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Genetic diversity and gene flow of the threatened Brazilian endemic parrotfish *Scarus trispinosus* (Valenciennes, 1840)

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ABSTRACT

The greenback parrotfish, *Scarus trispinosus*, is the largest herbivorous fish inhabiting Southwestern Atlantic reefs, and was recently included in the IUCN red list of threatened species as endangered due to the over-exploitation of their populations. The aim of this work was to evaluate the existence of structured populations (i.e. genetic unities) along a coast of approximately 2000 km of the NE Brazilian coast. The transferability of 17 primers synthesized for *Scarus rubroviolaceus* was tested for *S. trispinosus* and five transferable loci were validated and used. Two localities within the Abrolhos Bank, off the Central Brazilian coast (Corumbau and Caravelas) and in close proximity to the MPA, which encompasses the largest remnants of the *S. trispinosus* population, exhibited higher levels of genetic richness. Remaining locations, Pernambuco, Porto Seguro and Rio Grande do Norte exhibited lower genetic diversity. We found no genetic differences among sampled localities however, when those samples were gathered into latitudinal groups (northern vs southern) a subtle but significant genetic substructuring was revealed. It is proposed that a combination of high local individual admixture favoured by habitat connectivity drived genetic homogeneity at regional scales while larval dispersal contributed to heterogeneities observed at large scales maintaining gene flow through oceanographic currents.

1. Introduction

A key process driving connectivity in marine fish populations is the pelagic and potential dispersive larval phase (Bernardi, 2011). This phase is essential for species to colonize new habitats and to maintain fish populations by increasing genetic connectivity among locations (Leis and McCormick, 2002). Oceanic currents and coastal winds display a central role in population sub-structuring of fish species (Cowen et al., 2006, 2007) as they can enhance (or hinder) dispersal of individuals (Félix-Hackradt et al., 2013a). Added to these phenomena, the migration of adults and juveniles can contribute significantly to this homogenization (Galarza et al., 2009). On the contrary, there is increasing evidence for larval retention to natal environments through active swimming behaviour and/or local oceanographic conditions (Swearer et al., 1999; Jones et al., 2005; Abreu et al., 2014). A detailed understanding of such processes and on the degree of genetic variation as evolutionary potential are crucial for the development and implementation of strategies for effective management of exploited species and the conservation of threatened fish species, as fishing pressure and destruction of essential fish habitat can lead to genetic isolation of populations (Craig et al., 2011; Allendorf et al., 2014; Pinsky and Palumbi, 2014).

Genetic and demographic connectivity are highly variable at multiple spatial scales to a degree where few methodologies can elucidate (Cowen et al., 2007). Contemporary genetic tools, such as microsatellites markers, makes it possible to study complex populations (Karl

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et al., 2012). These markers are being largely used in studies on population dynamics due to their codominance, which combined with their hypervariability and easy replication, makes them into ideal tools for genomic screening and historical population relationships (Saenz-Agudelo et al., 2009; Calò et al., 2013), including fish populations (Mai et al., 2014; Ceballos et al., 2016). Once isolated and genotyped, is possible to get cross-amplification on some microsatellite loci between species very closed related (Primmer et al., 1996), and therefore reducing development costs. Importantly this strategy has been largely used to reveal its potential using in studies of fish population genetics (Priest et al., 2015; Portnoy et al., 2016).

Parrotfishes (Labridae: Scarinae) represent the dominant group (in numbers) among herbivorous reef fish and, together with surgeonfish (Acanthuridae), are of significant ecological importance in tropical seas, greatly increasing resistance and resilience of coral reef ecosystems (Choat et al., 2002; Hoey and Bellwood, 2008). Their intense feeding behaviour (Bellwood, 1994) and robust jaw apparatus allow them to scrape and excavate the substratum and consequently shape the habitat structure of benthic reef assemblages by controlling algal growth and facilitating coral growth (Mumby, 2006).

The greenback parrotfish, *Scarus trispinosus*, is an herbivorous species that is endemic to Brazilian coast, occurring from Santa Catarina through Rio Grande do Norte (Floeter et al., 2005) reaching 60m deep (Feitoza et al., 2005). This species is the largest Atlantic parrotfish reaching 60 cm mean total length (Cardozo-Ferreira and Joyeux, 2016) and it is a protogynous hermaphrodite (Sadovy, 2001). In terms of exploitation, a systematic selected removal of the bigger specimens can lead to a fast population loss of structure (Coleman et al., 2000; Sadovy, 2001). These biological and ecological characteristics combined with overexploitation in the last 30 years due to spearfishing (Francini-Filho and Moura, 2008), resulted in its inclusion on the international list of threatened species of International Union for Conservation of Nature (IUCN) as endangered. In Brazil, a ministerial order (MMA 445/2014) from the Environmental Ministry (MMA) has forbidden fishing, landing and commercialization of these species throughout the Brazilian territory since 2014 (Brasil, 2014). However, due to local fisher pressure, an updated order in 2017 (MMA 161/2017) has allowed its capture and selling until April of 2018, after which further fishing activity will be conditioned to the implementation of recovery plans (Brasil, 2017).

