A GENETIC ASSESSMENT OF POPULATION CONNECTIVITY IN MUTTON SNAPPER, *LUTJANUS ANALIS*

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ABSTRACT

Elucidating patterns of marine population connectivity is a central concern among biologists. Genetic markers are commonly utilized to quantify patterns of connectivity since they can reveal information about the exchange of genes, and thus migration events. The development of microsatellites as polymorphic, high resolution markers has significantly contributed to our understanding of population genetic structure in marine organisms. In this study, microsatellite loci were isolated from an enriched genomic library for the mutton snapper, *Lutjanus analis*, a commercially and recreationally valuable fish. This species is of particular interest to both conservationists and fishery managers, as the last known mutton snapper spawning aggregation in United States' waters was recently targeted for the establishment of the Dry Tortugas Ecological Reserve (DTER). In order to evaluate the potential utility of the reserve as a source population for fisheries of the southeastern United States, mutton snapper from five locations around the Caribbean were genotyped at eight loci to estimate interpopulation gene flow. Analyses ranging from population-based F-statistics to individual-based assignment indicated that population genetic substructure was largely absent from the five sample locations. The only evidence for structure identified the population from the west coast of Puerto Rico as most distinct, suggesting that future work on populations in the eastern Caribbean is warranted. The genetic similarity of mutton snapper populations implies free gene flow between populations; however, because the genetic composition of each sample was so similar, it is impossible to discern between the relative contributions of potential source populations. Thus we cannot rule out the possibility that the DTER serves as a significant source of recruits to the southeastern United States. Yet based on the results of this study we cannot confirm that is does.

Further research will be required to properly evaluate the utility of the DTER and to clarify corridors of connectivity across both the eastern and western portions of the Caribbean.

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INTRODUCTION

Elucidating patterns of population connectivity is a central concern among marine biologists. The actual extent of interpopulation exchange is a crucial element in understanding ecological and evolutionary processes and in determining the utility of management and conservation strategies. The marine environment provides a particularly complex backdrop to studies of connectivity, as absolute barriers to dispersal are rare and ocean currents can be temporally and spatially heterogeneous. The majority of marine organisms release tens to millions of tiny pelagic propagules (Thorson 1950; Sale 1991), precluding the direct observation of migration events involving the larval stage. Thus, our understanding of population connectivity in marine systems remains incomplete.

For decades, the paradigm in population biology of benthic organisms has been one of sessile or relatively sedentary adults interconnected over large geographic expanses by pelagic eggs and (or) larvae (Ehrlich 1975), with the degree of connectivity determined by a combination of current systems and larval dispersal capabilities (Roberts 1997). Genetic markers are commonly utilized to quantify patterns of connectivity since they can reveal information about the exchange of genes, and thus migration events. Population genetic studies have reported a variety of results, ranging from extensive genetic structure to a complete lack of structure, even over oceanic ranges. However, upon review of the existing literature, three broad patterns become apparent. First, genetic homogeneity across large geographic ranges is common in marine organisms, supporting the notion of extensive connectivity through larval dispersal or adult migration. Second, genetic breaks that coincide with known zoogeographic boundaries or historical barriers to dispersal are present across a variety of taxa, revealing the influence of historical events on current genetic patterns. Finally, in the event that significant population

structure is present, genetic differentiation often correlates with geographic distance, unique geographic features such as isolated oceanic islands, or life history traits that limit dispersal.

Genetic Homogeneity among Locations

The earliest genetic studies used protein electrophoresis techniques and eventually mitochondrial (mt) DNA surveys to examine population structure in marine organisms. One of the first applications of protein electrophoresis to a marine fish revealed genetic homogeneity over as much as 10,000 km for the milkfish, *Chanos chanos*, a euryhaline species distributed throughout the tropical Pacific and Indian Oceans (Winans 1980). Similarly, Vawter et al. (1980) reported low intraoceanic genetic distances for thirteen species of reef fishes collected from the Pacific coast of Panama and Gulf of California as well as the Atlantic coast of Panama and eastern Caribbean. In a survey of mtDNA, a lack of sequence divergence was revealed for skipjack tuna, *Katsuwonus pelamis*, between the Atlantic and Pacific Oceans, implying extensive interoceanic gene flow (Graves et al. 1984). A similar result was obtained for the American eel, *Anguilla rostrata*, which exhibited a complete lack of genetic divergence between populations ranging from Maine to Louisiana, along approximately 4,000 km of coastline (Avise et al. 1986). More recently, high levels of gene flow at both protein and mtDNA loci were revealed between populations of the sea urchin, *Echinothrix diadema*, across the eastern Pacific barrier (Lessios et al. 1998). Pelagic larvae of this sea urchin were hypothesized to traverse this 5,400 km expanse of deep oceanic water during strong El Niño years.

Such examples only comprise a subset of the vast body of literature reporting genetic homogeneity. Additional examples include a variety of shore fishes (Shaklee 1984; Rosenblatt and Waples 1986; Lacson 1992), several benthic invertebrates (Berger 1973; Levinton and

Suchanek 1978; Palumbi and Wilson 1990; Palumbi and Kessing 1991) and decapods with high adult vagility and exceptionally long pelagic larval durations (Ovenden et al. 1992; McMillen-Jackson et al. 1994; Silberman et al. 1994). The existence of wide-ranging populations is also supported indirectly by surveys in which the larvae of coral reef fishes (Leis 1983) and a variety of benthic invertebrates (Scheltema 1988) have been collected from the waters of the eastern Pacific barrier, a presumed obstacle to dispersal. This large body of evidence supporting panmixia in marine organisms has historically been claimed to coincide with a lack of absolute barriers to dispersal and the presence of long-lived pelagic larvae and (or) highly mobile adults.

Genetic Breaks and Historical Events

Although genetic homogeneity is common in marine organisms, numerous studies, particularly those employing mtDNA markers, have revealed distinct genetic breaks coinciding with historical barriers to dispersal and often known zoogeographic boundaries. The southeastern United States has been particularly well-studied in this respect, with concordant genetic patterns apparent across disparate taxa. The horseshoe crab, *Limulus polyphemus* (Saunders et al. 1986), oyster and gulf toadfish, *Opsanus tau* and *O. beta* (Avise et al. 1987), American oyster, *Crassostrea virginica* (Reeb and Avise 1990), black sea bass, *Centropristis striata* (Bowen and Avise 1990), and diamondback terrapin, *Malaclemys terrapin* (Lamb and Avise 1992) all exhibit genetic breaks coinciding with well-recognized zoogeographic boundaries (Briggs 1974) along the eastern coast of the United States. On the west coast of the United States, the copepod, *Tigriopus californicus*, displays a deep genetic break coinciding with Point Conception, California (Burton and Lee 1994). The mitochondrial sequence divergence observed at this biogeographic boundary (Briggs 1974) is among the highest reported for

conspecific populations and its location coincides with a break in nuclear DNA sequences (Burton and Lee 1994).

In the Pacific Ocean, genetic patterns in three species of giant clam, *Tridacna gigas* (Benzie and Williams 1995), *T. derasa* (Macaranas et al. 1992), and *T. maxima* (Benzie and Williams 1997) as well as the pearl oyster, *Pinctada margaritifera* (Benzie and Ballment 1994), run perpendicular to present-day surface currents. Genetic structure is instead concordant with hypothetical current patterns historically associated with low sea level stands and is consistent with speciation patterns in the region (Springer 1982). In such instances, where genetic breaks across various taxa do not coincide with any present-day barriers to dispersal, it appears that signatures of historical isolation remain in spite of the potential for contemporary gene flow.

Genetic Structure, Geography and Life History

Of the genetic studies that report significant population structure, there is often some aspect of geography or life history that decreases the potential for dispersal, and thus gene flow, between populations. In an examination of ten species of marine shore fishes with life histories varying form viviparity to an extended larval stage, populations of the live-bearing black perch, *Embiotoca jacksoni*, from southern and Baja California exhibited high levels of genetic differentiation (Waples 1987). The remaining nine species, all possessing a pelagic larval stage, exhibited low interpopulation distances that did, however, correlate with dispersal capability as estimated by larval duration and larval capture data. The crown-of-thorns starfish, *Acanthaster planci*, had exhibited genetic homogeneity across its range in various studies; however, significant genetic structure was reported between populations at the isolated extremes of the geographic range (Benzie and Stoddart 1992). Populations of the convict surgeonfish,

Acanthurus triostegus, among five island groups in French Polynesia separated by as little as 250 km exhibited significant genetic differentiation despite a pelagic larval stage lasting 60 to 70 days (Planes 1993). The genetic pattern is in concordance with prevailing current systems and may be strengthened by the unique geomorphology of French Polynesia and its isolated oceanic islands. The cleaner goby *Elacatinus evelynae* was shown to exhibit high levels of divergence between mtDNA haplotypes, and even reciprocal monophyly, on a scale much less extensive than that predicted by the presence of long-lived pelagic larvae (Taylor and Hellberg 2003). However, the over-representation of these larvae in inshore plankton samples, coupled with the speciose nature of this taxanomic group, suggests the presence of retention mechanisms and mating behaviors that enhance genetic divergence.

An Emerging View of Connectivity

In the past decade, accumulating evidence of genetic differentiation and local-recruitment has altered the way in which marine populations are viewed. The case of *Tigriopus californicus,* a tidepool copepod, starkly contrasts with scenarios in which geographic or life history characteristics can be readily employed to explain genetic patterns (Burton and Lee 1994). This species exhibits extensive interpopulation divergence across protein, mitochondrial and nuclear DNA markers in spite of the potential for extensive gene flow by both adult and larval stages.

The persistence of rich faunal assemblages and high levels of endemism on isolated oceanic islands also suggests that self-recruitment must be substantial in at least some instances and may be influenced by factors such as local current patterns and larval behavior (Robertson 2001).

Improvements in natural tagging techniques have increased our ability to track individuals in the marine environment. The exploitation of fish otoliths has been crucial in revealing information about elusive life stages; this technique is based on the theoretical assumption that the chemical composition of the otolith will be determined by that of the water mass in which it was formed (e.g. the site of larval or juvenile development). Using this technique, natal homing has been detected in an estuarine-spawing marine fish (Thorrold et al. 2001) and significant levels of self-recruitment have been revealed for a Caribbean reef fish (Swearer et al. 1999). In a similar application, Jones et al. (1999) were able to fluorescently label the otoliths of developing embryos on the northern Great Barrier Reef. Significant levels of self-recruitment were subsequently estimated after the recapture of marked individuals. The development of microsatellites as polymorphic, high resolution genetic markers has also significantly contributed to our understanding of population structure in marine organisms. These presumably neutral and rapidly evolving stretches of nuclear DNA have revealed previously undetected population structure for Atlantic cod, *Gadus morhua* (Bentzen et al. 1996; Ruzzante et al. 1998), Atlantic salmon, *Salmo salar* (McConnell et al. 1997), and the veined squid, *Loligo forbesi*, (Shaw et al. 1999).

