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Original Article

Genetic and biophysical modelling evidence of generational connectivity in the intensively exploited, Western North Atlantic red grouper (*Epinephelus morio*)

Andrea M. Bernard [1], Matthew W. Johnston[1], Rocío Pérez-Portela[2‡], Marjorie F. Oleksiak[2], Felicia C. Coleman[3], and Mahmood S. Shivji[1]*

[1]*Guy Harvey Research Institute, Nova Southeastern University, 8000 North Ocean Drive, Dania Beach, FL 33004, USA*
[2]*Department of Marine Biology and Ecology, Rosenstiel School of Marine and Atmospheric Science, University of Miami, 4600 Rickenbacker Causeway, Miami, FL 33149, USA*
[3]*Coastal and Marine Laboratory, Florida State University, 3618 Coastal Highway, St. Teresa, FL 32358, USA*

**Corresponding author: tel: + 1 954 262 3653; fax: + 1 954 262 4098; e-mail: mahmood@nova.edu.*
[‡]*Present address: King Juan Carlos University, C/Tulipán s/n, 28933 Móstoles (Madrid), Spain.*

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Understanding the connectivity of reef organisms is important to assist in the conservation of biological diversity and to facilitate sustainable fisheries in these ecosystems. Common methods to assess reef connectivity include both population genetics and biophysical modelling. Individually, these techniques can offer insight into population structure; however, the information acquired by any singular analysis is often subject to limitations, underscoring the need for a multi-faceted approach. To assess the connectivity dynamics of the red grouper (*Epinephelus morio*), an economically important reef fish species found throughout the Gulf of Mexico and USA western Atlantic, we utilized two sets of genetic markers (12 microsatellite loci and 632 single nucleotide polymorphisms) to resolve this species' population genetic structure, along with biophysical modelling to deliver a spatial forecast of potential larval "sources" and "sinks" across these same regions and spatial scale. Our genetic survey indicates little, if any, evidence of population genetic structure and modelling efforts indicate the potential for ecological connectivity between sampled regions over multiple generations. We offer that using a dual empirical and theoretical approach lessens the error associated with the use of any single method and provides an important step towards the validation of either of these methodologies.

Keywords: Atlantic Ocean, biophysical modelling, connectivity, grouper, Gulf of Mexico, microsatellite DNA, single nucleotide polymorphism

Introduction

The conservation and management of highly exploited taxa requires a thorough understanding of their population connectivity dynamics—that is, the rate of dispersal or recruitment occurring among groups of individuals across spatial scales (Hedgecock, 2010; Treml *et al.*, 2015). Connectivity not only serves to ensure the stability and resilience of populations, but also drives long-term patterns of biodiversity and the evolution of species (Thorrold, 2006; Kapralova *et al.*, 2011; Hock *et al.*, 2017), making the extent to which populations exchange individuals of great interest to managers, conservationists, and evolutionary biologists alike. Nonetheless, the underlying mechanisms driving patterns of connectivity are complex and may vary considerably across taxa and ecosystems (Cowen *et al.*, 2000; 2006; Mora and Sale, 2002; Cowen and Sponaugle, 2009). For many marine species where juveniles and adults exhibit limited dispersal and show

strong habitat associations (i.e. coral reefs, benthic, coastal, or intertidal zones), connectivity is largely determined by the extent and magnitude of pelagic larval dispersal (Cowen *et al.*, 2006; Cooke *et al.*, 2016; Klein *et al.*, 2016). Consequently, those drivers influencing larval dispersal potential [e.g. oceanographic conditions (White *et al.*, 2010), larval behaviour and movement (Cowen *et al.*, 2006; Paris *et al.*, 2007), mortality (Cowen *et al.*, 2000; 2006; Treml *et al.*, 2015), adult spawning behaviour, timing, and site location (Karnauskas *et al.*, 2011; Portnoy *et al.*, 2013; Donahue *et al.*, 2015), and pelagic larval duration (PLD) (Treml *et al.*, 2015)] are key to shaping the overall genetic population structure and the demographic linkages of such species.

Indirect population genetic approaches (i.e. *F*-statistics) have been widely adopted by researchers to infer spatial connectivity. However, using these data to define management units for a species is often quite difficult, as the relationship between levels of gene flow and demographic connectivity is complex. For instance, a key complexity is determining the minimum amount of gene flow that can occur between two groups of individuals that is sufficient to homogenize allele frequencies, while at the same time allows each group to maintain its demographic independence (Reviewed in Waples and Gaggiotti, 2006; Pallsbøll *et al.*, 2007; Hedgecock, 2010; Lowe and Allendorf 2010). Thus, with indirect genetic approaches, an absence of genetic differentiation between sites may only indicate that gene flow has occurred across evolutionary time-scales between groups, and not that the surveyed groups comprise a single, contemporary, demographically linked population (Reviewed in Waples and Gaggiotti, 2006; Hedgecock, 2010; Lowe and Allendorf, 2010). Consequently, researchers have begun to combine population genetics data with biophysical modelling of larval diffusion and advection to serve as a complementary approach to forecasting connectivity (Galindo *et al.*, 2006; Foster *et al.*, 2012; Truelove *et al.*, 2017; Mertens *et al.*, 2018). These genetics-biophysical modelling approaches are often quite feasible and may shed light on to the drivers (e.g. oceanographic, ecological, and biological) of dispersal and gene flow shaping connectivity (Hellberg, 2007; Hedgecock, 2010), while also serving to cross-validate approaches, providing a more holistic view of connectivity (including the relative magnitude of contemporary gene flow and any potential ecological drivers) (Baltazar-Soares *et al.*, 2018).

The red grouper (*Epinephelus morio*) is widely distributed throughout the western Atlantic (from North Carolina, USA to southern Brazil), the Gulf of Mexico, and the Caribbean (Garcia-Moliner and Eklund, 2004) and is an economically important coral reef-associated species targeted intensively by commercial and recreational fishers. Red grouper are protogynous hermaphrodites, with asynchronous broadcast spawning occurring largely in the winter and early spring, in small, spatially continuous, polygynous groups (rather than large temporally synced aggregations typical of other Atlantic groupers) (Brulé *et al.*, 1999; Coleman *et al.*, 1996, 2011; Burgos *et al.*, 2007). Red grouper are known to undergo ontogenetic shifts in habitat utilization (Moe, 1969; Burns and Robbins, 2006), with larvae possessing a pelagic phase lasting >40 d (Coleman and Koenig, 2010), juveniles occupying inshore shallow-water hard bottom habitats, and adults inhabiting offshore coral reefs and areas with karst topography (Coleman *et al.*, 1996, 2010, 2011). Dispersal of red grouper across its range is likely dependent on larval diffusion, as adult red grouper are largely sedentary (Coleman *et al.*, 2011). Adults do not appear to undertake spawning migrations and show strong site-fidelity to limestone solution holes, which they have been directly observed to actively maintain and excavate (Coleman *et al.*, 2010). These solution holes provide habitat and refuge for other taxa and increase local biodiversity, underscoring the red grouper's ecological importance to the community dynamics of reef ecosystems (Coleman and Williams, 2002; Coleman *et al.*, 2010; 2011; Ellis *et al.*, 2017).

