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REPORT

The ups and downs of coral reef fishes: the genetic characteristics of a formerly severely overfished but currently recovering Nassau grouper fish spawning aggregation

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Abstract The Nassau grouper (*Epinephelus striatus*) has sustained large declines across its distribution, including extirpation of many of its fish spawning aggregations (FSAs). Within US Virgin Islands (USVI) waters, Nassau grouper FSAs were overfished until their disappearance in the 1970s and 1980s. In the early 2000s, however, Nassau grouper were found gathering at Grammanik Bank, USVI, a mesophotic coral reef adjacent to one of the extinct aggregation sites, and regulatory protective measures were implemented to protect this fledgling FSA. The population genetic dynamics of this rapid FSA deterioration followed by protection-facilitated, incipient recovery are unknown. We addressed two objectives: (1) we explored which factors (i.e., local vs. external recruitment) might be key in shaping the USVI FSA recovery; and (2) we examined the consequences of severe past overfishing on this FSA's current genetic status. We genotyped individuals (15 microsatellites) from the USVI FSA comprising three successive spawning years (2008–2010), as well as individuals from a much larger, presumably less impacted, Nassau grouper FSA in the Cayman Islands, to assess their comparative population dynamics. No population structure was detected between the USVI and Cayman FSAs ($F_{ST} = -0.0004$); however, a temporally waning, genetic bottleneck signal was detected in the USVI FSA. Parentage analysis failed to identify any parent–offspring matches between USVI FSA adults and nearby juveniles, and relatedness analysis showed low levels of genetic relatedness among USVI FSA individuals. Genetic diversity across USVI FSA temporal collections was relatively high, and no marked differences were found between the USVI and Cayman FSAs. These collective results suggest that external recruitment is an important driver of the USVI FSA recovery. Furthermore, despite an apparent genetic bottleneck, the genetic diversity of USVI Nassau grouper has not been severely compromised. Our findings also provide a baseline for future genetic monitoring of the nascent USVI aggregation.

Keywords Nassau grouper · Microsatellite · Fish spawning aggregation · Bottleneck · Population recovery

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Introduction

Widespread declines in coral reef ecosystem biodiversity and fish abundance have been reported across regions and taxa (Pandolfi et al. 2003; Paddack et al. 2009). In many

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cases, overfishing has been a key factor contributing to fish declines, with far-reaching effects including modifications to reef-fish species composition and sometimes even loss of entire functional groups (Roberts 1995; Mora 2008). One overfished species is the Nassau grouper (*Epinephelus striatus;* Serranidae), with a 60 % decline in the number of fish spawning aggregations (FSAs) reported for this species in the Caribbean (Sadovy de Mitcheson et al. 2008). The FSAs comprise the reproductive biomass for this species. In winter months, adults may travel hundreds of kilometers (Bolden 2000) to gather at specific locations to spawn, forming large, highly transient, yet also highly synchronized (temporally and spatially) aggregations which historically ranged up to tens of thousands of individuals (Smith 1972). It is while aggregated that the Nassau grouper and other FSA-forming reef fishes become extremely vulnerable to overfishing (Sadovy de Mitcheson et al. 2008). As a result of FSA-targeted overexploitation, the International Union for Conservation of Nature (IUCN) Red List of Threatened Species has classified the Nassau grouper as Endangered throughout its range (Cornish and Eklund 2003), and it is currently under consideration for listing under the US Endangered Species Act (NOAA 2014).

Nassau grouper overfishing is well documented in the waters of the US Virgin Islands (USVI; Olsen and LaPlace 1979). Historically, two separate Nassau grouper FSAs formed within these waters during the winter months: one formed south of St. Thomas, USVI, in a region now designated as the Red Hind Marine Conservation District; and the second formed in the waters east of St. Croix, USVI, on the Lang Bank (Olsen and LaPlace 1979; Beets and Friedlander 1992). However, both aggregations were fished heavily in the 1970s and 1980s and were extirpated (Olsen and LaPlace 1979; Beets and Friedlander 1992; Sadovy 1999). After that, only very small and loose collections of Nassau groupers were anecdotally reported within USVI waters (Beets and Friedlander 1992). In the early 2000s, however, a small Nassau grouper FSA was discovered adjacent to the former St. Thomas aggregation site. This FSA was part of a multi-species (including *Mycteroperca venenosa*, yellowfin grouper) fish aggregation situated on a mesophotic coral reef called the Grammanik Bank (GB) (Fig. 1) (Nemeth et al. 2006). This discovery marked the first documented appearance of a Nassau grouper FSA within US waters since the mid-1970s (Kadison et al. 2009); however, commercial fishers were quick to target this new aggregation site and began to harvest both yellowfin and Nassau groupers (Nemeth et al. 2006). In 2005, the Caribbean Fisheries Management Council implemented an annual seasonal closure of GB to fishing (February to April). Since this closure, diver-based visual surveys suggest that Nassau grouper spawning biomass has increased at the aggregation site from a maximum abundance of ~30 individuals sighted per day in 2005, to ~100 in 2009 (Kadison et al. 2009). By 2013, a maximum abundance of 214 individuals was noted per day (Schärer-Umpierre et al. 2014).

