UNIVERSIDAD
NACIONAL
DE COLOMBIA

Genetic survey and variation of the populations of *Sparisoma chrysopterum* (Labriformes, Scarinae) in the southern and western Caribbean

María Katherine Ontiveros Ospina

Universidad Nacional de Colombia sede Caribe

Instituto de Estudios en Ciencias del Mar - CECIMAR

Santa Marta, Colombia

2023

Genetic survey and variation of the populations of *Sparisoma chrysopterum* (Labriformes, Scarinae) in the southern and western Caribbean

María Katherine Ontiveros Ospina

Tesis presentada como requisito parcial para optar al título de:

Magister en Ciencias – Biología

Director:

Arturo Acero Pizarro, Ph. D.

Codirector:

Jose J. Tavera, Ph. D.

Línea de Investigación: Biología Marina

Grupos de Investigación:

Fauna Marina Colombiana: Biodiversidad y Usos- Universidad Nacional de Colombia

Sistemática, Evolución Y Biogeografía Animal (SEyBA)- Universidad del Valle

Universidad Nacional de Colombia sede Caribe

Instituto de Estudios en Ciencias del Mar - CECIMAR

Santa Marta, Colombia

2023

“Dicen que antes de entrar en el mar, el río tiembla de miedo, mira para atrás, para ver su recorrido, para ver las cumbres y las montañas, para ver el largo y sinuoso camino que abrió entre selvas y poblados; y ve frente a sí un océano tan extenso que entrar en él solo puede ser desaparecer para siempre. Pero no hay otra manera: el río no puede volver, nadie puede volver, volver atrás es imposible en la existencia. El río precisa arriesgarse y entrar en el océano. Al entrar, el miedo desaparecerá, porque en ese momento sabrá que no se trata de desaparecer en él, sino de volverse océano.”

-Khalil Gibran-

Agradecimientos

No hay una mejor manera de describir este proceso que la frase de Gabriela Mistral: "*Dame la perseverancia de las olas del mar, que hacen de cada retroceso un punto de partida para un nuevo avance"*. En todos esos "ires y venires" siempre hubo alguien apoyándome, alentándome, acompañándome; por lo que sólo queda agradecer.

Al profe Arturo, por su infinita paciencia y bondad, a José por la confianza y el cariño, por abrirme las puertas del laboratorio y brindarme su amistad, a Tefi por ser mi polo a tierra y mi cómplice, a Anita por su calidez y disposición, a todos los del laboratorio por las alegrías siempre y a Elkin por la voluntad. A Julián Quintero por su guianza. A mis compañeros de la maestría por todas las aventuras y los buenos recuerdos, ha sido un orgullo verlos prosperar.

Esto no habría sido posible sin mi familia a quienes los honro por el ejemplo, la persistencia, por el apoyo y la honestidad. A la familia Pesqueira Caldas por su amor incondicional. A Carlos B. por las palabras de aliento siempre. A mi querido Omar, por ser mi luz en la oscuridad, por el cariño y las risas infinitas, por aguantarme en mis desesperos y en especial por la compañía.

A mis amigos, los de siempre, los viejos y los nuevos, los de la vida, los del colegio, la universidad, el trabajo, a todos aquellos que ya no están pero que me han hecho quien soy sepan que fueron un motor en este andar.

Finalmente quiero agradecer a la AUNAP, que hizo posible el desarrollo de este estudio mediante el convenio No. 000149 de 2015. Al laboratorio de Ictiología de la Universidad del Valle y a la Universidad Nacional de Colombia Sede Caribe.

Abstract

Genetic survey and variation of the populations of *Sparisoma chrysopterum* (Labriformes, Scarinae) in the southern and western Caribbean

Parrotfishes play a key role in marine ecosystems, especially on coral reefs, a highly threatened ecosystem of significant importance, as they host nearly 25% of the diversity of marine life. These fish have become the target of artisanal fishermen in the Caribbean and their decline in natural habitats has been noticeable. Despite the importance of these organisms, little is known about their populations in terms of their genetic diversity. Fishery resources generally do not acknowledge population structure or local adaptations; instead, they are prematurely considered panmictic stocks. Although parrotfishes are not classified as a fishing resource, they suffer from this pressure. Considering this, it is essential to know the diversity, population genetic structure, and connectivity as population indicators of their adaptive capacity, resilience, and survival in the face of present and future eventualities. *Sparisoma chrysopterum,* the redtail parrotfish, was selected as a model to evaluate genetic variation at the population level using single nucleotide polymorphisms (SNP) and its distribution in a longitudinal gradient that covers four areas of the southern and southwestern Caribbean (Margarita Island, Taganga, Cartagena, and San Andrés Island), locations in which the genetic flow can be interrupted by geographical barriers such as the Panama-Colombia countercurrent, Magdalena River plume, and Caribbean upwelling. There was no evidence of population structure in the southern Caribbean. The genetic diversity showed a pattern with low values, the heterozygosity was low in the four locations, and there were no barriers to gene flow. The general result suggests a *Sparisoma chrysopterum* in the southern Caribbean is a panmictic population.

Keywords: Population genomics, southern Caribbean, RADseq, SNP, redtail parrotfish

Resumen

Estado genético y variación de las poblaciones de *Sparisoma chrysopterum* (Labriformes, Scarinae) en el Caribe suroccidental

Los peces loro desempeñan un papel de vital importancia en los ecosistemas marinos, en especial en los arrecifes coralinos, un ecosistema altamente amenazado y de suma importancia pues alberga cerca de 25% de la diversidad de la vida marina. Estos peces se han convertido en objetivo de los pescadores artesanales del Caribe y su disminución en hábitats naturales ha sido notable. A pesar de la importancia de estos organismos, poco se conoce acerca de las poblaciones en términos de su diversidad genética. Los recursos pesqueros en general no toman en cuenta la estructura poblacional ni las adaptaciones locales, en cambio son consideradas, prematuramente, poblaciones panmícticas y, aunque los peces loro no están establecidos como recurso pesquero, sufren por esta presión. Teniendo esto en cuenta, se hace indispensable conocer la diversidad, estructura genética poblacional y conectividad como indicadores poblacionales de su capacidad adaptativa, resiliencia y supervivencia ante eventualidades presentes y futuras. Se seleccionó la especie *Sparisoma chrysopterum* como modelo para evaluar la variación genética a un nivel poblacional a través del estudio de polimorfismos de único nucleótido (SNP) y su distribución en un gradiente longitudinal que abarca cuatro áreas del Caribe sur y occidental (isla Margarita, Taganga, Cartagena e isla de San Andrés), áreas en las que el flujo genético puede verse interrumpido por las barreras geográficas de la contracorriente Panamá -Colombia, la pluma del Magdalena y la surgencia del Caribe. No hubo evidencia de estructura poblacional en el Caribe sur. La diversidad genética mostró un patrón con valores bajos, la heterocigosidad fue baja en las cuatro localidades y no se evidenciaron barreras para el flujo génico. El resultado general sugiere que *Sparisoma chrysopterum* en el Caribe sur es una población panmítica.

Palabras clave: Genómica poblacional, Caribe Sur, RADseq, SNP, Loro colirrojo

Table of content

Abstract ..... **V**

Resumen ..... **VI**

Introduction **8**

Study area **12**

Materials and Methods **14**
1 Sample collection 14
2 DNA extraction and RAD-seq library preparation 14
3 Raw data filtering and SNP calling 14
4 Genetic Diversity 15
5 Population structure analysis 16
6 Estimating connectivity 16

Results **17**
1 DNA extraction 17
2 RAD-seq library preparation 17
3 Genetic diversity 17
4 Population genetic structure 18
5 Connectivity and gene flow 21

Discussion **25**

Conclusions and recommendations **28**
Conclusions 28
Recommendations 29

References **30**

Introduction

Marine and coastal ecosystems have been considerably reduced and fragmented due to conflicts over their use, resulting in the loss of diversity. The constant growth and development of human coastal population have generated physical and chemical modifications of environments such as coastal wetlands, mangroves, and seagrasses, degrading and disrupting these habitats through overexploitation, dredging, and removal, as well as construction, pollution, and discharge, among others (Brown et al., 2018; Gray, 1997). Coastal ecosystems also maintain connectivity with coral reefs, which are the source of genetic stocks and serve as larval recruitment sites and nurseries for juveniles of many reef species that are important in coastal fishing activities (Cocheret De La Morinière et al., 2002; Dorenbosch et al., 2004; Nagelkerken et al., 2002).

Coral reefs are ecosystems of significant importance since they cover less than 1% of the ocean benthos yet host about 25% of marine life (Fisher et al., 2015; Knowlton et al., 2010). The three-dimensionality generated by reef structures serves as a refuge for many species that provide food for others, generating a truss of trophic and dynamic networks between species functioning in a delicate balance(Rogers et al., 2014). Additionally, they provide various ecosystem services such as protecting coastlines, regulating erosive processes,

biogeochemical services, fishing resources, and cultural services such as tourism (Elliff & Silva, 2017).

Herbivorous fish play an essential role in the prosperity of coral reefs (Adam et al., 2015; Bernardi et al., 2000; Comeros-Raynal et al., 2012; Cramer et al., 2017), as they feed on the fast-growing macroalgae that compete for space with the coral, skewing the competition in their favor, allowing greater recruitment (Jackson et al., 2014; Mumby, 2009a, 2009b; Steneck et al., 2014). In the last three decades, Caribbean coral reef ecosystems have been transforming into habitats dominated by algae (Swierts & Vermeij, 2016), greatly due to the decline in herbivore populations such as parrotfishes which influence these ecosystems, facilitating coral recruitment, growth and fecundity (Bernardi et al., 2000; Cramer et al., 2017; Hawkins & Roberts, 2004b, 2004a; Jackson et al., 2014; Mumby, 2009a, 2009b; Steneck et al., 2014) .

Parrotfishes are currently not only facing habitat loss but also pressure from local artisanal fisheries that have introduced them to their markets in the absence of large traditional commercial species (Hutchings, 2000; Hutchings & Reynolds, 2004; López-Angarita et al., 2021; Reynolds et al., 2005; Vallès & Oxenford, 2014), endangering these populations as both adults and juveniles are captured and the effect that this may cause is still little known (Enberg et al., 2009; Saad et al., 2013). Fishing can alter genetic diversity, since characteristics within a population are modified when fishing pressure is selective (Kenchington, 2013). Additionally, the management strategies for the use and exploitation of these resources are based on the biological and demographic information of the species, which is still vaguely known and rarely considers the most basic level of diversity, which is the genetic level, key aspect to determine conservation units (Dichmont et al., 2012; Funk et al., 2012; Nielsen et al., 2012; Rodríguez-Ezpeleta et al., 2016). For a long time, marine fishing resources have been managed assuming that populations are genetically homogeneous due to the lack of apparent or obvious barriers, a great capacity for dispersal of eggs and larvae, migrations, and large population sizes (Graves, 1998; Natasha et al., 2022; Nesbø et al., 2000; Puebla, 2009; Ward et al., 1994). However, possible biogeographic barriers have been identified (Robertson et al., 2006), which may interrupt the connectivity assumed in these populations; there are ‘hard’ and ‘soft’ barriers according to the isolation mechanism that defines them (Cowman & Bellwood, 2013). Hard barriers are those that physically prevent contact between populations. Soft barriers are those that

interrupt the mobility of adults or larval dispersal, but can become permeable, such as hydrological processes (e.g., currents, long distances) (Cowman & Bellwood, 2013; Luiz et al., 2012; Pelc et al., 2009). This study considered the possible impact of three soft barriers to the dispersal of *S. chrysopterum* in the southern Caribbean, first, the upwelling system in the Guajira (Colombia) and eastern Venezuela, the Magdalena River plume, and the Panama-Colombia Countercurrent (or Panama-Colombia gyre).