Considering the aspects related to the exploitation and conservation status of *Scarus trispinosus* the aim of this study was to (i) understand and assess population sub-structuring, and (ii) evaluate population connectivity, and (iii) assess levels of genetic diversity. These results can be useful to outline optimal management strategies, as well as to support the establishment of spatial management tools (such as Marine Protected Areas) and other appropriated fisheries management measures, which respects observed connectivity patterns on this species.

2. Material and methods

2.1. Study area

Samples of *S. trispinosus* were collected at five locations between 2014 and 2016, encompassing a geographic range of about 2000 km of Brazilian coast, extending from southern Bahia to Rio Grande do Norte: Rio do Fogo/RN (N = 44), Tamandaré/PE (N = 14), Porto Seguro/BA = 49), Corumbau/BA (N = 17) and Caravelas/BA (N = 52) (Fig. 1).

These sampling locations fall within the Brazilian tropical coast, which goes from Maranhão to Rio de Janeiro, and it is where the main coral reef formations are concentrated (Leão and Dominguez, 2000). Brazilian reef formations are especially unique, being distinguishable from the other reef systems found in the world due to (i) the growth structure in the form of mushrooms called "*chapeirões*", occurring in Abrolhos, (ii) the low diversity of reef-building species composed of relic species, but with a high degree of endemism (of the 23 coral species in the Brazilian coast, 25% are endemic), and (iii) high influence of silt-elastic sediments coming from river flows (Leão and Dominguez, 2000).

Although there are different types of reef formations in our study area, coastal reef benches predominate in shallow environments, which during low tides can form lagoons that connect to each other through meandering channels running through the dense reef structure (Leão et al., 2010). It is in this habitat where our target species can be found.

Additionally, adjacent to all sampling localities there was a number of marine protected areas - Coral Reef Environmental Protection Area (RN), Coral Coast Environmental Protection Area (PE), Natural Marine Park of Recife de Fora (BA), Corumbau Extractive Marine Reserve (BA), and Abrolhos National Marine Park (BA), from north to south - that were created, among other things, to contribute for coral reef conservation.

It is important to emphasize that the large gap between the sampled localities occurred because the species is currently threatened with extinction, therefore causing difficulties to obtain the desired number of samples in some localities (e.g. Corumbau and Tamandaré) and no samples at all in other places, despite sampling effort applied.

2.2. Sample collection, extraction and amplification

A total of 176 samples (a piece of tissue approximately 4 cm^2 from the anal fin) were obtained from local fishermen. Tamandaré (Pernambuco) was the locality with the lowest number of individuals, since this species is already under heavy fishing pressure, almost gone in this state (B. P. Ferreira pers. comm.). So, although there was a great effort, it was not possible to standardize the number of individuals sampled. The sampled tissues were conserved in 100% ethanol alcohol until extraction. DNA extractions were performed following the Qiagen® DNeasy Extraction Kit protocol. To verify the DNA integrity, an agarose gel electrophoresis (1%) was performed and DNA amount and quality were quantified by Picodrop®.

2.3. Amplification and genotyping

A total of 17 loci primers obtained for *Scarus rubroviolaceus* (Carlon and Lippé, 2007) were tested for transferability to *S. trispinosus* by the cross-amplification strategy. Of the 17 loci, only five successfully amplified and where thus retained (Sru-A8, Sru-A7, Sru-C127, Sru-A9 and Sru-D5 - see Carlon and Lippé, 2007). Multiplex PCRs were performed in 11 µl total volume, which included 1 µl (25–50 ng) DNA, 1X KCl buffer, 2.0 mM MgCl2, 0.08 mM of each dNTP, 0.18 mM of each primer, 0.5 U of Taq DNA Polymerase and ultrapure water. The reactions were performed under the following conditions: 94 °C for 5′, 10 cycles of 94 °C for 30", 60 °C for 1′ and 72 °C for 90", followed by 30 cycles of 94 °C for 30", 48 °C for 1′, 72 °C for 90" and a final extension of 72 °C for 10' (Gramacho et al., 2007). A 2 µl sample of each PCR product was run on 2% agarose gel stained with Gel RedTM/Uniscience and visualized under UV light. The PCR products of the duplex and triplex reactions were diluted 1:30 with ultrapure water. Subsequently, 1 µl of this mixture was diluted by adding 9 µl Liz-HiDi solution (0.2 µl Liz500 + 8.8 µl HiDi). The product of the amplifications was analysed on ABI 3500® Genetic Analyzer/Applied Biosystems. Allele scoring was performed using GeneMarker (Softgenetics, State College, PA, USA). A base genotype was used in all races as reference to avoid biased alleles. Alleles were sized and labelled by comparison to the genescan-500 LIZ internal size standard (Applied Biosystems, Inc.).