It remains unknown whether the ongoing GB FSA recovery is a result of aggregation at this site by (1) remnant individuals from the nearby overfished FSAs (i.e., St. Thomas and/or St. Croix), followed by expansion mainly by local reproduction, or (2) is mainly a consequence of immigration from more distant regions, with such migrants comprising either adult fish, or larvae dispersed from distant FSAs who have settled close-by, matured and recruited into the GB FSA. While conventional tagging and acoustic telemetry studies have found strong fidelity of Nassau grouper adults to spawning sites suggesting limited dispersal of adults across large spatial scales (Semmens et al. 2007; Kadison et al. 2009), little is known about the extent of local versus external larval recruitment and its effect on local FSA persistence. Previous studies on some other coral reef fishes have adopted molecular tools and parentage analyses and demonstrated that localized larval retention may be common (Jones et al. 2005, 2009; Puebla et al. 2012; Almany et al. 2013). If local retention is common in the Nassau grouper, genetic differentiation among FSAs may evolve over several generations due to their reproductive isolation, leading to genetic population structure among FSAs. Conversely, if immigration facilitated primarily via larvae generated from geographically distant FSAs is occurring, more complex patterns in population structure may be present.

Regulatory protection has led to the resurgence of some highly depleted grouper spawning aggregations elsewhere (Nemeth 2005; Heppell et al. 2012). However, biomass recovery by itself may not ensure the long-term health and persistence of a FSA. Loss of genetic diversity often accompanies severe declines in population biomass (i.e., a genetic bottleneck), and the retention of genetic diversity within populations is essential to prevent loss of fitness and evolutionary potential (Bouzat 2010; Pinksy and Palumbi 2014). The documented collapse in Nassau grouper FSA biomass in the USVI, followed by an incipient, presumably protection-based resurgence at GB, provides a novel opportunity to study the genetic consequences and characteristics of recovering reef fish populations.

Our study had two main objectives aimed at understanding the population genetic dynamics of a severely overfished but now incipiently recovering Nassau grouper FSA. First, we wished to explore which factors (local vs. external recruitment) might be key in shaping the FSA recovery at GB, and second, we aimed to examine the