Emerging evidence for high levels of self-recruitment and extensive genetic structure has led to a reconsideration of connectivity in the marine environment. Whereas populations were once assumed to be large and demographically open (Caley et al. 1996), the current paradigm acknowledges the variety of mechanisms that may enhance the retention of locally-produced offspring, thus creating geographically distinct, demographically closed populations. This emerging view has significant implications for management and conservation in marine systems. If, in fact, populations are independent entities with minimal exchange, the potential exists for

local adaptation, strong connections between larval recruitment and adult population dynamics, and genetic isolation increasing the prospect for speciation. Thus, this study addresses the crucial and fundamental issue of population connectivity by employing high resolution genetic markers to geographically widespread populations of a Caribbean reef fish, the mutton snapper, *Lutjanus analis*.

CHAPTER 1: ISOLATION OF MICROSATELLITE MARKERS

Introduction

Assays of genetic variation and structure in natural populations were initiated in the 1960s with the application of protein electrophoresis (Harris 1966; Hubby and Lewontin 1996; Lewontin and Hubby 1966). This technique is based on allelic differences in electrophoretic mobility, phenotypic manifestations of genetic variation in the encoding gene sequences. Protein electrophoresis facilitated the accumulation of an extensive genetic database for a wide variety of taxa and continues to be employed in systems for which enzyme and other protein markers have been well-developed. However, because this method is based on phenotypes, it is limited by its inability to detect the full range of variation at the DNA level (Kreitman 1983). In addition, protein markers often exhibit low levels of polymorphism rendering them uninformative for particular taxa (e.g. Atlantic salmon, *Salmo salar*) or in comparisons between closely-related populations.

Until the late 1970s, a DNA-based survey of genetic variation remained too costly and time-consuming to be practical in population-level studies. The application of restriction endonucleases to mitochondrial DNA (mtDNA) revealed DNA sequence variation in the form of restriction fragment length polymorphisms (RFLP), and were the first population-level studies to employ DNA-based markers (Avise et al. 1979b). Mitochondrial DNA proved powerful in the resolution of intraspecific relationships (Avise et al. 1979a) and revealed historical genetic breaks undetected by protein electrophoresis (Reeb and Avise 1990). The utility of these markers stems in part from unique characteristics of mitochondrial DNA, including its predominantly maternal inheritance, lack of recombination and rapid rate of evolution (Avise et al. 1987a). Mitochondrial markers continue to be employed in assays of genetic variation and

studies of zoogeographic boundaries and phylogenetic relationships. However, as with all genetic markers, there are limitations associated with mtDNA. Due to its maternal inheritance, male-mediated dispersal patterns cannot be detected. In addition, its nonrecombinant nature causes the mitochondrial molecule to act as a single locus, and being an organellar DNA, it is not clear how well mtDNA findings represent patterns in the nuclear DNA.

Various molecular techniques extended the survey of genetic variation to the nuclear genome. In particular, the identification of repetitive DNA as a source for molecular markers revolutionized population genetic studies. Repetitive DNA is ubiquitous across eukaryotes (Tautz 1989) and constitutes a substantial portion of the genome (Charlesworth et al. 1994). The utility of these regions is based on length polymorphisms resulting from variability in number of repeat units at a single locus. Minisatellite DNA, hypervariable regions consisting of tandem repeats 10 to 64 base pairs in length, was the first class of repetitive DNA to be widely applied. The advent of multilocus DNA fingerprinting in the mid 1980s (Jeffreys et al. 1985) was followed soon after by a variation on this technique targeting single minisatellite loci. Together, single- and multilocus fingerprints were applied to questions ranging from individual identification and paternity analysis (Quinn et al. 1987; Wetton et al. 1987) to examinations of population structure (Gilbert et al. 1990; Deka et al. 1991; Taylor et al. 1994). However, various characteristics of minisatellite repeats preclude them from being ideal genetic markers (O'Reilly and Wright 1995). First, the mechanisms of mutation affecting these sequences appear complex, hindering modeling efforts. Second, allelic length variants often do not differ by multiples of unit repeats which may lead to ambiguity in the sizing of bands. Finally, their large size can make it difficult to resolve alleles of similar lengths and may exclude PCR techniques.

Hypervariability in microsatellites, another class of repetitive DNA, was first reported in 1989 (Litt and Luty 1989; Tautz 1989; Weber and May 1989). In contrast to minisatellites, the tandem repeats of these regions range from 1-6 base pairs. Microsatellites embody numerous characteristics of an ideal genetic marker. Importantly, their size is highly amenable to PCR, offering the added advantage that very little DNA is required for their implementation. Since they are abundant in the genome and exhibit a range of polymorphism, markers can be specifically developed for various applications (e.g. paternity testing versus population studies). They exhibit codominant Mendelian inheritance permitting comparisons to Hardy-Weinberg and linkage expectations. Thus, microsatellite markers rapidly replaced minisatellites for most applications. The major limitation of microsatellites is the expense and time required for their isolation. Although many primers have been successfully employed across species boundaries (McConnell et al. 1995; Rico et al. 1996), there are reports of reduced polymorphism and increased homoplasy in cross-species amplifications (Moore et al. 1991; Bowcock et al. 1994; FitzSimmons et al. 1995; Garza et al. 1995). Since the mid 1990s, microsatellites have been the marker of choice for parentage, forensics, mapping efforts and population genetic studies. Technological advancements, in particular newer enrichment procedures (Ostrander et al. 1992), have significantly reduced the effort required for isolation of microsatellite loci. These highly polymorphic markers enable the assessment of genetic variation for taxa in which a lack of polymorphism hindered the use of more traditional markers (e.g. Hughes and Queller 1993) and the abundance of microsatellite loci facilitates a broad, multilocus survey of the genome. In this study, microsatellite loci were isolated from an enriched genomic library for the mutton snapper, *Lutjanus analis*, a commercially and recreationally valuable fish species. These markers were subsequently used to quantify genetic relatedness of populations across the Caribbean region in

the hopes that this information could contribute to improved conservation and management strategies.

Methods

Library Preparation

Microsatellite-enriched DNA libraries were created for the mutton snapper, *Lutjanus analis*. Genomic DNA of four individuals from Honduras was isolated according to the modified "Rapid Isolation of Mammalian DNA" protocol of Sambrook and Russell (2000) and enriched for microsatellites by the Savannah River Ecology Lab in Aiken, South Carolina according to the procedures of Glenn et al. (2005). Three unique mixtures of equimolar oligonucleotides were used to produce three separate enrichments, hereafter referred to as enrichment two, three and four, the numbers corresponding to the oligonucleotide mixtures used (Table 1). Enrichment elution products were amplified by the polymerase chain reaction (PCR) in 25 ul volumes containing 10 mM Tris-HCl, pH 8.3, 50 mM KCl, 1.9 mM $MgCl_2$, 0.01% (w/v) gelatin, 0.625 ug BSA, 2.5 mM each dNTP, 0.52 uM Super SNX-24 primer and 1 unit of AmpliTaq DNA Polymerase (Applied Biosystems (ABI), Foster City, CA). Cycling parameters included an initial denaturation step of 2 minutes at 95°C, followed by 25 cycles of 20 seconds at 95°C, 20 seconds at 60°C and 90 seconds at 72°C, with a final extension of 30 minutes at 72°C. PCR products were resolved and qualitatively assessed by gel electrophoresis, and then purified and ligated into pGEM-T Easy vector (Promega, Madison, WI). Ligation products were transformed into competent cells of *Escherichia coli*, plated on LB/ampicillin and incubated overnight at 37°C. The first library created in September 2003 exhibited a low yield of transformants, few of which were positive clones (i.e. those containing microsatellites), possibly

due to the age of the cloning kit employed. Therefore, a second library was created in November 2003 for enrichments one and three utilizing a new cloning kit.

Microsatellite Screening

Two-hundred colonies were picked in total, 100 from each library, and colonies were lysed in 20 ul of boiling water for five minutes at 100°C. Upon removal of cellular debris, cloned DNA was amplified in 25 or 30 ul reactions containing 10 mM Tris-HCl, pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$, 0.01% (w/v) gelatin, 2.5 mM each dNTP, 0.5 uM T7-Pro primer, 0.5 uM SP6 primer, and 0.75 units of AmpliTaq DNA Polymerase (Applied Biosystems). Cycling parameters were identical to those used in the library amplification. All PCR products were resolved by gel electrophoresis and products larger than 500 base pairs (bp) were selected for sequencing, as they are of the appropriate length to contain both the vector and the DNA insert. Selected PCR products were purified, sequenced using Big Dye kits (Applied Biosystems), and products of sequencing reactions were visualized on an ABI 3100. Sequences were screened for microsatellites (i.e. tandem repeats from two to six base pairs in length with a minimum of six repeat units) in Sequencher 4.2 and classified according to Weber (1990). Clones containing a microsatellite repeat and ample flanking sequence were chosen for sequencing in the opposite direction. Consensus sequences exported from Sequencher were used for primer design in the web-based program Primers 3 (Rozen and Skaletsky 2000). Reverse primers were PIG-tailed in order to force the A-tailing of all PCR products (Brownstein et al. 1996), thus reducing the ambiguity in sizing that can result from the tendency of *Taq* polymerase to add an A-tail to some products and not others.

Exploratory Genotyping

A total of ten individuals from Honduras, Belize and the Dry Tortugas were amplified with each set of unlabeled primers in 10 ul reactions using one of two PCR reactions that differed according to the DNA polymerase employed. The first reaction contained 10 mM Tris-HCl, pH 8.3, 50 mM KCl, 1.5 mM MgCl2, 0.01% (w/v) gelatin, 2.5 mM each dNTP, 0.5 uM each forward and reverse primer, and 0.25 units of AmpliTaq DNA Polymerase (Applied Biosystems). Cycling parameters included an initial denaturation step of 5 minutes at 95°C, followed by 35 cycles of 30 seconds at 94°C, 30 seconds at 58°C and 60 seconds at 72°C, with a final extension of 30 minutes at 72°C. The second PCR reaction included 15 mM Tris-HCl, pH 8.0, 50 mM KCl, 1.5 mM MgCl2, 2.5 mM each dNTP, 0.5 uM each forward and reverse primer, and 0.25 units of AmpliTaq Gold Polymerase (Applied Biosystems). Cycling parameters included a hot start step of 10 minutes at 95°C, followed by 35 cycles of 30 seconds at 94°C, 30 seconds at 58°C and 60 seconds at 72°C, with a final extension of 30 minutes at 72°C. AmpliTaq Gold Polymerase reduces non-specific amplification and increases the abundance of desired product by remaining in an inactive state prior to a 'hot start' denaturation step of 5-10 minutes at 95°C. This prevents the extension of mis-primed amplicons that commonly occurs at low temperatures (i.e. during the reaction set up). All PCR products were resolved by gel electrophoresis.