Although parrotfishes have been greatly studied for their ecological importance little is known about their genetics. Furthermore, few studies have addressed their genetic diversity, population structure, demographic history, and evolution (Bernardi et al., 2000; Loera-Padilla et al., 2021; Saad et al., 2013; Velasco-Montoya et al., 2022). However, recent demographic information, life history patterns, and reproductive biology have missing information that is essential to the conservation and management of these species. The genus *Sparisoma* is trans-Atlantic, with 15 species, eight in the Greater Caribbean, six of which are endemic. One of the latter is *Sparisoma chrysopterum*, the redtail parrotfish; at the moment the species threat is categorized in the IUCN red list as Least Concearn (Rocha et al., 2012) and it is not included in the Colombian red list assessment of marine fishes (Chasqui Velasco et al. 2017). Nonetheless, it is the third largest species of the genus in the Caribbean, reaching up to 46 cm in total length which makes it desirable as a food resource. It is found in a variety of habitats such as submerged vegetation, rocky and coral reefs, and soft bottoms like seagrass beds. This species is sexually dichromatic, has pelagic eggs, and is monandric as well as protogynous (Robertson & Van Tassell, 2019; Robertson & Warner, 1978). In the southern and western Caribbean, the consumption of these species has increased and a depletion in their natural habitats has been noticeable. This is a warning sign, given that parrotfishes are not considered a fishery resource and have no management or restrictions to their quotas, however, the devastating effects of their extinction in local habitats is greatly known (Bellwood et al., 2003; Cramer et al., 2017; Hawkins & Roberts, 2004b; Palumbi, 2008).

Determining the genetic diversity and gene flow for the redtail parrotfish between the different southern locations will provide valuable baseline information for conservation purposes and management processes of these populations. The insight to the genetic diversity present in this region may also reveal processes of local adaption (understood as

the specific genetic traits developed by populations resulting from natural selection to favor them in their local environment), and connectivity valuable to the resilience of the species, given the possible biogeographic barriers to genetic exchange (Brandrud et al., 2017; Nielsen et al., 2009; Picq et al., 2016; Silliman, 2019; Tiffin & Ross-Ibarra, 2014). On the other hand, the speciation processes in fish are considered to be determined by the life history of the organisms, larval dispersal, as well as other oceanographic factors such as currents (Bradbury et al., 2008; Loera-Padilla et al., 2021; Luiz et al., 2012; Pelc et al., 2009; Puebla, 2009a; Robertson et al., 2006). It is essential to establish whether geographic locations constitute an independent evolutionary unit (population) by evaluating the gene flow between them; small amounts of gene flow are sufficient to avoid the negative effects of genetic drift and inbreeding (processes exacerbated by fishing exploitation) which can lead to adaptive decline (Lowe & Allendorf, 2010). The RAD-seq technique allows to study a reduced representation of the genome of organisms, generating large numbers of molecular markers (single nucleotide polymorphisms, SNP) at a relative low cost. This technique has been able to resolve fine scale genetic structure that is not revealed by other genetic markers (Benestan et al., 2015; Nesbø et al., 2000; Rodríguez-Ezpeleta et al., 2016; Silliman, 2019). The RAD-seq technique was employed to obtain a SNP dataset to address the missing information and attempt to provide a genetic diversity description and evaluate possible evidence of microevolution processes that define the genetic structure in populations of *Sparisoma chrysopterum* in the southwestern region of the Caribbean Sea. That was the aim of this study as well as understanding this species from a genetic standpoint is important for improved conservation and management efforts.

Study area

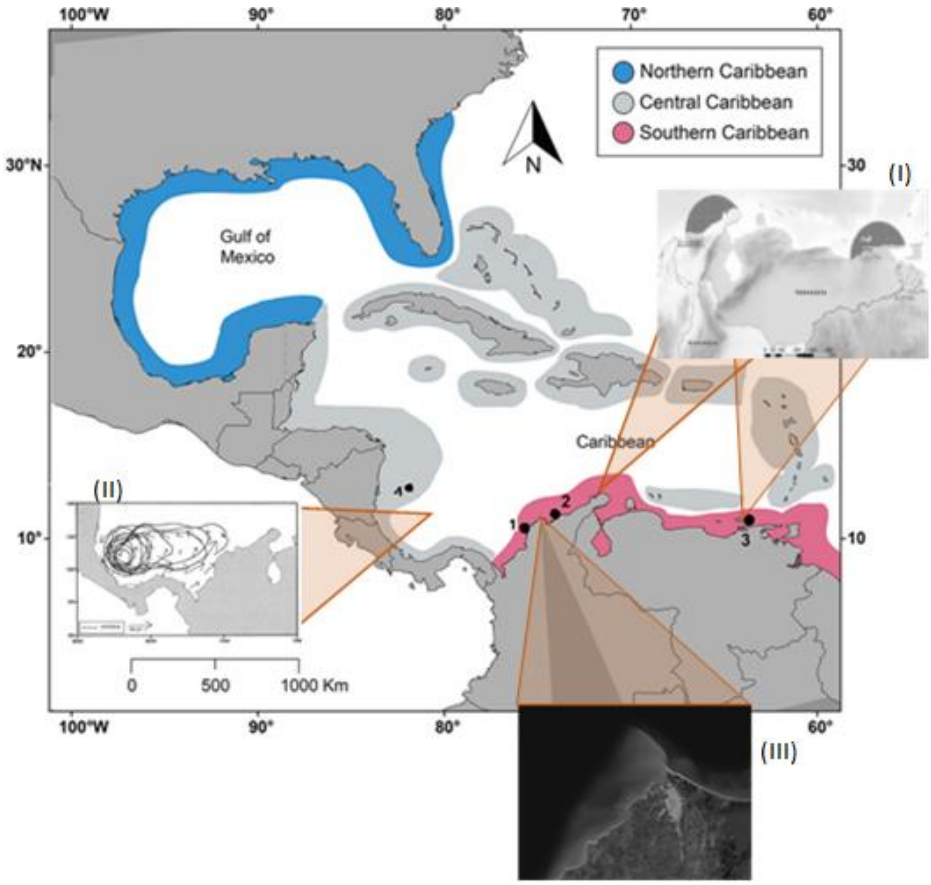
The tropical and subtropical western Atlantic is characterized as a high-diversity ocean, and one of the four major centers of tropical marine biodiversity in the world (Robertson & Cramer, 2014). It comprises three main subdivisions: A subtropical northern province (southeastern USA, Florida, and the Gulf of Mexico), a tropical central province (Central America, Bermuda, Bahamas, and the Antilles), and a southern province (continental shelf of northern South America). These are determined by environmental differences such as latitudinal variation in sea temperature, availability of major habitats and nutrient additions from upwelling and rivers (Robertson & Cramer, 2014).

This study includes locations in the southwestern portion of the central province (San Andrés Island) and in the southern province [Cartagena, Santa Marta (Taganga) and Venezuela (Margarita Island)]. The central province lacks freshwater input from large rivers, as well as significant upwelling systems. In this province, given the runoff in some areas and soft bottoms in coastlines, the formation of inshore reefs is hindered, but it is balanced by the vast continental shelf where offshore reefs support reef fish assemblages (Robertson & Cramer, 2014). On the contrary, the southern province is eutrophic; it has high nutrient input by large river outflows and coastal upwelling systems, which substantially influence in productivity as conditions such as temperature, salinity, and pH change. Another characteristic of the southern province is the presence of rocky shorelines and relatively uncommon structural coral reefs (Robertson & Cramer, 2014).

In the southern province three phenomena can be considered potential barriers to gene flow since they affect physical and chemical conditions of the marine environment. These are the Panama-Colombia Countercurrent (PCC), the Magdalena River Plume (MRP), and

the upwelling system at the northern South American Caribbean (Guajira and eastern Venezuela) (Figure 1-1). The PCC forms a gyre causing a relative encasement of the waters at the southwest corner of the Caribbean; this vortex rises the surface temperature of the water, generating greater rainfall characteristic of the Gulf of Darien region. This entails a decrease in the salinity of the waters in this region (Andrade, 2003). The second barrier is the outflow of the Magdalena River. This is the national Colombian river, and it carries vast amounts of sediments and nutrients within its waters. This freshwater input affects salinity, density, and water temperature (Restrepo et al., 2006, 2014; Rueda et al., 2016). Finally, the upwelling system at the Guajira and western Venezuelan region impacts the surface temperature distribution and salinity of these waters (Gómez Gaspar & Acero P., 2020; Paramo et al., 2011) .

Figure 1-1: Map of the sampling locations of *Sparisoma chrysopterum* for this study. Locations: (1) Cartagena, (2) Taganga, (3) Margarita Island, (4) San Andrés Island. The colored regions refer to biogeographic provinces as stated (Robertson & Cramer, 2014). Soft barriers to be assessed (I) Upwelling (Gómez Gaspar & Acero P., 2020), (II) Colombia- Panama Gyre (Andrade, 2001), (III) Magdalena River Plume *(Google, 2021)*. Map taken and modified from Loera-Padilla et al. (2021)



Materials and Methods

1 Sample collection

Samples were collected at four locations: two island locations (San Andrés and Margarita) and two mainland ones (Cartagena and Taganga). One was located in the central province (San Andrés Island) and three in the southern province (Cartagena, Taganga, and Margarita Island) of the Caribbean. Samples of *Sparisoma chrysopterum* fin clips were collected at local artisanal fish markets between 2015 and 2018. Fin clips were preserved in 96 % molecular grade ethanol and stored at -20 ºC. Ten samples from each location were processed for DNA extraction, and eight samples of each location were selected for the analyses.

2 DNA extraction and RAD-seq library preparation

High-quality (high-molecular-weight) genomic DNA extraction was performed using the Gentra Puregen Tissue Kit by QIAGEN, following the manufacturer's instructions. Genomic DNA integrity was assessed via electrophoresis using 1 % agarose gels. A total of 32 samples (eight individuals from each location) containing high molecular weight DNA (>10 kb) were normalized at a concentration of 20 ng/µL in a final volume of 50 µL. The RAD-seq library preparation by single digestion was carried out by Floragenex Inc.(www.floragenex.com) using the *SbfI* restriction enzyme. The final libraries were sequenced in two lanes of an Illumina Hi-Seq 2000 platform at the University of Oregon, using single-end 91 bp reads.