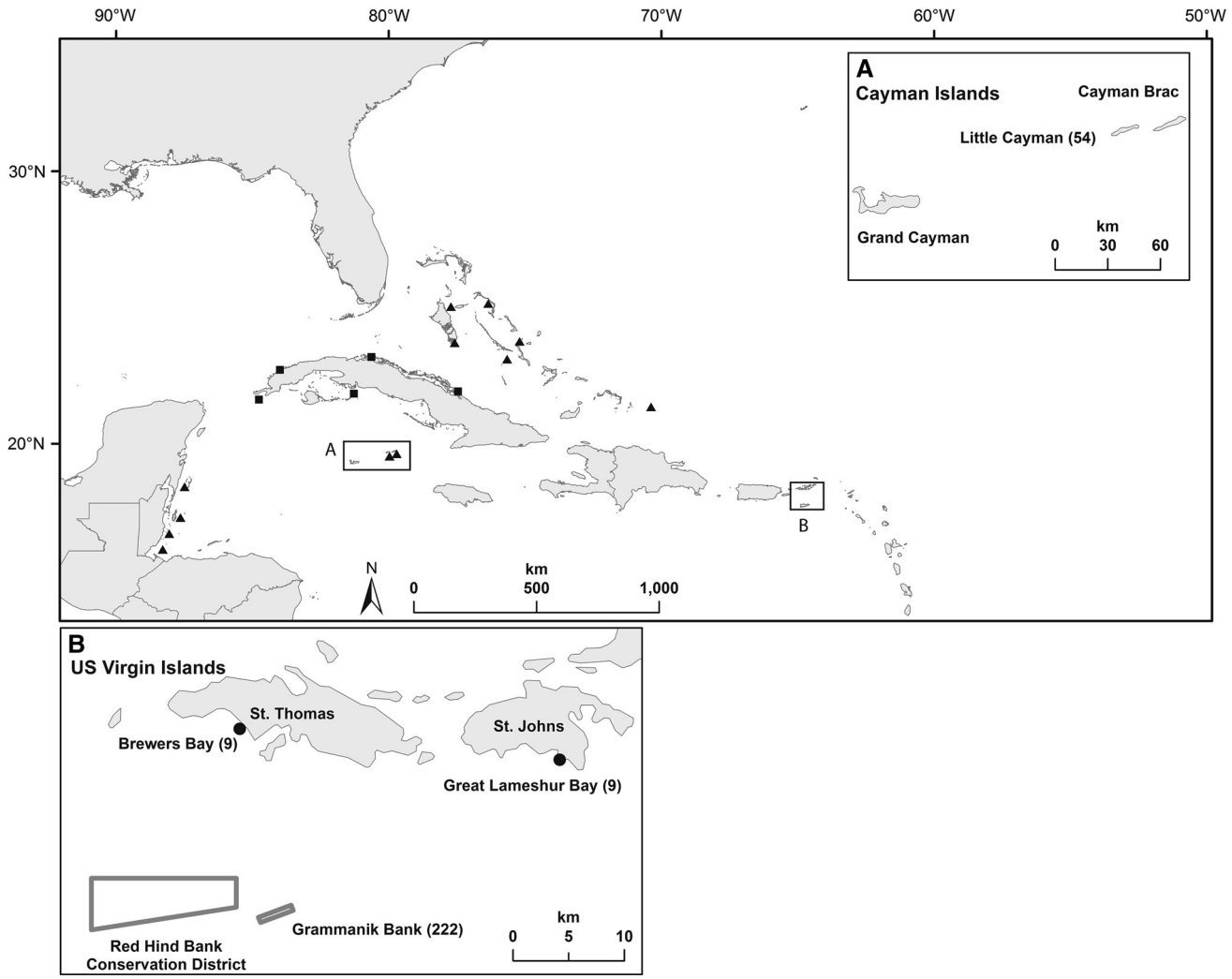

Fig. 1 Map of sampling locations for Nassau grouper (*Epinephelus striatus*). *Smaller map boxes* depict fish spawning aggregation sites (**A** Little Cayman, Cayman Islands; **B** Grammanik Bank, US Virgin Islands) and juvenile collection locations (**B** Brewers Bay and Great Lameshur Bay), with sample sizes in *brackets*. Adapted from Sadovy de Mitcheson et al. (2008): *triangles* indicate the locations of existing Nassau grouper FSAs believed to range in size from 100 to 3000 adult fish; *squares* indicate the sites within Cuban waters believed to produce small catches of Nassau grouper. Note, however, that there has been no direct assessment of these sites to determine whether they are spawning aggregations

consequences of severe overfishing on the current genetic status of this FSA. To address the first objective (the potential source of the ongoing recovery of the GB FSA), we examined three issues. First, we assessed the genetic connectivity of the GB Nassau grouper FSA to an aggregation occurring at Little Cayman (LC), Cayman Islands, located ~1600 km west of GB and in a separate Caribbean region (i.e., LC in the central Caribbean vs. GB in the eastern Caribbean; Jackson et al. 2014). The LC FSA was selected for comparison since it is one of the few remaining healthy aggregations (~2000–2500 individuals), and has been protected by seasonal fishing closures since 2003 (Heppell et al. 2012). While there are some other reports of small eastern Caribbean FSAs in closer proximity to GB (i.e., British Virgin Islands and Bajo de Sico, Puerto Rico), such aggregations are fledgling (Munro and Blok 2003; Schärer-Umpierre et al. 2014) and likely in the midst of recovering. Second, we tested the hypothesis that if recruitment were primarily local, relatedness among fish aggregating at GB would be higher than the relatedness of the GB fish to those from the LC FSA. Third, we assessed the origin of Nassau grouper juveniles captured within USVI waters via parentage and relatedness analyses to determine whether the GB FSA was the likely source of the sampled juveniles. To assess the second objective (the genetic consequences of Nassau grouper declines), the

genetic diversity and demographic history of the GB aggregation was compared to the much larger LC FSA.

Materials and methods

Study species, sample collection, DNA extraction, and genotyping

The Nassau grouper ranges throughout the tropical waters of the western Atlantic (Cornish and Eklund 2003). This species is primarily gonochoristic (separate sexes) and individuals reach sexual maturity between 4 and 8 yr (Sadovy and Colin 1995; Sadovy and Eklund 1999). Pelagic larval duration spans ~37–45 d (Colin et al. 1997), and grouper undergo ontogenetic shifts in habitat utilization. Juveniles are found in shallow sea grasses and macroalgae and eventually recruit to deeper waters and reef habitats (Sadovy and Eklund 1999). As adults, individuals aggregate to spawn, the timing of which appears to be linked to both lunar cycles and water temperature (Kobara et al. 2013).

Tissue collections were performed with approval (Letter of Authorizations) from the National Marine Fisheries Service and the Cayman Islands Conservation Board, and a collection permit was obtained from the National Parks Service (2008–2010; # VIIS-2008-SCI-0007). At the time of the field work (2005 and 2008–2010), the collecting agencies did not have an Animal Welfare Committee and an institutional IACUC permit was not required. Rather, all animal handling procedures were conducted using guidelines established by the American Fisheries Society and American Society of Ichthyology and Herpetology. Fin clip tissues were collected from a total of 276 adult Nassau groupers from two Caribbean FSAs located at: (1) LC, Cayman Islands (CI), $n = 54$ (January 2005); and (2) GB, St. Thomas, USVI, $n = 222$ collected in three spawning seasons (February–April): (2008, $n = 51$; 2009, $n = 93$; 2010, $n = 78$) (Fig. 1). Fish were collected using hook and line, fish traps, or by divers using hand nets. Percent of fin clips taken from captured GB Nassau grouper varied across sampling years (2008, ~60 %; 2009, ~69 %; 2010, ~98 %). In addition, fin clips from 18 juveniles were collected at two locations within USVI waters: Brewers Bay (18°20°N, 64°58°W), $n = 9$; Great Lameshur Bay (18°18°N, 64°43°W), $n = 9$ in June–July 2008 (Fig. 1). Tissue samples were stored in Sarkosyl–urea solution (1 % Sarcosyl, 8 M urea, 20 nM sodium phosphate, 1 mM EDTA, pH 6.8) (LC FSA) or 95 % ethanol (GB FSA) until genomic DNA extraction using the DNeasy Kit (Qiagen Inc., Valencia, CA). Individuals were genotyped at 15 species-specific microsatellite loci (Electronic Supplementary Material, ESM, Table S1) following Bernard et al. (2012).

Discrimination of spawning aggregation individuals and capture–recapture-based estimate of census size

Because samples from GB were collected as part of a FSA temporal monitoring project, numerous individuals were repeatedly sampled (within and among spawning years). To identify unique individuals for our subsequent population genetic analyses, the software CERVUS 3.0.3 (Kalinowski et al. 2007) was used to identify matching genotypes, and only unique genotypes were included in all subsequent analyses. The theoretical probability of identity between siblings [$P_{(ID)sib}$] as described in Waits et al. (2001) was estimated using CERVUS.

