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COASTWIDE STOCK STRUCTURE OF MONKFISH USING MICROSATELLITE DNA ANALYSIS

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ABSTRACT

The monkfish (*Lophius americanus*) fishery has been assessed and managed as two distinct units since the inception of its fishery management plan in 1999. In recent years, information on stock structure has begun to accumulate but there are still many uncertainties. Evidence suggests that the monkfish stock may be comprised of a single or multiple units, yet the fishery continues to be managed as two separate entities. In 2012 Cornell University Cooperative Extension Marine Program (CCE) received an RSA grant to help determine if the monkfish population is comprised of a single or of multiple stocks over their coast wide distribution from Newfoundland to North Carolina. The project used a sensitive genetic approach known as microsatellite DNA analysis to evaluate the coast wide structure of the stock. Utilizing a collaborative approach, monkfish biological samples were collected and the genetic structure of the monkfish population was analyzed. For the first time, this project empirically evaluated the coast wide monkfish stock structure using sensitive DNA markers. In summary our genetic analysis indicates that there are 2 and perhaps 3 genetic stocks of monkfish along their coastwide distribution and this differentiation may result from a latitudinal gradient in genetic differentiation. The delineation between genetically different stocks does not coincide with the current management division between the northern and southern management areas. Clearly, these genetic divisions do not respect the current 2 stock model by which monkfish are managed in U.S. waters today. However, the boundaries of these genetic stocks require further delineation and the temporal stability of these units between and with years requires confirmation before this new information can be effectively employed in a management context.

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The monkfish fishery is jointly managed by the New England Fishery Management Council (NEFMC) and the Mid-Atlantic Fishery Management Council (MAFMC), with the NEFMC having the administrative lead. The Councils manage the fishery as two stocks, with the Northern Fishery Management Area (NMA) covering the Gulf of Maine and northern part of Georges Bank and the Southern Fishery Management Area (SMA) extending from the southern flank of Georges Bank through the Mid-Atlantic Bight to North Carolina (NEFMC, 2011). Since 1999 this two-area Fishery Management Plan (FMP) has been in place to manage the resource jointly. The two assessment and management areas for monkfish were defined based on differences in temporal patterns of recruitment (estimated from NEFSC surveys), perceived differences in growth patterns, and differences in the contribution of fishing gear types (mainly trawl, gill net, and dredge) to the landings. (NEFSC, 2010). Current regulatory measures vary with type of permit including limited access permits, limitations on days at sea, mesh size restrictions, trip limits, minimum size limits, and other measures (NEFSC,2010). Currently, the bulk of evidence supports a single-stock hypothesis with the possibility of extensive movement between the management areas. Unfortunately, the extent of exchange that occurs and the pathways of migration are very poorly understood at present thus making it difficult to judge the implications of management as two separate stocks (MPDT, 2011). A definitive stock definition is crucial in order to determine best management practices for the future of this fishery.

Monkfish is also considered a Data Poor Stock. The 2007 Northeast Data Poor Stocks Working Group (DPWG) assessment developed new biomass reference points based on an updated age-based yield-per-recruit analysis and results of the SCALE model. The DPWG also concluded that uncertainties in historical catch data prohibited application of long-term models to estimate stock size. The managing councils adopted the DPWG's recommendations in December 2007, which resulted in revisions to the stock status in both areas. Based on these new biological reference points, the stocks were considered to be rebuilt and overfishing was not occurring. In 2010 an updated stock assessment, SARC 50, concluded that both stock units are above biomass thresholds indicating they are not overfished and overfishing is not occurring.

An important prerequisite of successful fisheries management is matching biologically relevant processes and management practices but unfortunately mismatches between biology and management actions often occur (Reiss et al. 2009). This results from an absence of data on these processes or ignoring their importance in crafting management policies. The stock is the primary unit of fisheries management, but little is known empirically about the coast wide stock structure of monkfish. It is generally agreed that fishes that are distributed over wide geographic areas should be managed on the basis of individual units of stock. That is because separate stocks often exhibit different critical life history characteristics such as growth, maturation, fecundity, and natural mortality rates. If multiple stocks exist within a species' distribution, it is assumed that each has evolved its own life history characteristics that are best suited for their environments and survival. Minimal intra-breeding within stocks does occur, but reproductive isolation among stocks maintains their integrity and allows for the development of stock-specific characteristics. These characteristics include differences in the frequencies of neutral genetic markers such as DNA microsatellites. If more than one stock exists within a species' distribution then each stock should be afforded protection consistent with its abundance and threats. Today, DNA-based genetic techniques are often used to determine stock structure in widely distributed marine species such as monkfish.

Recent empirical genetic results show that marine fishes exhibit significantly more stock structure than previously thought despite the absence of obvious physical barriers to mixing (Hauser and Carvalho 2008). Evidence now suggests that rather than being demographically open, marine fishes exhibit stock structure on fine geographic and temporal scales. This is due to processes that limit dispersal and promote self-replenishment of local populations and that promote spawning site fidelity, natal homing, egg and larval retention and local adaptation (Knutson et al. 2011). Frequently, this genetic patchiness results from environmental drivers such as sea surface temperature variation and differences in open ocean circulation patterns (Selkoe et al. 2010). Not only have molecular techniques revealed greater levels of heterogeneity of stock structure in marine fishes than previously thought, concomitant variation in ecologically important traits (growth and maturation rates, sex ratio) indicate the presence of extensive adaptive differentiation (Sala-Bozano et al. 2011). Recent developments in statistical analysis of population genetics data provide informative integration with ecological, environmental, and genetics data (Selkoe et al. 2008). For example, kinship models have been developed to estimate

current migration rates when genetic divergence is low, as typically seen in marine species (Palsboll et al. 2010).

There are several different DNA-based genetic approaches that have been used over recent decades to identify fish stocks. Some of these have proven much more informative and robust than others in delineating the stock structure of marine fishes. These approaches include analysis of maternally derived mitochondrial DNA (mtDNA), random amplified polymorphic DNA (RAPDs), and biparentally inherited microsatellite DNA analysis.

The RAPDs technique was popular because it was an easy to implement approach to study genetic variation in natural populations. Its appeal stemmed from the fact that no prior knowledge of the target organism's genome was needed prior to its application. Its shortcoming was its lack of reproducibility and its inapplicability in many statistical population genetics analyses. In fact, due to the problems associated with experiment reproducibility, many scientific journals no longer accept papers on natural populations that are based on RAPDs data. The RAPD study of monkfish (Chikarmane et al. 2000) is the most often cited study in support of the current stock model used in monkfish management. However, it should be noted that this study used the discredited RAPD technique and a limited number of samples and therefore may be misleading. Based on this extremely limited characterization of RAPD genotypes (small number of primer sets and very limited number of samples) the authors concluded that there was insufficient variation with this technique to define the stock structure of monkfish.