3 Raw data filtering and SNP calling

Raw sequences (Illumina reads) were demultiplexed and filtered with STACKS software v. 2.5.3 (Catchen et al., 2011, 2013; Rochette & Catchen, 2017) using the *process_radtags* pipeline setting the phred score to 25 for low quality data filtering. Demultiplexed and filtered sequences were initially tested, selecting a subset of the data by depth of coverage for each

location, following Rochette & Catchen (2017) protocol. Different values of *m* (minimum number of identical reads required to initiate a new putative allele), *M* (number of mismatches allowed between stacks within individuals) and *n* (number of mismatches allowed between stacks between individuals) parameters were tested to select the optimal parameter combination to proceed with the analyses.

The RADProc pipeline (Nadukkalam Ravindran et al., 2019) was executed selecting the eight individuals from each of the different locations with the highest number of reads. This program uses the same parameters defined by Stacks (*m*, *M*, and *n*) for assembling loci *de novo* and catalog building. To compare and identify optimal parameter settings, the RADProc program was run to sweep through the different parameter values for *m*, *M*, and *n*. Here, were combined *M* values from 1 to 8, *m* from 3 to 6, and *n* was set to a single value for each run (varying from 1 to 4). The parameter combination selected for running the *de novo* pipeline were m=3, M=4, n=2. The *populations* program was used to filter loci and variant sites. From the generated catalogue, loci were selected under the criteria of being shared in at least 80% (-R=0.8) of the individuals across the populations. The remaining sites also had to pass the constraints set for this module including a minimum allele frequency of 0.02 (*--min-maf 0.02*), and a maximum observed heterozygosity of 0.65 *(--max-obs-het 0.65*). In order to avoid paralogous loci, the command *--wirte-single-snp* was used to restrict data analysis to only the first SNP per locus. Finally, the parameter -p which is the minimum number of populations a locus must be present in to process a locus, was set to 1. Within this module, STACKS v. 2.5.3 can perform populations genetics statistics calculations, from which: divergence from Hardy-Weinberg equilibrium for each locus (*--hwe*), and SNP and haplotype-based F statistics (*--fstats*) were requested. The information was then exported in several formats for further analyses (VCF, GenePop, Structure, and PLINK). The output data in variant call format (VCF) files was used to conduct analyses. The output VCF file was converted into different formats using PGDSpider v2.1(Lischer & Excoffier, 2012).

4 Genetic Diversity

To determine genetic variation in between populations, F_{ST} per pair of populations was calculated following (Weir & Cockerham, 1984) formulation, implemented in adegenet with R v 4.0.5. Genetic diversity estimates, such as Observed Heterozygosity (H_o), Expected

Heterozygosity (H_e), and Inbreeding Coefficient (F_{IS}) were estimated with the package *hierfstats* (Goudet & Jombart, 2015) in R v 4.0.5 (R Core Team, 2019) and STACKS v.2.5.3 was used to estimate genetic diversity for each site.

5 Population structure analysis

To infer the degree of differentiation between sampled localities a pairwise F_{ST} test was performed in Arlequin v.3.5.2.2 software (Excoffier & Lischer, 2010) A principal component analysis was performed using *adegenet* package in R v 4.0.5 (Jombart & Ahmed, 2011; R Core Team, 2019), without any a priory population definition. A Bayesian clustering approach was implemented in STRUCTURE v 2.3.4 (Pritchard et al., 2000) to infer the most likely value of *K groups* (putative populations), based on admixture ancestry model. Estimations were carried out with an initial burn-in of 10,000 Markov Chain Monte Carlo (MCMC) iterations and followed by 500,000 iterations for each inferred cluster (*K* 1 to 5 with 10 replicates each) generations and a burn-in of 30,000. Optimal *K* was determined according to the Evanno method (Evanno et al., 2005) implemented in *StructureHarvester* (Earl & von Holdt, 2012; Jackson et al., 2014). The output was visualized using CLUMPAK (Kopelman et al., 2015).

A Discriminant Analysis of Principal Components (DAPC) was calculated using the R package *adegenet* v 2.1(Jombart, 2008; Jombart et al., 2010). Cross-validation was used to define the number of principal components (PCs) retained in the analysis, identifying the optimal point in the trade-off between dimensionality reduction and information loss.

To assess the degree of population structuring, a hierarchical analysis of molecular variance (AMOVA) was implemented with the function *poppr.amova* in the *poppr* package (Kamvar et al., 2014) in R (R Core Team, 2022).The AMOVA was performed using the ΦST statistic and making several hierarchical clusters: 1. Test 1: the four populations independently. Test 2: San Andrés Island in comparison with the other three populations. Test 3: Margarita Island (Venezuela) compared with the other three populations.

6 Estimating connectivity

A Mantel test was performed in GenAlEx 6.5 (Peakall & Smouse, 2012) to evaluate if genetic distances among populations increased as a linear function of geographic distances. The relative migration rates for every population were calculated using the R function, divMigrate from the diveRsity package (Keenan et al., 2013).

Results

1 DNA extraction

40 of the samples collected for *S. chrysopterum* were processed (10 for each location) for DNA extraction, the best 8 (DNA concentration > 50 ng/µL) of each location were selected for RAD-seq and further analyses.

2 RAD-seq library preparation

A total of 212,788 loci, composed of 19,371,147 sites were obtained from two sequencing lanes. After running the *process_radtags* pipeline in STACKS v 2.59 software to remove enzyme and barcode, and filtering for quality, 235,039 variant sites remained. However, due to the amount of missing data of variant sites a total of five individuals (three from Margarita and two from San Andrés) were removed from the analysis.

After the *de novo* assembly a total of 212,788 loci were obtained, composed of 316,573 sites. We used the *populations* pipeline to final data set contained 2,449 SNP singletons (SNPs). The *populations* pipeline was run afterwards, to calculate population genetics statistics and all files format used in different analyses. The parameters used for this program included a minimum minor allele frequency (*--min-maf*) of 0.02 to process a nucleotide site at a locus, and minimum percentage of individuals (80%) in a population required to process a locus for that population (*-r*).

3 Genetic diversity

Genetic variation parameters were calculated to determine the amount and distribution of genetic variation and possible population structure of the redtail parrotfish in the southwestern Caribbean. 27 samples of *S. chrysopterum* from four different locations were

used to obtain 2,449 SNPs, which revealed heterozygosity, both observed (Ho) and expected (He) to be similar in each location, and low evidence of inbreeding (F_{IS}) as well as nucleotide diversity (π) (**Table 3-1**).

Table 3-1: Genetic diversity indices per location.

Location	Number of Individuals	H_o	H_e	F_{IS}	π
Cartagena	8	0.00055	0.00098	0.00143	0.00106
San Andrés	6	0.00045	0.00097	0.00147	0.00109
Taganga	8	0.00053	0.00107	0.00171	0.00115
Margarita	5	0.00066	0.00098	0.00103	0.0011

Observed heterozygosity(H_o), Expected heterozygosity(H_e), Inbreeding coefficient(F_{IS}), Nucleotide diversity(π)

4 Population genetic structure

The Bayesian clustering approach and cross-validation error values were used to analyze individual clustering based on admixture ancestry model. The results indicated that there is likely only one putative genetic cluster present (K = 1) of redtail parrotfish across the southern Caribbean (Figure 4-1) as also shown by the Discriminant Analysis of Principal Components (DAPC) (Figure 4-4).

Cluster analysis (Admixture)

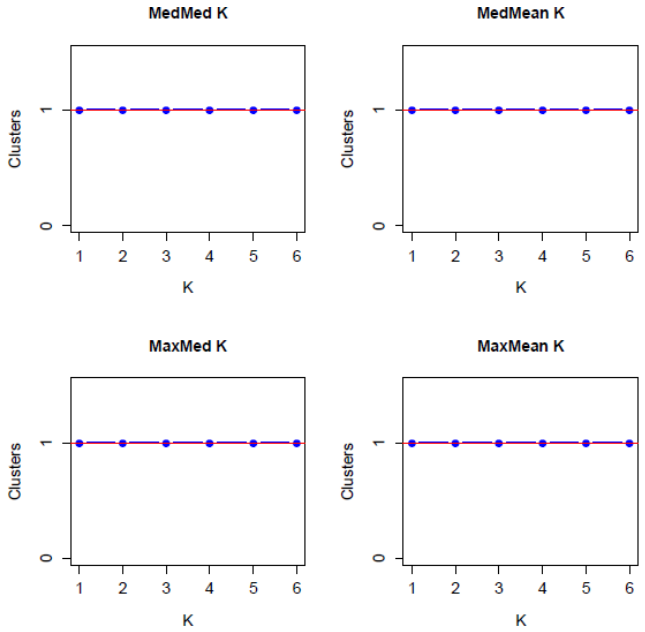


Figure 4-1 Clustering approach. A single cluster following (Puechmaille, 2016)

The Bayesian clustering approach to infer genetic ancestry shows a single genetic stock (blue), homogeneously distributed at the four locations, revealing no structure (Figure 4-2).

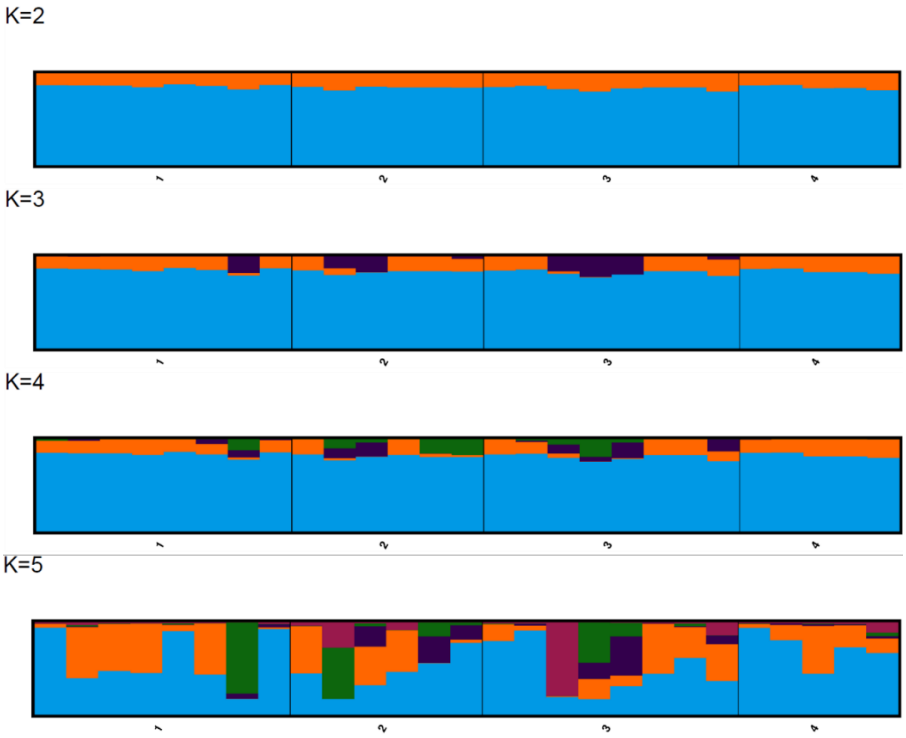


Figure 4-2 Graphical representation of the Bayesian clustering approach obtained from the dataset. Each bar represents an individual and each color, its inferred membership in each of the *K (2-5)* potential ancestral populations. 1) Cartagena 2) San Andrés Island 3) Taganga 4) Margarita Island.

