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Population genetic structure of Lemon Sharks in the Western Atlantic: is there evidence of gender-biased dispersal and differences between neutral and adaptive loci?

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Abstract of the Thesis

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Sampling difficulties and low genetic diversity have limited population genetic studies of large, vagile shark species. Through extensive sampling (580 individuals from 12 locations) and a multilocus approach involving two mitochondrial DNA loci (control region and ND2, composite sequence 1,730 bp) and eight microsatellites, I was able to delineate the population genetic structure of the lemon shark, *Negaprion brevirostris*, in the western Atlantic. I articulated two hypotheses to explain genetic structure in coastal sharks: (1) females exhibit natal philopatry to parturition sites, resulting in local population structure and (2) males are dispersive, resulting in high male-mediated gene flow. I predicted that maternally inherited mitochondrial genetic markers would be highly structured even on a local geographic scale, while bi-parentally inherited microsatellite markers would exhibit little to no genetic structure over the same range. Significant genetic structure was detected in the mitochondrial composite sequence of ND2 and control region (Φ_{ST} =0.293, p<0.000001), with at least seven distinct groups evident in the sampling area (East Peninsular Florida, West Peninsular Florida/Tiger Beach (Bahamas), Lower Florida Keys/Bimini (Bahamas)/Belize, Eleuthera (Bahamas), Louisiana, U.S. Virgin Islands and Brazil). Significant genetic structure was individually detected in both the coding ND2 (Φ_{ST} =0.293, p<0.000001) and the non-coding control region (Φ_{ST} =0.278, p<0.000001), but the ND2 gene was found to be an inappropriate locus to test for local adaptation in lemon sharks because all of the mutations were silent with the exception of a single mutation found in two sharks. Very weak genetic structure was also detected in nuclear microsatellites (F_{ST} =0.014; p<0.088), but only between the Brazilian population and all of the others. A Bayesian analysis of the microsatellite data failed to reject a null hypothesis that there is one population in the region. Both classes of genetic marker indicated that geographic distance between sampling areas was

correlated with genetic distance between them. All of these findings are consistent with my predictions and support the hypotheses of natal philopatry in females and high male-mediated gene flow. Fine-scale local genetic structure driven by behavior makes this species and others like it much more vulnerable to local fishing or habitat destruction than resource managers currently appreciate. I suggest future work should sequence larger regions of the mitochondrial genome to fully resolve population structure in this species and to investigate the possibility of local genetic adaptation in the coding regions.

Dedication Page

I dedicate this thesis to my family for a lifetime of support, my friends for their patience and my dearest, H. James Elisalda III, for his devotion. They have given me the strength and courage to accomplish great things.

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List of Abbreviations

Genetic Terms

- CR = mitochondrial control region
- ND2 = NADH dehydrogenase subunit 2
- PCR = Polymerase Chain Reaction
- A = adenine
- C = cytosine
- G = guanine
- T = thymine

Sampling Collections

- BIJ = Bimini, Bahamas juvenile lemon sharks
- BIS = Bimini, Bahamas subadult lemon sharks
- BZ = Belize subadult and adult lemon sharks
- EL = Eleuthera, Bahamas juvenile lemon sharks
- EV = Everglades, Florida subadult and adult lemon sharks
- GB = Gullivan Bay, Florida subadult lemon sharks
- JU = Jupiter, Florida subadult and adult lemon sharks
- KS = Cape Canaveral, Florida juvenile lemon sharks
- LA = Louisiana juvenile lemon sharks
- MQJ = Marquesas Key, Florida juvenile lemon sharks
- MQS = Marquesas Key, Florida subadult lemon sharks
- RO = Atol das Rocas, Brazil juvenile lemon sharks
- TB = Tiger Beach, Bahamas subadult and adult lemon sharks
- VI = U.S. Virgin Islands juvenile and adult lemon sharks

Age Classes

- JUV = juvenile lemon shark < 100 cm total length
- SUB = subadult lemon shark > 100 cm and < 200 cm total length
- ADU = adult lemon shark > 230 cm total length

Genetically Differentiated Subpopulations

- FK-SWB = Florida Keys and Southwest Bahamas subpopulation
- GS = Belize, Marquesas Key, Florida and Bimini, Bahamas Gulf Stream subpopulation
- EPF = East Peninsular Florida subpopulation
- WPF-TB = West Peninsular Florida and Tiger Beach, Bahamas subpopulation

Chapter 1

Introduction

Lemon sharks: a model species for studying the life history and ecology of large coastal sharks

Lemon sharks (*Negaprion brevirostris*) are large, warm temperate to tropical requiem sharks (F. Carcharhinidae) that occur in coastal areas in the Western Atlantic from New Jersey to Brazil (Figure 1). There are also small, genetically distinct populations of this species occurring in West Africa and the Eastern Pacific (Schultz et al. 2008). Lemon sharks mature at lengths of 230-240 cm, attained at ages of 10-12 years. They conform to the model coastal carcharhinoid shark life history pattern proposed by Springer (1967), where they are born in discrete coastal nursery areas and either remain there for several years (in subtropical and tropical areas, Chapman et al. 2009a) or return there on a regular basis after having seasonally migrated to avoid low water temperatures (in warm temperate areas, Reyier et al. 2008). Nursery areas are typically shallow, productive habitats such as estuaries or mangrove-fringed seagrass flats and occur in discrete patches along the coast of Florida (e.g., the Florida Keys, Biscayne Bay, the Everglades), the islands of the Bahamas (e.g., Bimini, Cat Island, Andros, Berry Islands, Nassau, Grand Bahama, Eleuthera) and other locations in the Caribbean and Central and South America. The northernmost limit for lemon shark breeding appears to be the estuaries and salt marshes of South Carolina (Castro 1987) and the southernmost nursery area is Atol das Rocas, Brazil (Feldheim et al. 2001). Larger subadult and adult animals live away from their natal nursery (Chapman et al. 2009a) and are generally more mobile than juveniles with individuals moving as far as 1,000 km from the site of tagging (Kohler et al. 1998, Reyier et al. 2008, Feldheim et al. 2001; Figure 2). Adult females typically only enter the nursery area to give birth and depart for deeper habitats such as reefs and offshore banks (Figures 3 and 4). Springer (1967) suggested that this basic model applied to many (but not all) coastal shark species, including many that are of conservation concern (Knip et al. 2010).

The lemon shark has widely been used as a model species for the study of large coastal shark life history and ecology because it fits the Springer (1967) model and is reasonably accessible to researchers (e.g., ontogeny, Barker et al. 2005; metabolism, Bushnell et al. 1989; survivorship, Aché de Freitas et al. 2009, Gruber et al. 2001; habitat selection, Morrissey and Gruber 1993; habitat utilization, Gruber et al. 1988; life history, Hoenig and Gruber 1990; anthropogenic influence on, Jennings et al. 2008; migration, Kessel et al. 2009; diet, Cortés and Gruber 1990; prey selection, Reeve et al. 2009). Genetic studies of this species have been conducted at Bimini, Bahamas and Marquesas Key, Florida and have revealed much about the breeding biology of large sharks. These studies have been facilitated by the ability to near exhaustively sample each cohort every year with sampling efforts of this nature having been completed each year since the mid- to late 1990s in these areas. The mating system was

characterized as one with frequent polyandry as more than 80% of females produced litters sired by more than one male (up to three sires; Feldheim et al. 2004, DiBattista et al. 2008). Approximately half of the adult females returned to give birth on a biennial cycle over six and eight breeding seasons in Bimini and Marquesas Key respectively (Feldheim et al. 2004, DiBattista et al. 2008). In contrast to females, males rarely sired more than one litter in the same nursery (Feldheim et al. 2004, DiBattista et al. 2008).

Preliminary evidence from Schultz et al. (2008) identified genetic population structure among western Atlantic lemon sharks. Comparisons between Bimini, Bahamas and Atol das Rocas, Brazil (> 6,000 km apart) found genetic differences between sites in both maternally inherited mitochondrial and bi-parentally inherited nuclear DNA. My thesis will build on this preliminary study of the population genetics of lemon sharks in the Western Atlantic and aims to test the following hypotheses: (1) females return to their birthplace to give birth (i.e., females practice "natal philopatry"), resulting in limited female-mediated genetic connectivity across their range; (2) males are more dispersive than females, resulting in a high degree of male-mediated genetic connectivity across their range; and (3) maternally inherited coding genes exhibit local adaptation to the natal environment.

Genetic Tools: genetic markers used to identify population structure

The mitochondrial genome is a maternally inherited, double-helical circle of approximately 16,700 base pairs that responds to selection because it codes for 13 proteins, 22 transfer RNAs and two ribosomal RNAs (Cao et al. 1998). These genes are critical for cellular respiration via oxidative phosphorylation and the production of adenosine triphosphate (ATP) and include one cytochrome reductase subunit, three cytochrome oxidase subunits, seven subunits of NADH-Q reductase, and two ATP synthase subunits (Stryer 1995). Mitochondrial DNA has a higher rate of base substitutions over nuclear DNA, and because of this, many mitochondrial genes have incurred intra-specific sequence variations that make them ideal for population genetic studies (Curole and Kocher 1999). One such gene is NADH dehydrogenase subunit 2 (hereafter referred to as "ND2"), which codes for the second subunit of NADH-ubiquinone oxidoreductase, responsible for electron transport in the production of ATP (Stryer 1995). ND2 in lemon sharks is 1041 base pairs and is located on the outer "heavy" strand of the mitochondrial genome between ND1 and cytochrome *c* oxidase I. ND2 was chosen for this study because of its intra-specific variation in shark species (Verissimo et al. 2010, Castilho et al. 2007, Farrel et al. 2009) and lemon sharks (preliminary data), thus ND2 has the potential to detect local adaptation within our sampled population. The other mitochondrial DNA marker used in my study, control region (hereafter referred to as "CR"), is a non-coding segment found on the heavy strand and is located between cytochrome *b* and the 12S small ribosomal subunit. CR is 1080 base pairs in lemon sharks and contains the origin of replication and origin of transcription for the mitochondrial genome (Schultz et al. 2008, Stryer 1995). It also demonstrates a significant level of intra-specific variation in many organisms including sharks. CR is commonly used in population genetic studies comparing mitochondrial and nuclear gene flow (e.g., Pardini et al. 2001, Portnoy et al. 2010).

Microsatellites are sequences with short tandem repeating segments of one to six base pairs found throughout organisms' genomes (e.g., [AC] dinucleotide, [AGAT] tetranucleotide, etc.; Goldstein and Schlotterer 1999). They are non-coding, co-dominant, and commonly used in

kinship and population structure studies (Feldheim et al. 2001, 2002; DiBattista et al. 2008). Mutations are most often caused by replication slippage resulting in the addition or deletion of one repeating unit, and occur at the rate of approximately once per 1000 generations (Goldstein and Schlotterer 1999). Primers to isolate microsatellites are developed for the flanking regions of the repeating sequences. Heterozygotes will yield two fragments after amplification (one allele from each parent), and homozygotes will yield one fragment (each parent contributed an identical allele). The numbers of bases are measured for each allele and used for data analysis.

Natal philopatry

The removal of large predators from coastal marine ecosystems is one of the most pervasive anthropogenic impacts on Earth's oceans and may have serious ecological repercussions (Heithaus et al. 2008). Large carcharhinoid sharks (e.g., requiem and hammerhead sharks) were some of the most common apex predators in coastal areas around the world until intense commercial shark fisheries developed, now largely driven by the market for dried shark fins (Clarke et al. 2006). A common assumption of resource managers is that many exploited sharks consist of large, widely distributed populations that are locally replenished by frequent mixing over large geographic areas. This view is incongruent with observations that shark fisheries established in a local coastal area frequently collapse shortly after they begin and are very slow to rebuild (Walker 1998, Hueter 1998, Hueter et al. 2005). Neighboring areas often maintain robust shark populations until fisheries develop there as well. This pattern of localized collapse of coastal shark fisheries has been documented for more than a century and has never been fully explained (Walker 1998, Hueter 1998, Hueter et al. 2005).

The leading explanation for the localized collapse of shark fisheries is the "natal philopatry hypothesis" (Hueter 1998, Hueter et al. 2005). Natal philopatry is defined as "the propensity to return or remain near the birthplace for reproduction". Among migratory marine animals natal philopatry has been documented in sea turtles (Bowen et al. 1989), anadromous fish (Quinn and Dittman 1990) and, most recently, certain marine fish (Thorrold et al. 2001, Rooker et al. 2010). Cury (1994) tentatively proposed that natal philopatry is a typical part of the reproductive strategy of mobile animals. Under this model, individuals imprint on environmental parameters within their natal area and relocate this site or these conditions when it is time for them to reproduce. In this manner they are able to deliver their offspring either to their own natal area or habitat that is very similar to it. Homing studies show that juvenile lemon sharks are capable of relocating their natal nursery when experimentally displaced from it into distant novel habitats (Edrén and Gruber 2005), which is consistent with the existence of an imprinting ability in this species. The natal philopatry hypothesis as it has been applied to sharks postulates that many coastal sharks imprint on and return to their birthplace when they are ready to give birth (Hueter 1998, Hueter et al. 2005). This leads to the development of metapopulations that are centered on discrete nursery areas, which are primarily replenished by parturition of females that were born there. Under this model, intense, localized fishing quickly removes juveniles living in and around the nursery area and their mothers when they come in to give birth, leading to a local collapse in the shark population (Hueter 1998, Hueter et al. 2005).

The natal philopatry hypothesis predicts that it would be possible to recapture sharks tagged as newborns in their natal nursery many years later when they have returned to give birth. While juvenile sharks tagged in their natal nursery area have been recaptured in the same

location a few years later (Hueter et al. 2005, Chapman et al. 2009a), the immense logistical challenges associated with recapturing these same sharks when they are giving birth many years later has precluded the collection of definitive evidence of natal philopatry. Another prediction of the natal philopatry hypothesis is that generations of females returning to their natal area to give birth will cause maternally inherited genetic variation (i.e., mitochondrial DNA) to become structured at local geographic scales. Genetic population structure has been documented among widely distributed coastal sharks, but differentiation has only been detected over large geographic distances (typically 1,000 km or more) due to sampling constraints or substantial barriers to dispersal such as oceanic expanses (Duncan et al. 2006, Schultz et al. 2008, Chapman et al. 2009b, Portnoy et al. 2010, Shivji 2010, Benavides et al. 2011). The most common genetic marker used in these studies is the mitochondrial CR, a non-coding portion of the mitochondrial genome. CR sequences are geographically partitioned along the Western Atlantic distribution of at least five large sharks: the scalloped hammerhead (*Sphyrna lewini*, Chapman et al. 2009b), the blacktip (*Carcharhinus limbatus*, Keeney et al. 2005), the bull (*Carcharhinus leucas*, Castro 2011), the dusky (*Carcharhinus obscurus*, Benavides et al. 2011) and the lemon shark (*Negaprion brevirostris*, Schultz et al. 2008). In all cases the authors have invoked natal philopatry by females to parturition sites as the mechanism underpinning structure, but only one of them were able to obtain samples from sites less than 1000 km apart or more. Even though Keeney et al. (2005) did sample some blacktip nursery areas that were in closer proximity (a few hundred kilometers) they only discovered structure between sampling locations that were 600-1,000 km or more apart. This study may have been limited by relatively low nucleotide diversity in the CR, which limited resolution. These genetic studies are consistent with the natal philopatry hypothesis but do not conclusively demonstrate that sharks usually return to their exact birthplace or even to areas within 1,000 km of it (Hueter et al. 2005). I therefore suggest that a more comprehensive approach, involving the sampling of more locations and with larger amounts of sequence data, is needed to test the natal philopatry hypothesis for sharks. I hypothesize that individual female lemon sharks practice natal philopatry to parturition sites and predict that maternally inherited genetic loci can be structured on local geographic scales (i.e., between locations separated by just hundreds of kilometers).

Male-biased dispersal

Mitochondrial genetic markers can provide important information on dispersal and reproductive mixing (i.e., "gene flow") within a species. Given their maternal mode of inheritance, however, mitochondrial genetic markers may provide a misleading picture of overall genetic connectivity if the magnitude of male-mediated gene flow differs from that of females ("sex-biased dispersal"). Sex-biased dispersal is common among vertebrates and occurs when individuals of one sex stay or return to their natal site (or group) to breed while individuals of the other sex are prone to disperse (Prugnolle and de Meeus 2002). For example, mammals commonly exhibit male-biased dispersal whereas birds exhibit female-biased dispersal (Handley and Perrin 2007, Arlt and Part 2008). Current hypotheses for the evolution of sex-biased dispersal are that it has the potential to reduce competition for resources within a given geographic area, reduce the competition for mates within a population and reduce the probability of inbreeding. Characterizing the magnitude of sex-biased dispersal is critical in exploited

species given the possibility of asymmetric exploitation of the dispersing or philopatric sex, leading to imbalanced sex ratios and reduced reproductive output (Handley and Perrin 2007).