Mitochondrial DNA polymorphisms are often successfully used to identify population structure in marine fishes. Mitochondrial DNA haplotypes are uniparentally inherited from one's mother and therefore provide maternally derived phylogenies. As practiced today, rather than characterize the entire 16 kb mtDNA molecule, researchers use the polymerase chain reaction (PCR) to amplify selected portions of the mtDNA molecule for sequence analysis. For population studies, PCR usually targets the control region or the most variable portion of mtDNA. One unpublished mtDNA study was recently conducted on monkfish collected from the Gulf of Maine to Cape Hatteras (Nguluwe et al. 2009; 2010). Rather than focus on the control region of mtDNA for analysis, this study sequenced the cytochrome C oxidase subunit 1 (COX-1) gene, a portion of mtDNA not noted for its variability and probably quite conservative because of functional constraints. The investigators concluded that assortment of mtDNA COX-1 haplotype clusters were not associated with management areas or geographic location. We feel that mtDNA COX-1 was an inappropriate target because of its conservativeness and as such the conclusions of these studies may be misleading in defining monkfish population structure.

Analysis of DNA microsatellites is overwhelmingly the method of choice to define genetic stock structure in marine fishes (Cadrin et al. 2005). This is because microsatellites evolve rapidly, even more so than the mtDNA control region mentioned above, and because microsatellites are biparentally inherited mixtures of identification that can be readily ascertained (unlike haploid mtDNA). Microsatellites are specifically targeted regions of neutral DNA which are co-dominantly inherited. Microsatellites are short, tandem repeated hypervariable DNA motifs (usually 2-4 base pairs in length) whose number of copies varies dramatically among individuals and presumably populations within a species. Typically, in population studies, 8-12 unlinked

microsatellite loci are screened for allelic variation. Composite genotypes are determined across all loci for each specimen and allelic frequencies are compared across all loci among collections for statistically significant differences. It is also possible to search for genetic structure among collections without a priori information as to where collections were made using recently developed Bayesian approaches. One drawback to the use of microsatellites is that loci must be isolated from the target species or very closely related congeners. Usually, microsatellites from the target species provide more reliable PCR amplification and higher levels of allelic variation than those from related species and thus the species-specific isolation effort pays dividends in the end. To date, there are no published studies on microsatellite diversity in *Lophius americanus*, however several studies have revealed low, but sometimes informative levels, of variation within European species of monkfish.

METHODS

Data Collection

The first step toward defining the monkfish stock structure was to collect biological samples that were representative of the range of monkfish. A collaborative network of participants was necessary to target and collect samples from Canada to North Carolina so that the area of the stock or stocks could be determined and then defined. Cornell Cooperative Extension (CCE) created and coordinated a Monkfish Sample Collection Network. This network represented stakeholders and scientists from both the Northern and Southern Management Areas (and two sites in Canada) and covered the entire range of the monkfish fishery. The main objective of this network was to develop a coast wide sampling program.

CCE recruited members of the fishing industry and scientists along the east coast to participate in our sample collection efforts. This allowed the entire range of monkfish to be examined for genetic identifiers. Commercial fishermen, dealers, wholesalers, and processors in ports across the Mid-Atlantic and New England regions and Canada were recruited to build this all-inclusive network. The network included participants from the Portland Fish Exchange of Portland, ME, the Whaling Auction of New Bedford, MA, Cape Ann Auction in Gloucester, MA, Town Dock in Pt. Judith, RI, Handrigan Seafood in Pt. Judith, RI, Inlet Seafood I Montauk, NY and Cor-J's in Hampton Bays, NY. Cooperating commercial fisherman are detailed in Table 1 below. CCE has coordinated with fellow scientists from NMFS- Northeast Fisheries Science Center (NEFSC) – Fall 2012 trawl survey, Virginia Institute of Marine Sciences (VIMS)-Northeast Area Monitoring and Assessment Program (NEAMAP)- Spring 2013 inshore trawl survey, Delaware State University, North Carolina Division of Marine Fisheries, Rutgers University and Canada Department of Fisheries and Oceans- Fall 2012 trawl survey. This network has allowed CCE to target monkfish from more specific project areas. Proper training was given to all participants to ensure data precision and proper sampling techniques. The network structure also served to strengthen relationships between industry, science and management.

Table 1

Cornell Monkfish Sample Collection Network Participants		
Fisherman	Vessel	State
Kelo S Pinkham	Jeanne C	Maine
Steven C Benner	High Roller	Maine
Lauren Dorothy Inc	Lauren Dorothy	Maine
Joseph B Leask	November Gal	Maine
Thomas Casamassa	Theresa Irene	Maine
Jordan Lynn Inc	Rachel T	Maine
Danny Boy Fisheries Inc	Danny Boy	Maine
Craig R Durant	Marie Ann	Maine
De Dee Mae II Inc	De Dee Mae II	Maine
Jordan Marine Inc	Jamie & Ashley	Maine
Atlantic Resource Corp	Capt. Joe	Maine
William McCann	Pilgrim	Massachusetts
Melon Fisheries	SS Melon	Massachusetts
James Santapaola	Amanda Leigh	Massachusetts
Island Queen Fishing Corp	Fair Wind 2	Massachusetts
Steven Welch	Holly and Abby	Massachusetts
Sammy Jo Fishing LLC	Sammy Joe	Massachusetts
Corvo LLC	Hera	Massachusetts
Ronald Gustafson	Cheryl Ann	Massachusetts
Phillip Lynch	Mary Elizabeth	Massachusetts
Nordic Fisheries	Tradition	Massachusetts
Lucinda Fishing Corp	Sao Marcos II	Massachusetts
New England Trawlers Inc	Morue	Massachusetts
Thomas Bell	Michael Brandon	Massachusetts
Boat Santa Rita III Inc	Maria Jo-Ann	Massachusetts
Kevin Shea	Endeavor	Massachusetts
Brian Bichrest	Safe Haven	Massachusetts
Tim Caldwell	CW Griswold	Rhode Island
Ted Platz	Louise	Rhode Island
John Stoltsgif	Martha Porter	Rhode Island
Todd Sutton	Sweet Misery	Rhode Island
Town Dock Inc	Lightning Bay	Rhode Island
Town Dock Inc	Excalibur	Rhode Island
Town Dock Inc	Determination	Rhode Island
Town Dock Inc	Rebecca Mary	Rhode Island
Town Dock Inc	Stephanie Bryan	Rhode Island
Jim Fox	Rayda Cheramie	Rhode Island
Scott Dudley	Atlantic Pearl	Rhode Island
Malcolm McClintock	Rhonda Denise	Rhode Island