Since direct census size estimates for the GB FSA were unavailable, census size was estimated from genetic capture–recapture data using the software *capwire* (Miller et al. 2005) and all capture–recapture information was pooled for all three spawning years. While the *capwire* software assumes that populations are closed to migration, this assumption may have been violated as visual qualitative surveys of the GB FSA noted a successive increase in the number of aggregating individuals across survey years (Kadison et al. 2009). Thus, the genetically derived FSA census size should be interpreted as a first-order estimate. The *capwire* analyses included default settings, implemented the likelihood ratio test to select the most appropriate capture model, and generated 95 % confidence intervals (CIs) (1000 parametric bootstraps).

Microsatellite loci summary statistics

Observed (H_O) and expected (H_E) heterozygosity and deviations from Hardy–Weinberg (HWE) and linkage equilibrium were analyzed using GENEPOP v. 4.0.10 (Raymond and Rousset 1995; Rousset 2008). Significance was estimated using an exact test, employing the Markov chain method (Guo and Thompson 1992; Rousset and Raymond 1995) and were adjusted using the sequential Bonferroni correction (Rice 1989). Allelic richness (R_S; El Mousadik and Petit 1996) was estimated for each locus and collection using FSTAT v. 2.9.3.2 (Goudet 2001). The frequency of null alleles was estimated using FreeNA (Chapuis and Estoup 2007). A nonparametric Kruskal–Wallis rank sum test was performed using R v. 2.7.2 to test for differences in genetic diversity (H_E, H_O, and R_S) among collections.

Genetic differentiation among FSA collections (temporal and spatial)

Pairwise values of genetic divergence between FSA collections were estimated using the programs FSTAT [F_{ST} (Weir and Cockerham 1984); 1000 iterations] and GenAlEx v.

6.501 (Peakall and Smouse 2006) [D_{est} (Jost 2008); G''_{ST} (Hedrick 2005; Meirmans and Hedrick 2011); 999 permutations]. Multiple measures of pairwise divergence were adopted to accommodate the high variability of microsatellites and the associated low values of divergence found using traditional estimates (i.e., F_{ST}) (see Meirmans and Hedrick 2011). To complement the population-level analysis, the individual-based program *Structure* v. 2.3.1 (Pritchard et al. 2000) was also utilized. *Structure* runs were performed including all unique adult individuals (GB, $n = 172$; LC, $n = 54$), and consisted of ten replicates assuming $K = 1$–5 (chain length and burn-in period = 300,000 and 100,000 iterations, respectively). *Structure* analyses assumed correlated allele frequencies (Falush et al. 2003) and the *locprior* model (Hubisz et al. 2009).

Relatedness and parentage analyses

To further assess whether local or external recruitment was driving the recovery of Nassau grouper at GB, both relatedness and parentage analyses were conducted. First, we tested the hypothesis that if local recruitment was the primary driver of recovery, relatedness among GB individuals would be higher than between GB and LC individuals. The mean symmetrical coefficient of relatedness (Queller and Goodnight 1989) among GB adults and between GB and LC adults was estimated using GenAlEx. A two-tailed t-test was performed (R 3.1.1) to test for differences between mean relatedness coefficients.

Second, to determine whether the juvenile Nassau groupers collected from surrounding USVI waters were progeny of the GB FSA spawning adults (i.e., consistent with local recruitment), parentage analyses were performed using the strict exclusionary and categorical likelihood-based approaches implemented in CERVUS. For the strict exclusionary method, a parent–offspring pair was identified if at least one allele per locus at each codominant marker was shared (per Mendelian inheritance in diploid organisms), while the categorical approach allows for parent–offspring allele mismatches to accommodate germ-line mutations, genotyping errors, and null alleles. Simulations were performed using all 15 microsatellite loci, 100,000 simulated offspring, and assuming 0.01 % genotyping errors. The proportion of candidate parents sampled for CERVUS parentage analyses was determined using the *capwire* estimated census size of the GB FSA. Statistical confidence was estimated using the LOD score (log-likelihood ratio), where paternity is assigned to an individual if the likelihood ratio is large relative to the likelihood ratios assigned to other candidate parents. Significance was assessed using strict 95 % CIs.

Third, as we were unable to exhaustively sample all candidate parents in the GB FSA, a complementary approach examining relatedness was implemented to assess the degree of local versus external recruitment. We tested the hypothesis that if local recruitment was the primary driver of recovery, relatedness between the USVI juveniles and the GB FSA adults would be significantly higher than between the USVI juveniles and the LC FSA adults. The mean symmetrical coefficients of relatedness between juvenile Nassau grouper and adults from both FSAs (GB and LC) were estimated using GenAlEx. A one-tailed t-test (assuming unequal variances) was performed to test for differences between mean relatedness coefficients.

