Genetic Connectivity of Nassau Grouper Aggregations in the Caribbean Sea

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ABSTRACT

Nassau grouper (*Epinephelus striatus*) form spatially and temporally predictable spawning aggregations that make them particularly susceptible to overfishing. It is the objective of this study to determine how sustainable aggregation-based fisheries are by determining the extent of genetic connectivity between aggregations. Genomic DNA was extracted from samples collected in aggregations in the Cayman Islands and U.S. Virgin Islands. Three mitochondrial markers (12S, ATP synthase, cytochrome b) were sequenced and 9 polymorphic microsatellite loci were isolated. Statistical analyses were performed on mitochondrial sequences to determine population structure and genetic connectivity. Preliminary results reveal high genetic connectivity and no statistically significant genetic structure at either the aggregation level or regional level between aggregations in the Cayman Islands and the U.S. Virgin Islands (fst = 0.01283, p-value = 0.74585). Such results suggest that spawning aggregations do not represent distinct populations.

KEY WORDS: Genetic connectivity, *Epinephelus striatus*, spawning aggregations, U.S. Virgin Islands, Cayman Islands

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PALABRAS CLAVE: Conectividad genética, *Epinephelus striatus,* agregaciones, Islas Vírgenes de E.U

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MOTS CLÉS: Connectivité génétique, *Epinephelus striatus,* agrégations, Îles Vierges Américaines

INTRODUCTION

Nassau grouper (*Epinephelus striatus*) form spatially and temporally predictable spawning aggregations, comprised of hundreds to thousands of fish, which are specifically targeted by small-scale fisheries (Smith 1972, Aguilar-Perera and Aguilar-Davila 1996, Sadovy and Eklund 1999, Ehrhardt and Deleveaux 2007). These ecologically important top predators regulate species composition and abundances of coral reef species. Studies also show that increased presence of groupers on reefs increases the likelihood of recruitment on reefs for other coral reef fish species (Stallings 2008). Thus increased fishing of Nassau grouper potentially makes marine communities more vulnerable to natural and anthropogenic disturbances (Friedlander and DeMartini 2002). Reports by fisherman since the 1980s have suggested depleted catch (Whaylen et al. 2006) and aggregation extinctions (Aguilar-Perera and Aguilar-Davila 1996, Aguilar-Perera 2006) despite limited understanding of – 1) ultimate loss of genetic diversity due to overfishing and 2) the extent of genetic connectivity between spawning aggregations. It is the objective of this study to determine how sustainable aggregation-based fisheries are for Nassau grouper. In order to address this question, the extent of genetic connectivity must be quantified to determine which aggregations are most at risk of extinction and which are supplying fish to other aggregations within the region. Analyses will determine at what spatial scale genetic variation is partitioned for Nassau grouper throughout the Caribbean. Increased knowledge of spawning aggregation dynamics will also benefit other species of aggregating marine fish, as reproductive aggregation behavior has been observed in at least 11 other families of reef fish (Domeier and Colin 1997).

MATERIALS AND METHODS

This study utilized samples collected from aggregations in the Cayman Islands and the U.S. Virgin Islands. The first set was collected from three aggregation sites in the Cayman Islands from 2005 to 2009. A total of 102 fin clips were collected, with 8 from the aggregation at Grand Cayman, 70 from the aggregation at Little Cayman and 27 from the aggregation at Cayman Brac, and stored in sarcosyl urea. The second set was collected from one aggregation site in the U.S. Virgin Islands in March 2010. A total of 78 fin clips were collected and stored in 95% ethanol.