Most of the USA red grouper fishery operates within the Gulf of Mexico along the West Florida Shelf, which spans the highly productive waters of the Florida Gulf coast (Schirripa *et al.*, 1999). Outside of the USA, the bulk of the red grouper fishery operates off Mexico within the waters north of the Yucatan Peninsula, along the Campeche Bank. Here, the resident red grouper population has been historically overfished (Burgos and Defeo, 2004; Lombardi-Carlson *et al.*, 2008). Given the red grouper's ecological importance as (i) an ecosystem engineer and integral community structuring member of hard bottom coastal and coral reef-associated ecosystems (Coleman and Williams, 2002) and (ii) a species of vast economic importance to both commercial and recreational fisheries, resolving the connectivity and demographic linkages between the West Florida Shelf and Campeche Bank remains a key management priority (Johnston and Bernard, 2017).

Previous population genetic surveys of red grouper in the western Atlantic and Gulf of Mexico have found no indications of genetic population structure (Richardson and Gold, 1997; Zatcoff *et al.*, 2004); however, these studies used only a handful of genetic markers with very limited statistical resolution. Given the ongoing management concerns for this economically important species (SEDAR, 2017) and advances in marker development, we investigated if the adoption of larger sets of polymorphic markers might allow for the detection of genetic differentiation of red grouper subpopulations where previous studies did not. Increased statistical resolution may also highlight the need for a more exhaustive population genetic study to resolve patterns of red grouper connectivity across varying life-stages and spatial scales. Herein, we examined red grouper population connectivity across three distinct management units: (i) the USA western Atlantic, a region managed by the South Atlantic Fishery Management Council (which includes the southeastern US seaboard through the Florida Keys with US Highway 1 as the boundary; SEDAR, 2019), (ii) the USA Gulf of Mexico, a region managed by the Gulf of Mexico Fishery Management Council (SEDAR, 2019), and (iii) the Mexican Gulf of Mexico, a region managed by Mexico's Federal Fisheries Commission (CONAPESCA). For our work, we focused on (i) whether red grouper sampled across the western North Atlantic and Gulf of Mexico demonstrate genetic population structure and (ii) understanding the potential source-sink dynamics of larval recruitment between geographically separated habitats through the use of biophysical modelling. To do this, we employed two sets of highly variable genetic markers—microsatellite DNA loci and single nucleotide polymorphisms (SNPs)—and employed *F*-statistics, and multi-locus clustering methods to infer red grouper population genetic dynamics. We then cross-validated our genetic findings using a particle tracking biophysical model to identify the potential oceanographic factors driving red grouper larval dispersal from these same locations (exact locations of red grouper tissue collections for microsatellite DNA analysis) over multiple generations. Using this multi-faceted approach, we explored the complexity of population connectivity dynamics for the red grouper—an ecosystem engineer and an important member of hard bottom coastal and coral reef associated ecosystems.

Methods

Sample collection

Red grouper tissue samples ($n = 315$) were collected between 2009 and 2015 from six locations across the USA western Atlantic and Gulf of Mexico. Within the USA western Atlantic (WAT), samples were collected from the coastal waters of Florida and the Carolinas ($n = 49$). In the Gulf of Mexico, red grouper were sampled from five locations: (i) the waters surrounding the Dry Tortugas National Park (DRT, $n = 47$)—a coral reef ecosystem ~100 km west of Key West; (ii) Florida Bay—5 km North of Marathon, Florida within the waters of the Florida Keys (FLK, $n = 35$); (iii) the western Florida Shelf (WFS, $n = 62$)—a highly productive area of the continental shelf stretching along Florida's Gulf coast; (iv) Campeche Bank, Mexico (CB, $n = 78$)—the southeastern platform of the Gulf of Mexico (exact sampling locations unknown), stretching from the Yucatan Straits to the Tabasco-Campeche Basin; and (v) Pulley Ridge (PRI, $n = 44$)—a submerged mesophotic (60–90 m) ridge, 100 km in length, and the deepest known hermatypic coral reef in USA waters (Jarrett *et al.*, 2005). PRI has been designated a Habitat Area of Particular Concern and is located ~250 km west of the Florida shelf (Figure 1). All samples were stored in 99% ethanol or a solution of sodium dodecyl sulphate (SDS; WAT only), and genomic DNA was extracted using the Qiagen DNeasy Kit (QIAGEN Inc., Valencia, CA).

Microsatellite DNA loci

A total of 304 red grouper samples were successfully genotyped at 13 microsatellite loci (see Figure 1 and Table 1 for final collection location sample numbers). Of these 13 markers, 11 were originally isolated from the Nassau grouper (*Epinephelus striatus*) (Bernard *et al.*, 2012), and the remaining 2 were isolated from gag (*Mycteroperca microlepis*) (Gag45; Chapman *et al.*, 1999) and black grouper (*Mycteroperca bonaci*) (Mbo66; Zatcoff *et al.*, 2002). Amplification and genotyping conditions of loci were consistent with methods outlined in Bernard *et al.* (2012) using a 5′-M13 labelled Forward primer and a fluorescently labelled universal M13 primer (5′-TGTAAAACGACGGCCAGT-3′) (Schuelke, 2000). Electrophoresis of all microsatellite loci was performed on an ABI 3130 genetic analyser and allele sizes scored using the software GeneMapper 3.7 (Applied Biosystems Inc., Foster City, CA, USA). GENEPOP input files were generated using the Excel MS toolkit (Park, 2001), and the resultant multi-locus genotypes were checked for duplicates using the R (3.5.0; R Core Team, 2018) package strataG 2.02 (Archer *et al.*, 2017).