Chris Roebuck	Karen Elizabeth	Rhode Island
Tim Froelich	Liberty & Miss Independence	New York
Richard Larocca Jr	Double Vision & Doubled Vision	New York
Vincent Damm	Seaquel II	New York
Brian Rade	Donna May	New York
Gerald Herlihy	Longshot	New York
Charles Etzel	Clover	New York
Rick Stevenson	Sea Smoke	New York
William Grimm	Perception	New York
Tim Kriegsmann	Comprise	New Jersey
Eric Svelling	Edge Runner	New Jersey
Kevin Wark	Dana Christine	New Jersey
Roger Wooleyhan	Wooly Bully	Maryland
Jamie Wescott	Risky Business	North Carolina & Virginia
Tommy Danchise	Brandon Lake	North Carolina & Virginia
Chris Walker	Krists~Caleb~Morgan	Virginia

In addition to the vessels and fishermen listed above, other cooperating industry members included:

- NMFS and the Northeast Fisheries Science Center. Samples were obtained from both Dr. Anne Richards' 2009 Cooperative Monkfish survey and the R/V Bigelo 2013 trawl survey.
- Don Powers and the Canadian Fisheries Department.
- Delaware State University.
- Virginia Institute of Marine Science and the Northeast Area Monitoring and Assessment Program (NEAMAP).
- Agger fish which was formally one of the largest monkfish dealers on the east coast (see Problems Encountered section).

As identified in the monkfish fishery management plan, there are two broad areas used to manage and divide the fishery (see Fig. 1). We further divided these two areas in order to develop our own sampling strata that would provide representative samples from throughout the range of the fishery. We created a total of six large sampling areas which are color coded and indicated in Figure 2. Each of these six areas was further divided into strata which are also shown in Figure 2. Overall, twenty strata were designated for sample collection. This stratified system provided us with a distribution of sampling effort and a means to quantify stock structure throughout the entire range of the fishery as well as providing seasonal, depth, and latitudinal and longitudinal dimensions to the sampling effort. Both Figures 1 and 2 also include the current NMFS statistical areas. The strata CCE developed were based on the current management areas overlain on the NMFS statistical areas. This would help to provide stock structure by various management and statistical areas, if stock differences are shown to exist. CCE determined the

minimum sampling targets to be 50 individual samples per stratum distributed over the course of the year to account for temporal distribution.

Figure 1 - Monkfish FMP Management Areas

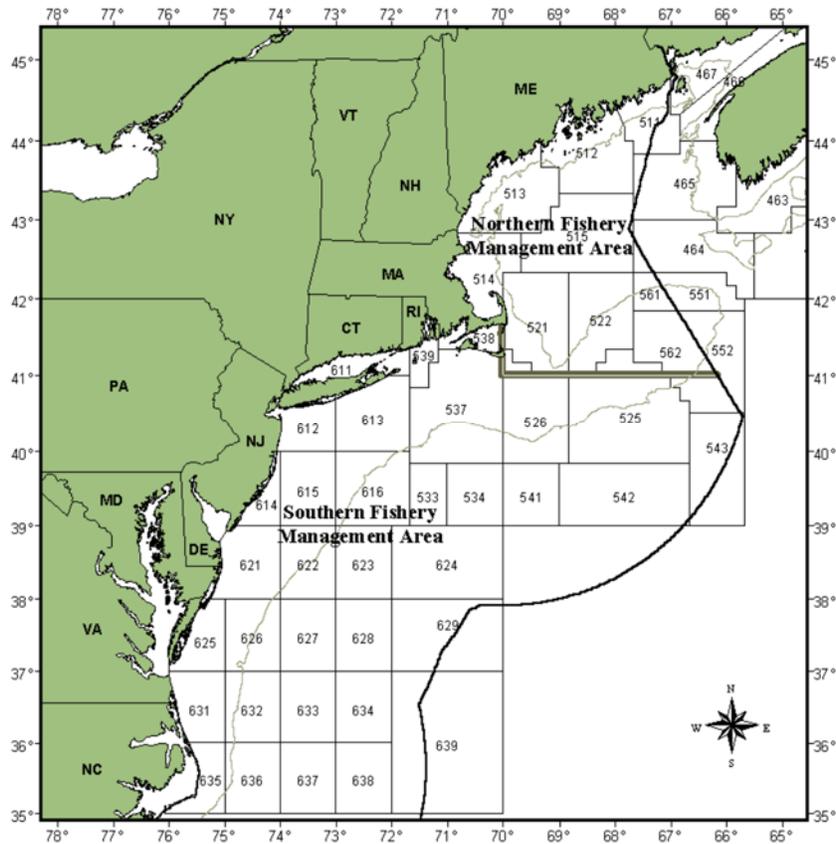
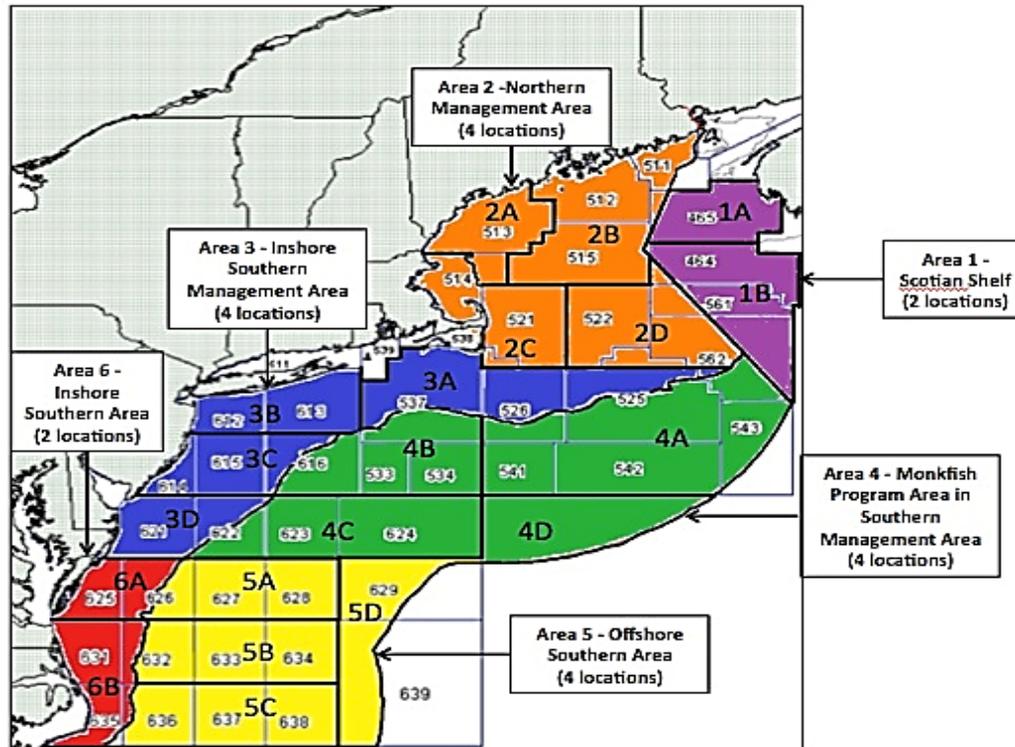


Figure 2 - CCE Sampling Strata



For this project, a “sample” consisted of a 1-inch clip of flesh from the tail fin of a single fish that was preserved in 95% ethanol. The information collected relative to each sample was date of harvest, area (specific latitude and longitude), depth, vessel trip report (VTR) number, and vessel name. Length/weight information was collected when possible as well as any anecdotal information from industry about noticeable fishing trends in relation to spawning, egg veils and larger size or quantity of fish.