Primer pairs that resulted in products with relatively distinct bands showing apparent length polymorphism were chosen for fluorescent dye-labeling. Genotyping on an ABI 3100 using the G5 dye system allowed four PCR products, fluorescently-labeled with PET, NED, 6FAM and VIC dyes, to be loaded with the size standard (LIZ; Applied Biosystems) into a single capillary. Using dye-labeled primers and the ABI 3100, the genotyping effort was expanded to

include thirty individuals, ten fish each from Belize, Honduras and Dry Tortugas. Reactions and cycling parameters were identical to those used for unlabeled primers. Peaks were analyzed against the size standard with GENESCAN 3.7 (Applied Biosystems) and assigned to size categories in GENOTYPER 3.7 (Applied Biosystems). Genotypes of the thirty individuals were tested for departure from Hardy-Weinberg equilibrium (HWE) at each locus in GENEPOP (Raymond and Rousset 1995a) using an exact probability test and a Markov chain method to obtain the unbiased exact P-value (Guo and Thompson 1992). A highly significant departure from HWE, calculated separately for each sampling location, was considered potential evidence for the presence of null alleles and thus that locus was dropped from the study or, in two cases, primers were redesigned. The annealing temperature for each remaining set of primers was optimized using an MJ Research thermal cycler with a gradient block. Genomic DNA from three individuals exhibiting a range in genotype quality (e.g. presence of non-specific amplification) was amplified with annealing temperatures ranging from 55 to 65°C. Primer pairs that produced unambiguous peaks, appropriate levels of polymorphism, and conformed well to the expectations of Mendelian markers (i.e. one or two peaks present in each individual) and HWE were chosen for large-scale genotyping.

Results

Library Characterization

Approximately 50 clones from the September 2003 library were sequenced in at least one direction and, of these, 36.7% were microsatellite-containing clones. Twenty-five percent of all isolated microsatellite repeats came from the September 2003 library, consisting entirely of di- and trinucleotides, as clones from enrichment two were targeted (Table 2). A greater success

rate was obtained with the November 2003 library with 58.1% of the approximately 100 sequenced clones containing microsatellites. Although twice as many clones were sequenced from the November 2003 library, it produced 75% of all isolated microsatellites, including di-, tri-, tetra- and pentanucleotide repeats; this library was created from enrichments two and four. The low success rate obtained with the earlier library was most likely due to the age of the cloning kit employed in its construction.

A total of 76 microsatellite repeat sequences were isolated from the enriched genomic libraries. These included 53 di-, three tri-, 18 tetra- and two pentanucleotide repeats (Table 3). Fifty-two of these repeat motifs were classified as perfect, 19 as imperfect, two as compound (perfect) and three as compound (imperfect). Six clones exhibited cryptic simplicity in which the arrangement of the repeat motif is scrambled and biased in nucleotide composition (Tautz et al. 1986). Although this class of repetitive DNA is reportedly widespread throughout the genome (Tautz et al. 1986; Wright and Bentzen 1994), it is not typically employed as a genetic marker and so these

regions were not included in the classification of microsatellites in this study. The most abundant repeat motif, $(AC)_n$, accounted for 59.2% of the isolated microsatellites; interestingly, the second most common motif (13.2%) was a tetranucleotide, $(AGAT)_n$

(Table 4). Repeat motifs ranged in size from 12 to 164 base pairs and in number of uninterrupted repats from six to 31.

The maximum number of uninterrupted repeats, rather than total repeat length, is a good proxy for predicting the level of polymorphism at a locus (Weber 1990). Substitutions within the tandem-repeat motif appear to hinder the process of slipped-strand mispairing during replication,

reducing the mutation rate and production of new alleles (Levinson and Gutman 1987); this is

Table 1. Oligonucleotide mixtures utilized in the enrichment procedure.

1	2	3
$(TG)_{12}$	$(AAAC)_6$	$(AAAT)_8$
$(AG)_{12}$	$(AAAG)_6$	$(AACT)_8$
$(AAG)_8$	$(AATC)_6$	$(AAGT)_8$
$(ATC)_8$	$(AATG)_6$	$(ACAT)_8$
$(AAC)_8$	$(ACCT)_6$	$(AGAT)_8$
$(AAT)_{12}$	$(ACAG)_6$	
$(ACT)_{12}$	$(ACTC)_6$	
	$(ACTG)_6$	

Table 2. Enriched genomic libraries.

Repeat Type	September 2003	November 2003
Dinucleotide	17	36
Trinucleotide	2	1
Tetranucleotide	0	18
Pentanucleotide	0	2
Total (%)	19 (25.0)	57 (75.0)

Table 3. Classification of microsatellite repeats.

Repeat Type	Perfect	Imperfect	Compound (perfect)	Compound (imperfect)	Total (%)
Dinucleotide	34	17	2	0	53 (70.0)
Trinucleotide	3	0	0	0	3 (4.0)
Tetranucleotide	13	2	0	3	18 (24.0)
Pentanucleotide	2	0	0	0	2 (3.0)
Total (%)	52 (68.0)	19 (25.0)	2 (3.0)	3 (4.0)	76

Table 4. Abundance and size distribution of repeat motifs.

	Size (bp)																		
Repeat Motif	12-15	16-19	20-23	24-27	28-31	32-35	36-39	40-43	44-47	48-51	52-55	56-59	60-63	64-67	68-71	72-75	76-79	= 80	Tot
AC	7	7	6	1	2	3	8	3	1	2	3		2						45
AGAT									1	1	1	1		1		2	1	2	10
AG			1	1		1	1			2									6
ATCC									1				1						2
AACT						1					1								2
AAG							2												2
AAAC				1															1
ATG				1															1
AGAAG						1					1								2
Total																			
AC/TC			1		1														2
AGAT/GGAT															1			1	2
AGAT/AGGT														1					1
Total	7	7	8	4	3	6	11	3	3	5	6	1	3	2	1	2	1	3	

consistent with reports of a correlation between repeat interruptions and locus polymorphism (Pepin et al. 1995). Motifs with an intermediate number of uninterrupted repeats were targeted for locus development, as short repeats are often monomorphic (Weber 1990) and excessively long repeats are less informative for population studies (O'Reilly and Wright 1995). In addition, tetranucleotides were preferentially chosen since stutter banding tends to be minimal or absent for this repeat type (O'Reilly and Wright 1995). Stutter bands, size variants of the repeat unit found adjacent to the true allele, are characteristic of microsatellite amplifications, often leading to ambiguity in peak sizing. For example, large stutter bands at a dinucleotide locus can cause the misclassification of a homozygote as a heterozygote with alleles separated by two base pairs.

Marker Development

A total of 30 sets of unlabeled primers were designed from a subset of the sequenced clones (Table 5). Twenty-six of these sets amplified well, producing bands across individuals that were easily visible on agarose gels (Figure 1). Four primer pairs only amplified for some individuals while two pairs produced no bands at all. Successful primer pairs were evaluated further by repeat motif and the appearance of polymorphism as estimated from the gels, and 18 were chosen for dye-labeling of the forward primer.

All eighteen sets of dye-labeled primers successfully amplified 10-30 individuals at target loci. Based on repeat motif, number of alleles, observed heterozygosity, conformation to H-W expectations, and the ease with which alleles could be identified and sized, eight of these loci were chosen for large-scale genotyping. Primer sets consisting of four loci each allow for the

most efficient utilization of the ABI 3100 since four dyes can be simultaneously loaded into each capillary. The loci chosen for final application (see Table 6) include two di- and six

Table 5. Loci targeted for unlabeled primer design.

Locus	Repeat motif of clone	Primer sequence (5' to 3')	Successful amplification
LaA-4	$(AAG)_{13}$	F: GGAAAAGAGAGGGGGAAAAA R: GTTTGGTGATCACAGACAGCAGGA	Y
LaA-11	$(AG)_{11}AC(AG)_2AC(AG)_{12}AC(AG)_2AC$ $(AG)_4\{AC(AG)_3\}_3AC(AG)_2AC(AG)_6$	F: GAGCGGCAAATAAAGGTGTT R: ACTGCTGTCTACCCCAGCAA	partial
LaA-12	$(CA)_{14}$	F: TCTGACAGCTGTTTGGTTGG R: GTTTAAAATGGGGTTGTGGTTGAG	Y
LaC-16	$(TC)_{11}(AC)_8$	F: GGTGTTGATTGGTCCTCTGG R: GTTTGGGGTTGGTATTCATCCAGT	Y
La8	$(CA)_{17}GA(CA)_{21}$	F: GCTTCACAAAACAGAACATGAA R: CAAGGCTGTGTGTGAAAAGTT	Y
La13	$(AG)_{24}CG(AG)_{21}$	F: TCCCATATAGATCAATGGCAAA R: GTTATGGCAGTGCTGACATTAACC	partial
La17	$(AC)_3TT(AC)_{19}$	F: ACATACACATGCCCACAGGA R: CTTGTGTTTGTGTCTCAGTTCTCA	Y
La21	$(CA)_{23}$	F: ACGTGTGGAATTGTGTTGTGA R: CAGGCCATTTGAGAGAGAGC	Y
La25 ‡	$(TAGA)_{19}$	F: GGAGGAACCTCCTGGAATGT R: GTTTGCACTTGAAGAAAAAGGGTGA	Y
La27	$(AAG)_{12}$	F: CAGGCTGATGGTTCAACTCC R: CCGGACCCTAATTTGAGTTTT	Y
La34	$(AC)_{18}$	F: AAAACGGGCTCCTAACCAAT R: GTTTACACCACGCAACATGAATTT	Y
La39	$(CA)_{11}$	F: TGCTGAGGAGCATTTGCTTT R: GTTTAAAGTCACATAAACGGGGACT	Y
La43	$(AC)_9$	F: GCTCTGTGCTCCCTGTGATT R: TACCATGTTCCCTCCCTCTG	Y
La46	$(AC)_{18}$	F: AATCTTGCGCTTGCTTCTGT R: ATGGGATCATAGCGTCCAAC	Y
La48	$(AC)_{18}$	F: TTTGTGTCCCCATGTGTGTT R: GTTTAATCCGCCAATGTATTCAGG	Y
La6a	$(ACTA)_{13}$	F: TGCAGGAAGTGTTGTGTTGAG R: CCAAACATTCGCCAAACATT	Y
La18a	$(GGAT)_5GGGT(GGAT)_2(AGAT)_{21}$	F: CCTCACTTTTGTGTGAGACAGC R: GTTATGATTGCTAGGAGCATCTGG	Y
La19a	$(GATA)_{12}$	F: GGGCTATCTTCACTGGGACT R: GTTTGAGTTTTTGGATGAATGGGTTC	partial
La20a	$(AGAT)_5AGGTAGAT(AGGT)_{16}$	F: CCTTCATCTGTGATCAGACAGC R: GTTTCCACTGACATGGAAAGAATTGA	Y
La23a	$(AGAT)_{13}$	F: TTTCTGCCTTCATTTTGCTG R: GTTTGGCTGTGATGACAAATTTCCT	Y
La26a	$(ATCC)_{11}$	F: TTCATCAGATGGCTGGTTCA R: GTTTGGAGTCATTCTCTGGGGTCA	N
La27a	$(AGAT)_{21}$	F: GCTGGCAACAATATTGAGCTT R: GTTTCTGTGTCCAAGGTCCATTGAT	Y
La28a	$(TAGA)_{17}TGAA(TGGA)_{16}(TAGA)_7$	F: TTTCCCAGTTTGGGATCAAT R: GTATTGTCTGAATCCAGGTCCTACA	N
La31a	$(AGAT)_{18}$	F: CTGTTCACATACGAGAAATCTGTG R: GTTTAACAAAGGACGCTCCATCAC	Y
La32a	$(AACT)_8$	F: ACAGTCACTTAATGTTATTGTTGTGTT R: GTTTCCCATGTCATGTGACTCAGC	Y
La34a	$(ATAG)_{14}$	F: TGTCTCTTCGAAATCAAACACAA R: GTTTGAGGCTTATCTGCCCCTCTC	Y
La36a	$(TAGA)_{26}$	F: TTGCACAAACTTCTGCTGCT R: GTTTCATGAGGATGAATTTGAGACCA	Y
La45a	$(CCAT)_{15}$	F: AACCACATCTGGCTCAATCA R: GTTTAGCCCCAGAGTAGGGTGAGA	Y
La49a	$(AGAT)_{11}$	F: GCTGAGGCAGAAATCACACA R: GTTATGTCCACTGATGCCTCAAAA	Y
La50a	$(CA)_{10}(CT)_{12}(CA)_{15}$	F: TGTGCTCATCTCTGCTCTGG R: GTTATGAATGCCATCAGTGCTTGT	partial