Principal component analysis (PCA) is a way to depict maximized similarities within groups. In contrast, the discriminant analysis of principal components (DAPC) creates a visual representation, using ordination, of the genetic variation among populations. The results obtained with this SNP dataset show genetic similarity among the dataset within the locations sampled (Figure 4-3, Figure 4-4).

The two principal components do not explain a significant portion (10%) of the variance Figure 4-3, which means those furthest individuals share the least similarities to the rest that are explained by these two components but may be explained by other components not represented in this plot. These two individuals can be considered possible outliers with unique data that do not fit the patterns shown by the other individuals, but further tests need to be carried out.

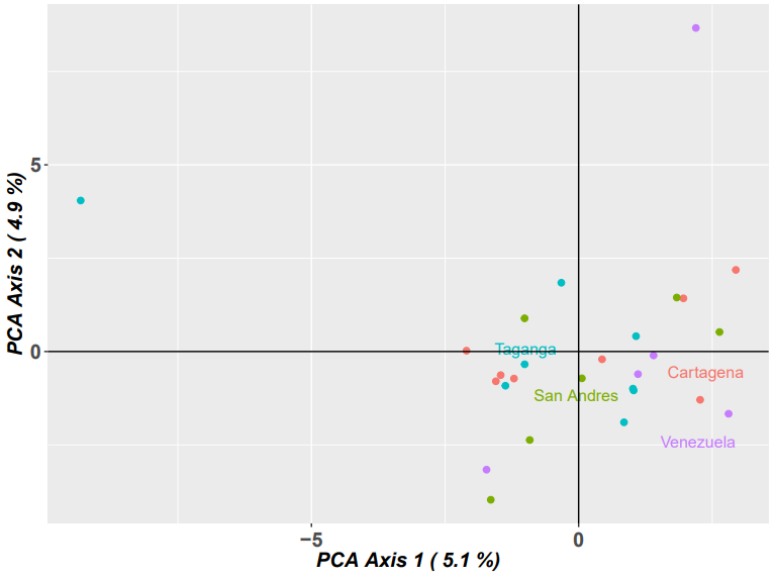


Figure 4-3 Principal component analysis (PCA)**.** The plot shows the first two principal components of the PCA obtained from the dataset, each dot represents one sample, and the color represents the location of origin.

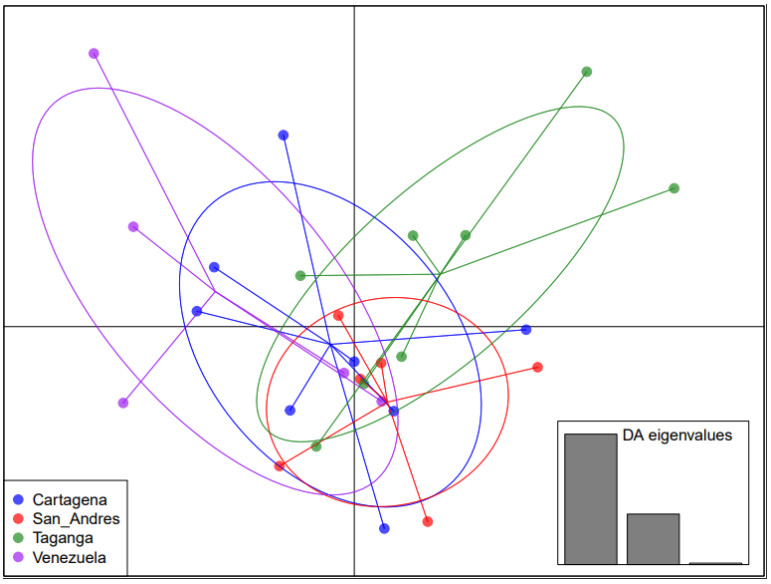


Figure 4-4 Discriminant analysis of principal components (DAPC) depicts the extent to which redtail parrotfish individuals overlap between population cluster.

5 Connectivity and gene flow

The pairwise F_{ST} index allows to measure the genetic differentiation between two subpopulations (locations). This index shows a moderate (0.05- 0.15) genetic differentiation between locations raging between 0.055 and 0.085, although none statistically significant (*p*= <0.05). Thus, there is no evidence of population structure (Table 5-1**¡Error! No se encuentra el origen de la referencia.**).

Table 5-1: Pairwise F_{ST}

	Cartagena	**San Andrés**	**Taganga**	**Margarita**
Cartagena	-	0.067856	0.055858	0.063938
San Andrés	-	-	0.069426	0.084872
Taganga	-	-	-	0.066336
Margarita	-	-	-	-

These results suggest that the greatest differentiation (0.085) between subpopulations (however non-significant), is between the two insular locations, San Andrés, and Margarita Islands. Followed by Cartagena and San Andrés (0.068) and Taganga and Margarita Island (0.066) and the lowest differentiation being between the closest locations Taganga and Cartagena (0.056).

Population specific F_{ST} measures deviation from an ancestral population (Table 5-2) (Kitada et al., 2021). The data shows that there is low to moderate (<0.05- 0.15) genetic differentiation between populations (locations) and those genetic differences are not of statistical significance. The data shows that the San Andres and Taganga locations have the most genetic differentiation (however, non-significant).

Table 5-2: Population-specific Fixation Index

	Cartagena	**San Andrés**	**Taganga**	**Margarita**
Cartagena	-	0.001384635	0.001407551	0.002560959
San Andrés	0.48	-	0.007053652	0.001049819
Taganga	0.38	0.90	-	0.001154736
Margarita	0.37	0.39	0.59	-

Above diagonal, Fixation index (F_{ST}) and associated P-values (below diagonal) between geographical population samples

AMOVA

The analysis of molecular variance showed that most of the genetic variation in the dataset was found among populations within the groups (F_{IS}) accounting for 55.7% of the total variation. The variation within individuals was significant as well, however, the variation among the population was not (Table 5-3).

Table 5-3: General AMOVA

Comparison of all four locations							
	Df	**SS**	**MS**	**Est. Var.**	**%**	**Phi**	**p value**
Among pops	3	1696.848	565.616	-0.906	-0.245		>0.725
Among individuals	23	13287.004	577.696	206.376	55.715		>0.001
Within individuals	27	4453.480	164.943	164.944	44.530		<0.001
Total	**53**	**19437.333**	**366.742**	**370.414**	**100.000**	**0.002**	

Table 5-4**Table 5-4** shows the same pattern as the general AMOVA, there is significant genetic differentiation among individuals within a population and within individuals but not among populations.

Table 5-4: AMOVA comparing San Andrés Island to the rest.

San Andres Island compared to all other locations							
	df	**SS**	**MS**	**Est. Var.**	**%**	**Phi**	**p value**
Among pops	1	593.208	593.208	0.9412	0.254		>0.284
Among individuals	25	14390.644	575.626	205.341	55.314		>0.001
Within individuals	27	4453.480	164.944	164.944	44.432		<0.001
Total	**53**	**19437.333**	**366.742**	**370.414**	**100.000**	**0.003**	

Comparing Margarita Island to the rest of locations (Table 5-5**Table 5-5**) results show the same pattern. Comparing the results of these three AMOVAs there is significant genetic differentiation within individuals and among individuals within populations, however, there was no significant genetic differentiation among populations.

Table 5-5: AMOVA comparing Margarita Island to the rest.

Margarita Island compared to all other locations							
	df	**SS**	**MS**	**Est. Var.**	**%**	**Phi**	**p value**
Among pops	1	546.336	546.336	-1.912	-0.518		>0.761
Among individuals	25	14437.517	577.501	206.278	55.855		>0.001
Within individuals	27	4453.480	164.944	164.944	44.627		<0.001
Total	**53**	**19437.333**	**366.742**	**369.310**	**100.000**	**-0.005**	

Although there are not significant differences among populations, the differences within and among individual can potentially evidence local adaptation.

Isolation by distance analysis (IBD)

There is no isolation effect by distance, the test was non-significant ($p > 0.1$). Yet the isolation by distance analysis shows a negative correlation between genetic distance and geographical distance (Figure 5-1), suggesting that the genetic flow is less restricted when populations are further apart (geographical distance increases). This seems counterintuitive; however, long-range dispersal and migration may support this result(Smith & Weissman, 2023).

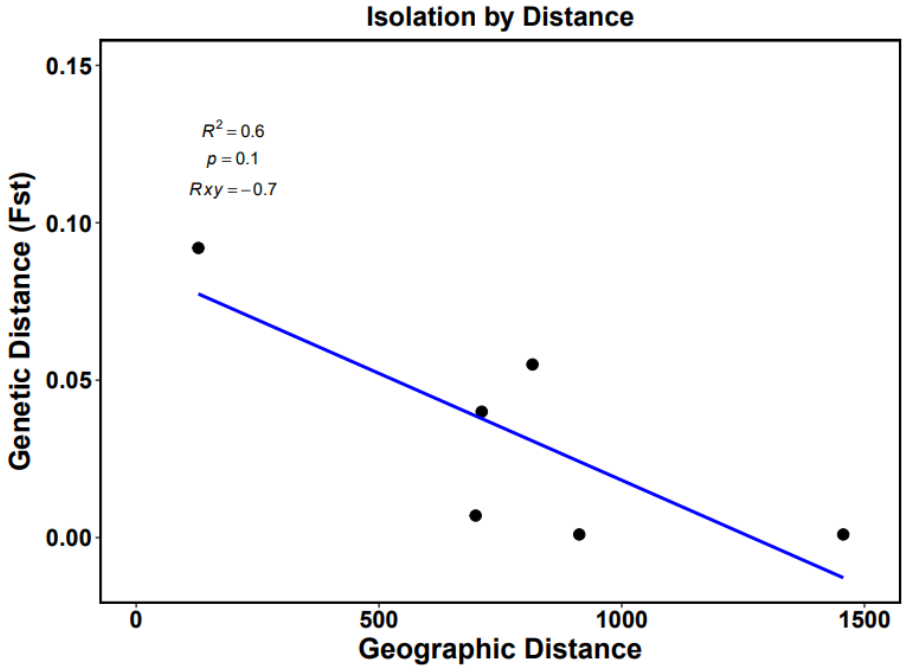


Figure 5-1 Correlation between Genetic distance (F_{ST}) and Geographical distance.

Relative Migration Network

The results of the diversity evaluate the connectivity between the four locations, a high connectivity was observed between all the locations. The migration patterns reveal a slight asymmetrical gene flow of the redtail parrotfish individuals between the sample locations with migration coefficients ranging from 0.5 to 1 (Figure 5-2). The highest genetic migration appears between the locations of Cartagena and Taganga.