The two most common methods employed to estimate levels of sex-biased dispersal are direct observational methods and genetic methods (Prugnolle and de Meeus 2002, Arlt and Part 2008, Bowen et al. 2005). It is difficult to make long term direct observations of large sharks in the field, which means that genetic methods have become the tool of choice for studying this behavior among sharks. Sex-biased dispersal in a population is generally inferred by comparing genetic population structure in a bi-parentally inherited marker (microsatellites or allozymes) and a uni-parentally inherited marker (mitochondrial DNA or Y-linked genes; Handley and Perrin 2007, Bowen et al. 2005). For instance, in a species in which only males disperse, maternally inherited markers from mitochondrial DNA will have moderate to high genetic structure and bi-parentally inherited markers like microsatellites will have very low or no genetic structure. Varying levels of sex-biased dispersal—typically with males being the dispersive sex—have been documented in other marine vertebrates using these genetic methods. Nesting colonies of loggerhead sea turtles (*Caretta caretta*) along the southeastern coast of the United States had strong genetic structure in mitochondrial DNA (Φ_{ST}=0.42, p<0.001) with lower bi-parentally inherited microsatellite structure (F_{ST}=0.002, p=0.05) demonstrating male-biased dispersal (Bowen et al. 2005). Chinook salmon (*Oncorhynchus tshawytscha*), another strongly philopatric marine species, are not usually thought to display sex-biased dispersal since both sexes must return to their natal site to breed via broadcast spawning. However, male-mediated dispersal was detected using microsatellite genotypes within nursery creeks among different gravel beds where females would deposit eggs in one bed and males would deliver sperm in multiple beds (Neville et al. 2006). In sharks, Pardini et al. (2001) detected highly differentiated populations with mitochondrial DNA and insignificant genetic differentiation with five microsatellite loci in white sharks (*Carcharodon carcharias*) from Australia and South Africa. Male-biased dispersal has also been seen in other shark species like the shortfin mako (*Isurus oxyrinchus*, Schrey and Heist 2003), sandbar shark (*Carcharhinus plumbeus*, Portnoy et al. 2010), and blacktip shark (*Carcharhinus limbatus*, Keeney et al. 2005). I hypothesize that lemon sharks, like these other species, will exhibit male-biased dispersal, and I predict that bi-parentally inherited microsatellites will show a lack of structure in the western Atlantic.

Local adaptation of mitochondrial genes

The vast majority of population genetic studies of marine fish survey non-coding genetic loci like CR and microsatellites (Conover et al. 2006). Marine fish often exhibit low levels of genetic structure at non-coding loci, which implies high gene flow and possibly limited adaptive divergence in local populations (Conover et al. 2006, Nielsen et al. 2009, Hemmer-Hansen et al. 2007). Although analysis of non-coding loci reveals important insights into the genetic structure of a population, the amount of gene flow necessary to geographically homogenize genetic variation over evolutionary timescales is relatively small (i.e., on the order of a single individual per generation). It is essential to also examine geographic variation in coding loci because they respond to the potentially stronger, diversifying force of local selection that can override the homogenizing force of gene flow. Relatively few studies have examined both non-coding and coding loci in marine fish, but the ones that do show local genetic adaptation is more common than previously thought. Adaptive population divergence and local adaptations at the DNA level

were found in the heat-shock cognate protein gene (Hsc70) of European flounder (*Platichthys flesus*) while microsatellite data indicated high gene flow within the same population (Hemmer-Hansen et al. 2007). Global population genetic studies of the sand goby (*Pomatoschistus minutus*) detected positive selection on the rhodopsin RH1 gene (Ebert and Andrew 2009, Larmuseau et al. 2009). High levels of polymorphism at three nucleotide positions in RH1 resulted in four amino acid variations adapted to specific environmental light conditions, and were incongruent with low neutral marker variation found in the same animals. Local directional selection has also been detected in Atlantic cod (*Gadus morhua*), another highly migratory marine species. Sequence variations in eight coding loci were found to correlate with temperature and/or salinity differences at spawning grounds during spawning times rather than geographic distance (Nielsen et al. 2009).

Mitochondria have their own haploid genome that encodes for a series of enzyme subunits critical to cellular respiration and electron transport (Stryer 1995). Many of these enzymes are affected by variations in temperature, and patterns of local adaptation may be detected in genes coding for the protein polymorphisms optimal to differing environmental conditions (Blier et al. 2001). Mitochondrial DNA has a higher rate of base substitution than most nuclear genes (Curole and Kocher 1999), and studies have shown environmental response to temperature of cytochrome *c* oxidase in Arctic charr (*Salvelinus fontinalis*, Blier and Lemieux 2001) and local adaptation in cytochrome *b* to climatic changes driven by temperature in the common vole (*Microtus arvalis*, Fink et al. 2004). Since respiration is affected by ambient environmental conditions like temperature, it is probable that mitochondrial genes and enzymes will evolve to suit the environment of the organism (Blier et al. 2001). In some widely distributed species mitochondrial DNA divergence among populations is associated with these enzyme adaptations to local or regional environmental conditions. For example, the circumglobally distributed killer whale (*Orcinus orca*) is now thought to consist of multiple species with mitochondrial genes that are adapted to regional environmental conditions (Foote et al. 2010).

Given predicted behavioral patterns of female philopatry in sharks, I have hypothesized that maternally inherited mitochondrial DNA will identify population genetic structure. Even though the adult sharks are migratory, juveniles may spend many years in their natal environment. If adult females return to their natal area, or an area very similar to it, for parturition, then selection could lead to local adaptation of maternally inherited proteins to local environmental conditions (such as temperature). Evidence for this phenomenon would be (a) mutations in coding regions of the mitochondrial genome that lead to changes in protein amino acid sequences and (b) geographic distribution of these coding sequences structured according to selection pressures of the local environment. As a result, patterns of population structure in coding loci may quantitatively or qualitatively differ from patterns in non-coding regions that respond only to genetic drift and gene flow. In a preliminary foray into this line of research for sharks, I will compare the magnitude and patterns of structure observed in a coding mitochondrial gene (ND2) and a non-coding mitochondrial locus (CR) in lemon sharks from my study area. No study to date has reported local adaptation in a shark species based on a mitochondrial gene, but intra-specific variation of ND2 in shark species provides the first opportunity to study this phenomenon (Verissimo et al. 2010, Castilho et al. 2007, Farrel et al. 2009).

Methods

Sample collection

Specimens were collected by various fishing methods. Juvenile lemon sharks were caught in two-meter cast nets from the shallows of Cape Canaveral, Florida (28.58°N, 80.55°W) periodically through the winter months. Nylon monofilament gill nets (180 m in length and 150 cm high) were used to capture neonates and juveniles following parturition in early summer from Bimini, Bahamas (25.73°N, 79.28°W), Marquesas Key, Florida (24.57°N, 82.12°W), and Atol das Rocas, Brazil (03.87°S, 33.82°W). Nets were deployed at dusk for 12 hours and monitored every 15 minutes for several nights consecutively. Adult sharks were caught with hook-and-line methods off of Tiger Beach, Bahamas (26.49°N, 78.70°W) and Jupiter, Florida (26.92°N, 80.06°W). Gillnets deployed by researchers or fishermen were used to sample sharks from Glover's Reef and Turneffe atoll, Belize (16.73°N, 87.80°W). Hand line fishing was used to capture juvenile and subadult sharks in other locations including the Florida Everglades (25.00°N, 80.99°W), Eleuthera, Bahamas (25.00°N, 76.33°W), off the coast of southern Louisiana (29.40°N, 91.40°W), and the United States Virgin Islands (18.33°N, 64.73°W). Bottom longline fishing gear was used to capture subadults from Gullivan Bay, Florida (26.88°N, 82.63°W), Bimini, Bahamas (25.73°N, 79.28°W) and Marquesas Key, Florida (24.57°N, 82.12°W).

Tissue samples from each shark were cut with scissors from the trailing edge of either dorsal fin or the lateral tip of pectoral or pelvic fins and stored in 90% ethanol or 20% DMSO. Various types of external or internal (Passive Integrated Transponder or PIT) tags were used to identify recaptured individuals, ensuring that all individuals in this study were only sampled once. Genomic DNA was extracted from ~20 mg of fin using the Qiagen DNEasy Kit (Qiagen Inc., Valencia, CA, USA). All sharks with total length less than 100 cm were considered juveniles (JUV), sharks ranging in length from 100 cm to 200 cm were considered subadult (SUB), and sharks greater than 230 cm were considered adults (ADU, Figure 5). Age classes were established by total length measured at time of capture (Chapman et al. 2009a). With the exception of Marquesas Key, Florida, Bimini, Bahamas and U.S. Virgin Islands, all specimens from each site fell into one of two categories: less than 100 cm or greater than 100 cm. Since Marquesas Key and Bimini had a large number of individuals from both groups, samples from those sites were separated into two collections per site. Sharks sampled from the 12 locations were assigned to collections by geographic location and age class for subsequent analyses. The 12 sites with abbreviations for each of the 14 collections are listed in Table 1.

DNA sequence analysis

Sequences from the entire CR (1080 bp) were PCR amplified using CR proline transfer RNA light strand forward primer Pro-L (5′-AGGGRAAGGAGGGTCAAACT-3′) and ribosomal RNA heavy strand reverse primer 282 12S (5′-AAGGCTAGGACCAAACCT-3′) as described by Keeney et al. (2003). PCR reactions and cycling parameters were carried out according to Keeney et al. (2003) using a MultiGene thermalcycler (Labnet International, Inc., Woodbridge, NJ, USA). Full sequences (1041 bp) from 40 lemon sharks were used to identify polymorphic sites of the ND2 gene. All polymorphisms were within a 650 base pair region. Partial sequences

from this region of ND2 were amplified using custom forward (5′-TGTATTAACCATCCTAATTTCAAG-3′) and reverse (5′-GGTGTTAGGGCAGAAGGATGGATA-3′) primers designed from GenBank Accession # U91418. Amplification reactions were carried out in 50 μl volumes containing 1 μl DNA template (~20 ng), 1X CoralLoad PCR Buffer, 200 μM dNTPs, 1 U HotStar *Taq* DNA Polymerase (Qiagen), and 0.25 μM forward and reverse primers. Cycling parameters optimized for this locus included an enzyme activation step of 95.0°C for 15 minutes, 35 cycles with a 94.0°C denaturation for 1 minute, a 50.0°C annealing temperature for 1 minute and a 72.0°C extension temperature for 2 minutes, and a final extension at 72.0°C for 10 minutes with a MultiGene thermalcycler (Labnet International).
PCR products were purified with ExoSAP-IT (Affymetrix, Inc., Santa Clara, CA, USA) and sequenced using the Big Dye Terminator v3.1 cycle sequencing kit (Applied Biosystems, Inc., Foster City, CA, USA) with a Bio-Rad DYAD thermalcycler (Bio-Rad Laboratories, Hercules, CA, USA). The resulting products were processed with an ABI 3730 automated genetic analyzer (Applied Biosystems).

Microsatellite analysis

Eight microsatellite loci were PCR amplified for each sample using locus specific forward and reverse primers. Four polymorphic dinucleotide microsatellites were used: LS22, LS30, LS54, and LS75 (Feldheim et al. 2001, 2002), as well as four polymorphic tetranucleotide microsatellites: LS542, LS560, LS572, and LS596 (DiBattista et al. 2008). Extensive validation efforts for all eight loci have been conducted (DiBattista et al. 2008). PCR was performed with a Bio-Rad DYAD thermalcycler (Bio-Rad Laboratories) with cycling parameters specific to each locus (available upon request; Feldheim et al. 2001, 2002; DiBattista et al. 2008). PCR products were genotyped on an ABI 3730 automated genetic analyzer (Applied Biosystems). Alleles were scored manually with Peak Scanner v1.0. Individuals missing data at three or more loci were omitted from the analyses.

Statistical analysis: Mitochondrial sequences

Sequences were aligned in Geneious Pro 5.1.7 (Drummond et al. 2010) where haplotypes of CR and ND2 were identified manually. Once separate haplotypes of ND2 and CR were identified for each sample, a new "composite" sequence file was created with the contiguous sequence of the 650 nucleotides from ND2 followed by the 1080 nucleotides from CR for that individual. Haplotype diversity, h, and nucleotide diversity, π, were calculated for the entire dataset for ND2, CR and the composite in DnaSP 4.0 (Rozas et al. 2003). These indices were then calculated for each of the 14 collections using the composite sequence. Maximum parsimony networks of ND2, CR and composite haplotypes were constructed in TCS 1.21 at the 95% confidence level to show the evolutionary relationships between haplotypes (Clement et al. 2000). In order to assess the possibility that the ND2 locus is responding to local selection I translated the nucleotide sequence I obtained for each haplotype into an amino acid sequence using Geneious Pro. Since I only obtained a partial sequence, my haplotype sequences were aligned with GenBank sequence U91418 to obtain the correct start, amino acid residue, and

codon positions. Changes in the amino acid sequences between haplotypes were then identified manually.

Analysis of molecular variance (AMOVA) was used to partition divergence among and within collections in Arlequin 3.5.1.2 (Excoffier et al. 1992, Schneider et al. 2000). Global (i.e., range-wide) AMOVA was performed for ND2 and CR individually, as well as for the composite. AMOVA generates a global fixation index, Φ_{ST}, for each locus that measures the proportion of genetic divergence that is partitioned among as opposed to within collections and ranges from zero (i.e., no divergence among collections) to 1 (i.e., 100% of divergence occurs among rather than within collections). I assumed the Tamura and Nei model of sequence evolution, which was previously found by Schultz et al. (2008) to best fit the evolution of the lemon shark CR. Pairwise Φ_{ST} values between collections were also generated for the 14 individual collections using ND2, CR and the composite sequence. The significance of global and pairwise Φ_{ST} was assessed via 10,000 permutations of the raw haplotype data. I used an Exact test to test for significant differences ($p<0.05$) in haplotype frequencies between collections (Raymond and Rousset 1995). I also calculated global and pairwise Φ_{ST} for subsets of the Florida/western Bahamas dataset to see if there were differences in levels of population structure according to the life-stage. The first AMOVA compared collections composed entirely of juveniles (i.e., BIJ, EL, KS and MQJ) and the second compared collections composed of entirely subadults/adults (i.e., BIS, GB, JU, EV, MQS and TB).

Statistical analysis: Microsatellites

Each microsatellite locus was tested for Hardy-Weinberg equilibrium. The Hardy-Weinberg principle assumes that all allele or genotype frequencies within a population remain in equilibrium across generations and the sum of the proportions of homozygotes of each allele plus heterozygotes equal 100% of the population (Hartl 2000). The number of alleles, observed heterozygosity (H_O) and expected heterozygosity (H_E) were obtained for each collection at each locus. H_O is simply the number of heterozygotes at one locus divided by the total number of individuals surveyed, and H_E is the estimated fraction of all individuals who would be heterozygous at a locus assuming Hardy-Weinberg equilibrium. The probability that heterozygosity deviated from Hardy-Weinberg equilibrium in paired comparisons of (1) each collection and (2) of each collection for each locus was calculated in Arlequin using the Markov chain exact probability test (Guo and Thompson 1992) with 100,000 iterations and 1000 dememorization steps. FSTAT was used to perform all tests described for the global population at each locus. Significance level for all pairwise comparisons was corrected using the Bonferroni method to maintain a type I error rate of $\alpha<0.05$ for all comparisons (Sokal and Rohlf 1995).

Linkage disequilibrium was calculated in Arlequin for each collection at each locus and globally in FSTAT to test for the presence of linkage between microsatellite loci. Probabilities of the presence of linkage were determined using the maximum-likelihood method (Slatkin and Excoffier 1996) with 10,000 permutations. Significance level was corrected using the Bonferroni method.

Allelic richness (A_R) and private alleles were calculated to assess allelic diversity within each locus. A_R measures the distribution of allele frequencies within the collection or population at each locus, and private alleles are those found in only one collection of a global data set (Hartl 2000). The M-ratio was used to test for the presence of a bottleneck within each locus then

averaged for all eight loci (Garza and Williamson 2001). A population bottleneck is a severe temporary reduction in population size, and can be detected by dividing the total number of alleles at each locus by the number of possible alleles of a given size range and averaged for seven or more loci.

AMOVA was used to calculate fixation indices for microsatellite data, denoted as F_{ST}. F_{ST} is a measure of allele frequency divergence between subpopulations. This value is analogous to Φ_{ST} and can be used to compare genetic structure between microsatellite and haplotype data. Arlequin was used to generate F_{ST} values for each collection using the pairwise method setting and tests of significance generated from 10,000 permutations. Global F_{ST} and significance was calculated using FSTAT 2.9.3.2 (Goudet 1995). Structure 2.3 was also used to (1) estimate population allele frequencies of microsatellite data by a model-based clustering method, (2) infer the presence of distinct populations in the Western Atlantic and (3) assign individuals to populations (Pritchard et al. 2000). Allele frequencies at each microsatellite locus were used to characterize an unknown number of discrete genetic populations, K, from the total data set of all samples at all loci. Once all K populations were defined, Structure assigned individuals to each K. Structure assumes that all populations are in Hardy-Weinberg equilibrium and linkage equilibrium. Structure was run using the admixture model with correlated allele frequencies, simulating K=1-15 with 350,000 Markov Chain Monte Carlo steps proceeding after a burn-in period of 15,000 steps. No a-priori information about sampling location was used in the simulation and 8 independent runs for each value of K were conducted to check for convergence.

Isolation-by-distance regression analysis

Regression analysis estimates the relationship of variable Y with variable X by expressing Y in terms of a linear function of X (Sokal and Rohlf 1995). Genetic isolation caused by physical distance can be inferred using this method. Fixation indices Φ_{ST} and F_{ST} were assigned as ordinate values and regressed over two sets of abscissa values: direct geographic distance and coastal geographic distance. Direct geographic distance was defined as the shortest distance between sampling locations. Coastal geographic distance was defined as the shortest path between sampling locations that avoided crossing deep ocean expanses. Distances were measured using Google Earth 5.0 based on linear distance and coastal path between GPS coordinates listed in the sampling methods (Hayes et al. 2003). Regression values and probabilities were calculated in Microsoft Excel 2007 using standard regression equations and significance ($\alpha<0.01$) described in Sokal and Rohlf (1995).