‡ La25 also contains a dinucleotide repeat, $(AC)_{13}$, in the flanking region.

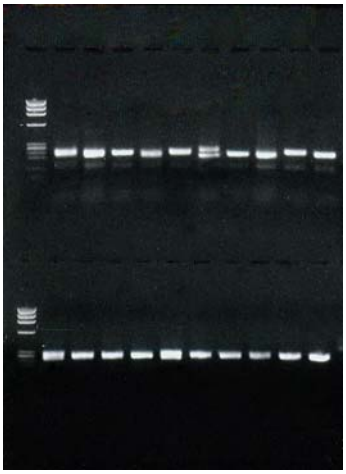

Figure 1. Successful amplification with unlabeled primers at isolated microsatellite loci. PCR products are shown for La45a (upper row of bands) and La49a (lower row of bands).

Table 6. Loci targeted for fluorescent dye-labeling. N = number of individuals genotyped per locus; H_E = expected heterozygosity; H_O = observed heterozygosity; test for HWE deviation: *** $P < 0.001$.

Locus	Repeat motif of clone	N	Number of Alleles	H_E	H_O	Comments
LaA-4	$(AAG)_{13}$					monomorphic
LaA-12	$(CA)_{14}$	30	7	0.626	0.600	
LaC-16	$(TC)_{11}(AC)_8$	33	10	0.890	0.939	
La8	$(CA)_{17}GA(CA)_{21}$	31	18	0.818	0.806***	null alleles
La13	$(AG)_{24}CG(AG)_{21}$					stutter bands
La25	$(TAGA)_{19}$	32	21	0.939	0.906	
La34	$(AC)_{18}$	32	23	0.946	0.969	
La39	$(CA)_{11}$	32	13	0.835	0.906	
La48	$(AC)_{18}$					stutter bands
La18a	$(GGAT)_5GGGT(GGAT)_2(AGAT)_{21}$	32	36	0.976	1.000	
La19a	$(GATA)_{12}$	29	12	0.848	0.345***	null alleles
La20a	$(AGAT)_5AGGTAGAT(AGGT)_{16}$					non-specific amplification
La23a	$(AGAT)_{13}$	41	13	0.868	0.634***	null alleles
La27a	$(AGAT)_{21}$	20	16	0.915	0.700***	null alleles
La27a ‡		20	17	0.923	0.700***	
La31a	$(AGAT)_{18}$	28	15	0.894	0.536***	null alleles
La36a	$(TAGA)_{26}$	10	5	0.784	0.200***	null alleles
La36a ‡		10	7	0.789	0.400***	
La45a	$(CCAT)_{15}$	26	12	0.883	0.962	
La49a	$(AGAT)_{11}$	22	12	0.910	0.955	

‡Denotes the redesigned primer sets.

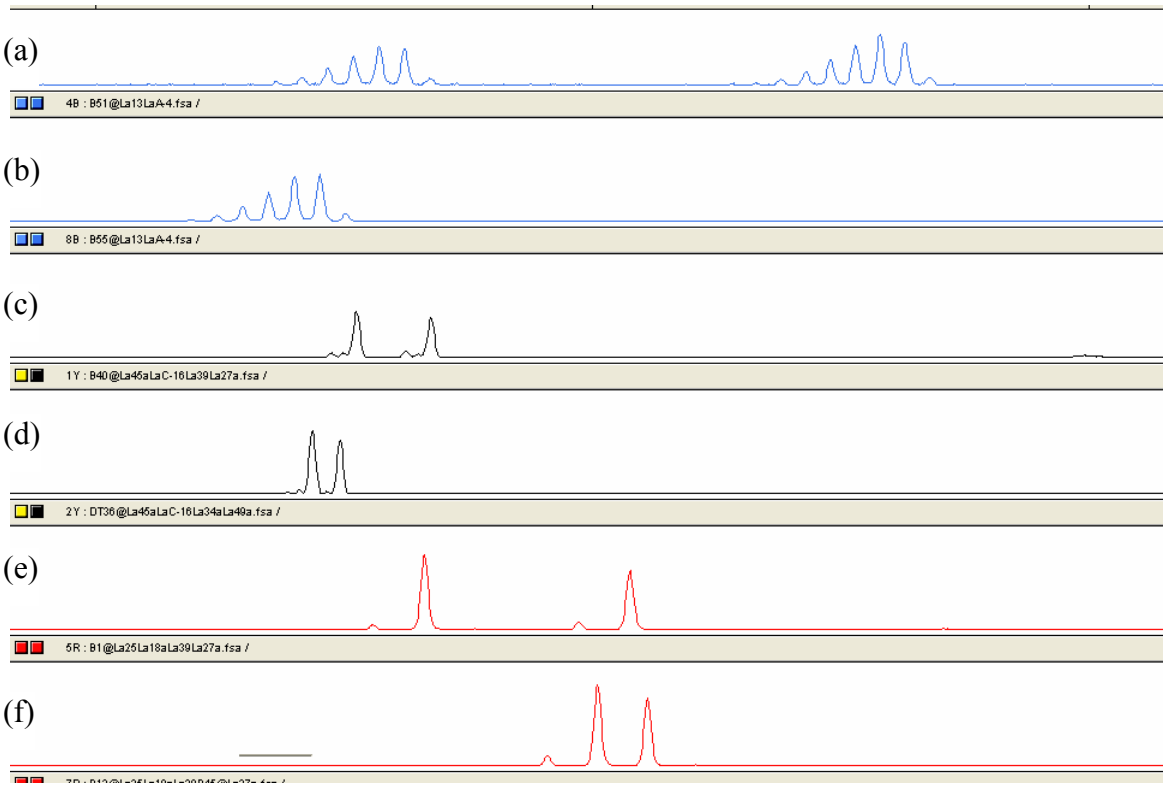

Figure 2. Stutter banding at locus La13 compared to peak morphologies at loci chosen for large-scale genotyping. (a) represents a heterozygote with an ambiguous allele, (b) a potential homozygote, (c) and (d) heterozygotes at LaC-16, a dinucleotide locus, and (e) and (f) heterozygotes at La25, a tetranucleotide locus.

tetranucleotides. These loci exhibited a range in polymorphism with 12 to 36 alleles at a locus and observed heterozygosities from 0.700 and 1.000. Despite the presence of significant deviations from HWE at La27a, this locus was chosen based on the repeat motif, relatively high observed heterozygosity, and the ease of allele identification and sizing. One final locus, LaA-12, was set aside for potential future application. Although it exhibited characteristics of a good marker, additional loci were not available to form a complete set of four.

The nine remaining loci were dropped from consideration for various reasons. Locus LaA-4 was monomorphic across 10 individuals for an allele at 94 base pairs (Table 6). Two additional loci, La48 and La13, exhibited an excess of stutter bands, preventing the unambiguous identification of alleles (see Figure 2). A high degree of non-specific amplification occurred at locus La20a with up to six peaks present for a single individual.

Finally, significant deviations from HWE were present at six loci (including La27a) in the form of heterozygote deficiencies. Several explanations for this departure from HWE exist, including population substructure and the presence of null alleles. Although tests were conducted separately for each location, it is not unexpected to find population substructure within a geographic sample simply due to chance events. Yet null alleles, alleles that fail to amplify due to mutations within priming sites, are reportedly common across microsatellite loci (Callen et al. 1993) and can occur in relatively high frequencies (Paetkau and Strobeck 1995). In addition, the presence of null alleles could significantly affect the results of a study. Therefore, in order to err on the side of caution, departures from HWE within a single location were considered evidence of null alleles. To address this issue further, two loci, La27a and La36a, were chosen for primer redesign (Table 6). A small sample of previously typed individuals was genotyped using a new set of primers for La27a and a new forward primer for La36a. At locus La27a, one previously

homozygous individual was typed as a heterozygote with redesigned primers and one previously heterozygous individual was typed as a homozygote. At locus La36a, two previously homozygous individuals were typed as heterozygotes with redesigned primers. Although the sample sizes employed were not large, this result establishes the presence of null alleles for at least two loci in this study. The frequency of nulls was estimated to be 0.116 and 0.330 at loci La27a and La36a, respectively (Brookfield et al. 1996).

Discussion

Microsatellite repeats were common in mutton snapper genomic DNA libraries, with a total of 76 repeats present in 150 sequenced clones. This result illustrates the success of the enrichment procedure and is consistent with reports of high abundance of microsatellites in other vertebrate genomes. The enrichment procedure prevents direct quantitative comparisons with studies in which microsatellites were isolated using alternative methods, such as hybridization analysis. However, it is valid to make comparisons of the relative abundance of repeat types and lengths as well as the levels of polymorphism.

The most common repeat motif in animal species, excluding mononucleotides, is $(AC)_n$. This motif accounted for 19% and 36% of all microsatellite repeats observed in humans and rats, respectively (Beckmann and Weber 1992). For obvious reasons, most isolation attempts concentrate on this motif, as is the case with the present study in which the enrichment procedure selected for only two dinucleotides, $(AC)_n$ and $(AG)_n$. $(AC)_n$ repeats accounted for 59.2% of microsatellites isolated for the mutton snapper and were seven times more abundant than $(AG)_n$. This is consistent with proportions of these repeat motifs reported for humans and rats (Beckmann and Weber 1992), brown trout (Estoup et al. 1993) and Atlantic cod (Brooker et al.