Sampling location summary statistics [number of alleles per locus (*a*), allelic richness (*Ar*), observed heterozygosity (H_O), and expected heterozygosity (H_E), inbreeding coefficient (F_{IS}), and testing for conformation to Hardy–Weinberg equilibrium (HWE) and linkage disequilibrium (LD)] were estimated using: (i) the R package diveRsity 1.9.90 (Keenan *et al.*, 2013) {*a*, *Ar*, H_O, H_E, F_{IS} [95% confidence intervals (95% *CIs*) generated with 1000 bootstraps; and population-level HWE testing using Fisher's exact test (1000 replicates)]} and using the function "divBasic", (ii) the R package pegas 0.11 (Paradis, 2010) (individual locus HWE testing within populations) using the "hw.test" function, and (iii) the program GENEPOP on the web (4.0.10; LD) (Rousset, 2008). The significance values for all tests (HWE and LD) were adjusted using the false discovery rate (FDR) method (Benjamini and Hochberg, 1995) as implemented in R. The overall percentage of missing data was estimated using the R package adegenet 2.1.1 (Jombart, 2008; Jombart and Ahmed, 2011). Micro-Checker (Van Oosterhout *et al.*, 2004) was used to test multi-locus microsatellite genotypes for the presence of null alleles, large allele dropout, and scoring errors due to stuttering, while FreeNa (Chapuis and Estoup, 2007) was used to estimate the frequency of null alleles occurring across loci.

To test for population structure, overall and pairwise estimates of genetic differentiation between pre-defined sampling locations were generated using two different metrics: (i) F_{ST} (Weir and Cockerham, 1984) using the R package strataG and (ii) *Dest* (Jost, 2008) using the program GenAlEx 6.503 (Peakall and Smouse, 2006, 2012); statistical significance was determined using 999 permutations and adjusted using the FDR correction. In addition, we adopted a set of hierarchical analysis of molecular variance (AMOVAs) to test for genetic differentiation among sample groups using the R package poppr 2.8.1 (Kamvar *et al.*, 2014, 2015). Sample collection locations were partitioned into varying hierarchical groups to test for significant genetic differentiation with the aim of maximizing between group (i.e. identified as "Pop" in poppr) genetic variance. Sample locations were grouped (i) according to National management jurisdiction [USA (WAT, DRT, FLK, WFS, PRI) vs. Mexico (CB)], (ii) official management zone [USA western South Atlantic (WAT; managed by the South Atlantic Fishery Management Council) vs. USA Gulf of Mexico (DRT, FLK, WFS, and PRI; managed by the Gulf of Mexico Fishery Management Council) vs. Mexico (CB)], and (iii) water body [Gulf of Mexico (DRT, FLK, WFS, PRI, CB) vs. Atlantic Ocean (WAT)]. Significance was determined using 1000 permutations and the quasieuclid correction method was used to correct for non-euclidean genetic distances. The program POWSIM 4.1 (Ryman and Palm, 2006) was used to assess the power of our microsatellite marker set and sampling scheme to resolve population-level differentiation between the six pre-defined sampling collection locations—assuming base (overall) red grouper allele frequencies across 12 microsatellite loci (see Results), and a range of effective population sizes ($N_E = 500, 1000, 2000, 5000, 10\,000$).

Clustering of individuals to identify genetic population structure was performed using STRUCTURE 2.3.4 (Pritchard *et al.*, 2000), an individual-based Bayesian clustering method. STRUCTURE analyses were performed using ten replicate chains for each value of K (1–6), assuming correlated allele frequencies (Falush *et al.*, 2003) and admixture. Markov chain Monte Carlo chain length and burn-in across runs were 300 000 and 100 000 repetitions, respectively. A second STRUCTURE analysis was performed assuming correlated allele frequencies, admixture, and the LOCPRIOR model (Hubisz *et al.*, 2009). The LOCPRIOR model was used as it has been shown to improve inference of population structure when weak population structure is present (Hubisz *et al.*, 2009). For this second LOCPRIOR run, *a priori* defined sample groupings corresponded to the six geographic sampling locations. The program STRUCTURE Harvester 0.6.94 (Earl and vonHoldt, 2012) was used to analyse STRUCTURE output and to estimate the metric ΔK (Evanno *et al.*, 2005). STRUCTURE results were visualized using the program CLUMPAK (Kopelman *et al.*, 2015).

Single nucleotide polymorphisms

Genotyping-by-sequencing (GBS) library construction was performed as outlined in Elshire *et al.* (2011) on 200 red grouper individuals. Of these 200 individuals, 176 (CB, $n = 26$; DRT, $n = 26$; FLK, $n = 24$; PRI, $n = 42$; WAT, $n = 20$; WFS, $n = 38$)