2.4. Data analysis

The total number of alleles (A) and private alleles (PA) as well observed (H_O) and expected heterozygosity (H_E) (Nei, 1978) for each locus and allele frequency were obtained with Genetix (Belkhir et al., 2004). The fixation indexes (F) and richness (R_S) was calculated using 10,000 permutations under FSTAT v.2.9.3.2 routine (Goudet, 2002)

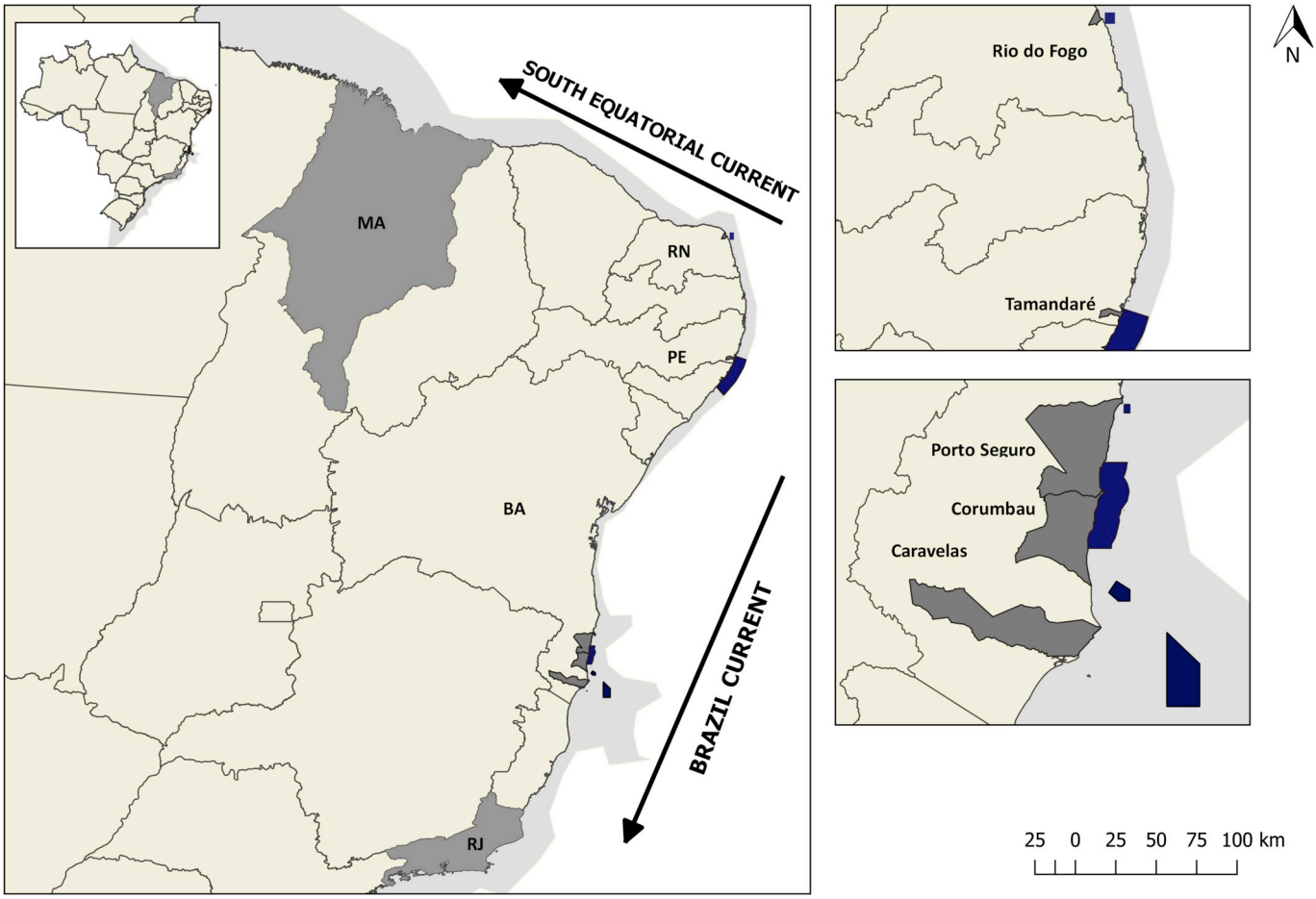

Fig. 1. Map showing the localities where *S. trispinosus* samples were collected (zoomed in on the right with the names of the municipalities), as well as the continental shelf width. The gray painted states represent the geographic distribution of the specie in Brazil. Marine Protected Areas are represented by dark blue area in the map: Coral Reef Environmental Protection Area (RN), Coral Coast Environmental Protection Area (PE), Natural Marine Park of Recife de Fora (BA), Corumbau Extractive Marine Reserve (BA), and Abrolhos National Marine Park (BA). Finally, the arrows represent ocean currents directions that influence the dynamics of coastal circulation. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