1994). Much less effort has been directed towards estimations of the abundance of tetranucleotide motifs in the genome. However, Beckmann and Weber (1992) reported a high occurrence of tetranucleotides for mammalian genomes, with $(AAAT)_n$ as the third and fourth most common microsatellite repeat in humans and rats, respectively.

Mutton snapper microsatellites were classified according to Weber (1990) as perfect, imperfect or compound repeats. Perfect repeats were most common (68.0%) followed by imperfect (25.0%) and compound (7.0%) repeats. The relative abundance of these repeat types is similar to estimates for Atlantic salmon (McConnell et al. 1995), rainbow trout (Morris et al. 1996), human (Weber 1990) and porcine genomes (Brooker et al. 1994). In contrast, Atlantic cod exhibited similar proportions of perfect and imperfect repeats (Brooker et al. 1994), and in an earlier study of Atlantic salmon, microsatellites were comprised mostly of perfect repeats, lacking compound repeats completely (Slettan et al. 1993).

Although particular motifs, such as $(AC)_n$ perfect repeats, are abundant across most vertebrate genomes, there appear to be significant differences in repeat length between taxa. Rat microsatellites were consistently longer than those found in humans (Beckmann and Weber 1992). Similarly, repeats in teleost fishes, including Atlantic cod, Atlantic salmon, rainbow trout and zebrafish, were significantly longer than those in a variety of mammalian genomes (Brooker et al. 1994). In particular, the most common repeat size in cod was almost twice that reported for mammals. The most common size class for microsatellite repeats in mutton snapper was 36-39 bp. This is higher than the most common size classes in humans and rats which are 20-23 and 20-27 bp, respectively (Beckmann and Weber 1992). However, the second most common size class in mutton snapper, 20-23 bp, was similar to that of humans and rats. In contrast, most Atlantic cod microsatellites were 12-22 bp and 60-70 bp, Atlantic salmon were 12-18 bp and 42-

48 bp, and rainbow trout were 48-58 bp. The abundance of smaller repeats in Atlantic cod and salmon were primarily due to the degenerate nature of longer repeats and an arbitrary classification system that required repeat motifs interrupted by more than three base pairs to be counted as individual repeats (Weber 1990). Thus, the most common size classes in cold-water fishes were consistently longer than those in mutton snapper. It has been hypothesized that the disparity in repeat size between cold-water fishes and mammals may be due to a higher propensity for slippage at the low and fluctuating temperatures experienced by poikilotherms (Brooker et al. 1994). The intermediate nature of the most common size classes in mutton snapper between those of mammals and cold-water fishes could simply be an artifact of the enrichment process that utilized oligonucleotides ranging from 24-32 bp in length. However, the largest repeats present in three mutton snapper tetranucleotides exceeded 80 bp; this is similar to the largest repeats in both Atlantic cod and salmon which exceeded 60 bp. Thus, regardless of any bias introduced by the enrichment or isolation procedures, the common occurrence of 36-39 bp repeats and the presence of repeats exceeding 80 bp in length are consistent with the trend in which repeat lengths in teleost fishes exceed those in mammalian genomes.

While microsatellites have been often been recognized for their high levels of polymorphism as compared to more traditional markers (e.g. allozymes and mtDNA), a useful characteristic of microsatellites is the variance in polymorphism across loci within species. For example, of seven loci isolated from Atlantic cod, the number of alleles ranged from one to 46 and heterozygosity from zero to 0.920 (Brooker et al. 1994). Six loci, originally isolated from various salmonids, exhibited a range of polymorphism in the Arctic char, with nine to 48 alleles and expected heterozygosity from 0.411 to 0.916 (Bernatchez et al. 1998). As expected, mutton snapper microsatellites also exhibited a range of levels of polymorphism. Eighteen loci were

amplified from 10-30 individuals using dye-labeled primers. The number of alleles present at a locus ranged from one to 36 and expected heterozygosity from zero to 0.976. This characteristic range of polymorphism of microsatellite loci underscores their suitability for a variety of applications. Parentage studies, in which individual identification is desirable, require highly polymorphic loci such as those exhibiting numerous alleles and high heterozygosities. However, such loci are not ideal for population studies which require less polymorphism, such that similarities within populations are not obscured by individual variation (Hedrick 1999).

To this end, microsatellite loci were chosen for the present study based on level of polymorphism. The eight chosen loci exhibited 10 to 36 alleles and expected heterozygosities from 0.818 to 0.976. Due to the large pool of potential loci, it was possible to apply additional criteria to the choice of loci, including ease of allele scoring and conformation to expectations of HWE. The characterization of mutton snapper microsatellite loci in this study illustrates the increased ease of isolation due to the enrichment procedure and supports the potential for the application of such markers in systems lacking previous genetic information.

CHAPTER 2: GENETIC ANALYSIS OF CONNECTIVITY

Introduction

Population Connectivity in the Caribbean

Since population structure in marine organisms was first examined, the Caribbean region has been the target of many studies. As noted earlier, one of the first measurements of genetic divergence in marine fish compared populations from the Atlantic coast of Panama to those from the Virgin Islands and Bermuda (Vawter et al. 1980). This early demonstration of low intraspecific genetic distances across large geographic expanses is consistent with the large body of evidence suggesting that many marine organisms are, in fact, open populations connected by the dispersal of a pelagic life stage. In a landmark study, Shulman and Bermingham (1995) sampled eight species of reef fish at six locations across the Caribbean, ranging from the Bahamas to Belize to Barbados. Fish species varied in early life history traits such as egg type (demersal and pelagic) and planktonic larval duration (PLD). Such characteristics are hypothesized to influence dispersal potential and, in turn, gene flow between populations. Employing mtDNA RFLP, Shulman and Bermingham (1995) found weak population structure for three and no structure for five of the eight species examined. In addition, genetic patterns did not correlate with life history traits or offshore surface current patterns. Thus, this study reinforced the presence of genetic homogeneity among populations across the Caribbean.

In the past decade, however, accumulating evidence of local recruitment and genetic differentiation, in part promoted by technological advances, has altered the way in which marine populations are viewed. Fish otoliths have become a crucial tool in the examination of elusive early life stages. Theoretically, the elemental composition should be similar to that of the water mass in which the otolith was formed (Thorrold et al. 2002). Under this assumption, Swearer et

al. (1999) were able to demonstrate local retention around St. Croix, U.S. Virgin Islands, with locally-retained larvae dominating recruitment events to leeward reefs. Similarly, improvements in modeling techniques have altered assumptions of dispersal potential. Recent modeling efforts taking into account the latest knowledge of surface current patterns and pelagic larval durations, as well as the effects of diffusion and mortality, have predicted a large reduction in dispersal potential for passive larvae (Cowen et al. 2000; Cowen et al. 2003). In a genetic study utilizing mtDNA sequences, Taylor and Hellberg (2003) reported significant haplotype divergence between populations across the Caribbean for the cleaner goby, *Elacatinus evelynae*. In spite of a pelagic larval period of three weeks, extremely restricted gene flow may be accounted for by the over-representation of these larvae in inshore plankton samples. This supports the potential roles of complex inshore current patterns and larval behavior in local retention.

The application of high resolution microsatellite markers has revealed significant genetic structure at various geographic scales in the western Atlantic and Caribbean Sea. Gutierrez-Rodriguez and Lasker (2004) identified three genetically distinct clusters of the gorgonian coral, *Pseudopterogorgia elisabethae*, within the Bahamas. Highly significant population structure in this gorgonian coral corresponds with a low dispersal potential, maintained by a reproductive strategy that includes brooding on the surface of the maternal colony. In a subsequent study targeting the entire Caribbean basin, Baums et al. (2005) identified two genetically distinct clusters of the elkhorn coral, *Acropora palmata*. The western cluster included populations from Panama, Mexico, Florida, the Bahamas, Navassa, Mona Island and Puerto Rico while the eastern cluster included the US Virgin Islands, St Vincent and the Grenadines, Bonaire and Curacao. This genetic pattern is concordant with biogeographic data recognizing a Caribbean province that coincides with western populations and a West Indian province with eastern populations (Briggs

1974). In a Bayesian clustering procedure, individuals from Puerto Rico and Mona Island were identified as having mixed ancestry between the two genetic clusters, a result in support of a proposed biogeographical break at Mona Channel. This large-scale examination of population structure across the Caribbean was the first such study to employ microsatellite markers.

Management and Conservation in the Marine Environment

The amount of actual dispersal and subsequent gene flow between populations has significant implications for both management and conservation practices (Roberts 1997). For example, managers of highly exploited areas that depend on alternative sources of recruits may overestimate the actual supply of larvae from geographically distant populations under the assumption of extensive connectivity. An emerging tool for both management and conservation in marine systems is the establishment of marine reserves, areas in which all exploitative practices are prohibited (Lubchenco et al. 2003). In order to be maximally effective, the placement of reserves must target (1) self-sustaining populations that are not dependent on recruitment from exploitted areas and (2) populations that can serve as sources for exploited areas through larval, juvenile or adult dispersal (Botsford et al. 2003).

In July 2001, the Dry Tortugas Ecological Reserve (DTER) was established approximately 70 miles southwest of Key West, Florida. The reserve is comprised of two areas exceeding 600 km^2; Tortugas North sits just west of Dry Tortugas National Park while Tortugas South lies 13 miles SW of the park and includes an area known as Riley's Hump. This topographic feature serves as a spawning site for various commercially and recreationally important snapper and grouper species, including the mutton snapper, gray snapper, cubera snapper, yellowtail snapper, dog snapper and black grouper. Riley's Hump served as a catalyst

for the establishment of a reserve. The high site fidelity and temporal stability exhibited by these spawning aggregations (Domeier and Colin, 1997) has led to their heavy exploitation and rapid decline (Burton 2002). In fact, this site represents the last known major spawning aggregation in US waters for the mutton snapper, making this species of particular interest to both conservationists and fishery managers.

The mutton snapper, *Lutjanus analis*, inhabits warm-temperate and tropical waters of the western Atlantic Ocean. Although generally a solitary fish, mutton snapper gather to form large spawning aggregations over the shelf edge from May to July that may persist for up to two weeks (Domeier and Colin 1997; Claro and Lindeman 2003). They exhibit high site-fidelity and also tend to aggregate at the same exact time each year, according to the lunar calendar (Domeier and Colin 1997). Historical aggregation sites have been reported around the Dry Tortugas, Cuba, Belize, Turks and Caicos and the U.S. Virgin Islands (Domeier and Colin 1997; Lindeman et al. 2000; Claro and Lindeman 2003). Actual spawning and courtship have not been observed for mutton snapper, but reports of ripe gonads in aggregating fish supports the occurrence of spawning after dark (Domeier and Colin 1997). Habitat in these aggregation sites does not appear to be unique or particularly pristine; however, sites do tend to correspond with current systems that may serve to retain or disperse larvae (Lee et al. 1994; Lindeman et al. 2000; Dahlgren et al. 2001; Claro and Lindeman 2003). In fact, the location of DTER at the juncture of several ocean currents that come together to form the Tortugas Gyre supports the potential for retention of locally-spawned larvae and their subsequent dispersal along the Florida reef tract (Lee et al. 1994; Criales and Lee 1995; Limouzy-Paris et al. 1997; Lindeman et al. 2001).