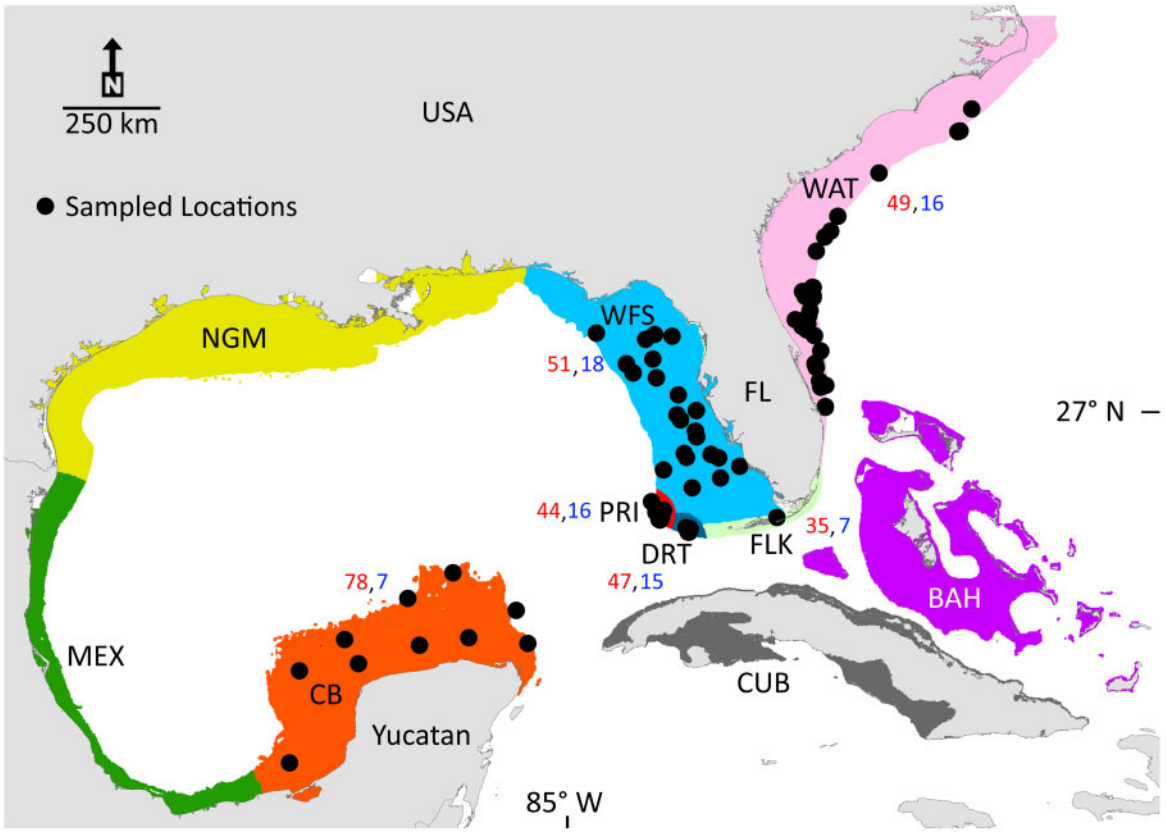

Figure 1. Map of the distribution of red grouper (*Epinephelus morio*) sampling locations. Inset shows the model domain and founder precincts for biophysical modelling. (●) represent collection sites of tissue samples for all locations save CB. For CB, exact collection locations were unknown and black circles represent the location of assumed founder locations for biophysical modelling. Values indicate overall sample sizes for each collection site (left = microsatellite sample size, right = SNP sample size). CB, Campeche Bank; MEX, Mexico; NGM, North Gulf of Mexico; WFS, western Florida Shelf; PRI, Pulley Ridge; DRT, Dry Tortugas; FLK, Florida Keys; WAT, US western Atlantic; CUB, Cuba; BAH, Bahamas.

Table 1. Genetic diversity summary statistics for 12 individual microsatellite loci and 632 SNPs across the 6 sampling locations for red grouper (*Epinephelus morio*): number of individuals genotyped (*n*), mean number of alleles (*a*), allelic richness (*Ar*), mean estimated frequency of null alleles (msat only; *Null*), inbreeding coefficient (F_{IS}) and associated 95% *CIs*, mean observed heterozygosity (H_O), and mean expected heterozygosity (H_E).

	Microsatellite DNA							SNPs				
Location	***n***	***a***	***Ar***	F_{IS} **(95% *CIs*)**	**Null**	H_O	H_E	***n***	***Ar***	F_{IS} **(95% *CIs*)**	H_O	H_E
CB	78	12.4	10.2	0.00 (−0.03, 0.03)	0.01	0.76	0.76	7	1.60	−0.14 (−0.28, 0.05)	0.29	0.26
DRT	47	10.8	9.6	−0.01 (−0.05, 0.03)	0.02	0.77	0.76	15	1.74	−0.04 (−0.10, 0.00[a])	0.29	0.28
FLK	35	9.9	8.9	−0.04 (−0.08, 0.00)	0.00	0.76	0.73	7	1.60	−0.12 (−0.25, −0.02)	0.28	0.25
PRI	44	11.2	9.7	−0.01 (−0.05, 0.03)	0.02	0.76	0.76[b]	16	1.67	0.01 (−0.06, 0.06)	0.26	0.27
WAT	49	11.8	9.8	0.04 (0.00, 0.07)	0.02	0.72	0.75	16	1.78	−0.05 (−0.10, −0.01)	0.29	0.28
WFS	51	11.3	9.9	−0.02 (−0.06, 0.01)	0.00	0.76	0.74	18	1.67	0.05 (−0.02, 0.09)	0.26	0.27

[a]Negative value rounded to zero.

[b]Heterozygosity deficiency compared with HWE at $p < 0.01$.

CB, Campeche Bank; DRT, Dry Tortugas; FLK, Florida keys; PRI, Pulley Ridge; WAT, USA western South Atlantic; WFS, western Florida Shelf.

were also genotyped at the 13 microsatellite loci. The remaining 24 samples (6 that appear in the final filtered dataset) were all collected from the WFS ($n_{\text{WFS-GBS total}} = 62$), but were not genotyped using the microsatellite panel. Final sample sizes after data quality filtering may be found in Figure 1 and Table 1. Genome complexity was reduced via digestion of 50 ng of genomic DNA using the restriction enzyme *AseI* and library sequencing was performed on a single lane of an Illumina HiSeq 2500 with 75 bp single end reads (Elim Biopharmaceuticals, Inc., Hayward, CA, USA). The TASSEL (Bradbury *et al.*, 2007) GBS non reference Universal Network-Enabled Analysis Kit pipeline (Lu *et al.*, 2013) was used to call SNPs (SNP discovery and subsequent filtering steps are listed in Supplementary Information S1).

SNP markers in the final filtered dataset were tested for conformation to HWE using the R package pegas and the "hw.test" function. Loci found to deviate from HWE proportions at a significance value of $p < 0.01$ in at least one of the surveyed sampling locations was discarded. Following HWE testing, remaining loci were tested for LD using the program Plink 1.90b6.7 (Purcell *et al.*, 2007). For each pair of loci showing an $r^2 > 0.5$, the locus with the highest amount of missing data was discarded. When a pair of SNPs possessed equal amounts of missing data, the locus selected for removal was via a random coin toss. For all SNP data, sample location summary statistics [*Ar*, H_O, H_E, testing for overall sample location HWE (Fisher's exact test using 1000 replicates), and F_{IS} and associated 95% *CIs* (1000 bootstraps)] were estimated using the R package diveRsity. Overall and pairwise estimates of differentiation (F_{ST}) between collection sites were generated using the R package strataG and significance was determine using 1000 permutations. Significance values were adjusted using the FDR approach as implemented in R. As described above, hierarchical genetic population structure was also assessed using a set of AMOVAs using the R package poppr, with sampling groupings and parameters identical to those outlined above. Significance was determined using 10 000 permutations and the quasieuclid correction method was used to correct for non-euclidean genetic distances. Further testing for population structure was performed using the program Admixture (Alexander *et al.*, 2009)—assuming default settings and the cross-validation procedure (CV) (assuming $K = 1$–10) to determine the most appropriate value of *K*.

Lagrangian biophysical modelling

To further assess red grouper connectivity throughout the study area, we ran a suite of simulations using a Lagrangian biophysical model that was previously employed to model connectivity of red grouper within Gulf of Mexico waters (Johnston and Bernard, 2017). The overall purpose of using the model was to quantify potential metapopulation connectivity between the red grouper genetic sampling locations and the broad geographic regions identified herein. See Supplementary Information S2 for a comprehensive description of the model logic using the Overview, Design concepts, and Design (ODD) protocol [as per Grimm *et al.* (2006; 2010)]. The following text is an abbreviated review of the model parameterization and the simulations that were employed for this study.