Results

A total of 580 sharks were sampled from 12 sites and assigned to 14 collections by geographic location and age class for data analyses (Table 1).

Genetic diversity: Mitochondrial loci

Control region sequences 1080 bp in length were obtained for 564 sharks and were composed of 31.6% adenine, 20.2% cytosine, 13.0% guanine, and 35.2% thymine. Eleven polymorphic sites consisted of 9 transitions and 2 insertion/deletions (indels) and resulted in 16 unique haplotypes, five of which were previously reported by Schultz et al. (2008) from GenBank Accession # FJ008700-FJ008704 (Table 2). Overall CR haplotype diversity, *h*, was 0.67618, and nucleotide diversity, π, was 0.00070. Partial ND2 sequences (650 bp) for 544 sharks were composed of 33.8% adenine, 29.5% cytosine, 9.2% guanine and 27.4% thymine. Eleven polymorphisms, all transition substitutions, defined nine unique haplotypes (Table 3). Overall ND2 *h* and π were 0.73798 and 0.00393 respectively. Composite sequences were constructed for 535 sharks. This composite sequence included the 22 polymorphic sites described for CR and ND2, and defined 38 unique haplotypes (Table 4). Overall *h* was 0.86424, and π was 0.00191. Sample size, number of haplotypes, sequence length, *h*, and π for each mitochondrial locus and for composite sequences of each collection are listed in Table 5.

Protein sequences of lemon shark ND2 haplotypes

One nucleotide polymorphism of the ND2 gene (base 51 of haplotype H6) resulted in an amino acid change in residue 46. A mutation from guanine to adenine in the second codon position produces histidine instead of arginine in the amino acid sequence. Haplotype H6 was only found in one adult shark from Jupiter and a subadult from the Everglades. These two samples were amplified and sequenced a second time to verify the nucleotide polymorphism. An additional mutation from thymine to cytosine in the first codon position of amino acid residue 234 between GenBank sequence U91418 and all of the haplotypes in our study (base 625) produces proline in our amino acid sequence instead of serine. All other nucleotide polymorphisms were synonymous.

Genetic diversity: Microsatellite loci

Genotypes of 523 lemon sharks were determined for eight microsatellite loci. Total number of alleles for each locus ranged from 5 to 22 with a mean of 13.5, and observed heterozygosities ranged from 0.488 to 0.976 with a mean of 0.785. Number of sharks analyzed and bottleneck tests (M-ratio) are listed for each collection and the global population in Table 6. Number of alleles (A), allelic richness (A_R), observed heterozygosity (H_O), expected heterozygosity (H_E) and number of private alleles (Private A) are listed for each locus and collection. Total number of alleles, mean A_R, H_O and H_E and number of private alleles are included for each locus of the total population (Table 6).

The M-ratio for the global population was greater than 0.68 (M-ratio=0.867) indicating that western Atlantic lemon sharks had not experienced a recent population bottleneck (Garza

and Williamson 2001). M-ratios for each of the 14 collections, however, were lower than the 0.68 threshold. This discrepancy can be attributed to two main factors. First, a significant proportion of rare alleles represented in < 2% of the global population were found in four loci (36% of alleles in LS22, 37% in LS30, 33% in LS75, and 25% in LS572). The abundance of rare or private alleles in only a few collections would decrease M-ratios for each site, but the global M-ratio would remain high. Second, historical gaps in the number of tandem repeats have been recorded in five out of the eight loci (Feldheim et al. 2001, 2002; DiBattista et al. 2008). These five loci (LS22, LS54, LS75, LS542 and LS572) are in violation of a key assumption for this model—microsatellite mutations are indels of only one repeat unit with no gaps (Garza and Williamson 2001). Gaps in the allele sequence would result in an underestimation of M-ratios (Garza and Williamson 2001). Once the gaps were removed and M-ratios re-calculated, only the U.S. Virgin Islands collection was below 0.68 indicating that this model may not be the most appropriate for my data set. In the case of U.S. Virgin Islands, limited sampling resulting in an incomplete representation of alleles is most likely the cause for its low M-ratio (0.405) with only 12 sharks analyzed. A minimum of 25 organisms should be sampled to evaluate a population bottleneck by this model (Garza and Williamson 2001).

Two collections deviated significantly from Hardy-Weinberg equilibrium at different loci (Cape Canaveral at LS22 and Atol das Rocas at LS560). All other collections and the global population were in equilibrium for each locus after Bonferroni correction (initial α=0.0005). Four out of 504 tests were significant for linkage disequilibrium after Bonferroni correction (initial α=0.0001): LS75/LS572 from Belize, LS22/LS596 and LS75/LS560 from Eleuthera, and LS542/LS572 from the juvenile Marquesas Key collection. No linkage disequilibrium was observed globally at any pair of loci.

Range-wide population structure

CR haplotype H02 was the most common in our study, found in each collection sampled and in 51% of all lemon sharks analyzed. Haplotype H01 was the next most common, found in 11 of the 14 collections and comprising 24% of all sharks. All other haplotypes were in rare in the global population (<7%) with private haplotypes found in the Bimini subadult population (H22), Eleuthera (H16 and H18), Gullivan Bay (H21), Jupiter (H20), and Louisiana (H13). Haplotype distribution by collection is listed in Table 7 for CR. Geographic distribution of CR haplotypes is shown in Figure 6 and haplotype network in Figure 7.

Three common haplotypes were found in ND2 sequences, each in approximately 29% of the sharks that were sampled (H1, H2 and H3). Haplotypes H1 and H2 were found in similar frequencies at each sample site, whereas haplotype H3 was mainly found in the Florida and Louisiana collections with the exception of Marquesas Key, where H3 was only found in one subadult. The remaining six haplotypes were all rare in the global population with private haplotypes occurring in Atol das Rocas (H8) and one shark from Jupiter (H9). All 44 sharks sampled from Atol das Rocas had the ND2 haplotype H8. No other haplotype displayed this type of geographic pattern, but Brazil is approximately 6,000 km from the majority of my sampling sites in Florida and the Bahamas. ND2 haplotype distribution by collection can be found in Table 8 and graphically in Figure 8 with haplotype network in Figure 9.

The two most common composite haplotypes reflect the distribution seen separately for each locus. Haplotypes H1-02 and H2-02 were found in 25% and 22% of the global population

respectively. The third most common haplotype (H3-01) was nearly exclusive to Florida collections with the exception again of Marquesas Key and was in 14% of all sharks analyzed. Haplotype H3-05 also followed this pattern at lower frequencies. Atol das Rocas had only two composite sequences (H8-01 and H8-02) and both were endemic to this site due to its private ND2 haplotype. Several sampling sites had rare haplotypes occurring at significantly higher frequencies than the rest of the population. For example, haplotypes H3-12 and H3-13 were common in Louisiana and extremely rare or undetected in other collections. Half of the U.S. Virgin Island collection (adults and juveniles) shared haplotype H2-12, which was otherwise found in only one subadult in Bimini. Haplotype H1-03 was exclusive to juveniles in Bimini, Eleuthera, and U.S. Virgin Islands. Adults from Jupiter (H3-14) and Tiger Beach (H1-17) each shared one rare haplotype with juveniles in Cape Canaveral. This pattern was also seen in one adult from Tiger Beach (H2-03), one subadult from Marquesas Key, and eight sharks from both age classes in Bimini. Composite haplotype distributions by collection are listed in Table 9 and displayed graphically in Figure 10 with the haplotype network in Figure 11.

I reject the null hypothesis of panmixia throughout the Western Atlantic in comparisons involving each mitochondrial locus alone and the composite sequence. Global AMOVA Φ_{ST} of the mitochondrial composite sequence (Φ_{ST} =0.293, p<0.000001; Table 10), for the coding ND2 (Φ_{ST} =0.293, p<0.000001) and non-coding CR (Φ_{ST} =0.278, p<0.000001) were all significantly different from zero and of a similar magnitude. Collections from Louisiana (Φ_{ST} range 0.273 to 0.873, p<0.000001) and Atol das Rocas (Φ_{ST} range 0.576 to 0.873, p<0.000001) exhibited consistently high pairwise Φ_{ST} values with all other collections using composite sequences (Table 11). The U.S. Virgin Islands exhibited significant pairwise Φ_{ST} values with most other collections, with the exception of those from Belize and the Bahamas (several of which were marginally non-significant). Belize exhibited significant pairwise Φ_{ST} values with all collections other than Marquesas Keys, the three collections in Bimini and Eleuthera (Bahamas) and the U.S. Virgin Islands. In contrast, global F_{ST} for microsatellites was low and insignificant (F_{ST} =0.014, p<0.088), and pairwise comparisons only detected significance between Atol das Rocas and all other collections (F_{ST} range 0.050 to 0.077, p<0.000001; Table 11). Bayesian cluster analysis of microsatellites run in Structure 2.3 also failed to detect population structure. The hypothesis that K=1 consistently exhibited a higher probability than K=2 to 15, and each individual was assigned to each of the assumed K populations in approximately equal proportions (i.e., apparently at random, Figure 12).

Delineation of matrilineal genetic structure within Florida and the Bahamas

There was clear evidence of population structure within Florida and the Bahamas from the composite haplotypes (Φ_{ST} = 0.136, p<0.000001). Collections separated into 2 sub-regions: peninsular Florida/Tiger Beach in the northern Bahamas and Marquesas Key (Florida Keys)/southwestern Bahamas. All pairwise Φ_{ST} values comparing collections between these sub-regions were significant. There was also evidence of population structure among collections in peninsular Florida, with the Everglades exhibiting significant pairwise Φ_{ST} values with Jupiter and Cape Canaveral, and Gullivan Bay exhibiting significant pairwise Φ_{ST} values with Cape Canaveral. Φ_{ST} for Florida and the Bahamas was only slightly higher for comparisons involving strictly juvenile collections (i.e., KS, MQJ, BIJ and EL; Φ_{ST} = 0.146, p <0.000001) than

comparisons between subadult/adult collections (i.e., BIS, MQS, EV, GB, JU and TB; Φ_{ST} = 0.128, p <0.000001).

Qualitative differences in population structure observed between ND2 and CR

Global Φ_{ST} values were significant and similar for ND2 (Φ_{ST} =0.293, p<0.000001) and CR (Φ_{ST} =0.278, p<0.000001); however, pairwise comparisons between these loci revealed differences in population structure (Table 12). Fixed differences were detected between Atol das Rocas and all collections for ND2, but no structure was seen in CR between Rocas and peninsular Florida sites (KS, JU, EV and GB). Structure was found in CR between western peninsular Florida collections (EV and GB) and southwestern Bahamas collections (BIJ, BIS and EL), but was absent in ND2. This same trend was seen in pairwise comparisons between the U.S. Virgin Islands and all three of the Belize and Bimini collections, where CR values were significant and ND2 values were not.

Correlation between genetic distance and geographic distance

Pairwise fixation indices of the composite sequence and microsatellites plotted against direct geographic distance (Figure 13A) and coastal distance (Figure 13B) detected significant associations of microsatellites with both direct distance (R^2=0.851, p=0.0095) and coastal distance (R^2=0.795, p=0.0031). Highly significant associations were also found in the mitochondrial composite sequences with direct (R^2=0.658, p<0.00001) and coastal (R^2=0.676, p<0.00001) distances. There was still a strong correlation between geographic distance and genetic distance when the most distant collection (Atol das Rocas) was removed (direct distance vs. Φ_{ST}, R^2 = 0.160, p<0.00005; coastal distance vs. Φ_{ST}, R^2 = 0.216, p<0.0004; direct distance vs. F_{ST}, R^2 = 0.027, p<0.00006; coastal distance vs. F_{ST}, R^2 = 0.004, p<0.00001). When all distant collections (BZ, LA, VI and RO) were removed regression statistics became non-significant for microsatellites (direct distance vs. F_{ST}, R^2 = 0.061, p=0.197; coastal distance vs. F_{ST}, R^2 = 0.014, p=0.016; Figures 14A and 14B). Values for mitochondrial sequences with coastal distance (R^2=0.011, p<0.004; Figure 14B) remained significant within the Florida and Bahamas collections, but were no longer significant with direct distance (R^2=0.011, p=0.014; Figure 14A).

Discussion

General description of range-wide genetic diversity and population structure

I examined the population genetic structure of lemon sharks throughout their western Atlantic distribution. They are partitioned into at least seven distinct subpopulations based on Exact tests for differentiation between collection pairs at mitochondrial loci (Table 13). This finding represents the largest number of genetically differentiated groups occurring within a continuous range yet documented in any shark species (Keeney et al. 2005, Duncan et al. 2006, Schultz et al. 2008, Chapman et al. 2009b, Portnoy et al. 2010, Shivji 2010, Benavides et al. 2011). Atol das Rocas (1), a small oceanic island off northeast Brazil, is the southernmost known nursery area for lemon sharks in the western Atlantic and is genetically distinct from all other collections at both the mitochondrial and nuclear loci. This indicates an absence of recent male or female mediated gene flow between this nursery and all of the other locations I examined. The collection from Louisiana (2) in the northern Gulf of Mexico was differentiated from all other collections, but only at the mitochondrial loci. The U.S. Virgin Islands (3) and Eleuthera, Bahamas (4) collections exhibited a similar pattern to Louisiana. Belize was differentiated from all other collections at the mitochondrial loci, except for the Marquesas Keys, Florida and Bimini, Bahamas collections. It therefore appears that lemon sharks exhibit the typical pattern for large coastal sharks in that populations separated by > 1,000-2,000 km are likely to be, but are not always, genetically differentiated at mitochondrial loci (Keeney et al. 2005, Duncan et al. 2006, Schultz et al. 2008, Chapman et al. 2009b, Portnoy et al. 2010, Shivji 2010, Benavides et al. 2011). These five collections are geographically connected by the Gulf Stream and will hereafter be referred to as the "GS" subpopulation (5). Atlantic coast Florida collections, Jupiter and Cape Canaveral, were significantly differentiated from all other collections at mitochondrial loci but not from each other, and hereafter referred to as the "East Peninsular Florida" (EPF) subpopulation (6). Finally, Gulf of Mexico coast Florida collections, Everglades and Gullivan Bay, were not significantly differentiated from each other but were to all other collections except for Tiger Beach, Bahamas at mitochondrial loci. I named this the "West Peninsular Florida-Tiger Beach" (WPF-TB) subpopulation (7). Tiger Beach was significantly differentiated from both GS and EPF despite its close proximity to Bimini, Bahamas (103 km) and Jupiter, Florida (152 km).

Local population structure and natal philopatry by females

Detecting population genetic structure in large migratory marine predators like sharks on a fine geographic scale (< 1000 km) is challenging for many reasons. First, these animals are difficult to sample. Researchers are commonly forced to pool individuals captured within a few hundred kilometers of one another to achieve adequate sample sizes for regional comparisons. Pooling then limits the opportunity to test for local population structure (e.g., Chapman et al. 2009b, Benavides et al. 2011). Local scale differentiation is also likely to be relatively weak given the sharks' mobility and therefore difficult to detect statistically, requiring high sample sizes and a large amount of DNA sequence information. Use of the mitochondrial CR, the most common genetic marker for shark population genetic studies, has previously been criticized for its lack of small-scale resolution in phylogeographic studies (DiBattista et al. 2008). I overcame

these obstacles by sampling a large number of individuals (average sample size of 41 sharks per collection) across a wide geographic range and by combining sequences from two polymorphic regions of the mitochondrial genome with eight nuclear microsatellites. I was able to examine local structure because 8 of the 12 sampling sites were within a 1,000 km diameter area, and the majority of sites in Florida in the Bahamas were less than 200 km from one another.

Genetic differentiation was detected at a geographic scale an order of magnitude smaller than reported in any previous population genetic study of a large migratory shark (i.e., between collections separated by hundreds rather than thousands of kilometers; Keeney et al. 2005, Duncan et al. 2006, Schultz et al. 2008, Chapman et al. 2009b, Portnoy et al. 2010, Shivji 2010, Benavides et al. 2011). I found there are at least three genetically differentiated groups of lemon sharks in Florida and the western Bahamas, all of which are based on significant differences in composite mitochondrial haplotype frequencies (i.e., via exact tests, Table 13) or significant pairwise fixation indices (i.e., via permutation tests, Table 11). The first differentiated group, EPF, exhibited significantly different composite haplotype frequencies to all other collections in the area and pairwise fixation indices that were significantly different from zero. The second group, WPF-TB, was also differentiated from EPF, and I suggest that additional work should look into the relationships between EPF and WPF-TB, employing both additional sampling locations (especially nursery areas, which should harbor the strongest signal of differentiation) and additional mitochondrial sequence data (e.g., ND4). The third distinct group in this region consisted of Marquesas Keys (Florida)/Bimini/Eleuthera based on pairwise fixation indices. This group was highly distinct from both EPF and WPF-TB, despite the close geographic proximity to them (as little as 100-200 km). I call them the "Florida Keys/Southwest Bahamas" group (FK-SWB). There is evidence of female-mediated gene flow between this group and Belize/U.S. Virgin Islands, although there were significant haplotype frequency differences between some of these collections.

I found that genetic distance between collections based on mitochondrial sequence data is highly correlated with the geographic distance between them. This was driven by large genetic distances between collections in Florida/Bahamas and more distant sites, including LA, BZ, VI and especially RO. In all locations I found that there was no difference how genetic distance correlated with direct and coastal geographic distance, which implies that the relatively narrow oceanic expanses separating some of these collections (e.g., the Florida straits) are not a physical barrier to female lemon sharks. The fact that distance influences female-mediated gene flow in lemon sharks suggests that straying by females most likely occurs between proximate breeding areas.