In order to evaluate the potential utility of the reserve, it is crucial to understand the extent of population connectivity between the Dry Tortugas and the Florida reef tract relative to

populations across the Caribbean. This study applies high resolution microsatellite markers to mutton snapper collected from five locations around the Caribbean in order to estimate interpopulation gene flow.

Methods

Sample Collection and DNA Isolation

Samples of adult fish were obtained between May 2003 and February 2005 and stored in salt-saturated dimethyl sulfoxide (DMSO). Sampling locations included Gladden Spit, Belize (BZ), Roatan, Honduras (HN), Dry Tortugas, Florida (DT) and Mayaguez, Puerto Rico (PR) (Figure 3; Michael Burton). In addition, a sample of juvenile fish was obtained from Jupiter, Florida (JP) in October 2004 (Michael Burton). Genomic DNA was isolated according to a modification of the "Rapid Isolation of Mammalian DNA" protocol of Sambrook and Russell (2000) and examined quantitatively and qualitatively by spectrophotometry and gel electrophoresis. The qualitative assessment proved to be a better indicator of amplification success, as extractions sometimes produced pure and concentrated DNA that was degraded and did not reliably PCR-amplify.

Genotyping

Approximately 245 individuals were genotyped at eight loci (Table 7). Amplifications were 15 ul in volume and contained 5-20 ng template DNA, 15 mM Tris-HCl, pH 8.0, 50 mM KCl, 1.5 mM MgCl2, 2.5 mM each dNTP, 0.5 uM each primer, and 0.75 U of AmpliTaq Gold (Applied Biosystems). Cycling parameters began with a hot start of 10 minutes at 95°C,

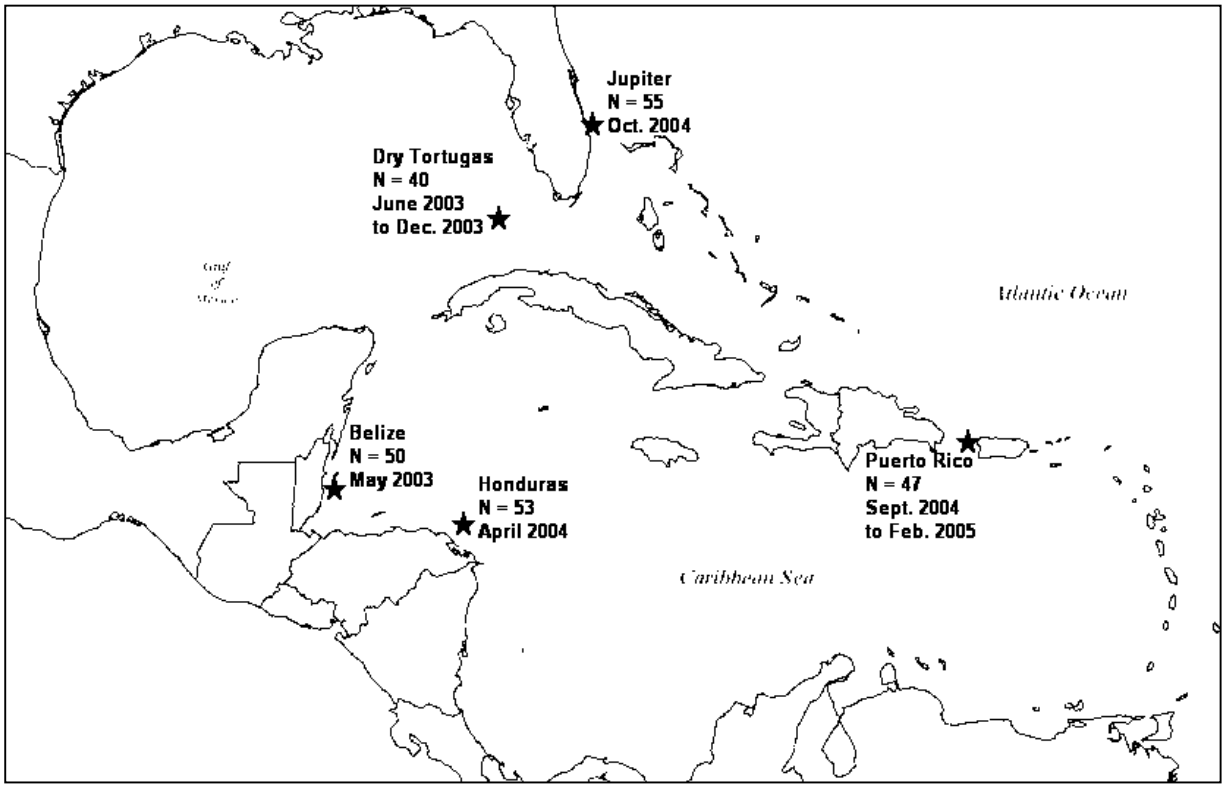

Figure 3. A map of sampling locations, including sample sizes (N) and dates.

Table 7. Mutton snapper microsatellite loci. Tm = annealing temperature; N = number of individuals genotyped; A = number of alleles; H_E = expected heterozygosity; H_O = observed heterozygosity; test for HWE: * $P < 0.05$, *** $P < 0.001$.

Locus	Repeat motif of clone	Primer sequences (5' to 3')	Tm (°C)	Product size range (bp)	N	A	H_E	$H_$
La25	$(ATCT)_{19}$	**F:** GGA GGA ACC TCC TGG AAT GT **R:** GTT TGC ACT TGA AGA AAA AGG GTG A	63	137-235	241	36	0.942	0.
La39	$(TG)_{11}$	**F:** TGC TGA GGA GCA TTT GCT TT **R:** GTT TAA AGT CAC ATA AAC GGG GAC T	65	143-179	226	15	0.802	0.
La18a	$(ATGG)_6GTGGATGG(ATAG)_{21}$	**F:** CCT CAC TTT TGT GTG AGA CAG C **R:** GTT ATG ATT GCT AGG AGC ATC TGG	65	188-340	239	37	0.964	0.
La27a	$(AGAT)_{22}$	**F:** CTT AGC AAG CCA ACA AAC AAT G **R:** GTT TCC AAG GTC CAT TGA TCT TTA GTG	65	176-312	184	39	0.939	0.
La34a	$(AGAT)_{14}(AGTG)(GAGT)(GAGA)$	**F:** TGT CTC TTC GAA ATC AAA CAC AA **R:** GTT TGA GGC TTA TCT GCC CCT CTC	57	239-303	235	17	0.914	0.
La45a	$(TCCA)_{15}$	**F:** AAC CAC ATC TGG CTC AAT CA **R:** GTT TAG CCC CAG AGT AGG GTG AGA	62	194-254	238	15	0.880	0.
La49a	$(TATC)_{12}(CATC)(TATC)$	**F:** GCT GAG GCA GAA ATC ACA C **R:** GTT ATG TCC ACT GAT GCC TCA AAA	65	224-312	235	19	0.905	0.
LaC-16	$(TC)_{11}(AC)_8$	**F:** GGT GTT GAT TGG TCC TCT GG **R:** GTT TGG GGT TGG TAT TCA TCC AGT	66	146-164	238	10	0.853	0.

followed by 35 cycles of 30 seconds at 94°C, 30 seconds at the designated annealing temperature (Table 7), and 60 seconds at 72°C, with a final extension of 30 minutes at 72°C. PCR products were visualized on an ABI 3100 and analyzed with GENESCAN 3.7. In GENOTYPER 3.7 peaks were labeled and binned into size categories corresponding to allelic length in base pairs.

Summary Statistics

Each population was tested for departures from HWE at each locus in GENEPOP (Raymond and Rousset 1995a) using a probability test and a Markov chain method to obtain the unbiased exact *P*-value (Guo and Thompson 1992). In a similar manner, all locus pairs were tested for linkage equilibrium within each population and across all populations. Since sample sizes varied from 38 to 55 individuals, allelic richness, the number of alleles present in populations independent of sample size, was calculated for each population-locus combination as well as overall using FSTAT (Goudet 1995). This calculation estimated the expected number of alleles for a sub-sample of genes equal in size to that of the smallest sample.

Population Structure

Population-based analyses were utilized to test the null hypothesis that all five mutton snapper samples comprise a genetically homogenous, panmictic population. First, in order to assess allelic and genotypic distributions across populations, an exact probability test (Raymond and Rousset 1995b) and a log-likelihood based exact test (Goudet et al. 1996) were performed in GENEPOP to evaluate genic and genotypic differentiation, respectively. Both tests employed a Markov chain method to calculate an unbiased estimate of the *P*-value.

A second evaluation, Wright's (1921) fixation index, is based on heterozygote deficiencies that result from non-random mating. An unbiased estimator, θ (Weir and Cockerham 1984), of F_{ST}, a measure of among population subdivision, was calculated in GENETIX (Belkhir et al. 1996-2002), as were pairwise F_{ST}s with permutation tests (1000 randomizations) to estimate *P*-values. An estimator of R_{ST}, ρ (Slatkin 1995), was also calculated using FSTAT (Goudet 1995). This analog of F_{ST} takes into account allelic size by assuming alleles of a similar size are more closely related, given that loci adhere to a stepwise mutation model (Slatkin 1995). An analysis of molecular variance (AMOVA, Excoffier et al. 1992) based on the number of different alleles (F_{ST}) was performed in ARLEQUIN (Schneider et al. 2000). This hierarchical analysis distributes the observed variance in heterozygosity into within population, between population and between group components. Finally, genetic chord distance, D_{CE} (Cavalli-Sforza and Edwards 1967), between each population pair was calculated in GENETIX with permutation tests (1000 randomizations) to estimate *P*-values.

Population Assignment

Individual-based analyses were also used to assess population structure in the five mutton snapper samples. These analyses exploit more of the information contained in each individual multilocus genotype, in contrast to population-based analyses that primarily utilize allele frequencies and heterozygosities calculated for each population.

Two fundamentally different procedures were used to assign individuals to source populations based on their multilocus genotypes: frequency-based and Bayesian assignment. The frequency-based method of Paetkau et al. (1995) was implemented in GENECLASS (Piry et al. 2004). Populations were determined *a priori* based on sampling locations, and GENECLASS generated

allele frequencies for each population excluding the individual to be assigned (Waser and Strobeck 1998). The expected frequency of each individual's genotype at each locus across all populations was calculated and each individual was assigned to the population from which its multilocus genotype most likely originated. A frequency of 0.001 was designated to alleles that were absent from a population. In order to generate probabilities rather than likelihood values, GENECLASS performs a Markov Chain Monte Carlo (MCMC) resampling procedure with 10,000 simulated individuals (Paetkau et al. 2004).