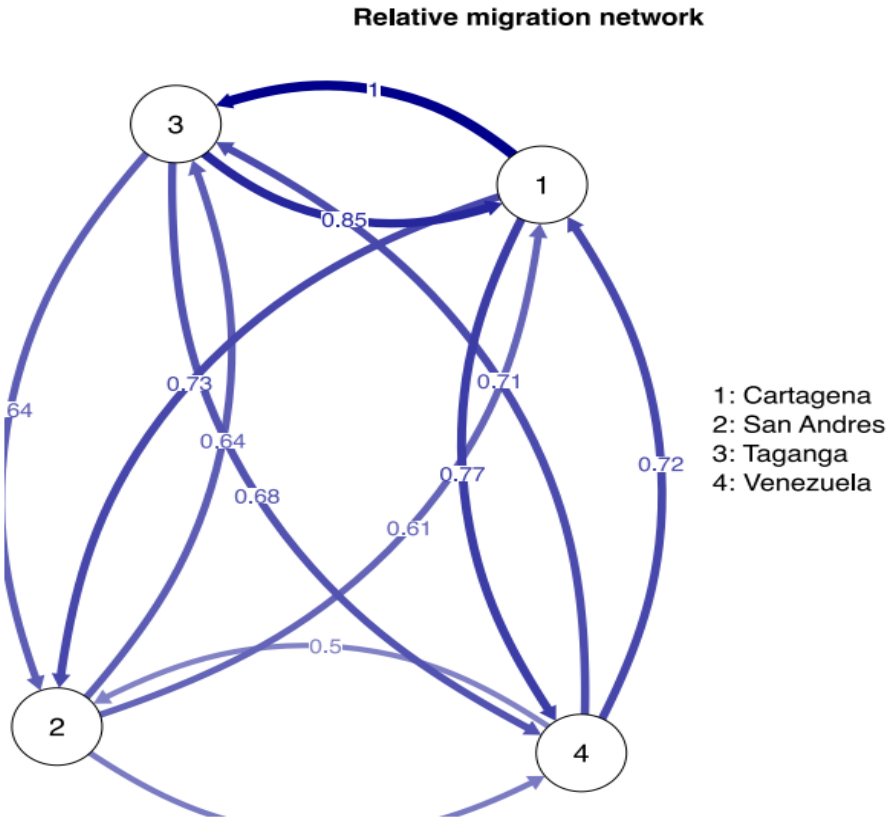


Figure 5-2 Directional relative migration network for sampled redtail parrotfish. Directional relative migration estimated using Nm migration statistic which represents the relative amount of gene flow from one population to another through migration. Arrows indicate the direction of gene flow and numbers show relative migration values (raging from zero to one), length, shading and thickness are determined by the relative strength of gene flow (Sundqvist et al., 2016).

Discussion

Parrotfish across the southern and western Caribbean represent important species for biodiversity, artisanal fisheries, and a significant role in marine ecosystems. In this study we focus in four main localities in southern Caribbean using Rad-seq analysis to examine and characterize the genetic diversity and barriers to gene flow. This study presents novel information regarding genetic diversity and population structure in the redtail parrotfish, demonstrating that the possible barriers to gene flow do not represent an impediment for genetic connectivity among the locations studied. The dataset revealed that there is no evidence of population subdivision or genetic structure across the southern Caribbean, and similar levels of genetic diversity were observed in the different locations. This species could be considered as a panmictic population for southern Caribbean.

The assessment of the genetic diversity reveals that both the observed as well as the expected heterozygosity are low, indicating there is limited genetic diversity (Table 3-1). However, since the observed heterozygosity is similar to the expected heterozygosity it suggests that the population is somehow in genetic equilibrium, contrary to Hoarau et al. (2005), who determined in overexploited flounders the evidence of a heterozygosity deficiency. Although there is no significant inbreeding (F_{IS}), positive values indicate a slight deficit of heterozygotes compared to the expected frequencies under Hardy-Weingberg equilibrium (Kardos et al., 2016). This is an alarm signal, meaning that there is some level of inbreeding inside the populations, that may lead to further loss of heterozygosity and genetic diversity. The nucleotide diversity is also low, and this is equivalent to measuring the allele diversity within a locus; this means few gene variants are present, indicating low genetic diversity. The minimum sample size was used in this study ((Nazareno et al., 2017) small sample size may bias these results, therefore,a larger cample size is needed to confirm these findings.

In terms of population structure, the analyses show that it is likely to find a single cluster (Figure 4-2) of redtail parrotfish along the southern Caribbean region. The results show a similar pattern to Loera-Padilla et al. (2021). They did not find genetic structure in *Sparisoma viride*, and neither Velasco-Montoya et al. (2022) in *Sparisoma aurofrenatum.* This was also evidenced by the PCA (**Figure *4-3***) and DAPC (**Figure *4-4***), where clusters cannot be clearly distinguished, unveiling similar genetic characteristics among the groups evaluated, supporting the idea of genetic connectivity among the locations. In broader

studies this would suggest panmixia, however, a panmictic population cannot be claimed since this study partially covers the distribution of *Sparisoma chrysopterum*. Other analyses such as de AMOVA and Pairwise F_{ST} indicate a low to moderate level of genetic differentiation between subpopulations, supporting previous findings, and indicating high levels of gene flow between these subpopulations.

Genetic connectivity in marine species may be affected by biophysical factors such as distance (geographical separation), currents, salinity, life history traits, and migration (Cowen et al., 2000, 2007; Kough & Paris, 2015; Nielsen et al., 2009; Palumbi, 1994; Rivera et al., 2011; White et al., 2010) and, as stated by Cowen & Sponaugle (2009) for most benthinc marine species with complex life cycles, connectivity occurs primarily during the pelagic larval stage. This study considered three possible barriers that impact those biophysical factors mentioned before, the upwelling in the Guajira and eastern Venezuela region, the Magdalena River plume, and the Panama-Colombia gyre; however, these affections were not significant to constrain the genetic flow between the areas. The ocean currents have been found to have a significant influence on the potential for marine larvae to disperse over long distances (White et al., 2010). In the Caribbean region genetic studies of various marine lifeforms, such as shrimp (Atencia-Galindo et al., 2021), fish (Wallace & Tringali, 2016), corals (Porto-Hannes et al., 2015), and gastropods (Díaz-Ferguson et al., 2010), have shown that the direction of ocean currents correlate to some species having a distinct genetic structure, while others do not show any genetic structure over both long and short distances. Studies such as the ones in gastropods (Díaz-Ferguson et al., 2012; Johnston et al., 2012), common octopus by Puentes Sayo (2021), parrotfishes (Loera-Padilla et al., 2021; Tovar Verba et al., 2022; Velasco-Montoya et al., 2022), reveal the importance of ocean currents as a major factor leading to connectivity. The findings in this study indicate that the biogeographic barriers evaluated do not represent an impairment to genetic flow, on the contrary, it evidences that the Panama- Colombia countercurrent may enhance the genetic flow among the southern Caribbean. The high genetic connectivity of the locations through currents is as well result of the potential of larval dispersal (Gilg & Hilbish, 2003; Richards et al., 2015; Rivera et al., 2011; Swearer et al., 2019; Taylor & Hellberg, 2003; Weersing & Toonen, 2009), which in terms of duration has been reported in other species of the genus *Sparisoma*, to last up to 90 days, and average between 57 and 60 days (Robertson et al., 2006). Usually, but not always (Taylor & Hellberg, 2003), a long larval duration implies high dispersal capability, which can increase the genetic flow and therefore genetic connectivity among subpopulations leading to genetically homogeneous distributions. However, keeping in mind, as mentioned by Cowen & Sponaugle (2009) the pelagic larvae duration (PLD) is taxon specific and influenced by the environment, causality should not be assumed but seems the most plausible explanation in this study for the high connectivity.

The redtail parrotfish is a herbivorous fish commonly associated with rubble and seagrass beds near coral reefs. It is the third largest species of its genus, growing up to 46 cm long, making it very attractive as food resource in local markets (artisanal fisheries). It is a

sexually dichromatic species, monandric with protogynous hermaphroditism, meaning only a few become terminal phase males, reducing the available gene pool when mating. They do not exhibit permanent territoriality, but they do defend temporal territories. They breed during both main seasons and probably throughout the year. As a large species, they do not spawn in small territories of dense grass beds, they are pelagic spawners (Smith, 1997). Its reproduction strategy is not haremic. However, multimale spawning is not the predominant mode of reproduction in this species (Robertson & Van Tassell, 2019; Robertson & Warner, 1978). Most of these biological characteristics may act as limiting factors to the genetic diversity of the species in the region; however, the process of larval dispersal appears to be a major determinant on the establishment and connectivity, therefore expansion, of this marine species (Cowen & Sponaugle, 2009; Taylor & Hellberg, 2003). Connectivity among populations is determinant, so much so that ecological processes, including local and metapopulation dynamics, community dynamics and structure, genetic diversity, and the ability of populations to withstand human exploitation, are crucially influenced by it. (Cowen et al., 2007).

Even though the analyses conducted in the present study are robust (with a decent number of SNPs recovered), these results need to be interpreted cautiously since the sampling size (five-eight individuals per location) is relatively small (the bare minimum) and may restrict the generalization of the findings and introduce potential biases. Furthermore, the locations selected for sampling do not encompass the complete distribution of the species under investigation, which could lead to incomplete insights into its genetic patterns and population dynamics. However, our results allow us to have a baseline of the genetic status of *Sparisoma chrysopterum* in the southern Caribbean. The results show a high connectivity supported by the species' larval development time. On the other hand, being a population with low genetic diversity and high connectivity, it can be affected by environmental changes, and the response to high selection pressures could cause a deterioration of the genetic pool. This should be evaluated in future studies.

Conclusions and recommendations

Conclusions

The current study represents the first genomic approach using RADseq to examine the genetic composition of the redtail parrotfish in the southern and western Caribbean. The findings reveal that *Sparisoma chrysopterum* exhibits high genetic connectivity among the studied locations, indicating no evidence of population divergence in the region regardless of the glimpse (possible evidence) of local adaptation; to determine panmixia a broader study must be conducted.

Despite the apparent equilibrium, low genetic diversity is observed in the species. Additionally, it appears to be at risk of further genetic diversity loss due to positive inbreeding values. Although the inbreeding coefficients are non-significant, indicating that inbreeding may not be the primary driver of diversity reduction, the positive values suggest a trend towards closely related mating. This raises concerns as continued inbreeding can contribute to a gradual decline in genetic variability and lead to the expression of deleterious alleles, potentially compromising the species' long-term adaptability and resilience to environmental challenges.

The study reveals that potential biogeographic barriers are, in fact, no impediment to gene flow between these locations, but it highlights the importance of larval dispersal and ocean currents in facilitating genetic connectivity, counteracting the potential limitations imposed by biophysical factors. This is suggested by a low to moderate level of genetic differentiation between subpopulations, supporting the notion of high gene flow in the species.