Local population genetic structure and isolation-by-distance observed in lemon sharks are consistent with three hypotheses pertaining to the movements of females: (a) they are sedentary (i.e., they settle and therefore breed in close proximity to their natal site), (b) there are physical barriers to dispersal that prevent them from using other potential nursery sites for parturition and (c) they frequently exhibit natal philopatry. There is already direct evidence of natal philopatry in this species. Long-term pedigree studies of lemon sharks conducted at Marquesas Key and Bimini identified long-term philopatry by gravid females to parturition sites (Feldheim et al. 2002, 2004; DiBattista et al. 2008). More recently, females tagged as neonates in Bimini in the mid-1990s have been recaptured pregnant, apparently returning to their natal site to give birth after many years at liberty (Feldheim, Gruber and Chapman, unpublished data). If this behavior has been reasonably common across many generations in the broader study area, it would

explain local population structure at maternally inherited loci. The competing hypotheses that local population structure is driven by sedentary behavior or physical barriers to dispersal are weak. There are potential barriers in this region, including the Florida Straits/Florida Current and large tracts of urbanized coastline in South Florida that may have fragmented lemon shark habitat. However, external tagging data (Kohler et al. 1998, Feldheim et al. 2001) and telemetry tracking (Kessel et al. unpublished) show frequent movements across these barriers (Figures 2 and 3). Thus, there are no physical barriers preventing females from giving birth in any of the nursery areas available to them. The competing hypothesis that lemon sharks are sedentary and use their natal nursery for parturition due to convenience (i.e., individuals "settle" in very close proximity to their natal area) is also flawed. Microsatellite genotyping in conjunction with lack of PIT tags from the annual census shows that nearly all (> 90%) of the subadult sharks (male and female) captured around Bimini originated from other nursery areas, demonstrating that they are mobile (Chapman et al. 2009a). Moreover, females only use Bimini for parturition and depart soon after for deeper reef habitats, indicating that they must repeatedly relocate nursery areas as opposed to never leaving them (Feldheim et al. unpublished, Figure 4). I therefore conclude that the local population structure observed in this region is more likely to be due to females and their offspring actively returning to their natal sites rather than sedentary behavior or the existence of physical barriers to dispersal.

Natal philopatry can be an advantageous reproductive strategy to large sharks that heavily invest in their progeny with small litter sizes and fully developed young (Keeney et al. 2005). Pregnant females could give their litters a higher chance of survival by giving birth to them in an environment with a history of successful recruitment (i.e., their own survival). This would also be true if high quality birthing grounds are scarce or spatially dispersed (DiBattista et al. 2008). The degree of natal philopatry may regulate the optimal level of inbreeding at a particular site if a female is leaving the parturition site to find mates elsewhere. Despite the benefits, DiBattista et al. (2008) and Feldheim et al. (2004) reported that only about half of the females using Bimini for parturition were definitely philopatric (i.e., the remaining females only gave birth at Bimini in one season). Although it is possible that some of these seemingly non-philopatric females may be those whose progeny were not sampled in subsequent years or females that experienced mortality prior to returning, these observations indicate that straying between nursery areas may occur. It is typical in most species that practice natal philopatry for some proportion of individuals to stray on occasion (e.g., Thorrold et al. 2001). Straying by adult female lemon sharks may, for example, represent relocation errors. Juvenile lemon sharks seem to possess an innate sense of direction relying on olfactory sensory cues in the water current, water depth gradient orientation and infrasound to relocate home ranges with high precision (Edrén and Gruber 2005), but the efficiency of this behavior in adults has yet to be studied. Females may also stray on occasion by choice. They may locate a non-natal nursery that is environmentally similar to their natal nursery and use it instead. Natal philopatry may also be condition-dependent, with poor condition individuals choosing to use proximate non-natal nursery areas for parturition rather than spending energy on relocating their natal area.

Male-biased dispersal

I found little to no genetic structure in nuclear microsatellite loci within western Atlantic lemon sharks, in sharp contrast to the highly significant structure (at least seven groups)

observed in mitochondrial loci. These findings indicate that male mediated gene flow in this region is substantially higher than female mediated gene flow and is congruent with observations that males rarely sire offspring in the same nursery area in different years (DiBattista et al. 2008, Feldheim et al. 2004). Isolation-by-distance was evident in the microsatellite data but only when the Atol das Rocas collection was included. This suggests that male-mediated gene flow is effective over all but the largest distances and is congruent with observations of males up to 1,000 km from the site of their birth (Feldheim et al. 2001, see Figure 2). Male-biased dispersal in lemon sharks might evolve to reduce mate competition or inbreeding (Handley and Perrin 2007). It is also possible that male-biased dispersal simply reflects the fact that it is far easier for males to stray and become reproductively integrated into distant populations than it is for females. Females are tied to coastal nursery areas for parturition and have to synchronize mating, gestation and parturition with the optimal time to release their offspring into an appropriate nursery environment. I suggest that this greatly reduces their ability to move between distant locations without compromising their reproductive fitness, at least in the short term. In contrast, males only need to move into a new area and locate females to be able to copulate and reproduce. Males can therefore be much more flexible about where they mate relative to their birthplace than females.

Comparison between coding and neutral mitochondrial loci

The existence of natal philopatry by females and of local population genetic structure also implies that it may be possible for sharks to exhibit local adaptation to the natal nursery area. This in turn could generate a selective positive feedback loop that enhances both local adaptation and natal philopatry over time. Although male-mediated dispersal (e.g., Schultz et al. 2008, Portnoy et al. 2010) may dampen local adaptation of nuclear genes it is possible that strong local selection could overwhelm modest levels of gene flow. Selection pressure for the heritable traits of length, mass and growth rate was evident in comparing juvenile lemon sharks between Bimini and Marquesas Key, where smaller size and lower growth rates were selected for in individuals from the Bimini nursery (DiBattista et al. 2008).

I detected nearly identical levels of genetic structure in the ND2 gene (Φ_{ST} =0.293, $p<0.000001$) and the non-coding CR (Φ_{ST} =0.278, $p<0.000001$) in lemon sharks. There were, however, some obvious qualitative differences in the patterns of population structure elucidated by the two markers. Most notably, Atol das Rocas lemon sharks had a private ND2 haplotype whereas they exhibited the same CR haplotypes as individuals sampled in Florida. Although differences in population structure revealed by coding and non-coding loci can be caused by divergent local selection, this cannot be occurring in this instance. Eight of the nine ND2 haplotypes in my study were characterized by silent mutations (i.e., ones that did not change the amino acid sequence of the protein), which indicate that selection was not acting on them directly. Qualitative differences in CR and ND2 population structure are probably due to the fact that both loci have relatively low nucleotide diversity and thus on their own do not have a lot of power for resolving subpopulations. I cannot, however, rule out that local adaptive divergence isn't at least partially responsible for the high degree of local population structure I observed overall. It is possible that there are mutations responding to local divergent selection elsewhere in the mitochondrial genome, possibly even in the unsequenced portion of ND2. The population genetic structure in the non-coding CR and the observed portion of the ND2 may be driven by

local selection for as yet unidentified mutations, and the neutral composite haplotypes I used as a marker are simply being carried along with them due to physical linkage. I suggest future studies could sequence the entire mitochondrial genome of lemon sharks from across my sampling range to see if there are any loci that may be responding to local selection and helping to drive the high degree of local population structure in this species.

Management implications

What are the management implications of my study for lemon sharks in the western Atlantic and for other coastal sharks with a similar life history pattern? I have demonstrated the existence of at least seven distinct genetic groups of lemon sharks in the Western Atlantic. Although these groups are not "stocks" in the classic fisheries sense due to a high degree of male-mediated genetic connectivity, they meet the criteria for being distinct "management units" (MUs, Moritz 1994). These groups represent adult females that are in a sense "anchored" to particular geographic areas for reproduction, which means they can be overfished in a local area as opposed to being replenished by range-wide mixing. This is especially true if females are targeted when they concentrate in the coastal zone to give birth. Mitochondrial sequence data suggests that straying females are not dispersing far enough from their natal sites to adequately replenish depleted stocks either. In addition, the high prevalence of rare alleles in nuclear loci leaves both males and females susceptible to fishing pressures. I conclude that recent decisions to protect lemon sharks in Florida state waters and to prohibit all shark fishing and export in the Bahamas are likely to enhance locally occurring lemon shark MUs by reducing mortality of adult females and juveniles in and around the nursery areas.

My findings also provide new support for the general hypothesis that rapid, localized collapse of coastal shark fisheries occurs because there is more localized population structure in coastal sharks than is traditionally thought to exist for large, mobile apex predators, due at least in part to natal philopatry (Hueter et al. 1998, Hueter et al. 2005). Given ever-increasing concern about the possibility of ecosystem change stemming from the removal of these apex predators (Heithaus et al. 2008), I suggest that such ecological perturbations may occur at a much more local geographic scale than resource managers are currently anticipating. I also suggest that individual countries may not need to wait for complex international regulations to be established for coastal shark conservation. To date, a truly comprehensive international response to the decline of coastal sharks has failed to materialize (e.g., the failure of many nations to draft a National Plan of Action for sharks as recommended by the Food and Agriculture Organization of the United Nations (FAO); the defeat of recent shark proposals at the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES)). Remote island nations and countries with long coastlines could employ local or national scale management actions in the interim to help conserve at least some of their coastal sharks. The overfishing of these apex predators has become a global environmental problem (Heithaus et al. 2008), but, like the conservation of other taxa that exhibit natal philopatry such as sea turtles and salmonids (Bowen et al. 2005, Neville et al. 2006), I suggest that local or national scale policies can be a significant part of the solution.

Future research directions

My study highlights the value of (a) sampling many locations, including proximate ones and (b) using multiple genetic markers, especially sequencing more of the mitochondrial genome than just the CR. I recommend that future shark population genetic studies should emulate this approach in order to better define local population structure and to delineate key barriers between differentiated populations. In particular I suggest that new DNA sequencing approaches (e.g., 454 sequencing, Ellegren 2008) should be used to conduct whole mitochondrial genome comparisons between collections. This will not only provide extremely high resolution for population genetic studies, but it will also elucidate the importance of local adaptive divergence in shaping the geographic distribution of different mitochondrial genomes. I hypothesize that large-scale mitogenome sequencing of neonate lemon sharks in the western Atlantic will resolve population structure on an even finer geographic scale (i.e., between adjacent nursery areas), thus providing even more compelling evidence of natal philopatry in this species.

My study has further resolved our understanding of the breeding biology of female sharks. Other than knowing that males are more dispersive than females, we still know very little about the breeding biology of male sharks. Natal philopatry is known to occur in male fish when both sexes home to their natal area for spawning. In viviparous species, like sharks, males need not home back to their natal nursery area because these areas are not necessarily where mating takes place. How male sharks locate mating grounds and how these areas relate to nursery areas are important questions. I hypothesize that regional mating grounds exist for large sharks and are used by females originating from many different nursery areas. Males exhibit philopatry to these areas, but their genes are dispersed over a wide area because the different females they fertilize then segregate into their own natal nursery areas. I suggest that surveys of stable carbon and oxygen isotope signatures between lemon sharks and localized primary producers could link males back to specific locations (Thorrold et al. 2001). Population studies based on nuclear coding genes like rhodopsin, heat shock proteins or the major histocompatibility complex, all associated with environmental variations, could delineate dispersal patterns in males or local adaptation in nuclear genes (Ebert and Andrew 2009, Hemmer-Hansen el al. 2007, Ohta et al. 2002).

Tables

Table 1: Number and age class of lemon sharks sampled for each collection ("Total") and number used for analysis of each genetic marker (ND2, CR, ND2-CR and Microsatellites). Each collection location is shown in Figure 1 and is assigned a code (in parentheses) that is used in all tables hereafter.

Sample Site	Age Class	ND2 mtDNA	CR mtDNA	ND2-CR mtDNA	Micro-satellites	Total
Bimini, Bahamas (BIJ)	JUV	49	55	48	56	56
Bimini, Bahamas (BIS)	SUB	42	49	42	49	49
Glover's Reef, Belize and Turneffe atoll, Belize (BZ)	SUB/ADU	36	36	36	37	42
Eleuthera, Bahamas (EL)	JUV	45	45	45	43	45
Everglades, Florida (EV)	SUB/ADU	49	59	49	47	59
Gullivan Bay, Florida (GB)	SUB	35	30	30	32	35
Jupiter, Florida (JU)	SUB/ADU	40	40	40	40	40
Cape Canaveral, Florida (KS)	JUV	42	43	42	28	43
Louisiana (LA)	JUV	41	40	40	37	42
Marquesas Key, Florida (MQJ)	JUV	41	40	40	41	41
Marquesas Key, Florida (MQS)	SUB	44	45	44	45	45
Atol das Rocas, Brazil (RO)	JUV	44	44	44	30	44
Tiger Beach, Bahamas (TB)	SUB/ADU	24	26	24	26	26
U.S. Virgin Islands (VI)	JUV/ADU	12	12	11	12	13
Total		**544**	**564**	**535**	**523**	**580**

Table 2: Polymorphic nucleotide positions for 16 lemon shark mitochondrial control region (CR) haplotypes. Haplotype designations are listed in the left column, and polymorphic nucleotide positions are listed across the top row. Indels are indicated with dashes (-).

CR	**Nucleotide Position**										
Haplotypes	**162**	**285**	**300**	**362**	**367**	**429**	**599**	**863**	**882**	**926**	**1074**
H01	T	T	T	C	T	C	T	C	-	-	G
H02	C	T	T	C	T	C	T	C	-	-	G
H03	C	T	T	C	T	C	T	C	-	T	G
H04	T	T	T	C	T	C	T	C	-	T	G
H05	T	T	T	T	T	C	T	C	-	-	G
H12	C	T	T	C	T	T	T	C	-	-	G
H13	T	T	T	C	T	T	T	C	-	-	G
H14	T	T	T	C	T	C	C	C	-	-	G
H15	T	T	T	C	C	C	T	C	-	-	G
H16	C	T	T	C	T	C	T	T	-	-	G
H17	C	C	T	C	T	C	T	C	-	-	G
H18	C	T	T	C	T	C	T	C	T	-	G
H19	C	T	T	T	T	C	T	C	-	-	G
H20	C	T	T	C	C	C	T	C	-	-	G
H21	C	T	T	C	T	C	T	C	-	T	A
H22	C	T	C	C	T	C	T	C	-	-	G

Table 3: Polymorphic nucleotide positions for 9 lemon shark mitochondrial ND2 haplotypes. Haplotype designations are listed in the left column, and polymorphic nucleotide positions are listed across the top row.

ND2	**Nucleotide Position**										
Haplotypes	**22**	**31**	**51**	**100**	**220**	**277**	**371**	**374**	**472**	**571**	**614**
H1	C	C	G	T	T	T	C	T	C	T	T
H2	T	T	G	C	T	T	T	T	T	T	T
H3	C	C	G	T	T	T	C	T	T	T	T
H4	C	C	G	T	T	C	C	T	C	T	T
H5	C	C	G	T	C	T	C	T	T	T	T
H6	C	C	A	T	T	T	C	T	T	T	T
H7	T	T	G	C	T	T	T	T	T	C	T
H8	T	T	G	C	T	T	T	C	T	T	T
H9	C	C	G	T	T	T	C	T	T	T	C

Table 4: Polymorphic nucleotide positions for 38 lemon shark haplotypes constructed from the composite mitochondrial ND2 and control region sequences (ND2-CR). Haplotype designations are listed in the left column, and polymorphic nucleotide positions are listed across the top row. Indels are indicated with dashes (-).