CCE coordinated with the cooperating members of the Monkfish Sample Collection Network to provide the sampling opportunities. Prior to commencing sampling, CCE trained participants in the network on the proper procedure for collecting samples. A detailed instruction manual and an instructional video was developed and distributed to all participants to reference. The instructional video can be viewed by following the included hyperlink; <http://ccesuffolk.org/Monkfish/>. CCE assembled sampling kits and distributed them to all of those included in the network. The sampling kit contained fin clip collection vials, preserving liquid (EtOH), scissors, markers, pencils, data sheets, labels, vial stands, and instruction manual. Self-addressed stamped envelopes were also included for easy return of samples to CCE.

Communication with all industry partners was made in person for an initial program introduction and training. CCE contacted network partners on a regular basis by phone or email to closely monitor both landings and sampling activity. This was done to ensure sufficient opportunities for sample collection that were representative of both time and strata. CCE coordinated the

collection of samples from network partners according to both fishing season and area to ensure sampling targets covered the entire range of the monkfish fishery. Follow-up in person visits with members of the Sample Collection Network occurred periodically during the project. Contact was also made with both the NMFS-NEFSC bottom trawl survey and the Canadian Dept. of Fisheries and Oceans bottom trawl survey to collect samples from Georges Banks, Grand Banks, and the Scotian Shelf. Members of the CCE staff coordinated with NMFS-Northeast Fisheries Science Center (NEFSC) in Woods Hole, MA to collect monkfish samples from the R/V Bigelow- Spring 2013 trawl survey. CCE targeted samples that would help fulfill the coast-wide data requirements of the project by sorting through the Spring 2013 trawl survey data before the visit. All samples collected from the Bigelow were small clips of monkfish vertebra tissue.

Members of the CCE staff were also responsible for dockside sample collection in the New York/Long Island region. This region spanned from Montauk Point on the east end of Long Island west to Fulton Fish Market in the borough of Bronx, New York City. CCE samplers are well versed and experienced in fin clip sample collection and dockside/at-sea biological sampling. CCE staff members also conducted periodic dockside sampling at ports in Maine, Massachusetts and Rhode Island. Rutgers University provided support by conducting dockside sampling in Barnegate, NJ. Contact with fishermen and NC Division of Marine Fisheries staff was made in efforts to collect southern samples from North Carolina and Virginia. Due to limited access of fishing and cold temperatures very few samples were collected from this area. (See Problems Encountered)

CCE developed a sampling database to target, track, and verify all the samples collected. CCE coordinated and tracked sampling targets on a weekly basis to ensure that project goals were met. All samples collected were received by CCE and each individual sample was logged, visually inspected, and organized for shipment for DNA analysis. An updated sample database was included with each sample mailing sent to NYU.

CCE staff members were also responsible for sample validation and verification in order to ensure the highest standards of quality control. The sample collection database was audited on a weekly basis. CCE and NYU maintained communication through conference calls to ensure the quality of the collected samples and data meet all required specifications. The vessel trip report (VTR) number linked to each sample was used to verify the details associated with that sample. Most importantly this included the area of harvest which could be verified by the latitude and longitude that also is included on the VTR. The accuracy of this information is critical to determining stock area.

DNA Analysis

Dr. Isaac Wirgin of New York University (NYU) in collaboration with CCE utilized a sensitive genetic approach, microsatellite DNA analysis, to empirically determine if monkfish constitute a single or multiple stocks over their coast wide distribution and most importantly between the northern and southern management areas.

Hypotheses:

- **H₀**- There are two genetic stocks of monkfish in U.S. waters that correspond to the management model now in effect; one north of Georges Bank and one south of Georges Bank.
 - **H₁**- There is only a single stock of monkfish in U.S. waters and coastwide
 - **H₂**- There are more than two stocks of monkfish in U.S. waters and coastwide that are spatially and/or temporally separated.
- **H₀**- Allelic frequencies are temporally stable over several years within individual genetic stocks.

The specific tasks of the DNA analysis were done at NYU and are listed below followed by a description and summary of the procedure for completing each task:

1 - Isolate genomic DNA from monkfish tissues from each of 1000-1200 monkfish fin clips.

In total, we successfully isolated and analyzed DNAs from 1,329 monkfish specimens that were collected from 19 statistical areas ranging from area 1A (Grand Banks, Newfoundland) to area 6B (coastal Virginia). Specimens were collected by the CCE Monkfish Sampling Network described elsewhere in this report. A sufficient number of specimens (>50) were analyzed from most of the 19 statistical areas to allow for robust statistical evaluation of the extent of genetic differentiation among them. However, for seven of them, our sample size was inadequate for a fair evaluation of their genetic discreteness—these included areas 4A (n=12), 4C (n=46), 5A (n=13), 5B (n=7), 5C (n=13), 5D (n=0), and 6B (n=34). Most of these areas are offshore and all are in the southern half of the species' distribution.

DNA Isolations

Skin biopsies, fin clips or muscle plugs from commercial fishermen were the primary sources of DNAs for this study. Additionally, we were successful in analyzing DNA from vertebra from frozen collections of monkfish from NEFSC of NOAA's fall and spring trawl surveys. In all cases, total DNA was isolated from EtOH-preserved tissue or frozen vertebra by their incubation in CTAB buffer (Saghai-Marroof et al. 1984) and digestion with proteinase K, followed by standard phenol-chloroform extractions and alcohol precipitations. DNA concentrations and purities were determined with a Nanodrop ND-1000 spectrophotometer. All DNAs were diluted to a final concentration of 50 ng/μl for standardization in subsequent PCR reactions.

2 - Identify at least 100 microsatellite loci from two monkfish DNA libraries that are enriched for two different microsatellite motifs using 454 technology to identify microsatellite and flanking primer sequences.

We were successful in isolating and characterizing a sufficient number of new microsatellite loci to sensitively determine the genetic stock structure of monkfish. We used Next Generation Sequencing in collaboration with Dr. Tim King of the USGS, Aquatic Ecology Branch, Leetown Science Center with a Life Technologies Ion Torrent semi-conductor sequencer to identify monkfish microsatellite loci and flanking single-copy sequences for PCR primer design. Initially, we prepared a monkfish DNA library using the following procedure. Monkfish genomic DNA was fragmented by using a DNA fragmentation Master Mix (New England Biolabs, MA) at 25 °C for 20 min. Library adaptors for the Ion Torrent were then ligated with the fragmented monkfish DNA by using T4 DNA ligase and ends were filled in with *Bst* DNA polymerase. Adaptor ligated DNA was then size fractionated using E-gel size select gels and 290-330 base pair (bp) range fragments were selected for 200 bp read lengths. Size fractionated, adaptor ligated DNA was then PCR amplified and the amplified library was purified using AMPure XP beads. An Agilent 2100 Bioanalyzer was used to ensure that the molecular size of the fragments was sufficient for subsequent sequencing steps.