following Weir and Cockerham (1984) and Gaggiotti et al. (1999). Furthermore, the presence of null alleles was confirmed by re-amplifications, following the same parameters of the previously analyses, in which all defective reactions were again amplified alongside a reference genotype that always performed correctly. Nevertheless, FreeNa (Chapuis and Estoup, 2007) was used to detect existing null alleles, and compare global and pairwise F_{ST} between our raw microsatellite data and after excluding null alleles (ENA) procedure with 1000 bootstrap repetitions. Deviations from Hardy-Weinberg equilibrium and estimates of linkage-disequilibrium per locus and per locality were determined using the randomization approach that applies Bonferroni corrections in Arlequin (Excoffier et al., 1992, 2005).

The differentiation among the sampled populations was quantified by pairwise F_{ST} and tested by 10,000 permutations with Genetix v.4.1 (Belkhir et al., 2004). Additionally, we used the program POWSIM v.4.0 (Ryman and Palm, 2006) to evaluate the statistical power of the microsatellite marker to detect genetic differentiation given the low number of polymorphic loci used and differences on sample sizes. Simulations were carried out for an effective population size of Ne = 1000 and Ne = 500 to yield F_{ST} values of 0.001, 0.0025, 0.005, 0.01, 0.02, 0.025 and 0.05. The parameters of the Markov Chain were fixed to 10,000, 1000 and 10,000 for, respectively, burn-ins, batches and iterations per run, for a total of 1000 runs each.

In addition, for investigate gene flow between sites, we used a Bayesian assignment test-based method implemented in BayesAss 1.3 (Wilson and Rannala, 2003). Using a Markov chain Monte Carlo (MCMC) procedure, the software estimates whether an individual is an immigrant using 30 million MCMC iterations with a burn-in of 10 million steps within a 95% of confidence interval. Also, the multivariate method DAPC (Jombart et al., 2010) was applied to identify genetic clusters of related individuals among sampled localities using R package adegenet. DAPC analysis was chosen instead of Structure procedure as it makes no assumptions about underlying genetic data structure and requires prior identification of groups using Bayesian information criteria (BIC) to maximize between-group variance. The alpha score was used to select the optimal number of principal components applied in the further analysis in which 9 PCs retained 89,2% of total genetic information (Figures A1 and A2 in Suppl. Mat. A). Finally, analysis of molecular variance (AMOVA) in Arlequin software v.3.5.1.2 (Excoffier et al., 1992, 2005) was performed in distinct ways: i) to evaluate the existence of genetic structure among sampled populations and ii) to infer genetic discontinuity between northern (PE + RN) and southern sampling localities (CA + CO + PS).

3. Results

3.1. Genetic variability

A total of 23 alleles were found in the five loci tested. The null allele frequency per locus and population ranged from 0 to 0.207 (Table A1 in Suppl. Mat. A), that according to Dakin and Avise (2004) is considered a rare/low frequency, and therefore we maintained all loci in further

Table 1
Summary statistics of five microsatellite loci and overall mean of the five sampling sites and all 176 individuals (N = number of samples, A = number of alleles, PA = number of private alleles, R_S = allelic richness, H_O = observed heterozygosity, H_E = expected heterozygosity, F_{IS} = coefficient of inbreeding). In bold significance results after Bonferroni correction.

Microsatellite loci						
Locations	Sru-A8	Sru-A7	Sru-C127	Sru-A9	Sru-D5	Average
Rio Grande do Norte (RN)						
N	41	41	39	36	35	38.4
A	2	5	8	1	2	3.6
PA	0	0	0	0	0	0
Rs	1.268	3.002	7.074	1.000	1.974	2.863
H_O	0.024	0.219	0.820	0.000	0.142	0.241
H_E	0.024	0.226	0.838	0.000	0.227	0.263
F_{IS}	0.000	0.032	0.022	NA	**0.375**	0.107
Pernambuco (PE)						
N	14	13	12	11	11	12.2
A	1	2	7	1	2	2.6
PA	0	0	0	0	0	0
Rs	1.000	1.846	6.909	1.000	2.000	2.551
H_O	0.000	0.076	0.916	0.000	0.090	0.216
H_E	0.000	0.076	0.833	0.000	0.506	0.283
F_{IS}	NA	−0.054	**0.290**	NA	**1.000**	0.412
PortoSeguro (PS)						
N	49	49	47	41	49	47
A	2	4	8	1	2	3.4
PA	0	0	0	0	0	0
Rs	1.224	3.035	6.946	1.000	1.979	2.836
H_O	0.020	0.285	0.680	0.000	0.081	0.213
H_E	0.020	0.260	0.836	0.000	0.247	0.272
F_{IS}	0.000	−0.084	0.082	NA	**0.697**	0.173
Corumbau (CO)						
N	17	17	16	17	16	16.6
A	2	4	8	1	2	3.4
PA	1	0	0	0	0	0
Rs	1.882	3.283	7.475	1.000	1.976	3.123
H_O	0	0.352	0.937	0	0.187	0.295
H_E	0.114	0.315	0.858	0	0.175	0.292
F_{IS}	**1.000**	−0.122	−0.094	NA	−0.071	0.177
Caravelas (CA)						
N	50	52	52	52	48	50.8
A	2	7	9	1	2	4.2
PA	0	2	1	0	0	0
Rs	1.393	5.041	6.769	1.000	1.993	3.239
H_O	0,000	0.480	0.788	0.000	0.062	0.266
H_E	0.396	0.517	0.792	0.000	0.294	0.400
F_{IS}	**1.000**	0.071	0.005	NA	**0.789**	0.466