	Nucleotide Position																					
Composite ND2-CR Haplotypes	**22**	**31**	**51**	**100**	**220**	**277**	**371**	**374**	**472**	**571**	**614**	**812**	**935**	**950**	**1012**	**1017**	**1079**	**1249**	**1513**	**1532**	**1576**	**1724**
H1-01	C	C	G	T	T	T	C	T	C	T	T	T	T	T	C	T	C	T	C	-	-	G
H1-02	C	C	G	T	T	T	C	T	C	T	T	C	T	T	C	T	C	T	C	-	-	G
H1-03	C	C	G	T	T	T	C	T	C	T	T	C	T	T	C	T	C	T	C	-	T	G
H1-04	C	C	G	T	T	T	C	T	C	T	T	T	T	T	C	T	C	T	C	-	T	G
H1-17	C	C	G	T	T	T	C	T	C	T	T	C	C	T	C	T	C	T	C	-	-	G
H1-18	C	C	G	T	T	T	C	T	C	T	T	C	T	T	C	T	C	T	C	T	-	G
H2-01	T	T	G	C	T	T	T	T	T	T	T	T	T	T	C	T	C	T	C	-	-	G
H2-02	T	T	G	C	T	T	T	T	T	T	T	C	T	T	C	T	C	T	C	-	-	G
H2-03	T	T	G	C	T	T	T	T	T	T	T	C	T	T	C	T	C	T	C	-	T	G
H2-05	T	T	G	C	T	T	T	T	T	T	T	T	T	T	T	T	C	T	C	-	-	G
H2-12	T	T	G	C	T	T	T	T	T	T	T	C	T	T	C	T	T	T	C	-	-	G
H2-15	T	T	G	C	T	T	T	T	T	T	T	T	T	T	C	C	C	T	C	-	-	G
H2-16	T	T	G	C	T	T	T	T	T	T	T	C	T	T	C	T	C	T	T	-	-	G
H2-19	T	T	G	C	T	T	T	T	T	T	T	C	T	T	T	T	C	T	C	-	-	G
H2-22	T	T	G	C	T	T	T	T	T	T	T	C	T	C	C	T	C	T	C	-	-	G
H3-01	C	C	G	T	T	T	C	T	T	T	T	T	T	T	C	T	C	T	C	-	-	G
H3-02	C	C	G	T	T	T	C	T	T	T	T	C	T	T	C	T	C	T	C	-	-	G
H3-04	C	C	G	T	T	T	C	T	T	T	T	T	T	T	C	T	C	T	C	-	T	G
H3-05	C	C	G	T	T	T	C	T	T	T	T	T	T	T	T	T	C	T	C	-	-	G
H3-12	C	C	G	T	T	T	C	T	T	T	T	C	T	T	C	T	T	T	C	-	-	G
H3-13	C	C	G	T	T	T	C	T	T	T	T	T	T	T	C	T	T	T	C	-	-	G
H3-14	C	C	G	T	T	T	C	T	T	T	T	T	T	T	C	T	C	C	C	-	-	G
H3-15	C	C	G	T	T	T	C	T	T	T	T	T	T	T	C	C	C	T	C	-	-	G
H3-19	C	C	G	T	T	T	C	T	T	T	T	C	T	T	T	T	C	T	C	-	-	G
H3-20	C	C	G	T	T	T	C	T	T	T	T	C	T	T	C	C	C	T	C	-	-	G
H3-21	C	C	G	T	T	T	C	T	T	T	T	C	T	T	C	T	C	T	C	-	T	A
H4-01	C	C	G	T	T	C	C	T	C	T	T	T	T	T	C	T	C	T	C	-	-	G
H4-02	C	C	G	T	T	C	C	T	C	T	T	C	T	T	C	T	C	T	C	-	-	G
H4-03	C	C	G	T	T	C	C	T	C	T	T	C	T	T	C	T	C	T	C	-	T	G
H5-01	C	C	G	T	C	T	C	T	T	T	T	T	T	T	C	T	C	T	C	-	-	G
H5-02	C	C	G	T	C	T	C	T	T	T	T	C	T	T	C	T	C	T	C	-	-	G
H6-01	C	C	A	T	T	T	C	T	T	T	T	T	T	T	C	T	C	T	C	-	-	G
H6-02	C	C	A	T	T	T	C	T	T	T	T	C	T	T	C	T	C	T	C	-	-	G
H7-02	T	T	G	C	T	T	T	T	T	C	T	C	T	T	C	T	C	T	C	-	-	G
H7-03	T	T	G	C	T	T	T	T	T	C	T	C	T	T	C	T	C	T	C	-	T	G
H8-01	T	T	G	C	T	T	T	C	T	T	T	T	T	T	C	T	C	T	C	-	-	G
H8-02	T	T	G	C	T	T	T	C	T	T	T	C	T	T	C	T	C	T	C	-	-	G
H9-04	C	C	G	T	T	T	C	T	T	T	C	T	T	T	C	T	C	T	C	-	T	G

Table 5: Summary of haplotype data for the entire data set and each collection for composite (ND2-CR) haplotypes. Mitochondrial loci are listed in the far left column, and collections are listed in the second column. Refer to Table 1 for collection abbreviations. N= number of individuals analyzed for each mitochondrial locus; Haplotypes= number of haplotypes; Seq Length= number of base pairs in the haplotype sequence; *h*= haplotype diversity; π= nucleotide diversity.

Locus	**Collection**	**N**	**Haplotypes**	**Seq Length**	***h***	***π***
ND2	Total	544	9	650	0.73798	0.00393
CR	Total	564	16	1080	0.67618	0.00070
Composite ND2-CR	Total	535	38	1730	0.86424	0.00191
	BIJ	48	8	1730	0.67908	0.00158
	BIS	42	11	1730	0.80139	0.00173
	BZ	36	5	1730	0.61587	0.00161
	EL	45	7	1730	0.75758	0.00157
	EV	49	16	1730	0.90136	0.00181
	GB	30	11	1730	0.87126	0.00156
	JU	40	11	1730	0.77821	0.00119
	KS	42	6	1730	0.58188	0.00086
	LA	40	4	1730	0.69615	0.00076
	MQJ	40	6	1730	0.64872	0.00163
	MQS	44	9	1730	0.73890	0.00176
	RO	44	2	1730	0.40592	0.00023
	TB	24	8	1730	0.79710	0.00155
	VI	11	4	1730	0.74545	0.00158

Table 6: Summary of microsatellite data for each collection and the entire data set. N= number of individuals analyzed for each collection; M-ratio= population bottleneck test for each collection; A= number of microsatellite alleles at each locus; H_O= observed microsatellite heterozygosity; H_E= expected microsatellite heterozygosity; A_R= allelic richness; Private A= number of private microsatellite alleles. Values marked with * deviate from Hardy-Weinberg expectations after correction of α using the Bonferroni method.

		Microsatellite Loci							
Collection	**Parameter**	**LS22**	**LS30**	**LS54**	**LS75**	**LS542**	**LS560**	**LS572**	**LS596**
BIJ	A	16	10	4	5	10	9	6	11
(N=56)	H_O	0.911	0.696	0.536	0.768	0.804	0.875	0.679	0.893
M-ratio=0.614	H_E	0.899	0.747	0.561	0.736	0.801	0.835	0.726	0.881
	A_R	9.992	6.759	3.181	4.341	6.394	6.808	4.828	8.588
	Private A	0	0	0	0	0	0	0	0
BIS	A	15	11	4	4	8	9	7	10
(N=49)	H_O	0.918	0.816	0.673	0.653	0.735	0.776	0.714	0.898
M-ratio=0.585	H_E	0.891	0.814	0.585	0.702	0.729	0.849	0.727	0.877
	A_R	9.310	7.491	3.818	3.923	5.587	7.236	5.132	8.116
	Private A	0	0	0	0	0	0	0	0
BZ	A	14	10	4	4	13	11	8	9
(N=37)	H_O	0.970	0.919	0.743	0.622	0.757	0.919	0.730	0.943
M-ratio=0.632	H_E	0.895	0.825	0.660	0.724	0.837	0.879	0.774	0.838
	A_R	9.785	7.07	3.682	3.986	7.66	8.408	5.946	7.442
	Private A	0	1	0	0	0	1	0	0
EL	A	14	10	4	6	12	9	6	11
(N=43)	H_O	0.974	0.767	0.721	0.907	0.765	0.814	0.941	0.837
M-ratio=0.634	H_E	0.879	0.735	0.679	0.748	0.845	0.840	0.744	0.866
	A_R	9.219	6.465	3.7	4.863	7.979	7.446	4.82	8.321
	Private A	0	0	0	1	2	0	0	0
EV	A	15	10	4	4	9	10	6	12
(N=47)	H_O	0.878	0.617	0.723	0.745	0.652	0.891	0.848	0.936
M-ratio=0.606	H_E	0.888	0.762	0.647	0.723	0.686	0.877	0.733	0.877
	A_R	9.52	6.556	3.55	3.974	5.546	7.872	4.88	8.748
	Private A	0	0	0	0	0	0	0	0
GB	A	11	10	4	4	9	8	5	9
(N=32)	H_O	0.793	0.875	0.594	0.688	0.750	0.935	0.875	0.875
M-ratio=0.525	H_E	0.875	0.829	0.674	0.749	0.737	0.872	0.734	0.851
	A_R	8.672	7.342	3.343	3.993	6.23	7.348	4.314	7.372
	Private A	0	0	0	0	0	0	0	0
JU	A	13	12	4	4	8	8	6	11
(N=40)	H_O	0.949	0.825	0.700	0.750	0.700	0.925	0.650	0.925
M-ratio=0.568	H_E	0.858	0.817	0.650	0.751	0.724	0.859	0.699	0.872
	A_R	8.683	6.804	3.475	3.995	5.51	7.237	4.826	8.263
	Private A	0	0	0	0	0	0	0	0

Table 6: Continued

Collection	Parameter	Microsatellite Loci							
		LS22	**LS30**	**LS54**	**LS75**	**LS542**	**LS560**	**LS572**	**LS596**
KS	A	13	9	4	5	6	8	7	10
(N=28)	H_O	0.565*	0.846	0.750	0.714	0.739	0.800	0.857	0.857
M-ratio=0.554	H_E	0.879	0.775	0.683	0.768	0.691	0.879	0.804	0.892
	A_R	9.556	6.827	3.873	4.778	5.132	7.655	5.891	8.476
	Private A	0	0	0	0	0	0	0	0
LA	A	13	10	4	4	7	10	5	11
(N=37)	H_O	0.946	0.719	0.649	0.889	0.730	0.917	0.784	0.784
M-ratio=0.561	H_E	0.897	0.774	0.652	0.739	0.770	0.863	0.737	0.856
	A_R	9.574	7.238	3.507	3.994	5.846	7.6	4.457	7.723
	Private A	0	0	0	0	0	0	0	0
MQJ	A	13	14	3	6	7	9	6	11
(N=41)	H_O	0.902	0.829	0.488	0.829	0.634	0.829	0.780	0.976
M-ratio=0.598	H_E	0.872	0.845	0.587	0.710	0.638	0.860	0.760	0.885
	A_R	9.287	8.217	2.995	4.665	5.131	7.388	4.861	8.441
	Private A	0	1	0	0	0	0	0	0
MQS	A	16	14	4	4	7	9	7	12
(N=45)	H_O	0.933	0.867	0.533	0.756	0.711	0.822	0.844	0.867
M-ratio=0.618	H_E	0.908	0.834	0.596	0.724	0.699	0.831	0.721	0.886
	A_R	10.592	8.358	3.565	3.974	5.95	6.946	5.014	8.934
	Private A	0	0	0	0	0	1	0	0
RO	A	10	8	5	4	8	9	4	11
(N=30)	H_O	0.700	0.714	0.567	0.533	0.767	0.800*	0.793	0.929
M-ratio=0.536	H_E	0.851	0.752	0.558	0.555	0.739	0.866	0.735	0.844
	A_R	8.122	6.344	4.084	3.736	6.377	7.44	3.99	8.192
	Private A	0	0	1	0	0	0	0	1
TB	A	13	9	4	4	7	9	5	9
(N=26)	H_O	0.920	0.640	0.731	0.577	0.692	0.808	0.808	0.885
M-ratio=0.525	H_E	0.885	0.749	0.639	0.579	0.718	0.857	0.777	0.865
	A_R	9.897	6.392	3.668	3.883	5.448	7.668	4.811	7.44
	Private A	0	0	0	0	0	0	0	0
VI	A	8	6	3	4	4	7	5	7
(N=12)	H_O	0.750	0.833	0.750	0.750	0.583	0.750	0.818	0.909
M-ratio=0.405	H_E	0.870	0.822	0.692	0.721	0.562	0.826	0.684	0.853
	A_R	7.826	5.993	3.000	3.917	3.913	6.830	5.000	7.000
	Private A	0	0	0	0	0	0	0	0
Total	A	22	21	5	7	16	12	8	13
(N=523)	Mean H_O	0.865	0.783	0.654	0.727	0.716	0.847	0.794	0.894
M-ratio=0.867	Mean H_E	0.882	0.792	0.633	0.709	0.727	0.856	0.738	0.866
	Mean A_R	9.813	7.246	3.585	4.339	6.240	7.594	5.007	8.413
	Private A	0	2	1	1	2	2	0	1

Table 7: Geographic distribution of lemon shark mitochondrial control region (CR) haplotypes. Haplotype designations are listed in the left column and collections are listed across the top row. See Table 1 for names associated with abbreviations.

CR	**Collection location**													
Haplotype	**BIJ**	**BIS**	**BZ**	**EL**	**EV**	**GB**	**JU**	**KS**	**LA**	**MQJ**	**MQS**	**RO**	**TB**	**VI**
H01	2	2	3	3	22	14	19	26	0	0	2	32	13	0
H02	40	29	33	30	19	7	11	12	13	34	33	12	7	5
H03	7	11	0	10	0	0	0	0	0	5	3	0	2	2
H04	3	3	0	0	3	0	1	0	0	1	2	0	0	0
H05	2	0	0	0	11	3	1	0	0	0	2	0	3	0
H12	1	0	0	0	0	3	0	0	15	0	0	0	0	5
H13	0	0	0	0	0	0	0	0	12	0	0	0	0	0
H14	0	0	0	0	0	0	5	1	0	0	0	0	0	0
H15	0	0	0	0	3	0	1	0	0	0	3	0	0	0
H16	0	0	0	1	0	0	0	0	0	0	0	0	0	0
H17	0	0	0	0	0	0	0	4	0	0	0	0	1	0
H18	0	0	0	1	0	0	0	0	0	0	0	0	0	0
H19	0	0	0	0	1	2	1	0	0	0	0	0	0	0
H20	0	0	0	0	0	0	1	0	0	0	0	0	0	0
H21	0	0	0	0	0	1	0	0	0	0	0	0	0	0
H22	0	4	0	0	0	0	0	0	0	0	0	0	0	0

Table 8: Geographic distribution of lemon shark mitochondrial ND2 haplotypes. Haplotype designations are listed in the left column and collections are listed across the top row. See Table 1 for names associated with abbreviations.

ND2	**Collection location**													
Haplo	**BIJ**	**BIS**	**BZ**	**EL**	**EV**	**GB**	**JU**	**KS**	**LA**	**MQJ**	**MQS**	**RO**	**TB**	**VI**
H1	27	14	17	22	3	2	7	12	13	22	19	0	2	3
H2	19	23	17	21	19	8	4	2	0	13	16	0	7	9
H3	2	1	1	2	23	21	26	28	28	1	8	0	15	0
H4	1	3	1	0	1	0	0	0	0	0	0	0	0	0
H5	0	0	0	0	2	4	1	0	0	0	0	0	0	0
H6	0	0	0	0	1	0	1	0	0	0	0	0	0	0
H7	0	1	0	0	0	0	0	0	0	5	1	0	0	0
H8	0	0	0	0	0	0	0	0	0	0	0	44	0	0
H9	0	0	0	0	0	0	1	0	0	0	0	0	0	0

Table 9: Geographic distribution of lemon shark haplotypes constructed from the composite mitochondrial ND2 and control region sequences (ND2-CR). Haplotype designations are listed in the left column and collections are listed across the top row. See Table 1 for names associated with abbreviations.

ND2-CR	**Collection location**													
Haplotype	**BIJ**	**BIS**	**BZ**	**EL**	**EV**	**GB**	**JU**	**KS**	**LA**	**MQJ**	**MQS**	**RO**	**TB**	**VI**
H1-01	0	0	0	0	0	1	0	0	0	0	0	0	0	0
H1-02	24	11	17	11	2	1	7	8	12	20	17	0	1	1
H1-03	1	1	0	10	0	0	0	0	0	1	0	0	0	2
H1-04	2	2	0	0	1	0	0	0	0	1	2	0	0	0
H1-17	0	0	0	0	0	0	0	4	0	0	0	0	1	0
H1-18	0	0	0	1	0	0	0	0	0	0	0	0	0	0
H2-01	0	1	2	3	6	1	0	0	0	0	0	0	2	0
H2-02	13	15	15	17	9	5	4	2	0	13	15	0	4	3
H2-03	4	4	0	0	0	0	0	0	0	0	1	0	1	0
H2-05	0	0	0	0	2	0	0	0	0	0	0	0	0	0
H2-12	1	0	0	0	0	0	0	0	0	0	0	0	0	5
H2-15	0	0	0	0	1	0	0	0	0	0	0	0	0	0
H2-16	0	0	0	1	0	0	0	0	0	0	0	0	0	0
H2-19	0	0	0	0	1	0	0	0	0	0	0	0	0	0
H2-22	0	3	0	0	0	0	0	0	0	0	0	0	0	0
H3-01	0	1	1	0	10	9	17	26	0	0	2	0	10	0
H3-02	0	0	0	2	4	1	0	1	1	0	1	0	2	0
H3-04	0	0	0	0	2	0	0	0	0	0	0	0	0	0
H3-05	2	0	0	0	5	3	1	0	0	0	2	0	3	0
H3-12	0	0	0	0	0	3	0	0	15	0	0	0	0	0
H3-13	0	0	0	0	0	0	0	0	12	0	0	0	0	0
H3-14	0	0	0	0	0	0	5	1	0	0	0	0	0	0
H3-15	0	0	0	0	2	0	1	0	0	0	3	0	0	0
H3-19	0	0	0	0	0	2	1	0	0	0	0	0	0	0
H3-20	0	0	0	0	0	0	1	0	0	0	0	0	0	0
H3-21	0	0	0	0	0	1	0	0	0	0	0	0	0	0
H4-01	0	0	0	0	1	0	0	0	0	0	0	0	0	0
H4-02	0	1	1	0	0	0	0	0	0	0	0	0	0	0
H4-03	1	2	0	0	0	0	0	0	0	0	0	0	0	0
H5-01	0	0	0	0	1	3	1	0	0	0	0	0	0	0
H5-02	0	0	0	0	1	0	0	0	0	0	0	0	0	0
H6-01	0	0	0	0	0	0	1	0	0	0	0	0	0	0
H6-02	0	0	0	0	1	0	0	0	0	0	0	0	0	0
H7-02	0	0	0	0	0	0	0	0	0	1	0	0	0	0
H7-03	0	1	0	0	0	0	0	0	0	4	1	0	0	0
H8-01	0	0	0	0	0	0	0	0	0	0	0	32	0	0
H8-02	0	0	0	0	0	0	0	0	0	0	0	12	0	0
H9-04	0	0	0	0	0	0	1	0	0	0	0	0	0	0

Table 10: Global analysis of molecular variance (AMOVA). AMOVA statistics within and among the global population, and global fixation indices for the composite mitochondrial sequence (ND2-CR), ND2 and control region (CR).