The primary "model domain" was defined as 98–76.5°W longitude and 18–35°N latitude to encompass the entire Gulf of Mexico and the Eastern Florida Shelf (i.e. western Atlantic) northward to 35°N (Figure 1). The model incorporated ocean condition data from the Gulf of Mexico 1/25° resolution Hybrid Current Ocean Model (HYCOM) and life history traits of red grouper (e.g. fecundity, mortality, maturity—see Supplementary Table S1 for all life history parameters used in the model) to produce forecasts of larval diffusion and recruitment. Data from the HYCOM were chosen as they are high-resolution and robust approximations of regional and global water flow that have shown to be useful in similar particle tracking simulations (Paris *et al.*, 2007; Kool *et al.*, 2010; Johnston and Purkis, 2015). We used daily snapshot (i.e. at 00: 00 UTM) HYCOM data from 2012 and 2013 as proxy years for the top 300 m of the water column, though red grouper larvae are likely primarily contained within the upper 20–30 m of the water column, similar to most reef organism larvae. Boundary conditions outside of the primary domain were sourced from the global 1/12° resolution HYCOM for surface waters only. The HYCOM data for years 2012 and 2013 were alternated randomly each year (i.e. via a coin toss using a random number generator) over the span of each simulation.

Potential habitat for red grouper was defined as all locations within the domain where water depths were 100 m or shallower, based on the literature-accepted depth tolerances of red grouper (Supplementary Table S1). This maximum depth value was based on red grouper catches from the Southeast Area Monitoring and Assessment Program (SEAMAP) and National Marine Fisheries Service (NMFS) fishery-independent Reef Fish and Bottom Longline resource surveys throughout the US Gulf of Mexico, as > 99% of catches in these programs occurred at depths of 100 m or shallower (Scott-Denton *et al.*, 2011). Such habitat was segregated into ten distinct "precincts" sourced from prior biophysical modelling studies in the region (e.g. Johnston *et al.*, 2017); however, the West Florida Shelf (WFS) was split into three precincts, to allow for the connectivity of DRT and PRI to be modelled separately. Though Johnston and Bernard (2017) suggest a connectivity break between the western and eastern Campeche Bank and the rest of the GOM, in this analysis we did not split the Bank into west and east as the sampled locations were unknown. The domain was divided into precincts to allow for connectivity to be qualitatively assessed over generations using a connectivity matrix.

Since red grouper samples were obtained from six of the ten precincts, six separate simulations were produced. Initial "founder populations"—that is, breeding adult females—were positioned geographically at the exact same locations where specimens were collected for the microsatellite genetic work ($n = 99$ distinct locations). Collection sites on the CB were unknown and therefore ten random locations were chosen to host founder populations on the Bank (Figure 1). Each founder population comprised five adult breeding females and each simulation was run over a period of ten years with larval production occurring from February through June of each year, corresponding to the breeding season of red grouper (Fitzhugh *et al.*, 2006; Lowerre-Barbieri *et al.*, 2014). Given that red grouper mature in two-to-three years (34 months was used in the study), ten years provided ample time for connectivity to become apparent over five generations of simulated grouper populations. For each designated simulation, the trajectories of larvae at hourly time-steps were plotted and colour-coded by PLD and the location of successful recruits plotted and colour-coded by generational cohort in a geographic information system (Figure 2, see Supplementary Information S3 for a 3 D animation of a ten-year simulation from the Pulley Ridge precinct). Finally, a transition matrix was assembled that plots founder locations (x-axis) vs. recruitment (y-axis) positions of red grouper larvae. The matrix is useful to describe connectivity among precincts in the study area.

Results

Microsatellite DNA

Multi-locus microsatellite genotypes were obtained from 304 red grouper samples from across the 6 sampling locations. Indications of null alleles were found at 3 of the 13 surveyed microsatellite loci and are not uncommon when cross-amplifying markers developed for other species as mutations may occur in

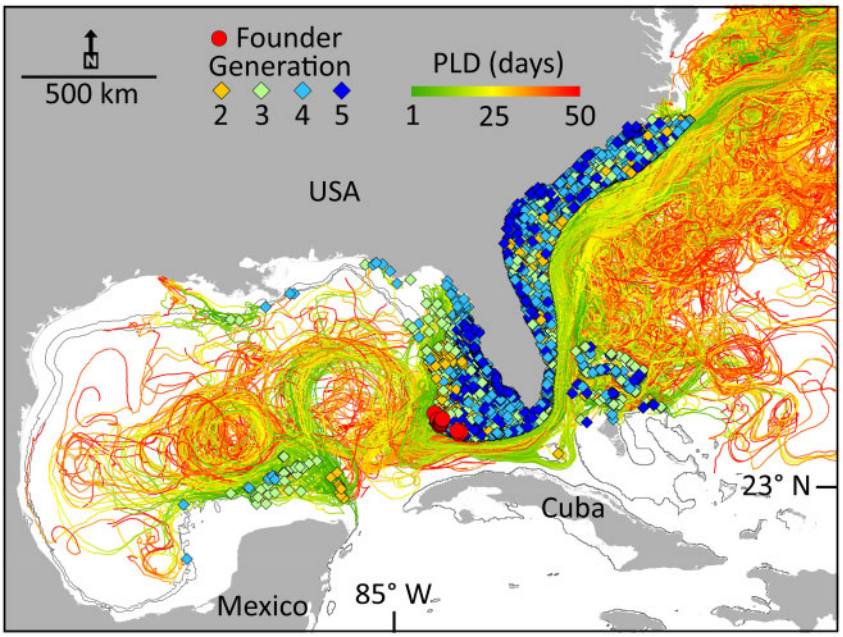

Figure 2. Predicted diffusion and recruitment patterns of red grouper (*Epinephelus morio*) larvae over five generations and ten years from the regions surrounding Pulley Ridge and the Dry Tortugas. Larval diffusion paths are coded by length of the PLD from 1 to 50 d - please see the online text for a color-coded version of the figure. Recruitment positions are indicated by diamonds, coded by generation. Founder generations are illustrated by circles. Up is north.

the primer binding sites. Micro-Checker indicated the likely presence of null alleles at locus Est265 within all six sample groupings (Supplementary Table S2), and likewise, the program FreeNa estimated a null allele frequency for this locus ranging between 10 and 17% across locations (Supplementary Table S2). As such, this locus was discarded from all downstream analyses. Although Micro-Checker also indicated the presence of null alleles at locus Est262 (WAT, DRT, and PRI) and Est376 (DRT only), these loci were retained for all subsequent analyses as their frequency did not exceed 5% (save WAT Est262) (Supplementary Table S2) and was indicated in only a subset of the surveyed locations (Supplementary Table S2). No evidence of large allele drop-out or scoring errors due to stuttering were found by Micro-Checker in the final 12-locus dataset; however, 4 of the 12 loci showed evidence of imperfect microsatellite repeat motifs (Est49B, Est92, Est267, and Est376), with allele sizes deviating from the standard base pair repeat interval.