FSA genetic diversity and demographic analyses

To assess the demographic history of the Nassau grouper collections, we (1) estimated the relative effective population sizes (N_E) and (2) tested for evidence of genetic bottlenecks in both FSAs (GB and LC). Maximum likelihood (ML) estimates of the long-term (weighted mean over $4N_E$ generations) effective population size (θ, where $\theta = 4N_E\mu$, and μ = per locus mutation rate) were generated for each temporal (GB) and spatial (GB and LC) FSA collection using the software LAMARC v. 2.1.3 (Kuhner 2006). Estimates of θ were generated using a subset of 10 microsatellites: loci exhibiting allele sizes not concordant with integers of the repeat size were discarded (loci Est373 and Est376) due to the LAMARC model assumptions. Furthermore, to alleviate any additional bias due to null alleles and/or linkage disequilibria, three additional loci were also omitted (Est265, Est360, and Est420). Random subsets of 20 individuals were selected from each temporal and spatial collection to decrease computation time (Kuhner 2006). Analyses consisted of two parallel chains, an adaptive heating scheme, and assumed the Brownian stepwise mutational model (SMM). Each run consisted of 10 initial chains (80,000 iterations), followed by three final chains (4,000,000 iterations). Multiple runs were performed and starting values were adjusted until convergence was obtained. Final estimates of θ and associated 95 % CIs were generated from three replicates.

To test for genetic evidence of a population decline (i.e., a genetic bottleneck) within GB and LC collections, the programs BOTTLENECK 1.2.02 (Cornuet and Luikart 1996; Piry et al. 1999) and M-ratio (Garza and Williamson 2001) were employed. For BOTTLENECK analyses, tests were performed assuming three mutational models (infinite allele model, IAM; SMM; and two-phased model, TPM). The TPM was set to consist of largely single-step stepwise mutations; however, as estimates of the exact mutation rate and model for serranid fishes are lacking, a range of assumed percentages of single-step mutations (% SMM) and associated variances (σ^2) were utilized: % SMM 70 %, $\sigma^2 = 30$; 80 %, $\sigma^2 = 20$; 90 %, $\sigma^2 = 12$; and

78 %, $\sigma^2 = 12$ (as suggested in Peery et al. 2012). Statistical significance of heterozygosity excesses was determined using the Wilcoxon signed-rank test (10,000 replications).

To test for bottlenecks using M-ratio, critical values (M_c) were generated assuming the mutational TPM parameters suggested by Garza and Williamson (2001) (% SMM = 90, average size of multi-step mutations = 3.5) and Peery et al. (2012) (78 % and 3.1). A range of pre-bottleneck values of θ were incorporated ranging from $\theta = 0.5$ to the LAMARC-derived FSA estimates of θ (see "Results"). As M-ratio assumes all loci conform to the SMM, those markers that demonstrated allele sizes that were not concordant with integers of the repeat size were discarded from the analysis (i.e., Est373 and Est376). Loci demonstrating minor evidence of imperfect repeats ($\sim$5 %) were still utilized; however, imperfect alleles were coded as missing data.

Results

Discrimination of individuals and genetic capture–recapture-based estimate of Grammanik Bank FSA census size

Of the 222 Nassau grouper adults collected from the GB FSA over three spawning years, CERVUS analysis of genotypes identified 172 unique genotypes. Of those, 135 were captured only once (1×), while 37 were recaptured on multiple occasions (29 individuals twice, four individuals three times, three individuals four times and one individual five times). Of the recaptures at GB, 12 recaptures occurred only within a single spawning season (2008, $n = 2$; 2009, $n = 8$; 2010, $n = 2$), 19 recaptures occurred across two spawning seasons (2008 and 2009, $n = 6$; 2009 and 2010, $n = 10$; 2008 and 2010, $n = 3$), and six were recaptured across all three spawning seasons (ESM Fig. S1). When matching genotypes were found, CERVUS estimated a very low 15 locus $P_{(ID)sib}$ of $\leq 1.61 \times 10^{-5}$, suggesting that identical multi-locus genotypes found within the dataset could be confidently identified as recaptures of the same individual (Waits et al. 2001). The software *capwire* identified the two innate rate models as the most appropriate capturability model for the dataset, and estimated the GB FSA census size at 541 individuals (95 % CI 387–608).

Microsatellite loci summary statistics

Little evidence of significant deviation from HWE was found among loci, as only a single locus (and collection) deviated significantly after correction for multiple pairwise tests ($\alpha = 0.05/15$; locus Est265; Pooled GB; ESM Table S1). Within collections (temporal or spatial), linkage disequilibrium (LD) was found to be significant among several pairs of loci after correction ($\alpha = 0.05/105$) (2008: Est265–Est376; 2009: Est340–Est420; 2010: Est340–Est420; Pooled GB: Est92–Est360, Est92–Est376); however, no clear pattern of association was present among loci, suggesting that the markers do sort independently. Overall, the proportion of null alleles was small (<5 %); however, the frequency of null alleles was larger for two markers (Est33a and Est265: 5.05–7.15 %) within collections. To eliminate the confounding effects of LD and the presence of null alleles, most analyses (F_{ST}, *Structure*, BOTTLENECK, M-ratio) were performed with (15 loci) and without (11 loci) the affected markers Est265, Est376, Est420, and Est360. As patterns of variation and demographic trends were consistent for both sets of loci, we report only those results generated using all 15 loci. The Kruskal–Wallis rank sum test revealed no significant differences in H_E, H_O, and R_S among FSA collections (temporal and spatial) at $P < 0.05$ (ESM Table S1).