Φ-Statistics ND2-CR Source of Variation	**d.f.**	**Sum of Squares**	**Variance Components**	**Percentage of Variation**
Among Populations	13	267.29	0.50855	29.29
Within Populations	521	639.641	1.22772	70.71
Total	534	906.931	1.73627	
Fixation Index (Φ_{ST})	0.29290, p<0.000001			
Φ-Statistics ND2 Source of Variation	**d.f.**	**Sum of Squares**	**Variance Components**	**Percentage of Variation**
Among Populations	13	206.1	0.38648	29.26
Within Populations	529	494.311	0.93443	70.74
Total	542	700.411	1.3209	
Fixation Index (Φ_{ST})	0.29259, p<0.000001			
Φ-Statistics CR Source of Variation	**d.f.**	**Sum of Squares**	**Variance Components**	**Percentage of Variation**
Among Populations	13	64.779	0.11688	27.8
Within Populations	550	166.958	0.30356	72.2
Total	563	231.737	0.42044	
Fixation Index (Φ_{ST})	0.27800, p<0.000001			

Table 11: Pairwise Φ_{ST} comparisons for composite sequences and microsatellites. Φ_{ST} values above the diagonal are for mitochondrial composite sequences using the Tamura & Nei distance method, and F_{ST} values below the diagonal are for microsatellite loci using the pairwise difference distance method. Values marked with * are significant after correction of α using the Bonferroni method.

	BZ	**BIJ**	**BIS**	**EL**	**EV**	**GB**	**JU**	**KS**	**LA**	**MQJ**	**MQS**	**RO**	**TB**	**VI**
BZ	-	-0.00727	-0.00646	-0.02198	0.13533	0.19984*	0.28820*	0.33268*	0.40844*	-0.01806	-0.00586	0.65155*	0.15936	0.11581
BIJ	0.00282	-	0.04455	-0.00395	0.14941*	0.17658*	0.24444*	0.26963*	0.33936*	-0.00659	-0.00650	0.67015*	0.15411	0.18953
BIS	0.00429	0.00063	-	-0.00101	0.14060*	0.23203*	0.33017*	0.38109*	0.44937*	0.01165	0.03100	0.57649*	0.17939	0.05525
EL	0.00118	0.00186	0.00748	-	0.12649*	0.17484*	0.25378*	0.28785*	0.35315*	-0.01387	-0.00280	0.58148*	0.13763	0.09235
EV	0.00992	0.00512	0.00051	0.00725	-	0.02812	0.10562	0.17304*	0.34136*	0.16387*	0.07674	0.57738*	0.57738	0.24079
GB	0.00804	0.01015	0.00783	0.00687	0.00033	-	0.01913	0.06967	0.24017*	0.21615*	0.11126	0.73176*	-0.01466	0.35364*
JU	0.00469	0.00616	0.00471	0.00981	0.00172	-0.00252	-	0.00420	0.27307*	0.29760*	0.18209*	0.78263*	0.04559	0.49492*
KS	0.00337	0.00478	-0.00051	0.00454	-0.00100	-0.00413	0.00003	-	0.28481*	0.33474*	0.21807*	0.83519*	0.10982	0.57981*
LA	0.00632	0.00251	0.00359	0.00301	-0.00235	-0.00421	0.00222	-0.00190	-	0.39913*	0.31805*	0.87281*	0.33228*	0.57525*
MQJ	0.00716	0.00669	0.00030	0.00904	-0.00108	0.00123	-0.00064	-0.00043	0.00539	-	0.00327	0.65852*	0.18270	0.13742
MQS	0.00825	0.00487	-0.00193	0.01072	0.00026	0.00633	0.00438	0.00119	0.00275	0.00066	-	0.63058*	0.07979	0.16238
RO	0.04978*	0.06662*	0.07347*	0.05331*	0.07229*	0.05749*	0.0629*	0.06638*	0.06412*	0.07734*	0.07799*	-	0.71502*	0.70961*
TB	0.01311	0.00855	0.00944	0.00567	0.00186	0.01262	0.01195	0.00933	0.00203	0.00385	0.00666	0.06005*	-	0.31362
VI	0.01006	0.01545	0.00904	0.01422	0.00315	-0.00378	0.00075	-0.00043	0.00828	0.00248	0.00724	0.06666*	0.01353	-

Table 12: Pairwise Φ_{ST} comparisons for ND2 and control region (CR) sequences using the Tamura & Nei distance method. Φ_{ST} values above the diagonal are for mitochondrial ND2 sequences, and Φ_{ST} values below the diagonal are for mitochondrial control region (CR) sequences. Values marked with * are significant after correction of α using the Bonferroni method.

	BZ	**BIJ**	**BIS**	**EL**	**EV**	**GB**	**JU**	**KS**	**LA**	**MQJ**	**MQS**	**RO**	**TB**	**VI**
BZ	-	-0.01094	-0.01077	-0.02523	0.05693	0.14765	0.24639*	0.30669*	0.30669*	-0.01858	-0.01161	0.66552*	0.08642	0.08619
BIJ	-0.00950	-	0.03389	-0.00857	0.07486	0.12835	0.12835*	0.22844*	0.28783*	-0.01215	-0.01360	0.68202*	0.08086	0.17379
BIS	0.00260	0.00729	-	-0.00556	0.06836	0.18908	0.30895*	0.37594*	0.44285*	0.01019	0.02540	0.60001*	0.11968	0.01548
EL	-0.00893	0.00366	0.00550	-	0.05527	0.14229	0.23615*	0.29217*	0.35794*	-0.01602	-0.01094	0.64611*	0.08283	0.09240
EV	0.37374*	0.33019*	0.35546*	0.29198*	-	0.02745	0.14527	0.23053*	0.30536*	0.06651	0.03016	0.67693*	-0.01279	0.16557
GB	0.31251*	0.25758*	0.28764*	0.20729*	0.00045	-	0.03335	0.11744	0.19946*	0.13862	0.08268	0.81255*	-0.01913	0.35981*
JU	0.40110*	0.35437*	0.37249*	0.28472*	0.02331	0.02789	-	0.00121	0.04972	0.21915*	0.15775	0.88379*	0.07063	0.54161*
KS	0.39402*	0.33725*	0.35680*	0.26675*	0.04519	0.03232	0.00944	-	-0.00779	0.26858*	0.20996*	0.92475*	0.16962	0.64535*
LA	0.47578*	0.44456*	0.45637*	0.34250*	0.40978*	0.29761*	0.40520*	0.41350*	-	0.33167*	0.27648*	0.96019*	0.27343*	0.73750*
MQJ	0.00739	0.03359	0.03584	-0.00300	0.44350*	0.40059*	0.48423*	0.48473*	0.52769*	-	-0.01160	0.65657*	0.08394	0.11655
MQS	0.02820	0.00354	0.02991	0.02125	0.23302*	0.16231	0.24437*	0.22973*	0.39419*	0.08738	-	0.68235*	0.03500	0.15364
RO	0.58050*	0.49174*	0.51218*	0.37458*	0.04860	0.06478	0.01313	0.01155	0.49679*	0.67323*	0.36554*	-	0.82055*	0.75534*
TB	0.40497*	0.32858*	0.35527*	0.24132*	-0.01152	-0.01716	0.00113	-0.00760	0.39253*	0.51224*	0.20718*	0.01913	-	0.27768
VI	0.40398*	0.30059*	0.31340*	0.13088	0.42617*	0.30525*	0.43344*	0.44997*	0.12363	0.51583	0.26247	0.61219*	0.42700*	-

Table 13: Exact tests for differentiation between pairs of collections based on composite CR-ND2 haplotype frequencies. * = Pair is significantly differentiated after Bonferroni correction (α''=0.05, initial α=0.0005), NS= not significant.

BZ	BZ													
BIJ	NS	BIJ												
BIS	NS	NS	BIS											
EL	*	*	*	EL										
EV	*	*	*	*	EV									
GB	*	*	*	*	NS	GB								
JU	*	*	*	*	*	*	JU							
KS	*	*	*	*	*	*	NS	KS						
LA	*	*	*	*	*	*	*	*	LA					
MQJ	NS	NS	*	*	*	*	*	*	*	MQJ				
MQS	NS	NS	NS	*	*	*	*	*	*	NS	MQS			
RO	*	*	*	*	*	*	*	*	*	*	*	RO		
TB	*	*	*	*	NS	NS	*	*	*	*	*	*	TB	
VI	*	*	*	*	*	*	*	*	*	*	*	*	*	VI

Figures

Figure 1: Western Atlantic distribution of lemon sharks, showing sampling locations for the current study (blue dots). Numbers represent collections: 1=BIJ and BIS, 2=BZ, 3=EL, 4=EV, 5=GB, 6=JU, 7=KS, 8=LA, 9=MQJ and MQS, 10=RO, 11=TB and 12=VI. See Table 1 for names associated with abbreviations. Photo inset (A) is a juvenile lemon shark, *Negaprion brevirostris*. Inset (B) is a map of the global distribution of the lemon shark with a frame around the location of the magnified sampling range. The map in inset (C) indentifies South American sampling site Atol das Rocas, Brazil with a frame around the location of the magnified sampling range.

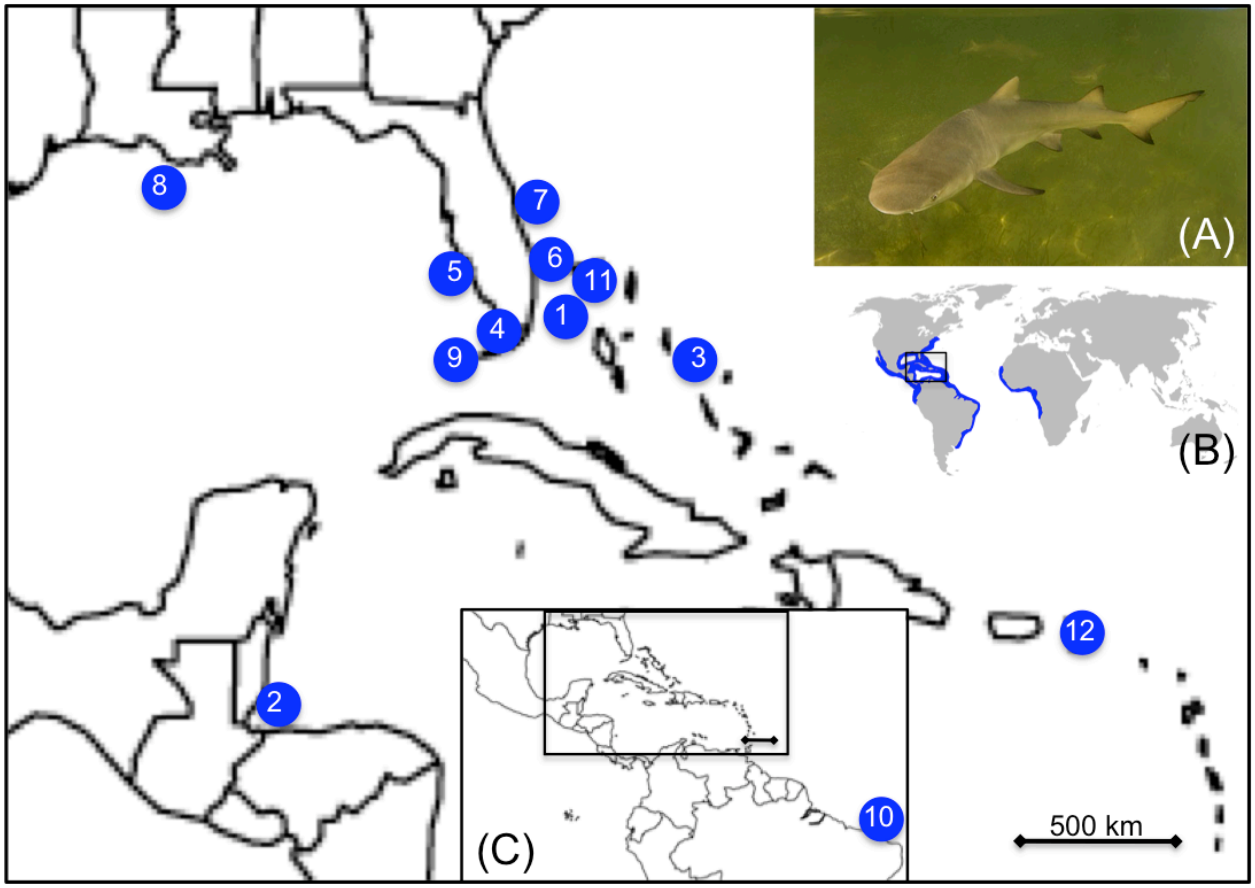


Figure 2: Movements of lemon sharks in Florida and the Western Bahamas based on external tags and acoustic transmitters. 1= Movement of a male lemon shark from its birthplace in Bimini, Bahamas (BI) to the Florida panhandle, where it was recaptured as an adult (Feldheim et al. 2001). 2= Movement by tagged juvenile lemon shark across the Gulf Stream from Bimini to the Florida Keys. Grey boxes show the number of adult female lemon sharks fitted with acoustic transmitters in Jupiter, Florida (JU) that were later detected in Georgia and South Carolina and Cape Canaveral (CC). Remaining repcapture locations show movements of sharks from JU and BI documented by satellite tags (data from Kessel et al. unpublished).

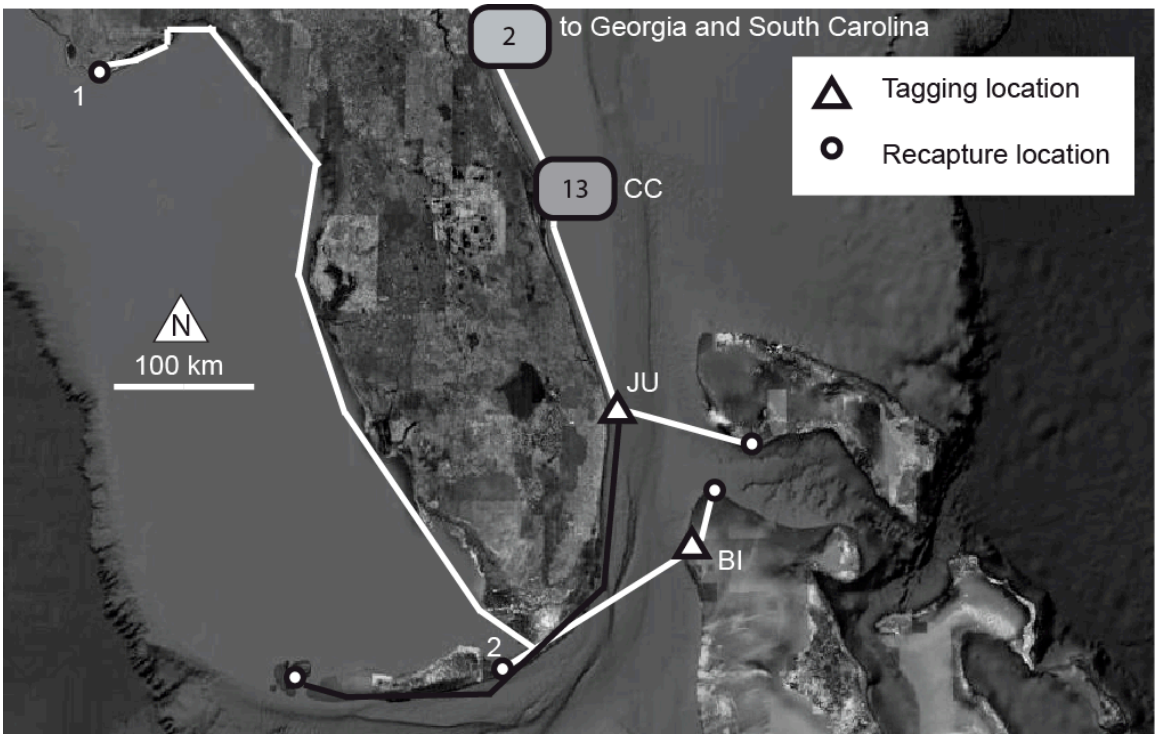


Figure 3: Movements of a satellite-tagged postpartum female lemon shark that was captured while giving birth at Bimini. The solid black triangle represents the date the shark was tagged, and the open triangle represents the date the transmitter detached from the shark and started transmitting depth data. Each small solid triangle represents a depth reading. Large grey inverted triangles indicate periods where the shark dove below 20 m. Inset shows the position of the shark at release (solid triangle) and at transmitter detachment (open triangle). Data from Feldheim et al. (unpublished).