Two runs were conducted on the Ion Torrent to identify di, tri, and tetranucleotide microsatellite repeats in the library. After the first two runs, more than one thousand di, tri and tetra nucleotide repeats were identified. A third run was also conducted which successfully identified another 1,774 sequences containing putative microsatellite and flanking regions. In total, using the Ion Torrent we characterized 118 unique microsatellite sequences with sufficient sequence for primer design.

3 - Test a subset of 25 microsatellites for their reproducibility in PCR amplification.

Over the course of the project, we purchased and empirically tested 72 microsatellite primer pairs identified by Next Generation Sequencing (Table 2) for their abilities to reliably amplify products from monkfish genomic DNAs and their usefulness in identifying informative genetic polymorphisms among these samples. We were restricted to only those sequences that contained sufficient flanking sequences on both ends (>50 base pairs) to allow for design of effective PCR primers. We also tested 14 PCR primer pairs previously isolated from a European monkfish species (*Lophius budegassa* (Blanco et al. 2006) and confirmed to amplify DNA from a second European monkfish *L. piscatorius* (Garoia et al. 2003) for their abilities to PCR amplify *L. americanus* genomic DNA.

Table 2

Seventy-Two Monkfish Microsatellite Loci that Were Identified by Next-Generation-Sequencing, Purchased, and Tested in this Project		
Microsatellite Locus Name	Primers Sequence	Description of Primers
Monk 397_1437_FP4	TATGGGTGGGAGAAGAGGTG	Left primer for Monk microsatellite 2RHWM:397:1437
Monk 397_1437_RP4	TTCTAGGACGAAAGGCTGGA	Right primer for Monk microsatellite 2RHWM:397:1437
Monk 468_2042_FP3	TCATGAAACGGGTCATTCTG	Left primer for Monk microsatellite 2RHWM:468:2042
Monk 468_2042_RP3	GACCTTCTGGCTCCTTTTCA	Right primer for Monk microsatellite 2RHWM:468:2042
Monk 829_555_FP1	AACGGCGTAATAACCGATGT	Left primer for Monk microsatellite 2RHWM:829:555
Monk 829_555_RP1	ACACCGTCTGTTCATCACT	Right primer for Monk microsatellite 2RHWM:829:555
Monk 881_1526_FP5	TAGAAACGTCCAATCAGGGG	Left primer for Monk microsatellite 2RHWM:881:1526
Monk 881_1526_RP5	CGGTGCTTTCATAACTACT	Right primer for Monk microsatellite 2RHWM:881:1526
Monk 918_2594_FP1	TCTGGGTTATTGGTTCTGCTG	Left primer for Monk microsatellite 2RHWM:918:2594
Monk 918_2594_RP1	TTCACGACAGTAGACGAGCG	Right primer for Monk microsatellite 2RHWM:918:2594
Monk 1629_309_FP1	GAGTTTGTGCAGGACGATGA	Left primer for Monk microsatellite 2RHWM:1629:309
Monk 1629_309_RP1	TCCAATCGCCATTCTAAC	Right primer for Monk microsatellite 2RHWM:1629:309
Monk 2135_1809_FP5	TTGAACATGTGATGATCCTGC	Left primer for Monk microsatellite 2RHWM:2135:1809
Monk 2135_1809_RP5	AGTATTGACAATCGTCGGCG	Right primer for Monk microsatellite 2RHWM:2135:1809
Monk 2211_2626_FP4	TAAAACCACTCACGGACC	Left primer for Monk microsatellite 2RHWM:881:1526
Monk 2211_2626_RP4	GGACCGATATCCAAAAGTTGA	Right primer for Monk microsatellite 2RHWM:881:1526
Monk 2227_1439_FP2	AAGTCTGAGAGGAGGGAGGG	Left primer for Monk microsatellite 2RHWM:2227:1439
Monk 2227_1439_RP2	GTGTCGGACCTCAATCACCT	Right primer for Monk microsatellite 2RHWM:2227:1439
Monk 2242_2322_FP2	AATTCTATGAAATAAATGCGAAACA	Left primer for Monk microsatellite 2RHWM:2242:2322
Monk 2242_2322_RP2	TGCGTTCACTCAACATGGA	Right primer for Monk microsatellite 2RHWM:2242:2322
Monk 2312_1529_FP1	CCCGTTTCCATTCTCACTA	Left primer for Monk microsatellite 2RHWM:2312:1529
Monk 2312_1529_RP1	CCGTCCTTCGATGGTAATTG	Right primer for Monk microsatellite 2RHWM:2312:1529
Monk 2357_1081_FP4	GGGAACATGAATGGAGGA	Left primer for Monk microsatellite 2RHWM:2357:1081
Monk 2357_1081_RP4	GTCAATGAGTATTTGATGAAATTTG	Right primer for Monk microsatellite 2RHWM:2357:1081
Monk 2460_2708_FP1	TCACCTTAAACATCGACCACC	Left primer for Monk microsatellite 2RHWM:2460:2708
Monk 2460_2708_RP1	GCGACAGACTTGAGAGGAGC	Right primer for Monk microsatellite 2RHWM:2460:2708
Monk 201_1512_FP1	AAAAGAAAACACATGTTGCG	Left primer for Monk microsatellite 2RHWM:201:1512
Monk 201_1512_RP1	ATGGCATCGAATTCGAAGTT	Right primer for Monk microsatellite 2RHWM:201:1512