Genetic differentiation among FSA collections (temporal and spatial)

Pairwise estimates of genetic differentiation (F_{ST}, D_{est}, and G''_{ST}) suggested high connectivity and no differences among Nassau grouper collections from GB over time, nor between the GB and LC FSAs (Table 1). A post-hoc power analysis using the program POWSIM (Ryman and Palm 2006) demonstrated that the variability across microsatellite loci was sufficient to detect fine-scale differences between collections (ESM Fig. S2). *Structure* analyses also showed genetic homogeneity among GB and LC sites, with log-likelihood values peaking at $K = 1$ [Ln $P(D) = -13{,}496.7$] (ESM Fig. S3).

Relatedness and parentage analyses

Estimates of the mean symmetrical coefficient of relatedness among GB adults (-0.006 ± 0.113, mean $\pm$ SD) was significantly lower ($P = 0.012$) than the mean relatedness between GB and LC aggregates (-0.002 ± 0.115). However, the frequency distributions of the relatedness estimates showed substantial overlap (ESM Fig. S4).

Parentage analyses (strict exclusionary and categorical likelihood) failed to identify a single parent–offspring match. CERVUS detected a two to five locus mismatch between each best parent–offspring pair and no single GB adult was confidently assigned (>95 %) as a likely parent to any of the juveniles.

Comparative analysis of the relatedness of the 18 USVI juveniles to adults from each of the two surveyed FSAs (GB vs. LC) demonstrated a significantly higher

Table 1 Pairwise values of divergence across temporal and spatial collections from Nassau grouper fish (*Epinephelus striatus*) spawning aggregations (FSAs) across 15 microsatellite loci

FSA comparison	F_{ST}	D_{est}	G''_{ST}
GB 2008 v LC	0.0000 (0.76)	0.000 (0.48)	0.000 (0.50)
GB 2009 v LC	0.0002 (0.68)	0.001 (0.43)	0.001 (0.42)
GB 2010 v LC	−0.0006 (0.95)	−0.003 (0.64)	−0.003 (0.65)
GB ALL v LC	−0.0004 (0.80)	−0.002 (0.61)	−0.002 (0.62)
GB 2008 v GB 2009	−0.0020 (0.99)	−0.009 (0.95)	−0.011 (0.95)
GB 2008 v GB 2010	−0.0006 (0.83)	−0.002 (0.63)	−0.003 (0.63)
GB 2009 v GB 2010	0.0003 (0.78)	0.001 (0.40)	0.001 (0.40)

Values in parentheses are P values

GB: Grammanik Bank; LC: Little Cayman; F_{ST}: Weir and Cockerham's (1984) estimator; D_{est}: Jost's (2008) estimator; G''_{ST}: Hedrick's (2005) analog of G_{ST} corrected for sampling bias (as described in Meirmans and Hedrick 2011)

($P = 0.011$) mean symmetrical relatedness coefficient between the juveniles and GB adults (-0.007 ± 0.116) than LC adults (-0.017 ± 0.120) (ESM Fig. S5).

FSA genetic diversity and demographic analyses

LAMARC's estimates of θ yielded largely similar estimates for both FSAs (Pooled GB vs. LC) (Table 2). Notably, however, ML estimates of θ from the temporal GB collections increased across years from 2008 to 2010, and 95 % CIs of 2008 and 2010 estimates did not overlap.

Under the IAM mutational model, the program BOTTLENECK detected the presence of a genetic bottleneck ($P < 0.001$) in all GB samples and the much larger LC FSA (Table 2). Results assuming the TPM were variable (Table 2), but most parameter scenarios under the TPM provided a significant bottleneck signal for GB. No bottleneck signal was found assuming the SMM (Table 2). No signal of a genetic bottleneck was detected using the M-ratio under any combination of parameters (data not shown).

Discussion

As declines of coral reef-associated taxa continue to occur globally, understanding the genetic consequences of such severe declines and the genetic characteristics of demographic recovery can help guide conservation and recovery planning efforts. The incipient recovery of Nassau grouper at GB provided a unique opportunity to examine the potential mechanisms responsible for this recovery (e.g., self- vs. external recruitment), and to study the genetic dynamics associated with the FSA demographic collapse and subsequent, ongoing re-establishment.

Genetic connectivity and recruitment to the Grammanik Bank FSA

Our finding of no genetic differentiation between GB and LC FSAs, combined with other Nassau grouper population genetic data, suggest the presence of contemporary genetic connectivity. Previously, Caribbean-wide research has identified the presence of regional genetic differences

Table 2 Effective population size estimates (θ) and BOTTLENECK significance values (probability values) for each implemented mutational model for spatial and temporal FSA collections of Nassau grouper

Fish spawning aggregation (FSA)	LAMARC	BOTTLENECK					
	θ_{MLE}	IAM	SMM	TPM $_{70,30}$	TPM $_{78,12}$	TPM $_{80,20}$	TPM$_{90,10}$
LC	17.488 (15.331–20.237)	0.0001**	0.9823	0.0319*	0.1514	0.1147	0.5765
GB ALL	17.279 (14.789–20.540)	0.0000**	0.9949	0.0051*	0.0938	0.0365*	0.5765
GB 2008	13.525 (11.753–15.843)	0.0000**	0.8961	0.0000**	0.0004**	0.0001**	0.0844
GB 2009	16.223 (14.158–18.718)	0.0000**	0.9823	0.0013*	0.0177*	0.0075*	0.2271
GB 2010	20.569 (16.837–22.808)	0.0000**	0.9635	0.0017*	0.0677	0.0416*	0.4670

GB: Grammanik Bank; LC: Little Cayman; θ_{MLE}: LAMARC-derived maximum likelihood estimate of effective population size, 95 % confidence intervals in parentheses. IAM: infinite allele model; SMM: stepwise mutation model; TPM: two-phase model (subscripts indicate the assumed percentage of SMM and the associated variance); * $P < 0.05$; ** $P < 0.001$

(Jackson et al. 2014). Yet, despite such broad-scale genetic differences, these authors did not detect pairwise genetic differentiation between the GB and LC FSAs using two sets of genetic markers (microsatellites, $F_{ST} = 0.000$; mitochondrial DNA, $\Phi_{ST} = -0.003$). These matching conclusions obtained using different suites of molecular tools, underscore the likelihood of contemporary gene flow between these two FSAs.