analysis. However, global F_{ST} for all loci and pairwise F_{ST} values were all higher using ENA correction (Chapuis and Estoup, 2007) when compared to ENA allele frequencies (Table 2 and Table A2 in Suppl. Mat. A), however neither were statically significant. This suggests that null alleles have no or minor influence on our analysis; therefore, all further tests were performed with uncorrected allele frequencies.

The number of alleles per locus varied from 1 (Sru-A9) to 9 (Sru-C127), being the Sru-C127 the one that contributing the most to total gene diversity (H_E = 0.858) (Table 1). The observed average heterozygosity and allelic richness were highest in Corumbau and Caravelas (H_O = 3.123, R_S = 0.295; H_O = 3.239, R_S = 0.266, respectively). Pernambuco was the locality with the lowest number of alleles (14), and together with Porto Seguro showed the lowest level of heterozygosity (H_O = 0.216 and 0.213, respectively) (Table 1). Caravelas and Pernambuco had also the highest values of inbreeding coefficient (0.46 and 0.41, respectively), while the lowest one was found on individuals from Rio Grande do Norte. There was no significant deviation from the Hardy-Weinberg equilibrium after Bonferroni correction in any of the studied populations. Evidence of linkage disequilibrium was found in two loci, Sru-A8 and Sru-C127, at Corumbau population.

Table 2
Comparison of pairwise F_{ST} derived from raw microsatellite data and after applying the excluding null alleles (ENA) correction method between sampled localities of *S. trispinosus* populations. No statistical significance was found using Bootstrap resampling over loci with 95% of confidence interval. Legend: CA = Caravelas, CO = Corumbau, RN = Rio Grande do Norte, PE = Pernambuco and PS = Porto Seguro.

	CA	CO	RN	PE	PS
CA	–	0.0184	0.0200	0.0304	0.0240
CO	0.018	–	0.0037	0.0093	0.0269
RN	0.020	0.003	–	−0.0053	0.0038
PE	0.030	0.009	−0.005	–	0.0058
PS	0.024	0.027	0.004	−0.006	

3.2. Population structure

Results of the POWSIM analysis revealed that, although with few loci, our marker had sufficient power to detected significant genetic differentiation above a F_{ST} value of 0.01 with 80% of probability (Figure A1 in Suppl. Mat. A) for both estimators used (Chi^2 = 0.8768 and Fisher = 0.8341).

The genetic structure among the sampled localities estimated by global (F_{ST} = 0.017; Table A2 in Suppl. Mat. A) and pairwise F_{ST} showed that after null alleles correction there are no significant differences among locations sampled (Table 2). The higher genetic difference was observed among Rio Grande do Norte/Pernambuco localities and Caravelas (F_{ST} = 0.02 and F_{ST} = 0.01, respectively) and the lowest registered between Corumbau/Porto Seguro and Rio Grande do Norte (both presenting F_{ST} = 0.003).

This result could also be verified by investigating the recent migration rate per generation among the sampled locations. The average percentage of immigrant individuals belonging to other locations varied from 4.7% (± 0.036 sd) to 11.9% (± 0.067 sd) being highest between Rio Grande do Norte and Pernambuco as source/recipient populations respectively (Table 3). Nevertheless, all sampled localities showed expected levels of self-recipient rates (≥66% of non-migrants, see BayesAss documentation) in which Pernambuco locality presented the highest proportion value, almost 75% (± 0.057 sd) (Table 3).