Monk 670_1612_FP4	CCGTGCAGAAAATGCTAGAAA	Left primer for Monk microsatellite 2RHWM:670:1612
Monk 670_1612_RP4	CACGTAGCCACTTCCAACAA	Right primer for Monk microsatellite 2RHWM:670:1612
Monk 715_1429_FP1	TTTGAAGCTAAACAATCGCTG	Left primer for Monk microsatellite 2RHWM:715:1429
Monk 715_1429_RP1	CCAACACTTAACTTTCAGGGCA	Right primer for Monk microsatellite 2RHWM:715:1429
Monk 812_2388_FP1	ATAAACGCGGAGAGAGAAGAA	Left primer for Monk microsatellite 2RHWM:812:2388
Monk 812_2388_RP1	TGAATTGGCATTCTTTCATTG	Right primer for Monk microsatellite 2RHWM:812:2388
Monk 957_499_FP1	AACCGTCTGAGACACCAACC	Left primer for Monk microsatellite 2RHWM:957:499
Monk 957_499_RP1	CCGTTTCAGTTTCGTTCCGTT	Right primer for Monk microsatellite 2RHWM:957:499
Monk 1211_1919_FP1	GACTAAATGTCCCTTTGGGG	Left primer for Monk microsatellite 2RHWM:1211:1919
Monk 1211_1919_RP1	GCTGACATCTAGGCAGACA	Right primer for Monk microsatellite 2RHWM:1211:1919
Monk 1363_1668_FP1	TCCGCCATAAATCAGGACTC	Left primer for Monk microsatellite 2RHWM:1363:1668
Monk 1363_1668_RP1	GATTGTTCTGTGCTAGGC	Right primer for Monk microsatellite 2RHWM:1363:1668
Monk 1742_1835_FP3	TTTTGTGCTGATGGTTGGAA	Left primer for Monk microsatellite 2RHWM:1742:1835
Monk 1742_1835_RP3	CACTCGGCAGTTGATCTGA	Right primer for Monk microsatellite 2RHWM:1742:1835
Monk 2183_2502_FP4	TGAAATGGACAAGAACCCCTG	Left primer for Monk microsatellite 2RHWM:2183:2502
Monk 2183_2502_RP4	TCCTCAATACTGAAACCGCC	Right primer for Monk microsatellite 2RHWM:2183:2502
Monk 2240_2021_FP4	TGTGAAATGCTGTCTGTGCTT	Left primer for Monk microsatellite 2RHWM:2240:2021
Monk 2240_2021_RP4	GTCTCTGTTTAAATCTGGAGCTG	Right primer for Monk microsatellite 2RHWM:2240:2021
Monk 2273_783_FP1	TACGATGCAGCTGTACGAGG	Left primer for Monk microsatellite 2RHWM:670:1612
Monk 2273_783_RP1	CAACGAATGTCATCAGTCAGG	Right primer for Monk microsatellite 2RHWM:670:1612
Monk_contig_4635_FP1	TTCCAGTTGGTCCATGCAAAA	Left primer for Monk microsatellite Lam_IT_reads_PG1-85_62112_contig_4635
Monk_contig_4635_RP1	CGTTTCTGGGTCGGTAGTGT	Right primer for Monk microsatellite Lam_IT_reads_PG1-85_62112_contig_4635
Monk_contig_826_FP1	TTGCTAGCCGTCAGTGAATG	Left primer for Monk microsatellite Lam_IT_reads_PG1-85_62112_contig_826
Monk_contig_826_RP1	CAAACATTCAGACCCCTCGCT	Right primer for Monk microsatellite Lam_IT_reads_PG1-85_62112_contig_826
Monk_contig_4162_FP1	CAAGCTGGAAAATCCGCTAGC	Left primer for Monk microsatellite Lam_IT_reads_PG1-85_62112_contig_4162
Monk_contig_4162_RP1	TTACCGTCTGTGGTTCTGTG	Right primer for Monk microsatellite Lam_IT_reads_PG1-85_62112_contig_4162
Monk_contig_1762_FP1	GACCGTTGATGCCTTTTCATC	Left primer for Monk microsatellite Lam_IT_reads_PG1-85_62112_contig_1762
Monk_contig_1762_RP1	TTTTATCCAGGCTGTTTGG	Right primer for Monk microsatellite Lam_IT_reads_PG1-85_62112_contig_1762
Monk_contig_4490_FP1	CCTCAGGCTTAGAGTGGTGC	Left primer for Monk microsatellite Lam_IT_reads_PG1-85_62112_contig_4490
Monk_contig_4490_RP1	GTTGTGCTGGCGTAAGGCT	Right primer for Monk microsatellite Lam_IT_reads_PG1-85_62112_contig_4490
Monk_contig_367_FP1	TGTTCTGCCTTTGAGAGGGT	Left primer for Monk microsatellite Lam_IT_reads_PG1-85_62112_contig_367
Monk_contig_367_RP1	AAATACTGACGGCTGCATC	Right primer for Monk microsatellite Lam_IT_reads_PG1-85_62112_contig_367
Monk_contig_1368_FP1	GGGAGAGAGAGAGGAGGAA	Left primer for Monk microsatellite Lam_IT_reads_PG1-85_62112_contig_1368
Monk_contig_1368_RP1	TTTTCGTTGTAAGTGCATGGAC	Right primer for Monk microsatellite Lam_IT_reads_PG1-85_62112_contig_1368
Monk_contig_9645_FP1	TGCCTTCCAAAACATGAGC	Left primer for Monk microsatellite Lam_pooled_contig_9645
Monk_contig_9645_RP1	CATTGCTTTGTAGCACTTTGAGA	Right primer for Monk microsatellite Lam_pooled_contig_9645
Monk_contig_12626_FP1	CCTTGTGCTGGTTCCGAT	Left primer for Monk microsatellite Lam_pooled_contig_12626
Monk_contig_12626_RP1	CAGGAGGGTCTGTGTGGAT	Right primer for Monk microsatellite Lam_pooled_contig_12626
Monk_contig_14752_FP1	AGAACAGCCCTTCCAGACT	Left primer for Monk microsatellite Lam_pooled_contig_14752
Monk_contig_14752_RP1	TGCTACAGCGCCGATCTAC	Right primer for Monk microsatellite Lam_pooled_contig_14752