This study provides a genetic baseline for management and conservation strategies regarding the redtail parrotfish; however, the limitations mentioned above should be considered when implementing any actions. Nonetheless, these results provide a valuable starting point to promote further research on this species. Consequently, monitoring and managing the species' genetic diversity are essential to mitigate the risks and safeguard its viability in the future.

Recommendations

Additional research with larger sample sizes, spatiotemporal samples of larvae, a wider range of locations covering the total distribution of the species, and supplementary demographic information and demographic history analyses are necessary to gain a more comprehensive understanding of the genetic patterns and population dynamics of the redtail parrotfish.

References

Adam, T. C., Kelley, M., Ruttenberg, B. I., & Burkepile, D. E. (2015). Resource partitioning along multiple niche axes drives functional diversity in parrotfishes on Caribbean coral reefs. Oecologia, 179(4), 1173–1185. https://doi.org/10.1007/S00442-015-3406-3/FIGURES/6

Andrade, C. A. (2001). Las corrientes superficiales en la cuenca de Colombia observadas con boyas de deriva. *Revista de La Academia Colombiana de Ciencias Exactas, Físicas y Naturales*, *25* (96), 321336.

Andrade, C. A. (2003). Evidence for an eastward flow along the Central and South American Caribbean Coast. *Journal of Geophysical Research*, *108*(C6), 3185. https://doi.org/10.1029/2002JC001549

Atencia-Galindo, M. A., Narvaéz, J. C., Ramírez, A., Paramo, J., & Aguire-Pabón, J. C. (2021). Genetic structure of the pink shrimp *Penaeus (Farfantepenaeus) notialis* (Pérez-Farfante, 1967) (Decapoda: Penaeidae) in the Colombian Caribbean. *Fisheries Research*, *243*, 106052. https://doi.org/10.1016/J.FISHRES.2021.106052

Bellwood, D. R., Hoey, A. S., & Choat, J. H. (2003). Limited functional redundancy in high diversity systems: resilience and ecosystem function on coral reefs. *Ecology Letters*, *6*(4), 281–285. https://doi.org/10.1046/j.1461-0248.2003.00432.x

Benestan, L., Gosselin, T., Perrier, C., Sainte-Marie, B., Rochette, R., & Bernatchez, L. (2015). RAD genotyping reveals fine-scale genetic structuring and provides powerful population assignment in a widely distributed marine species, the American lobster (*Homarus americanus*). *Molecular Ecology*, *24*(13), 3299–3315. https://doi.org/10.1111/mec.13245

Bernardi, G., Robertson, D. R., Clifton, K. E., & Azzurro, E. (2000). Molecular systematics, zoogeography, and evolutionary ecology of the Atlantic parrotfish genus *Sparisoma*. *Molecular Phylogenetics and Evolution*, *15*(2), 292–300. https://doi.org/10.1006/mpev.1999.0745

Bradbury, I. R., Laurel, B., Snelgrove, P. V. R., Bentzen, P., & Campana, S. E. (2008). Global patterns in marine dispersal estimates: the influence of geography, taxonomic category and life history. *Proceedings of the Royal Society B: Biological Sciences*, *275*(1644), 1803–1809. https://doi.org/10.1098/rspb.2008.0216

Brandrud, M. K., Paun, O., Lorenzo, M. T., Nordal, I., & Brysting, A. K. (2017). RADseq provides evidence for parallel ecotypic divergence in the autotetraploid *Cochlearia officinalis* in Northern Norway. *Scientific Reports*, *7*(1), 1–13. https://doi.org/10.1038/s41598-017-05794-z

Brown, K. T., Bender-Champ, D., Kubicek, A., van der Zande, R., Achlatis, M., Hoegh-Guldberg, O., & Dove, S. G. (2018). The dynamics of coral-algal interactions in space and time on the southern Great Barrier Reef. *Frontiers in Marine Science*, *5*(MAY), 1–13. https://doi.org/10.3389/fmars.2018.00181

Catchen, J. M., Amores, A., Hohenlohe, P., Cresko, W., & Postlethwait, J. H. (2011). Stacks: Building and genotyping loci de novo from short-read sequences. *G3: Genes, Genomes, Genetics*, *1*(3), 171–182. https://doi.org/10.1534/g3.111.000240

Catchen, J., Hohenlohe, P. A., Bassham, S., Amores, A., & Cresko, W. A. (2013). Stacks: an analysis tool set for population genomics. *Molecular Ecology*, *22*(11), 3124–3140. https://doi.org/10.1111/mec.12354

Chasqui Velasco, L. H., Polanco F., A., Acero P., A., Mejía Falla, P. A., Navia, A. F., Zapata, L. A., & Caldas, J. P. (2017). *Libro rojo de peces marinos de Colombia (2017).* INVEMAR. http://hdl.handle.net/1834/15894

Cocheret De La Morinière, E., Pollux, B. J. A., Nagelkerken, I., & Van Der Velde, G. (2002). Post-settlement life cycle migration patterns and habitat preference of coral reef fish that use seagrass and mangrove habitats as nurseries. *Estuarine, Coastal and Shelf Science*, *55*(2), 309–321. https://doi.org/10.1006/ECSS.2001.0907

Comeros-Raynal, M. T., Choat, J. H., Polidoro, B. A., Clements, K. D., Abesamis, R., Craig, M. T., Lazuardi, M. E., McIlwain, J., Muljadi, A., Myers, R. F., Nañola, C. L., Pardede, S., Rocha, L. A., Russell, B., Sanciangco, J. C., Stockwell, B., Harwell, H., & Carpenter, K. E. (2012). The likelihood of extinction of iconic and dominant herbivores and detritivores of coral reefs: The parrotfishes and surgeonfishes. *PLoS ONE*, *7*(7). https://doi.org/10.1371/journal.pone.0039825

Cowen, R. K., & Sponaugle, S. (2009). Larval dispersal and marine population connectivity. In *Annual Review of Marine Science* (Vol. 1). https://doi.org/10.1146/annurev.marine.010908.163757

Cowen, R. K., Lwiza, K. M. M., Sponaugle, S., Paris, C. B., & Olson, D. B. (2000). Connectivity of marine populations: Open or closed? *Science*, *287*(5454). https://doi.org/10.1126/science.287.5454.857

Cowen, R. K., Gawarkiewicz, G., Pineda, J., Thorrold, S. R., & Werner, F. E. (2007). Population connectivity in marine systems: An overview. *Oceanography*, *20*(SPL.ISS. 3). https://doi.org/10.5670/oceanog.2007.26

Cowman, P. F., & Bellwood, D. R. (2013). Vicariance across major marine biogeographic barriers: Temporal concordance and the relative intensity of hard versus soft barriers. *Proceedings of the Royal Society B: Biological Sciences*, *280*(1768). https://doi.org/10.1098/rspb.2013.1541

Cramer, K. L., O'Dea, A., Clark, T. R., Zhao, J., & Norris, R. D. (2017). Prehistorical and historical declines in Caribbean coral reef accretion rates driven by loss of parrotfish. *Nature Communications*, *8*, 14160. https://doi.org/10.1038/ncomms14160

Díaz-Ferguson, E., Haney, R., Wares, J., & Silliman, B. (2010). Population genetics of a trochid gastropod broadens picture of Caribbean Sea connectivity. *PLoS ONE*, *5*(9), 1–8. https://doi.org/10.1371/JOURNAL.PONE.0012675

Díaz-Ferguson, E., Haney, R. A., Wares, J. P., & Silliman, B. R. (2012). Genetic structure and connectivity patterns of two Caribbean rocky-intertidal gastropods. *Journal of Molluscan Studies*, *78*(1), 112–118. https://doi.org/10.1093/MOLLUS/EYR050

Dichmont, C. M., Ovenden, J. R., Berry, O., Welch, D. J., & Buckworth, R. C. (2012). *Scoping current and future genetic tools, their limitations and their applications for wild fisheries management Wealth from Oceans www.csiro.au*. www.csiro.au

Dorenbosch, M., Van Riel, M. C., Nagelkerken, I., & Van Der Velde, G. (2004). The relationship of reef fish densities to the proximity of mangrove and seagrass nurseries. *Estuarine, Coastal and Shelf Science*, *60*(1), 37–48. https://doi.org/10.1016/J.ECSS.2003.11.018

Earl, D. A., & vonHoldt, B. M. (2012). STRUCTURE HARVESTER: A website and program for visualizing STRUCTURE output and implementing the Evanno method. *Conservation Genetics Resources*, *4*(2), 359–361. https://doi.org/10.1007/s12686-011-9548-7

Elliff, C. I., & Silva, I. R. (2017). Coral reefs as the first line of defense: Shoreline protection in face of climate change. *Marine Environmental Research*, *127*, 148–154. https://doi.org/10.1016/j.marenvres.2017.03.007

Enberg, K., Jørgensen, C., Dunlop, E. S., Heino, M., & Dieckmann, U. (2009). Implications of fisheries-induced evolution for stock rebuilding and recovery. *Evolutionary Applications*, *2*(3), 394–414. https://doi.org/10.1111/j.1752-4571.2009.00077.x

Evanno, G., Regnaut, S., & Goudet, J. (2005). Detecting the number of clusters of individuals using the software STRUCTURE: A simulation study. *Molecular Ecology*, *14*(8), 2611–2620. https://doi.org/10.1111/j.1365-294X.2005.02553.x

Excoffier, L., & Lischer, H. E. L. (2010). Arlequin suite ver 3.5: A new series of programs to perform population genetics analyses under Linux and Windows. *Molecular Ecology Resources*, *10*(3), 564–567. https://doi.org/10.1111/j.1755-0998.2010.02847.x

Fisher, R., O'Leary, R. A., Low-Choy, S., Mengersen, K., Knowlton, N., Brainard, R. E., & Caley, M. J. (2015). Species richness on coral reefs and the pursuit of convergent global estimates. *Current Biology*, *25*(4), 500–505. https://doi.org/10.1016/j.cub.2014.12.022

Funk, W. C., McKay, J. K., Hohenlohe, P. A., & Allendorf, F. W. (2012). Harnessing genomics for delineating conservation units. *Trends in Ecology and Evolution*, *27*(9), 489–496. https://doi.org/10.1016/j.tree.2012.05.012

Gilg, M. R., & Hilbish, T. J. (2003). The geography of marine larval dispersal: Coupling genetics with fine-scale physical oceanography. *Ecology*, *84*(11). https://doi.org/10.1890/02-0498

Gómez Gaspar, A., & Acero P., A. (2020). Comparación de las surgencias de la Guajira colombiana y del oriente venezolano. *Boletín de Investigaciones Marinas y Costeras*, *49*(2), 131–172. https://doi.org/10.25268/bimc.invemar.2020.49.2.943

Goudet, J., & Jombart, T. (2015). hierfstat: estimation and tests of hierarchical F-statistics. In *R package version 0.04-22, 10.*

Graves, J. E. (1998). Molecular insight into the population structure of cosmopolitan marine fishes. *Journal of Heredity*, *89*(April), 427–437. https://doi.org/10.1093/jhered/89.5.427

Gray, J. S. (1997). *Marine biodiversity: patterns, threats and conservation needs*. *Biodiversity & Conservation*,*6*(1), 153–175.