Within the 12-locus dataset, no multi-locus duplicate genotypes were found, and the overall percentage of missing data was estimated as 1.37%. No single individual was missing data at more than 3 (of 12) loci. HWE locus testing identified three location-specific deviations following adjustment of statistical significance using FDR (within single sample collections) correction at $p < 0.05$: (i) DRT at locus Mbo66, (ii) WAT at locus Est262, and (iii) WFS at locus Est338 (Supplementary Table S2). No single locus pair was found to be in LD after FDR correction ($p < 0.05$) within any of the six surveyed sample locations; thus, all loci were considered to sort independently. Microsatellite loci showed comparable levels of polymorphism across collection sites (Table 1); however, considerable inter-locus variability was found. The number of alleles per locus overall ranged from 3 (Est267) to 31 (Est376) and the H_O ranged between 0.36 (Est267) and 0.94 (Gag45) (Supplementary Table S2). Mean *Ar* was highest within CB (10.2) and lowest within FLK (8.9) (Supplementary Table S2).

Overall and pairwise estimates of genetic differentiation (F_{ST} and *Dest*) revealed very limited evidence of genetic population structure between sampling sites (Table 2)—overall F_{ST} and *Dest* values were non-significant ($F_{ST} = -0.001$, $p = 0.72$; $Dest = -0.002$, $p = 0.70$) and only a single pairwise comparison yielded significant results prior to FDR correction (CB vs. FLK, both F_{ST} and *Dest*). The set of hierarchical AMOVAs also revealed an absence of population genetic structure across the surveyed area with nearly the entirety of the genetic variance found within samples (~99%) regardless of sample groupings (Supplementary Table S3). Among group (i.e. Pop in poppr) percent genetic variance did not exceed 0.13% across any of the three assayed sample groupings and was non-significant. Notably, power analyses showed that the sampling regime and microsatellite marker set used herein possessed the ability to resolve relatively low levels of genetic differentiation among red grouper collection sites ($F_{ST} = 0.005$, % significance = 100%) across all assumed N_Es (Supplementary Figure S1).

Individual-based STRUCTURE analyses of the microsatellite genotypes strongly supported the results of the pairwise estimates of differentiation and the hierarchical AMOVAs. For both STRUCTURE analyses, likelihood values peaked at $K = 1$ and ΔK values peaked at $K = 2$ (Supplementary Figure S2). For both STRUCTURE analyses, at $K > 1$ individuals were highly admixed and showed symmetrical assignment across inferred clusters ($q \sim 1/K$; Supplementary Figure S3a–d) and no single individual demonstrated strong assignment to any single cluster, indicating an absence of population structure (Pritchard *et al.*, 2009). Although both ΔK values were highest at $K = 2$, it is important to note that the metric ΔK does not allow for the consideration of $K = 1$ (see Janes *et al.*, 2017).

Table 2. Red grouper population-level pairwise values of differentiation for microsatellite loci (msat; F_{ST}, *Dest*) and SNPs (F_{ST}).

Collection location comparisons	Microsatellite DNA		SNPs
	F_{ST}	*Dest*	F_{ST}
CB vs. DRT	−0.001	−0.003	−0.001
CB vs. FLK	**0.005**	**0.015**	**0.019**
CB vs. PRI	−0.001	−0.002	−0.014
CB vs. WAT	−0.001	−0.003	−0.005
CB vs. WFS	−0.001	−0.002	−0.007
DRT vs. FLK	0.001	0.002	0.006
DRT vs. PRI	−0.003	−0.009	−0.001
DRT vs. WAT	−0.001	−0.002	**0.006**
DRT vs. WFS	−0.002	−0.006	**0.006**
FLK vs. PRI	−0.002	−0.007	0.006
FLK vs. WAT	0.001	0.003	**0.015***
FLK vs. WFS	0.002	0.006	0.007
PRI vs. WAT	−0.004	−0.012	0.000
PRI vs. WFS	0.000	0.000	0.004
WAT vs WFS	−0.002	−0.006	0.002

Bold values indicate significance at $p < 0.05$ prior to FDR correction.
CB, Campeche Bank; DRT, Dry Tortugas; FLK, Florida Keys; PRI, Pulley Ridge; WAT, USA western South Atlantic; WFS, western Florida Shelf.
*Significance at $p < 0.05$ after FDR correction.

Single nucleotide polymorphism

GBS library sequencing of 200 red grouper samples and subsequent data filtering yielded 675 SNPs across 79 individuals (for individual sampling location sample sizes see Table 1). A total of 36 loci showed significant deviations from HWE at $p < 0.01$ within one or more sample locations and were subsequently removed from the dataset. Following HWE testing, LD testing identified seven locus pairs with a $r^2 >$ than 0.5. As such, one locus from each of these pairs was omitted from all downstream analyses, yielding a dataset containing 632 SNPs genotyped across 79 individuals. The final dataset used for all subsequent analyses contained 27.66% missing data, overall. Summary statistics yielded similar estimates of genetic diversity (H_O, H_E, and *Ar*) across all six sampling locations. Fisher's exact testing for HWE showed no deviations from HWE across sampling locations, however, F_{IS} values for the DRT, FLK, and WAT collections were negative, and 95% *CI*s did not overlap zero (Table 1). The overall estimate of differentiation was low and non-significant ($F_{ST} = 0.002$; $p = 0.092$); however, several pairwise estimates of differentiation yielded low, but statistically significant genetic differentiation between sampling sites (Table 2). Four pairwise comparisons were significant prior to FDR correction, however, only a single comparison was significant after FDR correction (FLK vs. WAT, $F_{ST} = 0.015$; Table 2). Hierarchical assessments of genetic population structure using an AMOVA framework showed that nearly the entirety of the genetic variance was found within samples (∼96%) regardless of sample groupings (Supplementary Table S3). Among group (i.e. Pop in poppr) percent genetic variance did not exceed 0.06% across any of the three assayed sample groupings, but the variance between samples within defined subpopulations was consistently ∼4.0% and statistically significant (Supplementary Table S3). Admixture's CV method identified the optimal value of *K* for the red grouper SNP dataset as $K = 1$ (lowest CV = 0.532) (Supplementary Figure S4), indicating an absence of population genetic structure.

Lagrangian biophysical modelling

We found the potential for strong inter-precinct connectivity (as illustrated in the transition matrix), with larvae from several precincts transported throughout the entire model domain (Figure 3). For example, larvae sourced from the CB and PRI precincts were forecasted to recruit to all six precincts where samples were collected for the genetic work. The general trend of larval flow was from the southwest to the northeast, aided by the strong flowing Gulf Loop Current and Gulf Stream, as well as the long PLD of the fish (Figure 3). Interestingly, upstream connectivity (relative to the Gulf Loop Current) was forecasted between the PRI and CB precincts, suggesting the potential for bi-directional exchange of red grouper larvae between Mexican and United States red grouper habitat (Supplementary Information S2). All precincts demonstrated some level of larval retention (i.e. self-recruitment) as shown by larvae plotting on a diagonal from bottom left to top right within the matrix; however, larval retention was strongest in the WFS and WAT precincts. Finally, the WAT precinct received larvae from all precincts upstream (relative to the Gulf Loop Current) of the region but contributed only a few larvae to the Bahamas (BAH) precinct.