Monk_contig_17484_FP1	TTTCCAATGACCAAGAGTCG	Left primer for Monk microsatellite Lam_pooled_contig_17484
Monk_contig_17484_RP1	TGTTTCTGTTTTCATTTCGCTTT	Right primer for Monk microsatellite Lam_pooled_contig_17484
Monk_contig_25862_FP1	TTCCGCCCTCTGTTTGTCTCT	Left primer for Monk microsatellite Lam_pooled_contig_25862
Monk_contig_25862_RP1	TAAACGCTCCAAGGTCAAAG	Right primer for Monk microsatellite Lam_pooled_contig_25862
Monk_contig_31337_FP1	TTTTTATATGAGTTTCACACGCTTT	Left primer for Monk microsatellite Lam_pooled_contig_31337
Monk_contig_31337_RP1	TTTTAGCACTTTGAGATTTGATCC	Right primer for Monk microsatellite Lam_pooled_contig_31337
Monk_contig_47995_FP1	ATGGGAAAATGGATGGGAAT	Left primer for Monk microsatellite Lam_pooled_contig_47995
Monk_contig_47995_RP1	CATTTAGATCCAGCCCTGG	Right primer for Monk microsatellite Lam_pooled_contig_47995
Monk_contig_116899_FP1	AAGAACCAACTGAGGAGGATTC	Left primer for Monk microsatellite Lam_contig_116899
Monk_contig_116899_RP1	CATTGTGCATTCTTATCTTACAG	Right primer for Monk microsatellite Lam_contig_116899
Monk_contig_12808_FP1	TAAAGTTGTTAGCCATGTG	Left primer for Monk microsatellite Lam_contig_12808
Monk_contig_12808_RP1	GTTAGCACTTTGAGATTGATCC	Right primer for Monk microsatellite Lam_contig_12808
Monk_contig_132284_FP1	ATAGTGGATCCTCTGCTCCTAG	Left primer for Monk microsatellite Lam_contig_132284
Monk_contig_132284_RP1	TTCCCGAAGATAAACGTACACC	Right primer for Monk microsatellite Lam_contig_132284
Monk_contig_134777_FP1	ATGAAGCTGATTTGGGCAAAG	Left primer for Monk microsatellite Lam_contig_134777
Monk_contig_134777_RP1	TGGATTGATCAAATGTAAGAGGC	Right primer for Monk microsatellite Lam_contig_134777
Monk_contig_141975_FP1	AATGATTGTCACTGTCCACCTC	Left primer for Monk microsatellite Lam_contig_141975
Monk_contig_141975_RP1	GAGTCTCTCACATAGGAGTCG	Right primer for Monk microsatellite Lam_contig_141975
Monk_contig_167825_FP1	CCGGTAAAGAGGGTACATATC	Left primer for Monk microsatellite Lam_contig_167825
Monk_contig_167825_RP1	TGAACGTCAATGAAACATGTTAC	Right primer for Monk microsatellite Lam_contig_167825
Monk_contig_17706_FP1	TCCTGTGATGCTGATGATTGAC	Left primer for Monk microsatellite Lam_contig_17706
Monk_contig_17706_RP1	TGAGAGTGAGACCTCCTACAAC	Right primer for Monk microsatellite Lam_contig_17706
Monk_contig_17806_FP1	TAAGGAGAATCTGTGGACAACG	Left primer for Monk microsatellite Lam_contig_17806
Monk_contig_17806_RP1	TTCATACTTCCGATATTTATAGC	Right primer for Monk microsatellite Lam_contig_17806
Monk_contig_21932_FP1	GGTCTGAACATAAATGAGGCTC	Left primer for Monk microsatellite Lam_contig_21932
Monk_contig_21932_RP1	CTCATCCACACTATGTCTCTGC	Right primer for Monk microsatellite Lam_contig_21932
Monk_contig_29128_FP1	CTTTCATGCTCATCGATGTGTG	Left primer for Monk microsatellite Lam_contig_29128
Monk_contig_29128_RP1	CTCGATGTCCAATGATCACAAG	Right primer for Monk microsatellite Lam_contig_29128
Monk_contig_29581_FP1	CGGTGTGATTGAAGGTTGAAG	Left primer for Monk microsatellite Lam_contig_29581
Monk_contig_29581_RP1	ACAACACCTGAGATGCTTAGAC	Right primer for Monk microsatellite Lam_contig_29581
Monk_contig_30722_FP1	TCTTCTGTGAGGATCAACATGG	Left primer for Monk microsatellite Lam_contig_30722
Monk_contig_30722_RP1	TACTGCATCATCAATCGACATG	Right primer for Monk microsatellite Lam_contig_30722
Monk_contig_32328_FP1	CCTGAAAGACGTTATTTGGGTC	Left primer for Monk microsatellite Lam_contig_32328
Monk_contig_32328_RP1	GTGACCCGAGCGTGAAGAG	Right primer for Monk microsatellite Lam_contig_32328
Monk_contig_36702_FP1	ACCTCACAGACAATAATAGCCG	Left primer for Monk microsatellite Lam_contig_36702
Monk_contig_36702_RP1	TGGAATAAGTCTCTGGATTGGTC	Right primer for Monk microsatellite Lam_contig_36702
Monk_contig_42528_FP1	AGACAAGATGACACAAGATCAG	Left primer for Monk microsatellite Lam_contig_42528
Monk_contig_42528_RP1	TTAAGTAGAAGCAAGTGGAGCC	Right primer for Monk microsatellite Lam_contig_42528
Monk_contig_64199_FP1	GTACGTGTAAGCTATTTGGAAGG	Left primer for Monk microsatellite Lam_contig_64199
Monk_contig_64199_RP1	TTTTTAAACGTCGGAAGTGCTG	Right primer for Monk microsatellite Lam_contig_64199
Monk_contig_65401_FP1	GACACGTTATAAAGACACTGGTG	Left primer for Monk microsatellite Lam_contig_65401
Monk_contig_65401_RP1	CGGATGTTGCGTACTTTTACAG	Right primer for Monk microsatellite Lam_contig_65401
Monk_contig_93121_FP1	AGTCACTATAAATTCATCATTG	Left primer for Monk microsatellite Lam_contig_93121
Monk_contig_93121_RP1	GGAAATGCAAAATGGACACCTAC	Right primer for Monk microsatellite Lam_contig_93121
Monk_contig_115570_FP1	GGAGAAAATGGAGTTTGGTGGG	Left primer for Monk microsatellite Lam_contig_115570
Monk_contig_115570_RP1	CATCACAGAGACACTAGAGAGG	Right primer for Monk microsatellite Lam_contig_115570
Monk_contig_131899_FP1	GGTGTGATAAAAAGCAGCTTGAC	Left primer for Monk microsatellite Lam_contig_131899
Monk_contig_131899_RP1	GTGTGGCGTGCATACAAATATC	Right primer for Monk microsatellite Lam_contig_131899
Monk_contig_14651_FP1	GCTCTCCTCTTGCAACCTATAG	Left primer for Monk microsatellite Lam_contig_14651
Monk_contig_14651_RP1	TCCGTGTGATAAAGTGGCTC	Right primer for Monk microsatellite Lam_contig_14651
Monk_contig_152100_FP1	TAGAGGTCCTATTTCATCTGGG	Left primer for Monk microsatellite Lam_contig_152100
Monk_contig_152100_RP1	TGTCACTACATACCTCGCTATC	Right primer for Monk microsatellite Lam_contig_152100
Monk_contig_156116_FP1	TCATCTACATGCCTTTCAACAAG	Left primer for Monk microsatellite Lam_contig_156116
Monk_contig_156116_RP1	CTTTCACAGACCGTGAACATC	Right primer for Monk microsatellite Lam_contig_156116
Monk_contig_177332_FP1	TGGTCACTCTGAAGAACTCCAC	Left primer for Monk microsatellite Lam_contig_177332
Monk_contig_177332_RP1	CCACTTCCACATGCTGATTATG	Right primer for Monk microsatellite Lam_contig_177332
Monk_contig_178262_FP1	CGTTATTGCTTTGTCTGCGAC	Left primer for Monk microsatellite Lam_contig_178262
Monk_contig_178262_RP1	TCATGGGTTCTATCTGTCAGTC	Right primer for Monk microsatellite Lam_contig_178262
Monk_contig_2488_FP1	GAACTAGAGTCCAGTCAGAAGC	Left primer for Monk microsatellite Lam_contig_2488
Monk_contig_2488_RP1	GGTGGCCGACTAGAATCATTC	Right primer for Monk microsatellite Lam_contig_2488
Monk_contig_33936_FP1	AGTGGTAGTGTGTGCTGATAG	Left primer for Monk microsatellite Lam_contig_33936
Monk_contig_33936_RP1	TGGTTACAGAAAATCAAGGATGC	Right primer for Monk microsatellite Lam_contig_33936
Monk_contig_37120_FP1	GCCCGAAAACGATCTAATTGAAG	Left primer for Monk microsatellite Lam_contig_37120
Monk_contig_37120_RP1	GGTGTGGTGGAGCTGGAGTTC	Right primer for Monk microsatellite Lam_contig_37120
Monk_contig_4370_FP1	TCTCTGTTGGCTATTTGGTGAC	Left primer for Monk microsatellite Lam_contig_4370
Monk_contig_4370_RP1	AGACTGCAGAGAGTTTGTCTG	Right primer for Monk microsatellite Lam_contig_4370
Monk_contig_77819_FP1	AAACTACGAACCTCGGAAGTGG	Left primer for Monk microsatellite Lam_contig_77819
Monk_contig_77819_RP1	GCTCAAGAGGTGAGAGTCCAG	Right primer for Monk microsatellite Lam_contig_77819
Monk_contig_83223_FP1	GACTGTTGAATATTTCCAGCACCC	Left primer for Monk microsatellite Lam_contig_83223
Monk_contig_83223_RP1	TGAGAACCACCTGCTCTAATTGAG	Right primer for Monk microsatellite Lam_contig_83223
Monk_contig_84620_FP1	ATATTTAACAGTGCCTCCGGAG	Left primer for Monk microsatellite Lam_contig_84620
Monk_contig_84620_RP1	CCAATCGAAAACCGTCCCTCTTG	Right primer for Monk microsatellite Lam_contig_84620
Monk_contig_89951_FP1	CCATTAGTAGACGCTGTTGATG	Left primer for Monk microsatellite Lam_contig_89951
Monk_contig_89951_RP1	CAGATGATTCAGTAGCAGGAAAC	Right primer for Monk microsatellite Lam_contig_89951
Monk_contig_93858_FP1	GGCTTTGATTGCTGAACGTTG	Left primer for Monk microsatellite Lam_contig_93858
Monk_contig_93858_RP1	AGCTCAGTTCGACATTCTCAC	Right primer for Monk microsatellite Lam_contig_93858