Genotypes were also analyzed using a Bayesian assignment procedure implemented in the program STRUCTURE (Pritchard et al. 2000). STRUCTURE assumes Hardy-Weinberg and linkage equilibrium and uses a MCMC algorithm to infer population parameters that conform to these modeling assumptions. In order to determine the number of genetically distinct clusters (K) in the dataset, individual genotypes were used as the prior in the estimation of posterior probabilities for K = {1, 2,…5}. Five runs were performed at each value of K to ensure proper mixing in the chain and consistent results. All runs used a burnin of 10^6 followed by 10^6 MCMC iterations. Parameters assumed an admixture model in which individuals may have mixed ancestry, and correlated allele frequencies which could account for similarity between closely related populations. For each individual in the analysis, a probability of membership in each cluster was estimated.

Results

Summary Statistics

High levels of polymorphism were observed in all five populations of mutton snapper at the eight microsatellite loci. The expected and observed heterozygosities ranged from 0.794 to

Table 8. Genetic diversity at eight microsatellite loci in five populations of mutton snapper. N = number of genotyped individuals; A = number of alleles; Â = allelic richness; a = number of private alleles; H_E = expected heterozygosity; H_O = observed heterozygosity; test for HWE: * P < 0.05, ** P < 0.01, *** P ≤ 0.001.

	Locus								
Population	La25	La39	La18a	La27a	La34a	La45a	La49a	LaC-16	Overall
Belize									
N	49	44	47	39	48	49	48	48	
A	21	11	31	25	14	13	14	10	139
Â	18.9	9.6	27.0	22.3	13.4	11.5	13.0	9.4	125.1
a	-	-	3	2	-	-	2	-	7
H_E	0.943	0.806	0.968	0.945	0.910	0.856	0.911	0.864	
H_O	0.939	0.750	0.957	0.872*	0.875	0.898	0.896	0.896*	
Honduras									
N	53	53	53	40	52	52	52	53	
A	27	13	28	26	17	14	12	10	147
Â	21.5	11.3	24.5	22.3	15.6	12.3	11.5	9.5	128.5
a	5	1	-	2	-	-	-	-	8
H_E	0.942	0.813	0.962	0.939	0.926	0.887	0.905	0.847	
H_O	0.868	0.585***	0.962	0.825	0.942	0.865	0.827	0.830	
Dry Tortugas									
N	38	38	38	29	38	39	38	39	
A	24	11	31	19	16	13	12	9	135
Â	21.8	10.3	28.1	19.0	15.4	12.0	11.3	8.7	126.6
a	-	1	-	1	-	-	-	-	2
H_E	0.942	0.794	0.964	0.920	0.915	0.876	0.894	0.859	
H_O	0.895	0.500***	0.947	0.862	0.921	0.821	0.789	0.846	
Jupiter									
N	55	54	55	44	55	55	55	54	
A	25	11	32	29	16	12	14	10	149
Â	21.2	9.7	26.8	23.9	14.8	11.4	12.8	9.3	129.9
a	2	-	2	2	-	-	1	-	7
H_E	0.949	0.802	0.966	0.945	0.917	0.891	0.916	0.858	
H_O	0.964	0.537***	0.982	0.841	0.836	0.873	0.891	0.759	
Puerto Rico									
N	46	37	46	32	42	43	42	44	
A	25	12	27	22	16	12	15	10	139
Â	21.0	10.6	23.7	21.0	14.9	11.2	13.9	9.3	125.6
a	3	-	-	1	-	1	2	-	7
H_E	0.934	0.771	0.956	0.937	0.911	0.873	0.908	0.846	
H_O	0.957	0.622**	0.957	0.906	0.976	0.884	0.762*	0.841	

0.966 and 0.500 to 0.982, respectively (Table 8). The number of alleles detected per locus ranged from nine to 32. As can be expected, populations with larger sample sizes exhibited slightly increased levels of allelic diversity, with a total of 149 alleles present in JP and only 139 in DT. Also, only two private alleles were present in DT, while all other populations contained seven or eight. However, there were no apparent trends towards reduced heterozygosity in populations with smaller sample sizes,
and estimations of allelic richness indicated that no single population was particularly deficient in genetic diversity.

Seven out of 40 tests indicated significant departures from HWE ($0 < P < 0.05$). Four of these significant tests occurred at locus La39, while the remaining three were distributed across loci (Table 8). PR and BZ each exhibited significant deviations from HWE at two of the eight loci. All locus pairs exhibited linkage equilibrium when tested across populations. In contrast, pairwise locus comparisons within each individual population yielded six significant tests indicating linkage disequilibrium out of a total of 140 tests ($0.01 < P < 0.05$). Interestingly, five of these significant tests occurred in the PR sample.

Population Structure

Three out of 80 tests indicated significant heterogeneity in allelic distribution between population pairs ($0.01 < P < 0.05$). Each of these three tests included PR as compared to JP, BZ and HN, and heterogeneity was limited to La18a and La39 (Figure 4). Tests of genotypic distributions between population pairs indicated significant heterogeneity in two out of 80 tests ($0.01 < P < 0.05$).

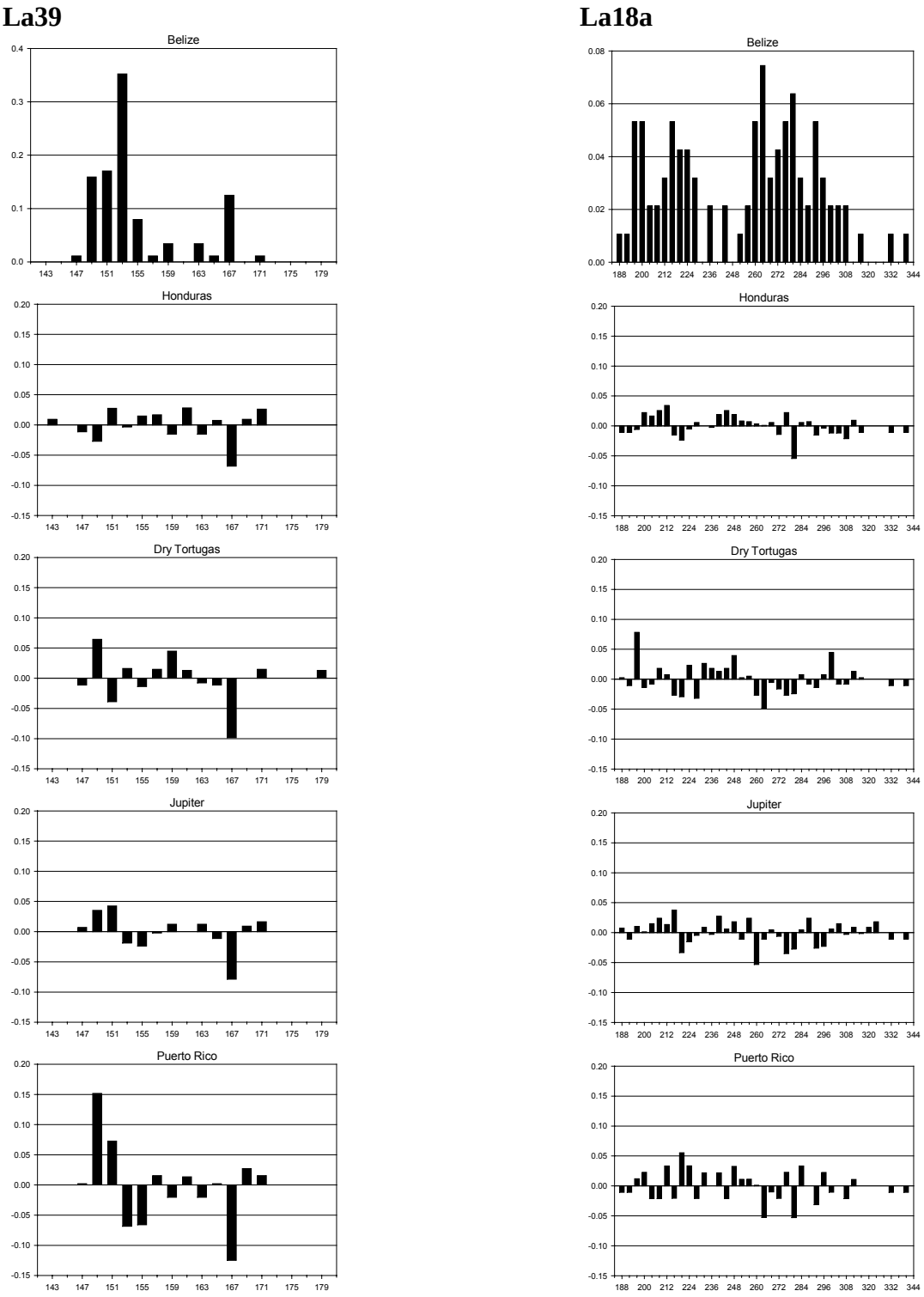

Figure 4. Allele frequencies at two microsatellite loci in five mutton snapper populations. The absolute allele frequencies are shown for BZ, while the other four populations are shown as the difference with the observed distribution in BZ. For each locus: x-axis = allele size (bp) and y-axis = allele frequencies.

Table 9. Population substructure across all populations estimated with θ (F_{ST}) and ρ (R_{ST}).

Locus	θ (F_{ST})	ρ (R_{ST})
La25	0.000	-0.003
La39	0.000	0.005
La18a	0.000	-0.005
La27a	-0.001	0.000
La34a	-0.002	-0.008
La45a	0.003	-0.003
La49a	-0.005	-0.009
LaC-16	-0.004	-0.009
Overall	-0.0011	-0.0041

Table 10. Pairwise comparisons of mutton snapper populations. Above the diagonal: F_{ST} (*P*-value); below the diagonal: D_{CE} (*P*-value).

	BZ	HN	DT	JP	PR
BZ		-0.0024 (0.926)	-0.0015 (0.748)	-0.0016 (0.844)	0.0007 (0.312)
HN	0.012 (0.921)		0.0004 (0.401)	-0.0035 (0.995)	-0.0009 (0.661)
DT	0.014 (0.842)	0.015 (0.686)		-0.0023 (0.878)	0.0022 (0.176)
JP	0.012 (0.792)	0.012 (0.900)	0.014 (0.913)		-0.0001 (0.468)
PR	0.017 (0.119)	0.014 (0.747)	0.018 (0.405)	0.017 (0.096)	

Table 11. Analysis of molecular variance (AMOVA) among groups and populations. All tests of F-statistics were non-significant.