Genomic DNA was extracted from sarcosyl urea

preserved tissues with the use of the Qiagen DNeasy mini kit using the manufacturer's protocols. Genomic DNA was extracted from ethanol preserved tissues using the standard chloroform extraction and isopropanol precipitation methods (Sambrook *et al*. 1989). Three mitochondrial markers were sequenced for each sample. A 357 bp fragment of 12S rRNA was amplified with primers 12Sal (5'AAACTGGGATTAGATACCCCACTAT-3') and 12Sbr (5'AGAGTGACGGGCGGTGTGT-3'). A 634 bp fragment of the ATP synthase units 6 and 8 were amplified with primers L8331 (5'-AAAGCRTYRGCCTTTTAAGC-3') and H9236 (5' GTTAGTGGTCAKGGGCTTGGRTC 3'). A 785 bp fragment of cytochrome b was amplified with primers gludgL (5'-TGAYTTGAARAACCAYCGTTG-3') and CB3H (5'GGCAAATAGGAARTATCATTC-3') (Palumbi *et al*. 1991). All markers were sequenced in the forward direction using an ABI 3700 sequencer. Nine polymorphic microsatellite loci were cross-amplified using primer pairs designed for *Epinephelus acanthistius*. Loci isolated represent di and tetra repeats of the following kind - $(CA)_x$, $(CATC)_x$, $(TACA)_x$ and $(TAGA)_x$. Sequencher 4.5 was used to align mitochondrial sequences. MacClade was used to identify informative sites. Arlequin (Excoffier *et al*. 2005) was used to perform an analysis of molecular variance (AMOVA) associated with population structure and genetic connectivity in order to generate fst values, and identify unique haplotypes. Additionally, Migrate-n was used to determine directional gene flow.

RESULTS AND DISCUSSION

Analysis of mitochondrial sequences identified 14 informative sites, providing enough power with which to recover statistically significant genetic differentiation if present. Informative sites defined 19 haplotypes, which exhibit little genetic divergence from one another and whose distribution is not associated with any geographic patterns (Figure 1). Preliminary results suggest a lack of genetic structure at either the aggregation level or at a regional level between the aggregations in the Cayman Islands and the U.S. Virgin Islands. The AMOVA revealed high levels of historical gene flow between aggregations based on non-significant fst values (fst = 0.01283, $p = 0.74585$), demonstrating genetic homogeneity and extensive interbreeding among aggregations. Output from Migrate-n revealed that observed panmixia has been facilitated by extensive directional gene flow from aggregations in the U.S. Virgin Islands to aggregations in the Cayman Islands (15,637 ± 3603 migrants). Lack of genetic structure between spawning aggregations has been observed in other population genetic studies of aggregation -forming species (Shulzitski et al. 2009, Shaw et al. 2010), and suggests that aggregations do not represent distinct populations. While genotyping of all samples for the nine isolated microsatellites could potentially resolve finer scale genetic structure among aggregations, studies have shown that microsatellites may not reveal any more structure than mitochondrial sequences (Haavie et al. 2000), in particular in a case where mitochondrial sequences are variable enough to determine genetic structure. These results are important for management, as aggregations of Nassau grouper continue to disappear throughout the Caribbean. If the species is panmictic throughout its range then aggregation-based fisheries could have less of an impact on loss of genetic diversity for the species than if aggregations represented genetically distinct populations.

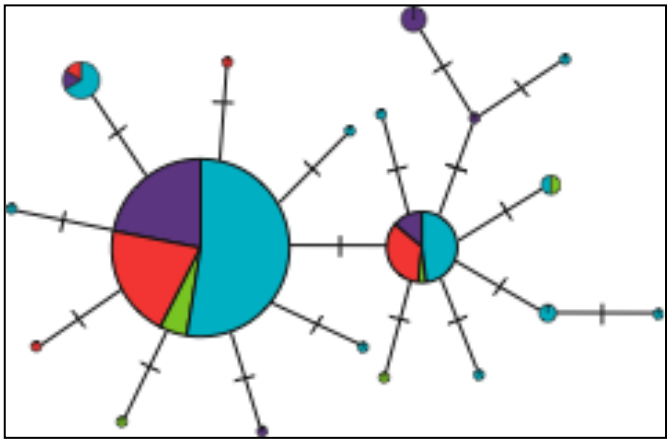

Figure 1. Haplotype Network for Nassau grouper (*Epinephelus striatus*) based upon 3 mitochondrial loci. Individual sequences from aggregations are color coded as follows – blue (Little Cayman), purple (U.S. Virgin Islands), green (Grand Cayman) and red (Cayman Brac). Each circle represents a unique haplotype and is proportional in size to the number of individuals from the dataset that possess it. Each notch mark represents a single mutational change.

CONCLUSIONS

If spawning aggregations are in fact the most appropriate biological unit by which to define subpopulations for Nassau grouper then preliminary results from this study suggest genetic homogeneity between aggregations in the Cayman Islands and the U.S. Virgin Islands. Expanding the current study to include spawning aggregations from other localities in the Caribbean will determine if there is any larger scale regional population structure revealed by aggregations; it will be important to determine if the species is actually panmictic throughout its range. All significant results of the final study should be incorporated to regional management plans for Nassau grouper.

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