Similarly, the discriminant analysis of principal components (DAPC) showed no clear genetic clustering our data revealed by a non-reachable lower BIC value for different maximum number of clusters simulated and low alpha scores obtained (Fig. A2 in Suppl. Mat. A). However, by determining an arbitrary cluster number (n = 5) which corresponds to 5 original localities sampled, it revealed that all inferred clusters formed were in fact formed by individuals of all sampled localities, indicating high level of admixture (Fig A3 in Suppl. Mat. A). AMOVA results corroborated the absence of genetic structure among *S. trispinosus* sampling localities (F_{ST} = 0.001; p = 0.959); however, when northern vs southern localities were considered (K = 2), a low but significant FSC value of 0.011 reveal a subtle level of sub structuring among populations within groups tested, although 97% of the total genetic variance occurred among the individuals within the sampled locations (Table 4).

4. Discussion

These are the first results of population genetics of an endangered parrotfish species at Brazilian coast. Despite belonging to the same genus, the obtained results showed low microsatellite transferability rate between *Scarus rubroviolaceus* and *S. trispinosus* (29,41%), resulting only in five loci. Although the scope of our observations may be limited due to the restricted number of markers obtained and considering the

Table 3
The mean migration rates ± standard deviation [95% confidence interval] calculated by BayesAss using the microsatellite data are included for all source/recipient population comparisons. Legend: Source populations are listed across the top row; populations receiving immigrants are listed in the left-hand column. Caravelas (CA), Corumbau (CO), Rio Grande do Norte (RN), Pernambuco (PE) and Porto Seguro (PS). Values along the diagonal (bold) line are the self-recipient rates into the source population.

	CA	sd	CO	sd	RN	sd	PE	sd	PS	sd
CA	**0.727 [0.667, 0.812]**	± 0.044	0.061 [0.000, 0.157]	± 0.049	0.056 [0.000, 0.166]	± 0.052	0.052 [0.000, 0.142]	± 0.044	0.049 [0.000, 0.134]	± 0.042
CO	0.065 [0.000, 0.161]	± 0.049	**0.707 [0.666, 0.779]**	± 0.036	0.070 [0.000, 0.183]	± 0.057	0.061 [0.000, 0.171]	± 0.054	0.047 [0.000, 0.136]	± 0.044
RN	0.075 [0.000, 0.182]	± 0.055	0.091 [0.000, 0.201]	± 0.059	**0.705 [0.666, 0.780]**	± 0.037	0.080 [0.000, 0.193]	± 0.058	0.075 [0.000, 0.185]	± 0.056
PE	0.080 [0.000, 0.179]	± 0.054	0.092 [0.000, 0.203]	± 0.060	0.119 [0.000, 0.234]	± 0.067	**0.756 [0.666, 0.861]**	± 0.057	0.083 [0.000, 0.196]	± 0.059
PS	0.050 [0.000, 0.124]	± 0.039	0.0473 [0.000, 0.122]	± 0.038	0.048 [0.000, 0.126]	± 0.040	0.047 [0.000, 0.116]	± 0.036	0.744 **[0.666, 0.837]**	± 0.051

fact that the use of cross-specific markers can cause loss of genetic diversity, important considerations can be made.

Samples obtained at Corumbau and Caravelas displayed higher mean heterozygosity levels and higher allelic richness than other areas. Those localities are inserted into Abrolhos reef bank, the largest coral reef formation of South Atlantic Ocean. It consists of shallow (average 30 m) continental shelf which extends from Caravela's city up to 250 km offshore (Marchioro and Nunes, 2003), in which numerous reef habitat formations could be seen such as fringing reefs, mushroom reef type (“*chapeirões*”) (Leão et al., 2003), rhodolits beds (Amado-Filho et al., 2012) as well large sinkholes (Bastos et al., 2013). This large amount of preferred habitat is probably the responsible for harbouring the largest remnant populations of *S. trispinosus* at Brazilian coast (Ferreira et al., 2001; Francini-Filho and Moura, 2008), and may therefore resulted in higher allelic richness. Population genetic analyses in two parrotfish species from the Australian Great Barrier Reef showed a relationship among size and diversity of habitats with higher genetic variation (Dudgeon et al., 2000). In fact, large population sizes and habitat heterogeneity are known to positively influence genetic diversity in marine fish (Mitton and Lewis, 1989).

In addition, two important Marine Protected Areas – Abrolhos Marine Park and Corumbau Extractive Reserve, could also have influenced regional genetic diversity indexes. Recent works have shown higher genetic diversity in regions closer to fully protected areas when compared to non-protected ones (that is, where fishing is allowed, see Pérez-Ruzafa et al., 2006; Félix-Hackradt et al., 2013b), revealing that MPAs are important tools to conserving ocean's genetic resources (Arrieta et al., 2010). The results obtained herein reinforce that no-take MPAs could act as a genetic reservoir thus contributing to enhancing genetic diversity of neighbouring areas. Thus, the higher genetic variation observed in *S. trispinosus* from Corumbau and Caravelas (Abrolhos Marine Park) could be a combined result of larger habitat heterogeneity in a protected area. According to this evidence we suggest another comparative population genetic study in fish species living at both restricted/large – protected/non-protected habitats in order to test for the defended hypothesis.