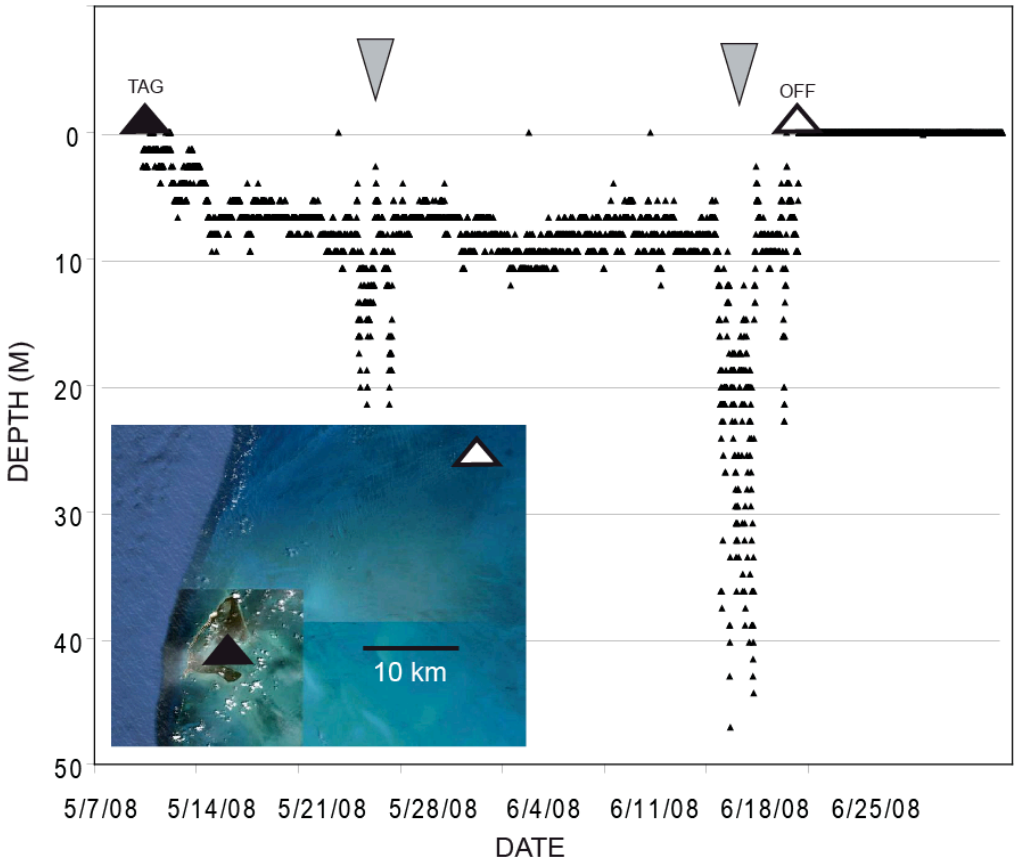


Figure 4: Monthly occurrence of adult female lemon sharks captured off Bimini, Bahamas from 1992-2010. Grey shaded months are those where neonate lemon sharks (defined as having an open or partially open umbilicus) are encountered. Data from Feldheim et al. (unpublished).

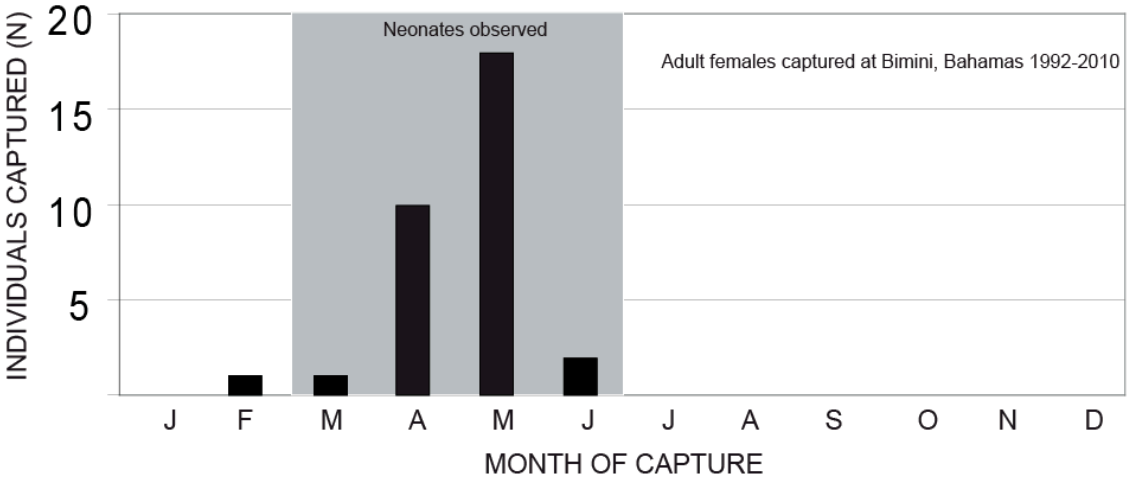


Figure 5: Length-frequency histogram of lemon sharks by age class. 'JUV' represents young-of-the-year and juveniles < 100cm, 'SUB' represents subadult sexually immature sharks from 100 cm to 200 cm. 'ADU' represents adults > 230cm.

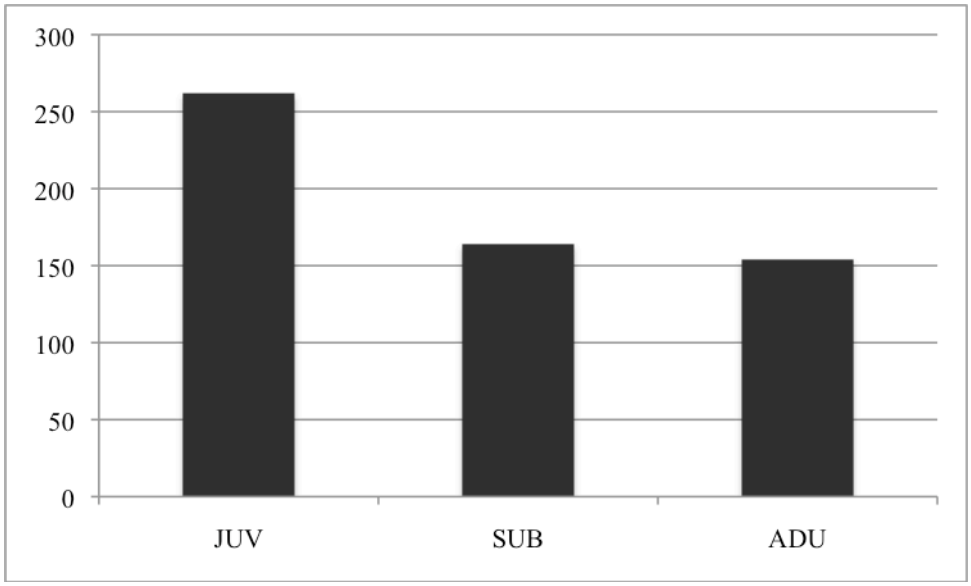


Figure 6: Distribution of mitochondrial control region (CR) haplotypes. Pie charts show the frequency of CR haplotypes in each collection. Haplotype color designations are listed in the key. For haplotype information refer to Tables 2 and 7. Collection abbreviation and number of individuals analyzed are noted in each pie chart. See Table 1 for names associated with abbreviations.

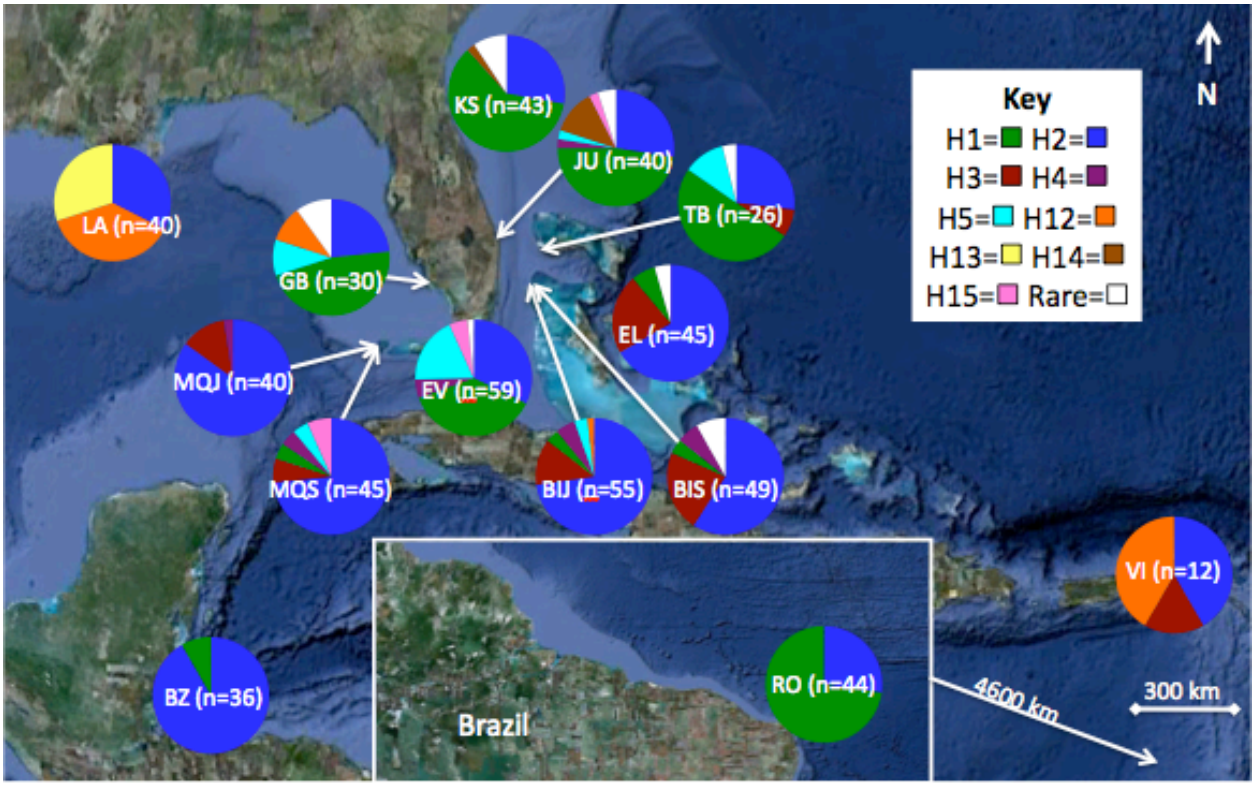


Figure 7: Mitochondrial control region (CR) haplotype network (95% confidence) with individual haplotype designations noted in each circle. For haplotype information, refer to Tables 2 and 7. Circle sizes are proportional to the frequency of haplotypes in the global population. Colors within each circle represent the 14 collections sampled as shown in the figure key. See Table 1 for names associated with abbreviations.

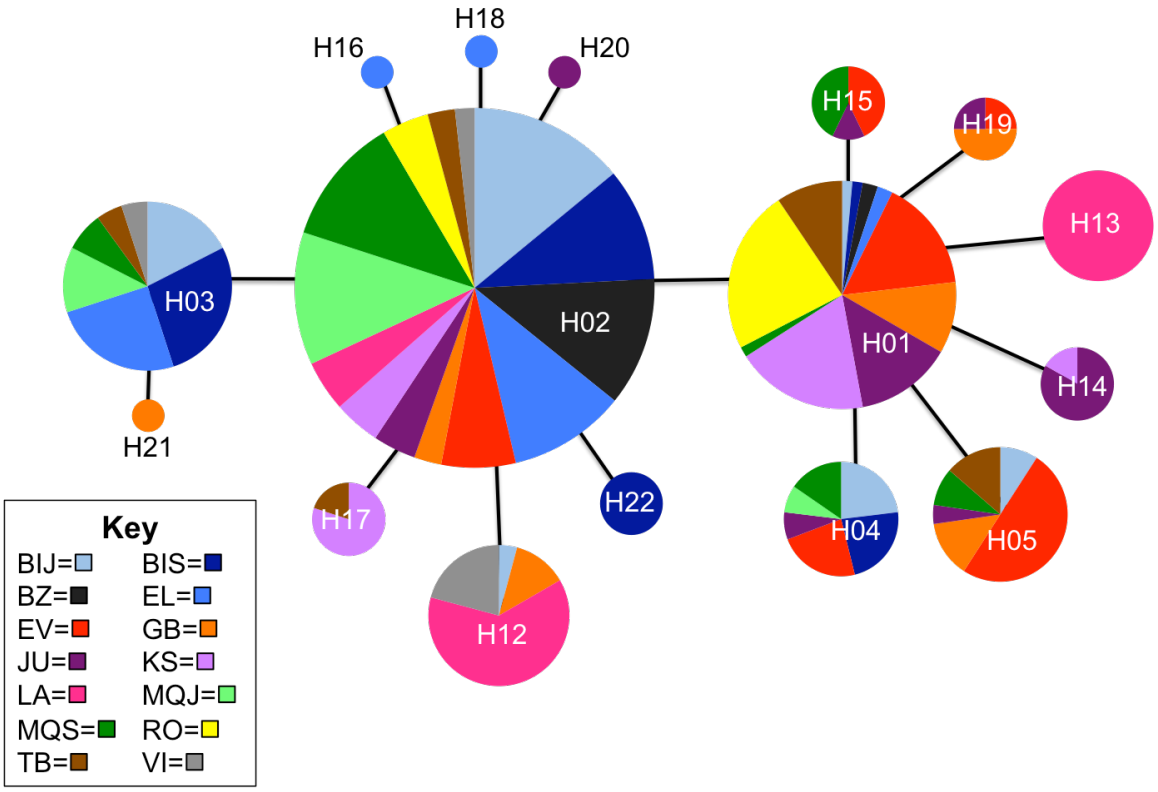


Figure 8: Geographical distribution of mitochondrial ND2 haplotypes. Pie charts represent the frequency of ND2 haplotypes in each collection. Haplotype color designations are listed in the figure key. For haplotype information, refer to Tables 3 and 8. Collection abbreviation and number of individuals analyzed are noted in each pie chart. See Table 1 for names associated with abbreviations.

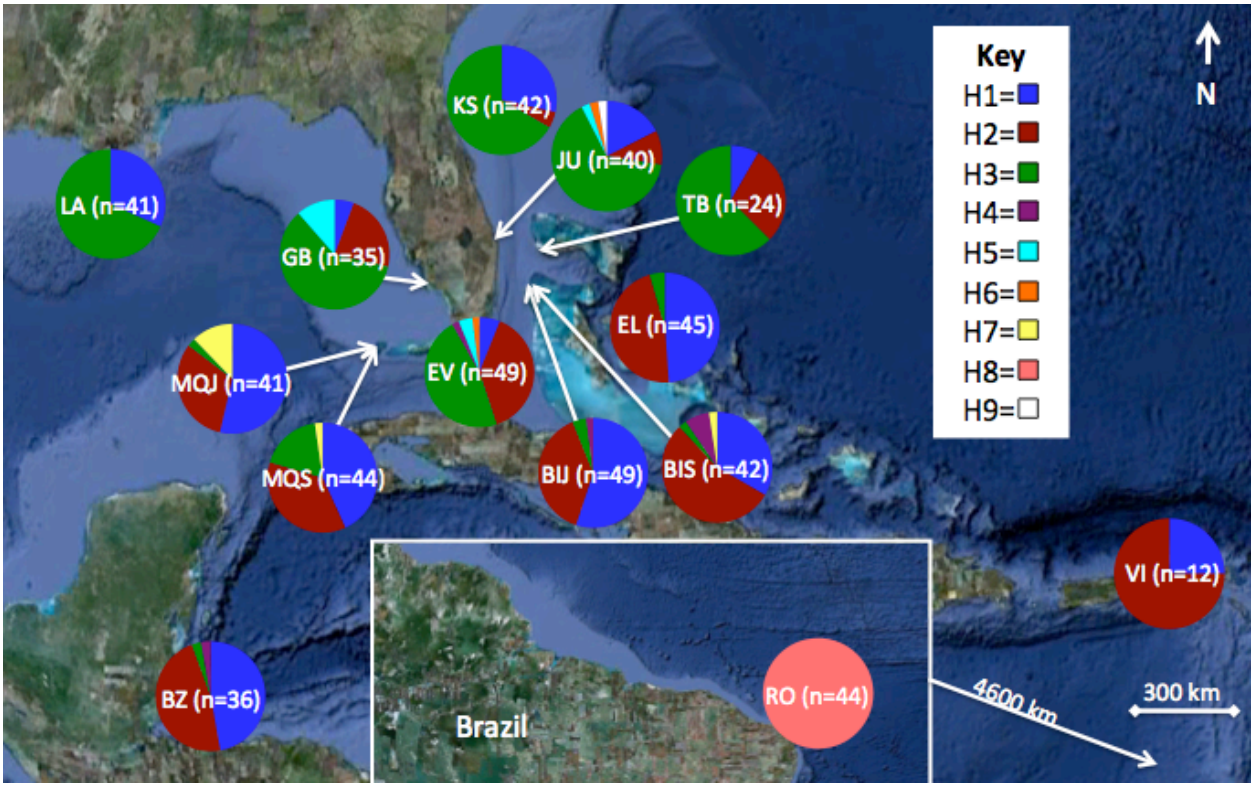


Figure 9: Mitochondrial ND2 haplotype network (95% confidence) with individual haplotype designations noted in each circle. For haplotype information, refer to Tables 3 and 8. Circle sizes are proportional to the frequency of haplotypes in the global population. Colors within each circle represent the 14 collections sampled as shown in the figure key. See Table 1 for names associated with abbreviations.