The lack of genetic differentiation between the two FSAs is surprising in some aspects [the large distance (~1600 km) separating the two sites and local oceanographic processes], but not others [e.g., the long pelagic larval duration of Nassau grouper (37–45 d; Colin et al. 1997) and regional oceanographic processes]. Recent work examining the local oceanographic processes at two Nassau grouper FSAs, including LC, have shown that local surface currents and eddies may favor the nearby retention of pelagic larvae (Heppell et al. 2009; Karnauskas et al. 2011). In contrast, the long pelagic larval duration of Nassau grouper leaves open the potential for long-distance dispersal, and biophysical modeling of Caribbean larval dispersal suggests that the central Caribbean may be a zone of mixing for larvae from diverse regional sources (Cowen et al. 2006). Furthermore, similar modeling has suggested that for several reef fishes, neighboring populations may serve as stepping stones to facilitate regional dispersal across multiple generations (Holstein et al. 2014), thereby linking habitats and homogenizing allele frequencies. Nevertheless, connectivity between the LC and GB FSAs is at odds with the hypothesis that local larval retention may be a key driver of the persistence of reef fish populations (e.g., Jones et al. 2005; Puebla et al. 2012; Almany et al. 2013). We note, however, that the degree to which local retention drives recruitment seems to vary, as vastly divergent levels of connectivity have been noted across marine taxa with pelagic larvae (Riginos and Liggins 2013; Selkoe et al. 2014), and little evidence exists to suggest that populations are exclusively self-recruiting (Jones et al. 2009).

Parentage and genetic relatedness analyses provided limited support for local larval retention and demographic independence of Nassau grouper at GB. Parentage analyses failed to identify a single parent–offspring match within our sample collection, although only a relatively small number of juveniles could be obtained for genotyping, and potential FSA parents were not exhaustively sampled. Additionally, the GB FSA fish showed significantly lower relatedness among each other compared to GB vs. LC fish, a finding inconsistent with the prediction that the GB FSA recovery was primarily driven by local recruitment. Unfortunately, negative mean estimates of relatedness and their associated high variances render interpretation of such results difficult. Conversely, the juvenile to adult mean relatedness coefficient was significantly higher for GB than LC fish; however, the average juvenile to adult GB relatedness was very low (−0.007), suggesting that on average, surveyed adults were unrelated to USVI juveniles and that external recruitment was likely contributing to Nassau grouper recovery at this site. Interestingly, the range of pairwise relatedness values for each comparison [LC vs. juveniles (−0.337–0.407), and GB vs. juveniles (−0.370–0.416)] included several relatively high values, indicating some form of biological relationship between individuals may be present (second to fourth order). Parentage analyses of additional USVI juveniles and exhaustive sampling of adults will be required to clarify this issue. Collectively, however, the parentage and relatedness results, as well as the negligible pairwise F_{ST} estimates between the LC and GB FSAs, suggest that at least some immigration is likely occurring into GB, and that recovery of Nassau grouper here is at least partially contingent on external sources.

With respect to adult Nassau grouper fidelity to spawning sites, the fish genotypes clearly demonstrated individuals returning to the FSA in more than one spawning year. Twenty-five individuals were recaptured in at least two seasons, and of these, six individuals were present in all three survey years, suggesting some adult long-term fidelity to the GB FSA. These results provide further support to previous tag-based findings of seasonal returns of individual Nassau grouper adults to specific FSAs (Semmens et al. 2007; Kadison et al. 2009; Heppell et al. 2012), and underscore the value of protecting these FSAs from fisheries (Grüss et al. 2014).

Genetic bottleneck and genetic diversity

The rapid, overfishing-driven collapse of Nassau grouper FSAs in the USVI would be predicted to cause both a reduction in genetic diversity and a genetic signature of this decline in the remaining animals. However, a number of demographic (e.g., time elapsed since population collapse, immigration) or statistical factors (e.g., surveyed sample size and marker number) may influence the genetic signature of, and power to detect, a population bottleneck (Garza and Williamson 2001; Williamson-Natesan 2005; Peery et al. 2012; Hoban et al. 2013).

Although the M-ratio test provided no evidence of bottlenecks, the heterozygosity excess test (BOTTLENECK) provided evidence of genetic bottlenecks in the GB FSA in all cases under the IAM and most cases under the TPM mutational models, but not under the SMM mutational model and TPM model 90 % parameter (Table 2). Concordant with previous studies, our results indicated that the power of the bottleneck tests appeared to be influenced by the assumed microsatellite mutational model and the

associated parameters (i.e., an over-susceptibility of the IAM and SMM models to Type I and Type II errors, respectively) (Cornuet and Luikart 1996; Luikart and Cornuet 1998; Peery et al. 2012), suggesting that the TPM may be the most robust to infer demographic changes. Furthermore, the power of the M-ratio test to detect genetic bottlenecks can be low for recent (<5 generations ago) population declines (Peery et al. 2012), and since the Nassau grouper FSA collapses occurred in the 1970s to 1980s (i.e., only a few generations ago; generation time ~8–9 yr), the heterozygosity excess test results may be more robust in this situation (Williamson-Natesan 2005). From the overall bottleneck testing results and documented near extirpation of USVI FSAs, an inference that a genetic bottleneck occurred in GB Nassau grouper seems reasonable.