Hawkins, J. P., & Roberts, C. M. (2004a). Effects of artisanal fishing on Caribbean coral reefs. *Conservation Biology*, *18*(1), 215–226. https://doi.org/10.1111/j.1523-1739.2004.00328.x

Hawkins, J. P., & Roberts, C. M. (2004b). Effects of fishing on sex-changing Caribbean parrotfishes. *Biological Conservation*, *115*(2), 213–226. https://doi.org/10.1016/S0006-3207(03)00119-8

Hoarau, G., Boon, E., Jongma, D. N., Ferber, S., Palsson, J., Van Der Veer, H. W., Rijnsdorp, A. D., Stam, W. T., & Olsen, J. L. (2005). Low effective population size and evidence for inbreeding in an overexploited flatfish, plaice (*Pleuronectes platessa* L.). *Proceedings of the Royal Society B: Biological Sciences*, *272*(1562). https://doi.org/10.1098/rspb.2004.2963

Hutchings, J. A. (2000). Collapse and recovery of marine fishes. *Nature*, *406*(6798). https://doi.org/10.1038/35022565

Hutchings, J. A., & Reynolds, J. D. (2004). Marine fish population collapses: Consequences for recovery and extinction risk. In *BioScience* (Vol. 54, Issue 4). https://doi.org/10.1641/0006-3568(2004)054[0297:MFPCCF]2.0.CO;2

Jackson, J. B., Donovan, M. K., Cramer, K., & Lam, V. (2014). Status and trends of Caribbean coral reefs: 1970-2012. *Global Coral Reef Monitoring Network*, 306.

Johnston, L., Miller, M. W., & Baums, I. B. (2012). Assessment of host-associated genetic differentiation among phenotypically divergent populations of a coral-eating gastropod across the Caribbean. *PLoS ONE*, *7*(11). https://doi.org/10.1371/journal.pone.0047630

Jombart, T. (2008). Adegenet: A R package for the multivariate analysis of genetic markers. *Bioinformatics*, *24*(11). https://doi.org/10.1093/bioinformatics/btn129

Jombart, T., & Ahmed, I. (2011). Adegenet 1.3-1: new tools for the analysis of genome-wide SNP data. *Bioinformatics*, *27*(21), 3070–3071. https://doi.org/10.1093/BIOINFORMATICS/BTR521

Jombart, T., Devillard, S., & Balloux, F. (2010). Discriminant analysis of principal components: A new method for the analysis of genetically structured populations. *BMC Genetics*, *11*. https://doi.org/10.1186/1471-2156-11-94

Kamvar, Z. N., Tabima, J. F., & Gŕunwald, N. J. (2014). Poppr: An R package for genetic analysis of populations with clonal, partially clonal, and/or sexual reproduction. *PeerJ*, *2014*(1). https://doi.org/10.7717/peerj.281

Kardos, M., Taylor, H. R., Ellegren, H., Luikart, G., & Allendorf, F. W. (2016). Genomics advances the study of inbreeding depression in the wild. In *Evolutionary Applications* (Vol. 9, Issue 10). https://doi.org/10.1111/eva.12414

Keenan, K., Mcginnity, P., Cross, T. F., Crozier, W. W., & Prodöhl, P. A. (2013). diveRsity: An R package for the estimation and exploration of population genetics parameters and their associated errors. *Methods in Ecology and Evolution*, *4*(8), 782–788. https://doi.org/10.1111/2041-210X.12067

Kenchington, E. (2013). The effects of fishing on species and genetic diversity. *Responsible Fisheries in the Marine Ecosystem*, 235–255. https://doi.org/10.1079/9780851996332.0235

Kitada, S., Nakamichi, R., & Kishino, H. (2021). Understanding population structure in an evolutionary context: population-specific FST and pairwise FST. *G3 Genes|Genomes|Genetics*, *11*(11). https://doi.org/10.1093/G3JOURNAL/JKAB316

Knowlton, N., Brainard, R. E., Fisher, R., Moews, M., Plaisance, L., & Caley, M. J. (2010). Coral reef biodiversity. *Life in the World's Oceans: Diversity, Distribution, and Abundance*, 65–78. https://doi.org/10.1002/9781444325508.CH4

Kopelman, N. M., Mayzel, J., Jakobsson, M., Rosenberg, N. A., & Mayrose, I. (2015). Clumpak: A program for identifying clustering modes and packaging population structure inferences across K. *Molecular Ecology Resources*, *15*(5), 1179–1191. https://doi.org/10.1111/1755-0998.12387

Kough, A. S., & Paris, C. B. (2015). The influence of spawning periodicity on population connectivity. *Coral Reefs*, *34*(3), 753–757. https://doi.org/10.1007/S00338-015-1311-1/METRICS

Loera-Padilla, F. J., Piñeros, V. J., Baldwin, C. C., Cox, C. E., Simoes, N., Ribeiro, E., Lasso-Alcalá, O. M., & Domínguez-Domínguez, O. (2021). Phylogeography, population connectivity and demographic history of the Stoplight parrotfish, *Sparisoma viride* (Teleostei: Labridae), in the Greater Caribbean. *Coral Reefs*, 1–13. https://doi.org/10.1007/s00338-020-02036-z

López-Angarita, J., Del Pilar, M., Orjuela, R., Peña, K. G., & Escobar, D. (2021). Results from a conservation initiative for parrotfishes in the Colombian Caribbean. *BioRxiv*, 2021.09.27.462015. https://doi.org/10.1101/2021.09.27.462015

Lowe, W. H., & Allendorf, F. W. (2010). What can genetics tell us about population connectivity? *Molecular Ecology*, *19*(15), 3038–3051. https://doi.org/10.1111/j.1365-294X.2010.04688.x

Luiz, O. J., Madin, J. S., Robertson, D.R., Rocha, L. A., Wirtz, P., & Floeter, S. R. (2012). Ecological traits influencing range expansion across large oceanic dispersal barriers: Insights from tropical Atlantic reef fishes. *Proceedings of the Royal Society B: Biological Sciences*, *279*(1730), 1033–1040. https://doi.org/10.1098/rspb.2011.1525

Mumby, P. J. (2009a). Herbivory versus corallivory: Are parrotfish good or bad for Caribbean coral reefs? *Coral Reefs*, *28*(3), 683–690. https://doi.org/10.1007/s00338-009-0501-0

Mumby, P. J. (2009b). Phase shifts and the stability of macroalgal communities on Caribbean coral reefs. *Coral Reefs*, *28*(3), 761–773. https://doi.org/10.1007/s00338-009-0506-8

Nadukkalam Ravindran, P., Bentzen, P., Bradbury, I. R., & Beiko, R. G. (2019). RADProc: A computationally efficient de novo locus assembler for population studies using RADseq data. *Molecular Ecology Resources*, *19*(1), 272–282. https://doi.org/10.1111/1755-0998.12954

Nagelkerken, I., Roberts, C. M., Van der Velde, G., Dorenbosch, M., Van Riel, M. C., Cocheret de la Morinière, E., & Nienhuis, P. H. (2002). How important are mangroves and seagrass beds for coral-reef fish? The nursery hypothesis tested on an island scale. *Marine Ecology Progress Series*, *244*, 299–305. https://doi.org/10.3354/meps244299

Natasha, J., Stockwell, B. L., Marie, A. D., Hampton, J., Smith, N., Nicol, S., & Rico, C. (2022). No population structure of bigeye tunas (*Thunnus obesus*) in the Western and Central Pacific Ocean indicated by single nucleotide polymorphisms. *Frontiers in Marine Science*, *9*, 371. https://doi.org/10.3389/FMARS.2022.799684/BIBTEX

Nazareno, A. G., Bemmels, J. B., Dick, C. W., & Lohmann, L. G. (2017). Minimum sample sizes for population genomics: an empirical study from an Amazonian plant species. *Molecular Ecology Resources*, *17*(6), 1136–1147. https://doi.org/10.1111/1755-0998.12654

Nesbø, C. L., Rueness, E. K., Iversen, S. A., Skagen, D. W., & Jakobsen, K. S. (2000). Phylogeography and population history of Atlantic mackerel (*Scomber scombrus* L.): a

genealogical approach reveals genetic structuring among the eastern Atlantic stocks. *Proceedings of the Royal Society B: Biological Sciences*, *267*(1440), 281–292. https://doi.org/10.1098/rspb.2000.0998

Nielsen, E. E., Hemmer-Hansen, J., Larsen, P. F., & Bekkevold, D. (2009). Population genomics of marine fishes: Identifying adaptive variation in space and time. *Molecular Ecology*, *18*(15), 3128–3150. https://doi.org/10.1111/j.1365-294X.2009.04272.x

Nielsen, E. E., Cariani, A., Aoidh, E. Mac, Maes, G. E., Milano, I., Ogden, R., Taylor, M., Hemmer-Hansen, J., Babbucci, M., Bargelloni, L., Bekkevold, D., Diopere, E., Grenfell, L., Helyar, S., Limborg, M. T., Martinsohn, J. T., McEwing, R., Panitz, F., Patarnello, T., … Carvalho, G. R. (2012). Gene-associated markers provide tools for tackling illegal fishing and false eco-certification. *Nature Communications*, *3*. https://doi.org/10.1038/ncomms1845

Palumbi, S. R. (1994). Genetic divergence, reproductive isolation, and marine speciation. *Annual Review of Ecology and Systematics*, *25*, 547–572. https://doi.org/10.1146/annurev.es.25.110194.002555

Palumbi, S. R. (2008). Humans as the world's greatest evolutionary force. *Urban Ecology: An International Perspective on the Interaction Between Humans and Nature*, *293*(September), 15–24. https://doi.org/10.1007/978-0-387-73412-5_2

Paramo, J., Correa, M., & Núñez, S. (2011). *Evidence of physical-biological mismatch in the Guajira upwelling system, Colombian Caribbean* *Revista de Biologia Marina y Oceanografia*,*46*(3), 421-430. http://podaac.jlp.nasa.gov/quickscat

Peakall, R., & Smouse, P. E. (2012). GenALEx 6.5: Genetic analysis in Excel. Population genetic software for teaching and research-an update. *Bioinformatics*, *28*(19). https://doi.org/10.1093/bioinformatics/bts460

Pelc, R. A., Warner, R. R., & Gaines, S. D. (2009). Geographical patterns of genetic structure in marine species with contrasting life histories. *Journal of Biogeography*, *36*(10), 1881–1890. https://doi.org/10.1111/j.1365-2699.2009.02138.x

Picq, S., Mcmillan, W. O., & Puebla, O. (2016). Population genomics of local adaptation versus speciation in coral reef fishes (*Hypoplectrus* spp, Serranidae). *Ecology and Evolution*, *6*(7), 2109–2124. https://doi.org/10.1002/ece3.2028

Porto-Hannes, I., Zubillaga, A. L., Shearer, T. L., Bastidas, C., Salazar, C., Coffroth, M. A., & Szmant, A. M. (2015). Population structure of the corals *Orbicella faveolata* and *Acropora palmata* in the Mesoamerican Barrier Reef System with comparisons over Caribbean basin-wide spatial scale. *Marine Biology*, *162*(1), 81–98. https://doi.org/10.1007/S00227-014-2560-1/METRICS

Pritchard, J. K., Stephens, M., & Donnelly, P. (2000). *Inference of Population Structure Using Multilocus Genotype Data*. http://www.stats.ox.ac.uk/pritch/home.html.