Source of variation (FST)	Degrees of freedom	Sum of squares	Variance components	Variation (%)
Among groups	1	1.714	-0.00341	-0.13
Among populations within groups	3	6.661	-0.00357	-0.14
Within populations	485	1247.596	2.57236	100.27
Total	489	1255.971	2.56538	

All estimates of θ (F_{ST}) and (R_{ST}) across populations were less than 0.005 and most were zero or negative values (Table 9). Pairwise comparisons of F_{ST} ranged from -0.0035 to 0.0022, and all values were non-significant. There was a slight trend in which pairwise comparisons involving PR exhibited the largest, albeit still small, F_{ST} values; the DT with HN comparison was the exception to this trend (Table 10). An AMOVA was performed with PR pre-defined as one group and the remaining populations comprising a second group. The definition of groups was based on the results of previous work that indicated Puerto Rico as a potential intermediate population between eastern and western Caribbean genetic clusters (Baums et al. 2005). One-hundred percent of the variance was partitioned to the within populations component (Table 11). The same result was obtained when the analysis was performed based on the sum of squared size differences (R_{ST}) and when HN was included in the first group with PR (data not shown). Pairwise genetic distances, D_{CE}, were all non-significant and ranged from, 0.012 to 0.018. Again, the largest distances were calculated for comparisons involving PR. The only discrepancy in this trend was the DT with HN comparison.

Although the results of these population-level analyses were not highly significant, the tendency of PR to be involved in pairwise comparisions exhibiting the largest differences between samples supports distinction of this geographic location.

Population Assignment

Only 35 individuals (14.3%) were correctly assigned to their source populations (Table 12). Individuals were assigned to the population in which their multilocus genotypes exhibited the highest probability of occurrence. However, assignment probabilities in the dataset ranged from 0.0011 to 0.9416 and, for most individuals, these values were similar across populations.

Table 12. Individual assignments of the frequency-based test. Values represent the number (%) of individuals from the source populations in each assigned population. Bold-faced values denote assignment to populations of origin.

	Assigned Population					
Source Population	BZ	DT	HN	JP	PR	Total
BZ	**3 (6.0)**	7 (14.0)	16 (32.0)	23 (46.0)	1 (2.0)	50 (20.4)
DT	4 (10.0)	**5 (12.5)**	13 (32.5)	15 (37.5)	3 (7.5)	40 (16.3)
HN	6 (11.3)	11 (20.8)	**10 (18.9)**	21.5 (40.6)	4.5 (8.5)	53 (21.6)
JP	9 (16.4)	11 (20.0)	18 (32.7)	**14 (25.5)**	3 (5.5)	55 (22.4)
PR	5 (10.6)	6 (12.8)	19 (40.4)	14 (29.8)	**3 (6.4)**	47 (19.2)
Total	27 (11.0)	40 (16.3)	76 (31.0)	87.5 (35.7)	14.5 (5.9)	245

Table 13. Probability of the number of populations (K) inferred by STRUCTURE. Log P(X|K) = ln probability of the data and log P (K|X) = posterior probability.

K	log P(XIK)	P(KIX)
1	-9631	1.0
2	-9914	~0.0
3	-10409	~0.0
4	-10544	~0.0
5	-11850	~0.0

Table 14. Proportion of membership of each pre-defined population in clusters inferred by STRUCTURE assuming two genetic clusters (a) and five genetic clusters (b).

Population ID	Two inferred clusters		Five inferred clusters				
	I	II	I	II	III	IV	V
BZ	0.500	0.500	0.204	0.199	0.198	0.200	0.200
HN	0.500	0.500	0.215	0.186	0.203	0.187	0.209
DT	0.500	0.500	0.192	0.208	0.198	0.207	0.195
JP	0.500	0.500	0.201	0.200	0.200	0.200	0.199
PR	0.500	0.500	0.198	0.207	0.195	0.204	0.197

Thus, this method was unsuccessful at detecting the origin of an individual based on its multilocus genotype and population allele frequencies. This is not surprising given the similarity in allele frequencies across population samples. In addition, sample size appeared to highly influence the assignmentprocedure, as the two largest populations, HN and JP, received the greatest number of assignments (Table 12).

STRUCTURE identified a single genetic cluster with a posterior probability of 1.0 (Table 13). Thus, in the absence of prior information regarding the origin of individual genotypes, posterior probabilities of the parameter, K, do not support the presence of genetic structure within the sample of 245 individuals. When the assignment procedure was implemented using five clusters, individuals from each population were assigned equally well to all five (Table 14). A similar result was obtained assuming two clusters for the assignment (Table 14). Again, this result is not particularly unexpected based on the results of previous tests indicating that the populations conformed well to expectations of H-W and linkage equilibrium.

Discussion

The null hypothesis, that sampled mutton snapper populations constitute a single panmictic population, cannot be rejected based on the genetic pattern observed at eight microsatellite loci across 245 individuals. Analyses ranging from population-based F-statistics to individual-based assignment indicate that population genetic substructure is absent from the five sample locations, ranging across approximately 2000 km.

Comparison with Other Studies

A lack of genetic differentiation across large distances is not unique to mutton snapper populations. Heist and Gold (2000) obtained similar results with five microsatellite loci across 1500 km of the Gulf of Mexico for the red snapper, *Lutjanus campechanus*. Genetic diversity was slightly higher in the present study; however, values of F- and R-statistics were similar for both red and mutton snapper populations. Larval dispersal has long been recognized as a homogenizing force in marine systems. The pelagic larval duration (PLD) of mutton snapper is 27 to 37 days based on otolith settlement marks (Lindeman et al. 2000), similar to that of the red snapper (28 to 30 days, Heist and Gold 2000). This 4- to 5-week period of time spent in the water column may facilitate long distance dispersal and, thus, genetic homogenization.

In contrast to the outcome of the present study, microsatellite loci have often been recognized for their ability to reveal subtle genetic structure. As was previously discussed, populations of the gorgonian coral, *Pseudopterogorgia elisabethae*, exhibited significant structure across small geographic distances at six microsatellite loci (Gutierrez-Rodriguez and Lasker 2004). Yet it must be noted that the life history of these brooding corals is quite distinct from that of mutton snapper, which broadcast gametes in large offshore spawning aggregations, resulting in relatively long-lived pelagic larvae. Significant genetic structure was also detected using microsatellites in the elkhorn coral, *Acropora palmata* (Baums et al. 2005). Although these corals broadcast gametes into the water column, complex inshore currents coupled with a five- to 20-day PLD may facilitate local retention. Thus, based on life history characteristics alone, it is not surprising that the five mutton snapper populations exhibited genetic homogeneity. However, it is important to recognize several caveats to the conclusion of panmixia for mutton snapper populations based on the present study.

Caveats to the Conclusion of Panmixia

The range of mutton snapper across the Caribbean, in particular its large spawning aggregations, was not sampled in its entirety. According to the only other study employing microsatellite markers to a similar geographic range, genetic differentiation was partitioned into eastern and western Caribbean clusters (Baums et al. 2005). Samples of elkhorn coral from Puerto Rico and Mona Island were intermediate between the two clusters. Mutton snapper populations belonging to the eastern cluster were not sampled, so we cannot evaluate divergence between eastern and western Caribbean populations. Interestingly, however, there was a non-significant trend in which PR was the most distinct of the five populations. This is apparent in the involvement of PR in all significant tests for deviations from H-W and linkage equilibrium, and for heterogeneity in allelic and genotypic distributions. The trend is also present in pairwise estimates of F_{ST} and genetic chord distance. Due to the tendency of larval dispersal to homogenize populations, the longer larval duration of the mutton snapper relative to that of elkhorn coral may cause genetic structure to be more subtle in the former. This being the case, the slight and non-significant differentiation of PR may be consistent with the results of Baums et al. (2005). However, no definitive conclusions can be made without the addition of eastern Caribbean populations to the mutton snapper dataset.

Although microsatellites have proven to exhibit high resolution in numerous studies, their application remains hindered by the disproportionate effect of very small levels of migration on genetic divergence (Slatkin 1987). Thus, the results of genetic surveys can greatly inflate estimations of dispersal between populations that are demographically closed on an ecological scale. This issue of scale is apparent in the conflicting results of studies employing alternative markers to measure local retention. For example, significant levels of natal homing were

revealed for weakfish along the eastern coast of the United States (Thorrold et al. 2001). Sixty to 81 % of individuals returned to spawn in the estuary of their birth. However, previous genetic studies employing allozymes and mtDNA failed to detect any genetic structure in weakfish sampled across a similar range. Thus, although genetic markers exhibit homogeneity among mutton snapper populations, alternative markers (e.g. elemental signatures or artificial tags) may reveal significant levels of local retention around spawning sites.

Management and Conservation Implications

The genetic similarity of mutton snapper populations revealed at eight microsatellite loci implies free gene flow between populations. However, because the genetic composition of each sample was so similar, it is impossible to discern between the relative contributions of Puerto Rico, Belize, Honduras and the Dry Tortugas as source populations for the Jupiter sample. Thus we cannot rule out the possibility that the DTER serves as a significant source of recruits to the southeastern United States. Yet based on the results of this study we cannot confirm that is does. It is logical to assume that the nearest source population (i.e. Dry Tortugas) supplies the majority of recruits to southeast Florida; however, in the absence of a higher resolution marker, this will remain unknown. Future attempts to estimate the relative contributions of mutton snapper spawning aggregations to adult populations may include an increase in the number of loci employed. This would be quite feasible given that enriched genomic libraries have already been constructed for the mutton snapper. An alternative strategy would involve a large increase in sample size. The addition of individuals could increase the resolution by revealing more rare alleles that are confined to particular locations. The development of alternative markers, such as single nucleotide polymorphisms (SNP) or nuclear sequences, is also possible, though much

more expensive and time-consuming. Finally, the implementation of mark-recapture studies using artificial tags could reveal the degree of site fidelity exhibited by adults as well as the geographic range of adults that utilize a particular aggregation. This avenue of research would also be feasible, as numerous adults could be marked at once in the spawning aggregation.

CONCLUSIONS

Future Directions for Understanding Connectivity

Here I propose two large-scale studies that may increase our ability to estimate and understand patterns of connectivity in marine systems. First, a study that simultaneously employs elemental tags and microsatellite markers will facilitate the direct comparison of the contrasting information produced by each technique. Thorrold et al. (2001) noted that previous genetic studies had, in fact, failed to detect genetic differentiation between populations of weakfish, *Cynoscion regalis*, in spite of the significant levels of natal homing revealed by elemental signatures. However, these previous studies had employed allozymes and mtDNA rather than high resolution microsatellite markers. Thus, it remains to be seen how the results of microsatellite surveys compare to those employing elemental tags.

A second study entails the survey of genetic variation at microsatellite loci for a variety of species sampled extensively across the Caribbean basin. In contrast to the application of mtDNA RFLPs in Shulman and Bermingham (1995), the use of high resolution markers will enable the detection of the more subtle population structure that can be expected for marine organisms with high dispersal potentials. The goal of such a study would be to understand the dominant dispersal corridors, most likely driven by the major surface current patterns. Species exhibiting a range of dispersal potentials, in particular species with minimal PLDs, should be incorporated into the study. The average of estimated PLDs surveyed in Shulman and Bermingham (1995) ranged from 15 to 81.5 days. In order to increase the potential for detecting genetic structure, the study should also include species that exhibit very low actual dispersal (e.g. gobies). Such species could be used, in turn, to infer broad patterns of connectivity that are

otherwise impossible to detect in other species due to the powerful homogenizing force of migration.

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