On the contrary, lowest allelic number and observed heterozygosity were found at Pernambuco and Porto Seguro localities, respectively. These results could be an outcome of a continued process of individual removal by fishing and consequently genetic pool erosion, facilitated by reef proximity to the coast and the fishing gear used by fishermen, the “speargun”. According to Nunes et al. (2012), this technique is highly employed at Bahia state targeting mainly piscivorous fishes. However, at southern Bahia, in which Abrolhos region is inserted, the herbivores can contribute to 15% of total catch, which are primarily constituted of *S. trispinosus*. In addition, the monitoring of landings of local fisheries at Porto Seguro revealed that the relative importance of *S. trispinosus* in total capture (in weight) ranged from 8,5–21% depending the fishery category (DallOrto et al., 2017). This indicates great fishing pressure over greenback parrotfish populations. In Tamandaré (Pernambuco) however, although the species is appreciated by fishers and thus under continued pressure, it is presently relatively rare in landings (B. P. Ferreira pers.comm).

Several studies have shown the fishing effects on genetic diversity in several commercially important fish species (Smith et al., 1991; Hauser et al., 2002). Specially, in a meta-analytic survey done with 72 overexploited fish species, Pinsky and Palumbi (2014) evidenced that overfishing is directly related to the population genetic diversity reduction. Besides, the fishing pressure targeting larger individuals can reduce larval output and thus connectivity, since fish fecundity tends to increase with fish body size (Birkeland and Dayton, 2005). Despite not being the aim of this work to determine the fishing effect over the greenback genetic diversity, it is possible to infer that if the fishing effort over *S. trispinosus* populations persists, this could lead to reduction of effective population size, as showed by Previero (2014). Consequently, a greater loss of important and rare alleles may occur (Allendorf et al., 2014), reducing the resilience of greenback parrotfish populations against disturbances such as climate changes.

Table 4
AMOVA test of statistical genetic differences between northern localities and southern ones, considering K = 2 and K = 1cluster groups. Significant result ($p < 0.05$) in bold. Df = degrees of freedom; SS = sum of squares.

	Source of variation	Df	SS	Variance components	Variation (%)	Fixation indices
K = 2	Among groups	1	0.597	−0.003	−0.54	F_{CT} = −0.005
	Among populations within groups	3	2.870	0.006	1.15	**F_{SC}=0.011**
	Among individuals within populations	169	94.539	0.010	1.73	F_{IS} = 0.017
	Within individuals	174	94.000	0.540	97.65	F_{IT} = 0.023
	Total	347	192.006	0.553		
K = 1	Among populations	1	0.474	−0.000	−0.11	F_{ST} = −0.001
	Among individuals within populations	172	97.532	0.013	2.42	F_{IS} = 0.024
	Within individuals	174	94.00	0.540	97.69	F_{IT} = 0.023
	Total	347	192.006	0.553		

Although not significant global and pairwise F_{ST} values showed low levels of genetic heterogeneity (ranging from 0.01 to 0.03) which was consistent with moderate migrant rates found between all geographical locations. This pattern might have been produced by the continued admixture of individuals in which adult and juvenile phases could have contributed to facilitate gene flow provided reef habitat continuity at regional scales (Palumbi, 1992; Mora and Sale, 2002; Weersing and Toonen, 2009). Studies that explored the movement patterns of several Scaridae species (Howard et al., 2013), showed a maximum home range of 43.000 m^2 for *Scarus rivulatus* (Welsh and Bellwood, 2012), while *S. rubroviolaceus* showed high habitat fidelity and a home range of 2.500 m^2 (Ong, 2007). These findings suggest a limited capacity of genetic homogenization at regional and local scales through fish movement, illustrating that larval dispersal is acting at larger spatial scales.

Other work that investigated the genetic structure of marine fish species were also unable of detecting significant differences among populations along Brazilian coast (*Scomberomorus cavalla*, Santa Brígida et al., 2007; *Ocyurus chrysurus*, Vasconcellos et al., 2008, Silva et al., 2015; *Chaetodon striatus* and *Pomacanthus paru*, Affonso and Galetti, 2007), between coastal reefs and oceanic islands (*Acanthurus chirurgus*, Rocha et al., 2002; *Cephalopholis fulva*, Freitas et al., 2003, Souza et al., 2015; Neves et al., 2016), through the employment of mitochondrial markers (lutjanids, Dias Junior et al., 2012; Pereira, 2016) and at larger temporal scales (*Cynoscion acoupa,* Rodrigues et al., 2008). Nevertheless, some studies revealed genetic divergence between Brazilian and Caribbean populations (*Acanthurus bahianus* and *Acanthurus coeruleus*, Rocha et al., 2002; *Ocyurus chrysurus,* Vasconcellos et al., 2008), among tropical and subtropical regions (*Macrodon ancylodon*, Santos et al., 2006; *Epinephelus itajara*, Abreu et al., 2014, Damasceno et al., 2015; *Chaetodipterus faber*, Machado et al., 2017).