Puebla, O. (2009). Ecological speciation in marine v. freshwater fishes. *Journal of Fish Biology*, *75*(5), 960–996. https://doi.org/10.1111/j.1095-8649.2009.02358.x

Puechmaille, S. J. (2016). The program structure does not reliably recover the correct population structure when sampling is uneven: Subsampling and new estimators alleviate the problem. *Molecular Ecology Resources*, *16*(3). https://doi.org/10.1111/1755-0998.12512

Puentes Sayo, P. A. (2021). *Patrón filogeográfico del pulpo común Octopus insularis en el Caribe de Colombia*. Universidad Nacional de Colombia. https://repositorio.unal.edu.co/handle/unal/81142

R Core Team. (2019). *R Core Team (2019) R A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. - References - Scientific Research Publishing*. https://www.scirp.org/(S(lz5mqp453edsnp55rrgjct55))/reference/ReferencesPapers.aspx?ReferenceID=2631126

R Core Team. (2022). *R: A Language and Environment for Statistical Computing* (3.4.4). R Foundation for Statistical Computing.

Restrepo, J. C., Ortíz, J. C., Pierini, J., Schrottke, K., Maza, M., Otero, L., & Aguirre, J. (2014). Freshwater discharge into the Caribbean Sea from the rivers of Northwestern South America (Colombia): Magnitude, variability and recent changes. *Journal of Hydrology*, *509*, 266–281. https://doi.org/10.1016/j.jhydrol.2013.11.045

Restrepo, J. D., Zapata, P., Díaz, J. M., Garzón-Ferreira, J., & García, C. B. (2006). Fluvial fluxes into the Caribbean Sea and their impact on coastal ecosystems: The Magdalena River, Colombia. *Global and Planetary Change*, *50*(1–2), 33–49. https://doi.org/10.1016/j.gloplacha.2005.09.002

Reynolds, J. D., Dulvy, N. K., Goodwin, N. B., & Hutchings, J. A. (2005). Biology of extinction risk in marine fishes. *Proceedings of the Royal Society B: Biological Sciences*, *272*(1579), 2337–2344. https://doi.org/10.1098/RSPB.2005.3281

Richards, V. P., DeBiasse, M. B., & Shivji, M. S. (2015). Genetic evidence supports larval retention in the western Caribbean for an invertebrate with high dispersal capability (*Ophiothrix suensonii*: Echinodermata, Ophiuroidea). *Coral Reefs*, *34*(1), 313–325. https://doi.org/10.1007/S00338-014-1237-Z/METRICS

Rivera, A. M. J., Andrews, K. R., Kobayashi, D. R., Wren, J. L. K., Kelley, C., Roderick, G. K., & Toonen, R. J. (2011). Genetic analyses and simulations of larval dispersal reveal distinct populations and directional connectivity across the range of the Hawaiian grouper

(*Epinephelus quernus*). *Journal of Marine Biology*, *2011*, 11. https://doi.org/10.1155/2011/765353

Robertson, D. R., & Cramer, K. L. (2014). Defining and dividing the Greater Caribbean: Insights from the biogeography of shorefishes. *PLoS ONE*, *9*(7). https://doi.org/10.1371/journal.pone.0102918

Robertson, D. R., & Van Tassell, J. (2019). *Shorefishes - Homepage*. Shorefishes of the Greater Caribbean: Online Information System. https://biogeodb.stri.si.edu/caribbean/en/pages

Robertson, R., & Warner, R. R. (1978). Sexual patterns in the labroid fishes of the Western Caribbean, II, the parrotfishes (Scaridae). *Smithsonian Contributions to Zoology*, *255*, 1–26. https://doi.org/10.5479/si.00810282.255

Robertson, D., Karg, F., Leao de Moura, R., Victor, B. C., & Bernardi, G. (2006). Mechanisms of speciation and faunal enrichment in Atlantic parrotfishes. *Molecular Phylogenetics and Evolution*, *40*(3), 795–807. https://doi.org/10.1016/j.ympev.2006.04.011

Rocha, L. A., Choat, J. H., Clements, K. D., Russell, B., Myers, R., Lazuardi, M. E., Muljadi, A., Pardede, S., & Rahardjo, P. (2012). Sparisoma chrysopterum*. The IUCN Red List of Threatened Species 2012: e.T190738A17788150.* https://doi.org/https://dx.doi.org/10.2305/IUCN.UK.2012.RLTS.T190738A17788150.en

Rochette, N. C., & Catchen, J. M. (2017). Deriving genotypes from RAD-seq short-read data using Stacks. *Nature Protocols*, *12*(12), 2640–2659. https://doi.org/10.1038/nprot.2017.123

Rodríguez-Ezpeleta, N., Bradbury, I. R., Mendibil, I., Álvarez, P., Cotano, U., & Irigoien, X. (2016). Population structure of Atlantic mackerel inferred from RAD-seq-derived SNP markers: effects of sequence clustering parameters and hierarchical SNP selection. *Molecular Ecology Resources*, *16*(4), 991–1001. https://doi.org/10.1111/1755-0998.12518

Rogers, A., Blanchard, J. L., & Mumby, P. J. (2014). Vulnerability of coral reef fisheries to a loss of structural complexity. *Current Biology*, *24*(9), 1000–1005. https://doi.org/10.1016/J.CUB.2014.03.026

Rueda, J., Rivillas-Ospina, G., Ávila, H., & Acuña, G. (2016). Dinámica estacional de la pluma turbia del delta Río Magdalena y su respuesta ante la hidrodinámica local, el viento y los flujos de calor Análisis intramensual. *Instituto de Estudios Hidráulicos y Ambientales*, *October 2015*, 23. https://www.researchgate.net/publication/283292433_Dinamica_estacional_de_la_pluma_turbia_del_delta_Rio_Magdalena_y_su_respuesta_ante_la_hidrodinamica_local_el_viento_y_los_flujos_de_calor_-_Analisis_intramensual

Saad, Y. M., AbuZinadah, O. A. H., & El-Domyati, M. F. (2013). Monitoring of genetic diversity in some parrotfish species based on inter simple sequence repeats polymorphism. *Life Science Journal*, *10*(4), 1841–1846.

Silliman, K. (2019). Population structure, genetic connectivity, and adaptation in the Olympia oyster (*Ostrea lurida*) along the west coast of North America. *Evolutionary Applications*, *12*(5), 923–939. https://doi.org/10.1111/eva.12766

Smith, C. L. (1997). *National Audubon Society Field Guide to Tropical Marine Fishes: Caribbean, Gulf of Mexico, Florida, Bahamas, Bermuda.* (Knopf Doubleday Publishing Group, Ed.). Knopf.

Smith, T. B., & Weissman, D. B. (2023). Isolation by distance in populations with power-law dispersal. *G3: Genes, Genomes, Genetics*, *13*(4). https://doi.org/10.1093/g3journal/jkad023

Steneck, R. S., Arnold, S. N., & Mumby, P. J. (2014). Experiment mimics fishing on parrotfish: insights on coral reef recovery and alternative attractors. *Marine Ecology Progress Series*, *506*, 115–127. https://doi.org/10.3354/MEPS10764

Sundqvist, L., Keenan, K., Zackrisson, M., Prodöhl, P., & Kleinhans, D. (2016). Directional genetic differentiation and relative migration. *Ecology and Evolution*, *6*(11), 3461–3475. https://doi.org/10.1002/ECE3.2096

Swearer, S. E., Treml, E. A., & Shima, J. S. (2019). A review of biophysical models of marine larval dispersal. In *Oceanography and Marine Biology* (Vol. 57, pp. 325–356). CRC Press. https://doi.org/10.1201/9780429026379-7

Swierts, T., & Vermeij, M. J. A. (2016). Competitive interactions between corals and turf algae depend on coral colony form. *PeerJ*, *2016*(5), 1–18. https://doi.org/10.7717/peerj.1984

Taylor, M. S., & Hellberg, M. E. (2003). Genetic evidence for local retention of pelagic larvae in a Caribbean reef fish. *Science*, *299*(5603). https://doi.org/10.1126/science.1079365

Tiffin, P., & Ross-Ibarra, J. (2014). Advances and limits of using population genetics to understand local adaptation. *Trends in Ecology and Evolution*, *29*(12), 673–680. https://doi.org/10.1016/j.tree.2014.10.004

Tovar Verba, J., Ferreira, C. E. L., Pennino, M. G., Hagberg, L., Lopes, P. F. M., Padovani Ferreira, B., Maia Queiroz Lima, S., & Stow, A. (2022). Genetic structure of the threatened gray parrotfish (*Sparisoma axillare*) in the southwestern Atlantic. *Coral Reefs 2022 42:1*, *42*(1), 105–117. https://doi.org/10.1007/S00338-022-02324-W

Vallès, H., & Oxenford, H. A. (2014). Parrotfish size: A simple yet useful alternative indicator of fishing effects on Caribbean reefs? *PLoS ONE*, *9*(1). https://doi.org/10.1371/journal.pone.0086291

Velasco-Montoya, D. A., Millán-Márquez, A. M., & Tavera, J. (2022). Genetic connectivity in *Sparisoma aurofrenatum* (redband parrotfish): an unexpected journey. *Hydrobiologia*, *849*(8), 1727–1741. https://doi.org/10.1007/S10750-022-04806-Y/METRICS

Wallace, E. M., & Tringali, M. D. (2016). Fishery composition and evidence of population structure and hybridization in the Atlantic bonefish species complex (*Albula* spp.). *Marine Biology*, *163*(6), 1–15. https://doi.org/10.1007/S00227-016-2915-X/METRICS

Ward, R. D., Woodwark, M., & Skibinski, D. O. F. (1994). A comparison of genetic diversity levels in marine, freshwater, and anadromous fishes. In *Journal of Fish Biology* (Vol. 44, Issue 2, pp. 213–232). https://doi.org/10.1111/j.1095-8649.1994.tb01200.x

Weersing, K., & Toonen, R. J. (2009). Population genetics, larval dispersal, and connectivity in marine systems. *Marine Ecology Progress Series*, *393*. https://doi.org/10.3354/meps08287

Weir, B. S., & Cockerham, C. C. (1984). Estimating F-statistics for the analysis of population structure. *Evolution*, *38*(6), 1358. https://doi.org/10.2307/2408641

White, C., Selkoe, K., Watson, J., Siegel, D., & Zacherl, D. (2010). Ocean currents help explain population genetic structure. *Proceedings of the Royal Society B-Biological Sciences*, *277*.