Interestingly, the ability to detect a genetic bottleneck within the GB temporal samples also decreased with each successive spawning season. For instance, while BOTTLENECK results of the 2008 spawning fish were highly significant ($P < 0.001$) or approached significance ($P = 0.08$) for all assumed TPM parameter percentages and variances, the 2010 collection generally demonstrated less evidence of a bottleneck utilizing these same parameters (Table 2). Combined with the findings of no genetic differentiation among the two FSAs (GB and LC), this bottleneck signal reduction across temporal replicates may be a consequence of immigration and/or recruitment into the GB FSA. For instance, as a population declines in size, the rate at which alleles are lost from the population is higher than the rate at which heterozygosity decreases (Piry et al. 1999). The program BOTTLENECK exploits this difference between metrics and identifies a genetic bottleneck when it detects an excess of heterozygosity relative to that expected under equilibrium. If new alleles are introduced into the population via immigration, this heterozygosity excess will decrease, thereby effectively removing genetic evidence of a bottleneck. While genetic rescue via immigration has been found to be an important factor contributing to the recovery of genetic diversity in many reduced populations (Keller et al. 2001; McEachern et al. 2011; Caplins et al. 2014), it is impossible to know whether the temporally waning bottleneck signal is a consequence of immigration. However, gene flow, even in small amounts, may be sufficient to buffer against the genetic consequences of population reductions and may erase evidence of past bottlenecks (Cornuet and Luikart 1996).

Recovery of the GB FSA was first noted in the early 2000s (Nemeth et al. 2006), yet genetic sampling of the population for the present study did not commence until 2008. Since populations may recover genetic diversity very quickly via immigration following a population decline, a much stronger genetic bottleneck signal may have been detected had sampling commenced several years prior. Additional metrics also suggest that population growth was occurring throughout the study period (2008–2010). Diver-based visual surveys of Nassau grouper at the GB FSA noted a successive increase in the number of aggregating adults (Kadison et al. 2009), and our genetic estimates of θ also demonstrated a successive increase across temporal replicates (Table 2). Moreover, a very strong recruitment pulse of larval Nassau grouper occurred in the USVI along the south coasts of St. Thomas and St. John in 2006 and 2007 (R Nemeth, personal observation). Since minimum age at sexual maturity is 4 yr (Sadovy and Eklund 1999), it is possible that by 2010 individuals from the 2006 cohort reached sexual maturity and may have recruited to the GB FAS as adults. However, we have no evidence to support this hypothesis at this time. Overall, the temporal changes in signals of genetic diversity (θ increasing from 2008 to 2010) and genetic bottlenecks (reduction in bottleneck signal from 2008 to 2010) at GB support the hypothesis that an influx of new genotypes (immigration) from neighboring connected FSAs may be occurring.

Interestingly, a bottleneck signal was also detected in the LC FSA samples, albeit in only two of the heterozygote excess tests (IAM and TPM—70 % parameter). Given the sensitivity of bottleneck tests to the mutational model used, this signal may be spurious. However, all five historically known Nassau grouper FSAs within the Cayman Islands have been fished heavily, leading to FSA extirpations or large declines (Heppell et al. 2012). While the LC FSA is one of the largest known remaining aggregations (Whaylen et al. 2004), it was estimated in 2001 to be up to four times larger (7000–8000 fish) than when sampled in 2005 (2000–2500 fish; Heppell et al. 2012). Although the LC FSA bottleneck signal was much weaker than that found in the GB FSA (Table 2), the possibility of a genetic bottleneck at the LC FSA remains and needs further investigation.

Remnant or recovering Nassau grouper FSAs have been reported elsewhere within the eastern Caribbean (British Virgin Islands and Bajo de Sico, Puerto Rico; Munro and Blok 2003; Schärer-Umpierre et al. 2014), suggesting that the GB FSA may only be one of several eastern Caribbean FSAs to recover if provided sufficient regulatory protections. The results of our temporal genetic characterization of the GB FSA are generally supportive of external sources of recruitment playing a major, although possibly not exclusive, role in its recovery (census size and genetic). However, we note that our conclusions are based on analysis of the putative recovery of a single Nassau grouper FSA, and the dynamics of recovery at other sites could be different. Nevertheless, if external recruitment is important to most other regional Nassau grouper FSAs in the

Caribbean, protection of individual FSAs should be a high priority for not just the specific FSA but also because recovery and persistence of Nassau grouper within the eastern Caribbean in general may be contingent on survival and growth of a metapopulation of the remaining, even if fledgling, FSAs. Furthermore, the similarity of genetic diversity levels between FSAs, despite a much larger apparent LC FSA census size (2000–2500 visually censused at LC vs. 541 genetically censused at GB) provides optimism for the ongoing recovery and evolutionary potential of the GB FSA. Finally, our findings also provide a unique baseline for future monitoring the genetic outcomes associated with recovery of an endangered marine species.

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