Discussion

This study provides a multi-faceted view of the population connectivity dynamics of an economically important species and key marine ecosystem engineer. Herein, we found red grouper genetic connectivity across both sets of genetic markers (microsatellite DNA and SNPs) throughout the surveyed area, indicating that gene flow has occurred across management units and National jurisdictions. Broad-scale spatial connectivity was also forecasted using a Lagrangian biophysical model to infer larval diffusion and advection throughout this same area, indicating that long-distance dispersal to distant red grouper sampling sites is possible, and may be facilitated through multi-generational spawning events (Figure 3). In addition, the model forecasted a general trend of larval flow from the southwest Gulf of Mexico to the northeast Gulf of Mexico and western Atlantic, and that larvae sourced from CB and PRI may potentially recruit to all six genetically surveyed precincts, underscoring their potential importance as larval "sources" to all other precincts and red grouper habitats throughout the region.

Herein, the two genetic marker datasets yielded largely congruent findings across analysis methods and metrics. Individual-based clustering analyses (STRUCTURE and Admixture), hierarchical AMOVAs, and the bulk of overall and pairwise estimates of genetic differentiation (F_{ST}), consistently failed to identify population genetic structure in red grouper; however, some subtle indications of population structure were identified within the SNP dataset, which we suggest should be interpreted cautiously. For instance, pairwise testing for genetic differentiation using the SNP dataset identified four comparisons that were statistically significant (CB vs. FLK, DRT vs. WAT, DRT vs. WFS, and FLK vs. WAT) before FDR correction; only FLK vs. WAT was significant after FDR correction (Table 2). In contrast, only one of the above four comparisons was significantly different ($p < 0.05$ prior to FDR correction) using the 12-locus microsatellite dataset (CB vs. FLK). Likewise, differences can also be seen when comparing the AMOVA results between marker sets with respect to the amount of genetic variance found between samples within subpopulations (Supplementary Table S3). This discrepancy between

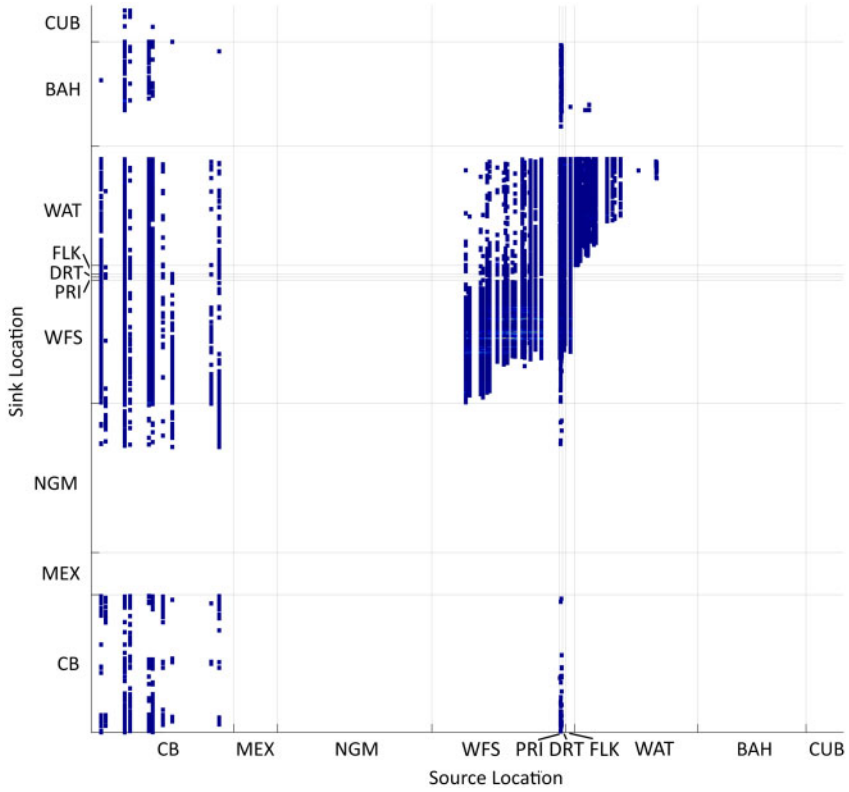

Figure 3. Transition matrix plotting red grouper (*Epinephelus morio*) founder locations (x-axis) against larval recruitment positions (y-axis) by precinct as forecast by the biophysical model. Squares indicate the geographic locations of larval recruitment. Squares positioned along the bottom-left to top-right diagonal indicate self-recruitment within a precinct. Horizontal and vertical lines delineate precinct boundaries. CB, Campeche Bank; MEX, Mexico; NGM, North Gulf of Mexico; WFS, western Florida Shelf; PRI, Pulley Ridge; DRT, Dry Tortugas; FLK, Florida Keys; WAT, US western Atlantic; CUB, Cuba; BAH, Bahamas.

marker sets could be an artefact of low sample numbers in the SNP dataset. After SNP quality filtering, location-specific sample sizes were greatly reduced (see Table 1, CB and FLK, $n=7$). When such low sample numbers are combined with a low number of resolved SNPs (as seen here), robust inferences of population differentiation may not be possible (see Willing *et al.*, 2012; Nazareno *et al.*, 2017; Flesch *et al.*, 2018), as collections may not be sufficient to capture all of the genetic variation present at those sites.

The broad spatial connectivity of red grouper found here likely results from a combination of this species' unique life history characters [i.e. large population size, extended PLD (>40 d; Coleman and Koenig, 2010), spawning and reproductive behaviour] and the ocean circulation dynamics of the Gulf of Mexico. Unlike some groupers that form temporally synced spawning aggregations, the location and timing of which are hypothesized to coincide with the formation of oceanographic conditions favouring local or nearshore larval retention (Heppell *et al.*, 2011; Karnauskas *et al.*, 2011), red grouper spawn asynchronously, in small groups, across a broad geographic area (Coleman *et al.*, 1996). This latter reproductive strategy may provide more opportunities for larval dispersal (and subsequent gene flow resulting in the low levels of genetic differentiation seen here); however, spawning behaviour alone (aggregating vs. non-aggregating) is only one of several factors (oceanographic or behavioural) that may shape species connectivity (Portnoy *et al.*, 2013). Additionally, across our model simulations, it is largely the fast-flowing Gulf Loop Current and the red grouper's lengthy PLD that, when combined, facilitate the wide forecasted diffusion of larvae throughout the study area. The Gulf Loop Current originates as the Caribbean current and flows northward into the Gulf of Mexico, where it loops clockwise, and then flows southward along the outer slope of the WFS until it exists the Florida Straits as the Florida and Gulf Stream Currents. Within our simulations, those larvae that become entrained in the Gulf Loop Current were transported and subsequently recruited to areas distant from their natal origin via single or a sequence of reproductive events over multiple generations. These multi-generational distribution networks have been described elsewhere (Holstein *et al.*, 2014). Recruitment by our model, however, does not guarantee larval settlement and reproductive success, two conditions necessary for gene flow and demographic linkages to occur between sites. Analogous connectivity patterns within the Gulf of Mexico (i.e. Gulf Loop Current facilitating dispersal) have been forecasted using similar biophysical models to study other coral reef-associated fishes (e.g. red snapper, lionfishes, and red grouper), and some of these efforts also forecast larval diffusion from the CB to the waters of the WFS and WAT at levels sufficient to potentially homogenize allele frequencies between locations (Johnson *et al.*, 2013; Johnston and Bernard, 2017).