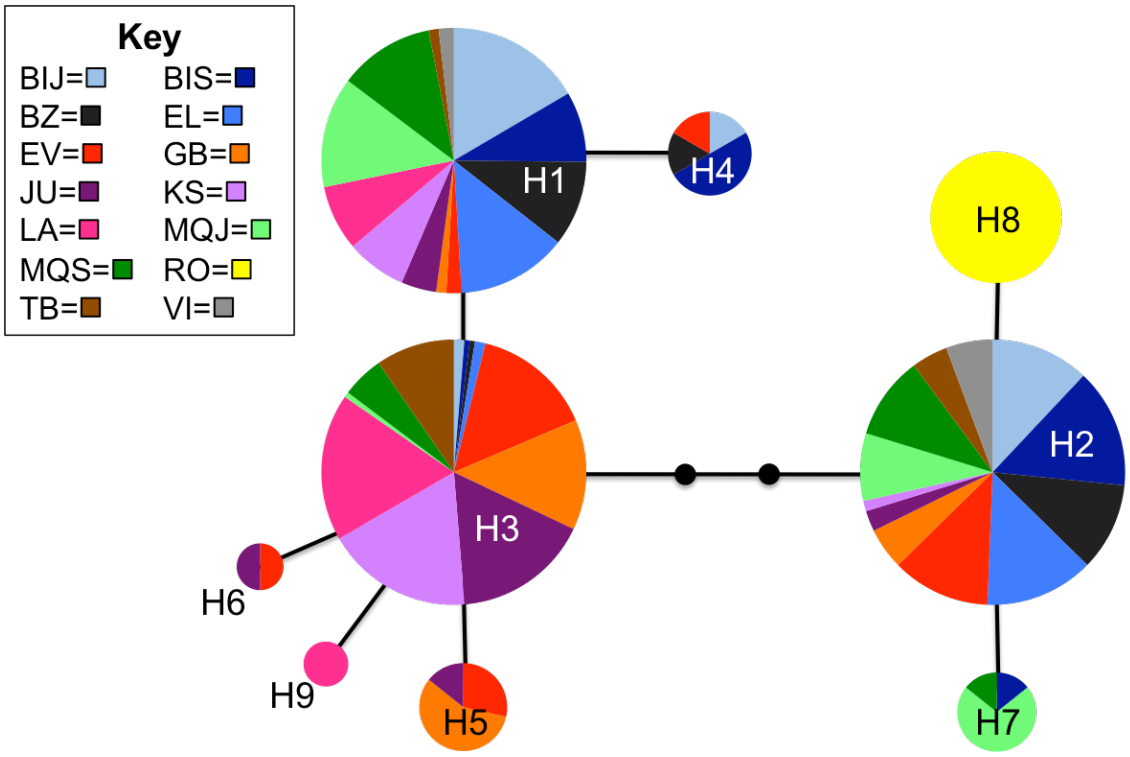


Figure 10: Geographical distribution of mitochondrial composite sequence haplotypes. Colors represent different haplotypes. Haplotype designations are listed in the figure key. For haplotype information, refer to Tables 4 and 9. Collection abbreviation and number of individuals analyzed are noted in each pie chart. See Table 1 for names associated with abbreviations.

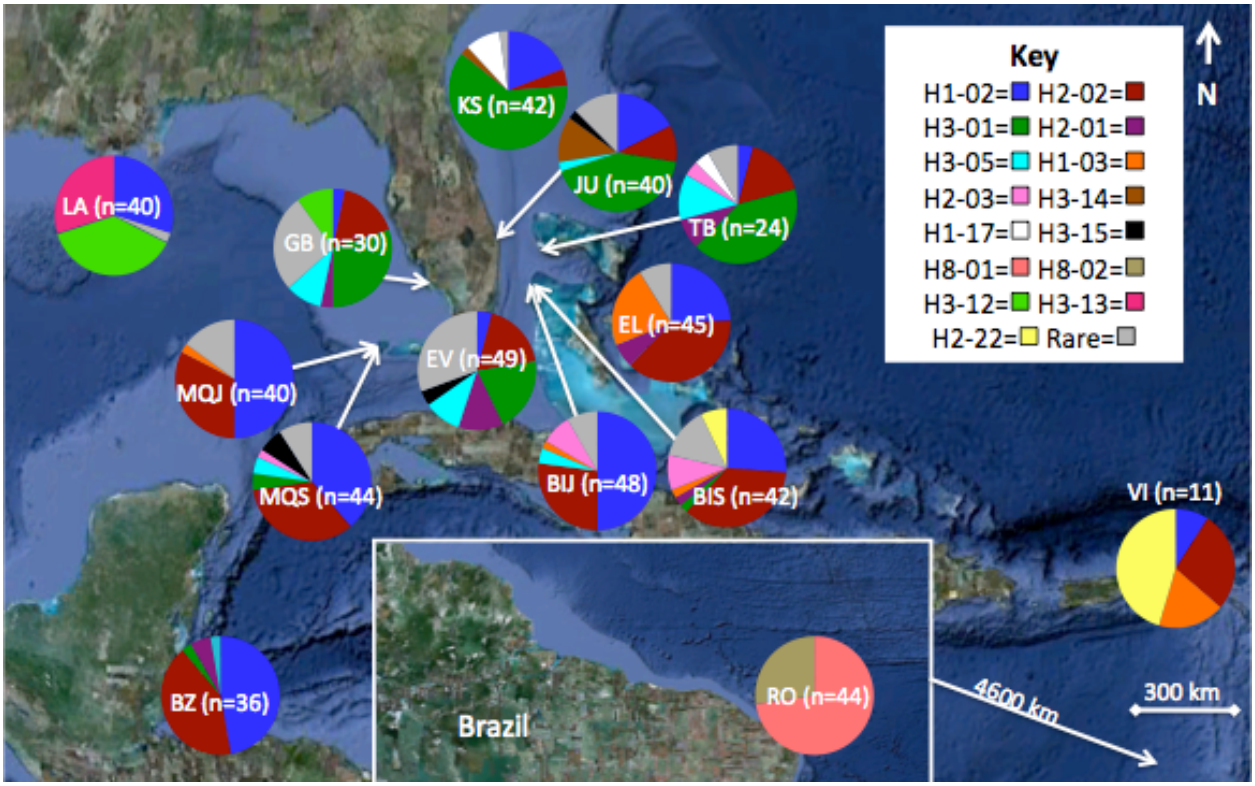


Figure 11: Mitochondrial composite sequence haplotype network (95% confidence) with individual haplotype designations noted in each circle. For haplotype information, refer to Tables 4 and 9. Circle sizes are proportional to the frequency of haplotypes in the global population. Colors within each circle represent the 14 collections sampled as shown in the figure key. See Table 1 for names associated with abbreviations.

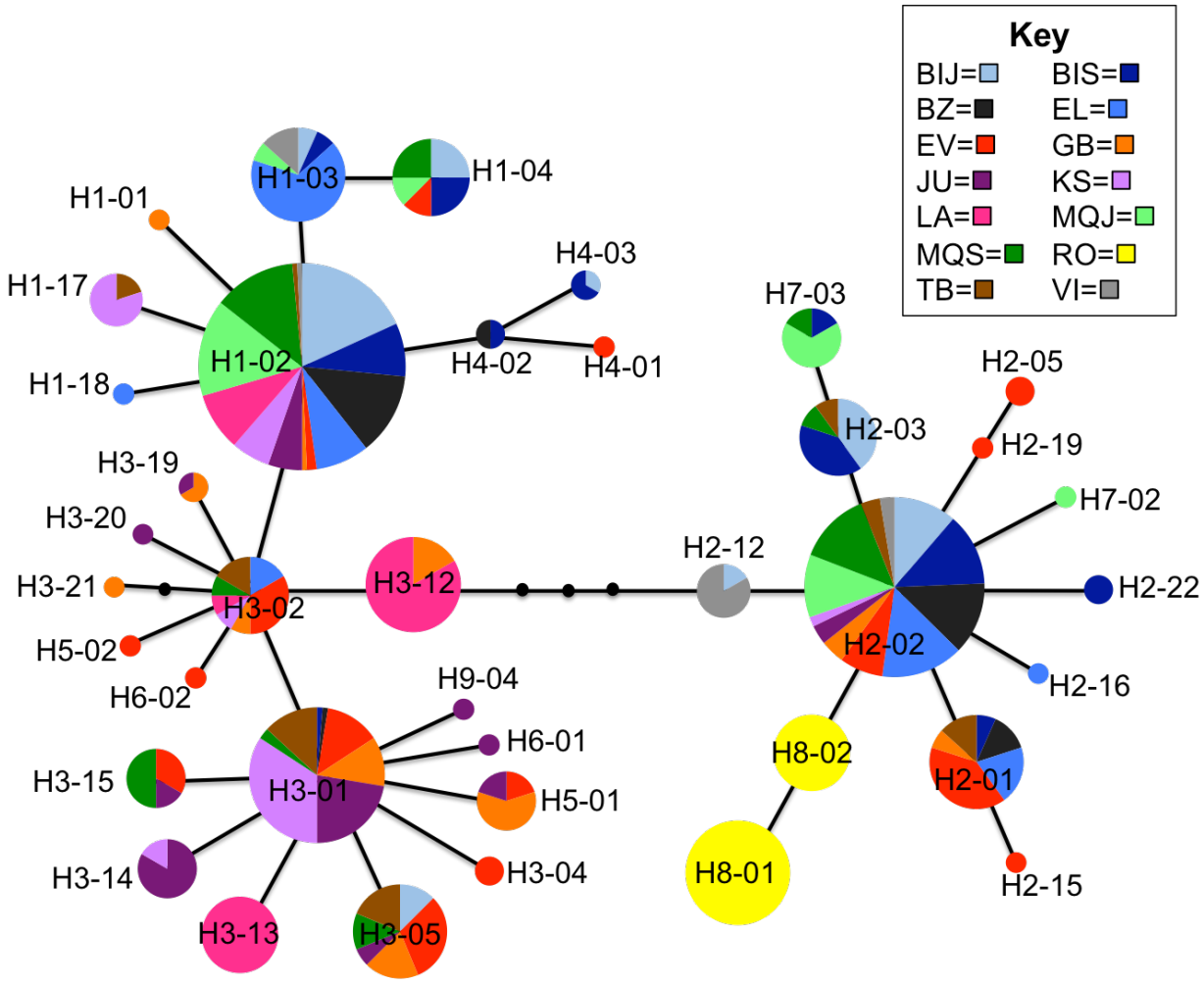


Figure 12: Cluster analysis run in Structure 2.3. Each individual in the data set is represented by a single vertical line, which is partitioned into clusters (colored segments). The Y-axis is the estimated membership fraction in each of the inferred clusters. The X-axis is the distribution of K in the sampled population. In this case, all clusters are equally distributed throughout the entire population sampled (i.e., only one population was identified, K=1).

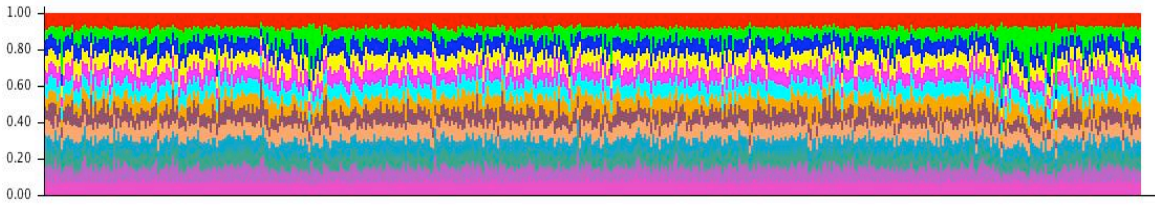


Figure 13: Isolation-by-distance graphs comparing (A) genetic distance and direct geographical distance between collections, and (B) genetic distance and coastal geographical distance between collections. Pairwise mitochondrial fixation indices are represented by grey squares, and black circles represent pairwise microsatellite fixation indices

Figure 13 A: Isolation-by-distance graph comparing genetic distance and direct geographical distance between collections.

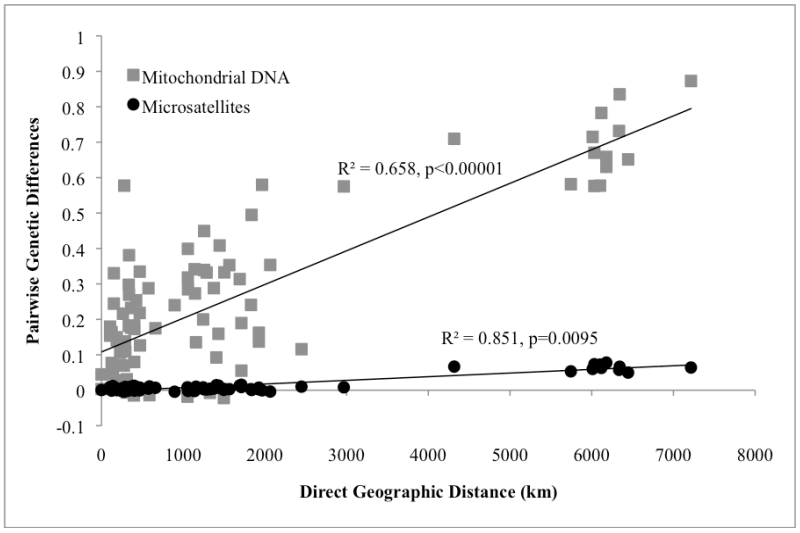


Figure 13 B: Isolation-by-distance graph comparing genetic distance and coastal geographical distance between collections.

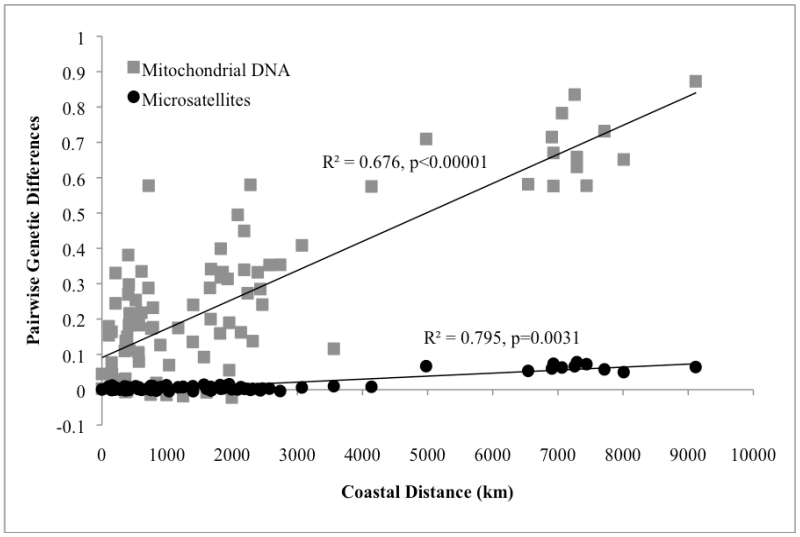


Figure 14: Isolation-by-distance graphs comparing (A) genetic distance and direct geographical distance between Florida and western Bahamas collections, and (B) genetic distance and coastal geographical distance between Florida and western Bahamas collections. Pairwise mitochondrial fixation indices are represented by grey squares, and black circles represent pairwise microsatellite fixation indices.

Figure 14 A: Isolation-by-distance graph comparing genetic distance and direct geographical distance between collections.

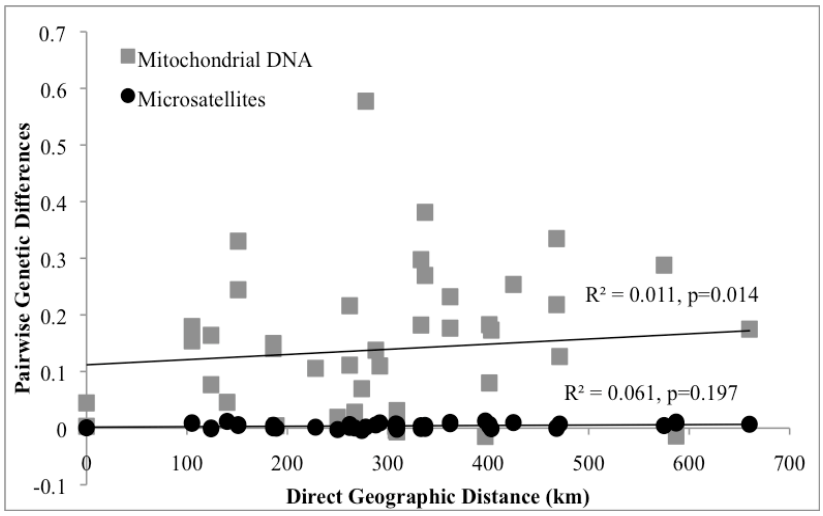
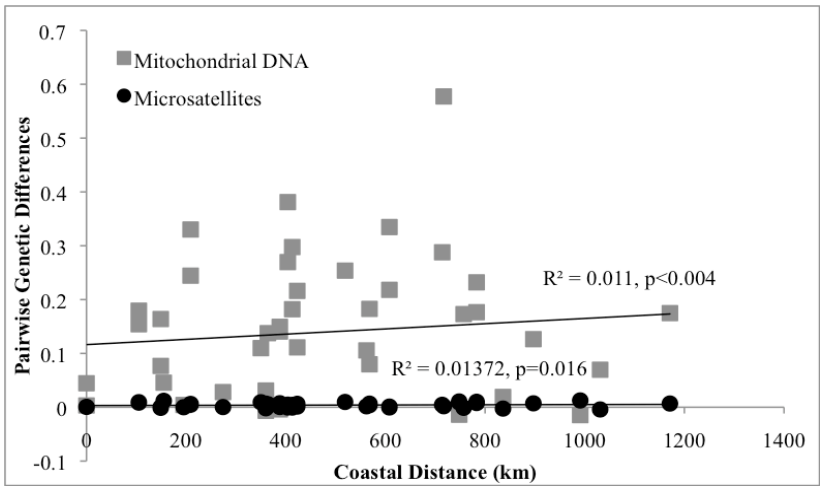


Figure 14 B: Isolation-by-distance graph comparing genetic distance and coastal geographical distance between collections.



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