tzeng, W.N., Chen, J.D., Knibb, W., Durand, J.D., 2010. Development and characterization of 13 GT/CA microsatellite loci in cosmopolitan flathead mullet *Mugil cephalus*. Mol. Ecol. Resour. 10 (6), 1098–1105.
Sherman, K., Duda, A.M., 1999. Large Marine ecosystems: an emerging paradigm for fishery sustainability. Fisheries 24 (12), 15–26.
Siccha-Ramirez, R., Menezes, N.A., Nirchio, M., Forestia, F., Oliveira, C., 2014. Molecular identification of mullet species of the Atlantic South Caribbean and South America and the phylogeographic analysis of *Mugil liza*. Rev. Fish. Sci. Aquac. 22 (1), 86–96.
van Oosterhout, C., Hutchinson, W.F., Wills, D.P.M., Shipley, P., 2004. MICRO-CHECKER: software for identifying and correcting genotyping errors in microsatellite data. Mol. Ecol. Notes 4, 535–538.
Vieira, J.P., 1991. Juvenile mullets (Pisces: Mugilidae) in the estuary of Lagoa dos Patos, RS, Brazil. Copeia 2, 409–418.
Vieira, J.P., Scalabrin, C., 1991. Migração reprodutiva da "Tainha" (*Mugil platanus*, Günther, 1880) no sul do Brasil. Atlântica 13 (1), 131–141.
Vieira, J.P., Garcia, A.M., Grimm, A.M., 2008. Preliminary evidences of El Niño effects on the mullet fishery of Patos Lagoon estuary (Brazil). Braz. Archives Biol. Technol. 51, 433–440.
Weir, B.S., Cockerham, C.C., 1984. Estimating F-statistics for the analysis of population structure. Evolution 38, 1358–1370.
Whitfield, A.K., Panfili, J., Durand, J.-D., 2012. A global review of the cosmopolitan flathead mullet *Mugil cephalus* Linnaeus 1758 (Teleostei: Mugilidae), with emphasis on the biology, genetics, ecology and fisheries aspects of this apparent species complex. Rev. Fish. Biol. Fish. 22 (3), 641–681.
Xu, T.-J., Sun, D.-Q., Shi, G., Wang, R.-X., 2010. Development and characterization of polymorphic microsatellite markers in the gray mullet (*Mugil cephalus*). Genet. Mol. Res. 9 (3), 1791–1795.
Yamaguti, N., 1979. Diferenciação geográfica de *Macrodon ancylodon* (Bloch & Schneider, 1801) na costa brasileira, entre as latitudes 18°36′S e 32°10′S: etapa I. Bol. Inst. Ocean. 28 (1), 53–118.