While genetic data do not support the population genetic differentiation (non-significant F_{ST} estimates across both sets of genetic markers) between red grouper populations inhabiting the western and eastern Florida shelves (two areas of high biological

and economic importance to USA fisheries), our biophysical modelling does forecast the potential for larval retention within these sites (Figure 3). Reliable estimates of precinct-specific larval retention rates, however, are not available from the output of this model due to the use of uneven sample sizes and irregular distributions across the study area. We offer that this model-predicted retention may be due to depth-driven variation in water flow across the Gulf of Mexico and western Atlantic continental shelves (Weisberg *et al.*, 2009; Liu and Weisberg, 2012), which may favour retention in nearshore waters. Within the Gulf of Mexico, larvae spawned within the inner shelf region may avoid entrainment and subsequent downstream transport in the Gulf Loop Current which flows along the outer, and deeper areas of the shelf. Likewise, inner- and mid-shelf waters that flow along the South Atlantic Bight (i.e. WAT) are largely wind driven and slow (i.e. which may promote retention), while water flow along the outer western Atlantic shelf may facilitate downstream connectivity via larval entrainment in the fast-moving Florida Gulf Stream Current (Blanton *et al.*, 2003).

The above potential mechanisms for larval retention are extremely important for the management of red grouper, as they indicate that despite our findings of genetic homogeneity between these two sites, populations of red grouper inhabiting the WFS and WAT have the potential for self-seeding and demographic independence. This model-derived hypothesis needs to be tested with a more exhaustive genetic survey of samples from these two areas to inform management of red grouper. We note that samples for our study were collected opportunistically over a span of six years and contained a mixture of fish age classes and life-stages. Such a sampling scheme may make it difficult to resolve subtle differences in population genetic structure, even with a robust marker set and adequate sample sizes such as we have with our microsatellite dataset. The adoption of a targeted sampling strategy that aims to obtain tissues from adults across large stretches of coastal habitat during a single spawning season, as well as young-of-the-year from these same geographic regions, may allow for the further elucidation of fine-scale patterns of population structure. A paired biophysical model incorporating an even sample size distribution could also be adopted, and simulation runs that incorporate an evenly distributed sample grid and *in situ* population estimates could be performed to generate improved predictions of larval retention and dispersal.

As off-shore mesophotic coral reef ecosystems are often hypothesized to experience fewer anthropogenic and/or climate-driven effects than other shallow-water, coastal environments, these systems may have the potential to serve as biodiversity or genetic refuges, and should, therefore be considered conservation priorities (Lesser *et al.*, 2009; Slattery *et al.*, 2011). However, within the Gulf of Mexico's PRI mesophotic coral reef ecosystem, surveys have demonstrated recent community changes, including a decline in coral cover and the increased abundance of the invasive lionfish (*Pterois volitans*), which have been found at PRI in red grouper solution holes (Reed *et al.*, 2015; Harter *et al.*, 2017). We found that red grouper inhabiting PRI do not show any evidence of genetic differentiation from other sampled collection sites, indicating that gene flow between PRI and other coastal red grouper habitats has occurred. Furthermore, through our model simulations, we found support for the hypothesis that PRI may serve as a deep-water genetic refuge, in that red grouper are predicted to export larvae to both upstream and downstream (relative to the Gulf Loop Current) shallow-water Gulf of Mexico (including CB) and Atlantic coastal regions. In our simulations, upstream connectivity (PRI to CB) was most likely achieved via entrainment of larvae in anticyclonic eddies spawned from the Gulf Loop Current in the south-central Gulf of Mexico. Connectivity of Pulley Ridge to neighbouring regions, including Dry Tortugas, Florida Keys, east Florida coast, and the Campeche Bank has been studied elsewhere (Olascoaga *et al.*, 2018). Connectivity between PRI and its neighbouring locations is of particular relevance for red grouper, given its critical role within ecosystems as engineers. As such, changes in red grouper abundance at PRI may consequentially affect opportunities for settlement and shelter for a variety of other marine fishes and invertebrates, including critical juvenile life-stages (Coleman and Williams, 2002; Coleman *et al.*, 2010; Ellis and Faletti, 2016).

Concluding remarks

Our study using microsatellite DNA and SNP markers, along with two other previous surveys (Richardson and Gold, 1997; Zatcoff *et al.*, 2004) have concordantly found little genetic population structure across large portions of the red grouper's USA (Gulf of Mexico and western Atlantic) distribution, and commercially important areas of its Mexican range. The combination of our multiple method approach, along with previous researcher's results, suggest that larval recruitment and gene flow have linked red grouper populations across nearly the entirety of the sampled region. Nonetheless, despite these multiple surveys, the magnitude and frequency of larval diffusion and gene flow occurring among spawning habitats remains unknown and accordingly, several details for refining the areal extent of red grouper management units requires further consideration. For instance, the occurrence of infrequent or episodic pulses of larval dispersal and subsequent recruitment across large spatial scales may work to homogenize red grouper allele frequencies, generating low and non-significant *F*-statistics (as largely seen here). However, such infrequent instances of larval dispersal may have only minor demographic implications regarding the transfer of individuals across management units. Conversely, frequent gene flow may create stronger dependencies on external sources of recruitment generating a single panmictic population for management purposes. While our work cannot differentiate between these two demographic scenarios (episodic vs. frequent gene flow), it does underscore the need for a more robust survey of the ecological and genetic connectivity of red grouper within some of its most biologically and economically important habitats. Presently, the CB and WAT red grouper management units are considered overfished, and the CB fishery remains largely unregulated (Burgos and Defeo, 2004; Lombardi-Carlson *et al.*, 2008; SEDAR, 2017). These circumstances underscore the need for continued exploration of the persistence and dynamics of recruitment across regions and generations to better understand the population and demographic structure and resiliency of this important commercial species.

Supplementary data

Supplementary material is available at the *ICESJMS* online version of the manuscript.

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