On the other hand, a weak but significant genetic heterogeneity was found when grouping northern *vs* southern locations (F_{CT} = 0.01), nevertheless more than 90% of the genetic variability relied within individuals within their localities. One possibility is that at such scales, larval transport is sufficient to maintain gene flow but restricted enough to provide subtle differences. Brazil current (BC) is the main oceanographic current acting at Brazilian coast which carries warm water from equatorial region southward until Brazil-Argentina border, where it meets the cold Malvina's current with opposite direction (Ekau and Knoppers, 1998; Lira et al., 2010). Owing to its great extension, the BC can be responsible for the large distance gene flow observed along 2000 km of coast, where local currents can sustain, along with adult and juvenile movement, high regional and local admixture, carrying fish larvae between adjacent reef systems.

Reef fish have relatively long pelagic larval duration (PLD) (~a month) (Victor and Wellington, 2000). There is little information for Scarinae PLD: 24 days for *Scarus iserti* (Clifton, 1995); between 29 and 41.8 days for five undetermined *Scarus* species from Okinawa, Japan (Ishihara and Tachihara, 2011); and larger mean PLD of 57 days (47–80 days) and 60 days (50–93 days) for *Sparisoma viride* and *Sparisoma radians* respectively at Panamá (Victor and Wellington, 2000). These estimates can explain the ability of reaching long distances in Scarinae. Notwithstanding, it is paramount that new studies focused in the identification and description of *S. trispinosus* larvae to proper evaluate its dispersal potential and its influence over connectivity patterns.

In a similar way, several studies related the genetic homogeneity to large PLDs (Rocha et al., 2002; Freitas et al., 2003; Santa Brígida et al., 2007), adult habitat preferences (Rocha et al., 2002), high mobility (Santa Brígida et al., 2007; Silva et al., 2015), and/or local coastal currents (Freitas et al., 2003; Vasconcellos et al., 2008; Silva et al., 2015). On the other hand, genetic differences were attributed to Amazon river discharges, isolating Caribbean fauna from Brazilian one (Rocha et al., 2002; Machado et al., 2017) and ecological factors associated to distinct local selective pressures (Affonso and Galetti, 2007).

DAPC analysis were not able to define genetic clusters; this outcome might happen if i) there is no genetic structure at all, ii) there are not enough information to disentangle different values of k, or iii) the method does not apply to analysed data (Jombart et al., 2010). Many studies using microsatellite markers have used properly DACP analysis (Rolshausen et al., 2015; Portnoy et al., 2016; Blankenship et al., 2017) indicating that the method is appropriate to such data. Another hypothesis is that our data set was not sufficient to detect clusters probably due to low loci number employed. Despite this might be expected, a POWSIM analysis revealed that our marker had enough power to detect low genetic structure (~0.01), similar the ones found in this study, with 80% of confidence depicted by either χ^2 and Fisher tests (Figure A1 in Suppl. Mat. A). Therefore, the most probable reason is that there is no genetically differentiation among geographical localities studied, although more studies using more (and polymorphic) microsatellite loci must be done.

Recently, several microsatellites have been identified for threatened species (Farias et al., 2003; Abdul Muneer et al., 2009), which can be useful tools in the development of management programs for species population recovery, by indicating genetic diversity hotspots that can be used as source for population restocking (Lopera-Barrero et al., 2013). In our study, we depicted that the genetic structure of *S. trispinosus* populations varied at different spatial scales. Therefore, additional effort must be done on the development of species-specific molecular markers and to fulfil sampling gaps localities in order to promote genetically-assisted management actions to achieve successful conservation goals.

5. Conclusion

In this work we found no genetic differences among sampled localities however, when those samples were gathered into latitudinal groups (northern *vs* southern) a subtle but significant genetic substructuring were revealed using *Scarus trispinosus*, an endemic and endangered parrotfish species at Brazilian coast. It is proposed that a combination of high local individual admixture favoured by habitat connectivity drived genetic homogeneity at regional scales while larval dispersal contributed to heterogeneities observed at large scales maintaining gene flow through oceanographic currents. Indeed, by comparing genetic diversity among studied localities, we found evidences that high habitat availability provided by a large continental shelf combined with genetic reservoir inside fully marine protected areas can contribute significantly to increase genetic diversity in adjacent waters. Contrastingly, we observed that historically overfished areas harboured the smaller allelic richness and diversity values, suggesting genetic erosion due to fishing effect. Although limited, our results can be used as a baseline assessment for future works aiming conservation and recovery of *S. trispinosus*, however, for a better resolution, it is paramount to develop new molecular markers or the use of high-throughput molecular techniques.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.marenvres.2018.10.004.

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