All PCR amplifications were tested at four different annealing temperatures (52°, 56°, 60°, and 64° C) and with two different DNA polymerases (Taq DNA polymerase and KlenTaq) for each primer pair. In total, 34 of the 72 putative microsatellite loci identified by Next Generation Sequencing provided reliable PCR products under one of the amplification conditions described above. In addition, 7 of the 14 previously isolated microsatellites from *L. budegassa* provided reliable amplification of *Lophius americanus* genomic DNA. Thus, we had 41 primer pairs that we could test for their abilities to define population structure in American monkfish. We ordered oligonucleotide primers for all of these and tested their abilities to PCR amplify monkfish genomic DNA and reveal polymorphisms among 8 specimens from geographically distant areas (two from each of four areas).

4 - Evaluate the extent of allelic diversity at 25 microsatellites that reproducibly amplify in 5 specimens from each of 5 geographically or temporally distinct collections sites.

Of the 41 loci tested in 8 specimens from four geographically distant locales, 13 could be reliably PCR amplified, scored, and provided sufficient levels of genetic variation for population analysis. These 13 were selected for subsequent population screening.

5 - Characterize microsatellite variation in each of the samples collected at 12 diagnostic loci.

All specimens were characterized at the 13 microsatellite loci that are listed in Table 3 using the primers and annealing temperatures that are indicated. All loci showed moderate to high levels of allelic variation based on results from our studies (Table 4). PCR reactions in 20 µl volumes contained approximately 50 ng of template DNA, 1 x PCR buffer, 0.25 mM dNTPs, 0.05 µM forward and reverse primers (one was fluorescently labeled) and 0.1 U Taq DNA or KlenTaq polymerase. PCRs were either multiplexed or done as single reactions and then subsequently multi-pooled prior to analysis. Characterization of microsatellite genotypes was done at no charge to this project on a Beckman Coulter CEQ™8000 capillary-based DNA sequencer (Beckman Coulter, Fullerton, CA) housed across the hall from Wirgin's lab in the NYU NIEHS Molecular Facilities Core of which Wirgin is Co-Director. Multi-pooled PCR reactions were diluted up to 1:3 with Sample Loading Solution (Beckman Coulter), 0.5 to 2 µl of diluted PCR reactions were loaded onto 96 well plates along with 0.5 µl of CEQ DNA Size Standard-400 (Beckman Colter) and run with the FRAG 1 program (Beckman Coulter). MICRO-CHECKER (Oosterhout et al. 2004) was used to test for the presence of null alleles, errors due to microsatellite stuttering, and large allele dropout.

Therefore, in total in this study, we screened 17,277 loci (13 loci x 1,329 specimens). The number of diagnostic loci developed and screened and the number of specimens analyzed exceeded those identified in our original RSA proposal (12 loci and 1000-1200 specimens).

Table 3

Monkfish microsatellite loci isolated, characterized, and screened in 1329 monkfish specimens from 19 collection locales in this project and their primer sequences, repeat motifs, and primer annealing temperatures.

<u>Locus Name</u>	<u>Primers Used in PCR</u>	<u>Repeat Motif</u>	<u>Annealing Temp.</u>
MK2183	Forward: TTGAAATGGACAAGAACCCTG Reverse: TCCTCAATACTGAAACCGCC	(AATG) ⁿ	64° C
MK2242	Forward: AATTCTATGAAATAAATATGCGAAACA Reverse: TGCGTTCAGTCAACATGGA	(ATC) ⁿ	64° C
MK2227	Forward: AAGTCTGAGAGGAGGGAGGG Reverse: GTGTCGGACCTCAATCACCT	(GGA) ⁿ	56° C
MK4490	Forward: CCTCAGGCTTAGAGTGGTGC Reverse: GTTGTGTCTGGCGTAAGGCT	(TG) ⁿ	56° C
OVI-Lb-B20	Forward: CAGCCCATAGGAAATAGACTG Reverse: AGAAAAGTGAAAGCAACACAA	(CT) ⁿ	64° C
MK25862	Forward: TTCGCCCTCTGTTTGTCTCT Reverse: TAACGCTCCCAAGGTCAAAG	(TCTG) ⁿ	64° C
MK17706	Forward: TCCTGTGATGCTGATGATTGAC Reverse: TGAGAGTGAGACCTCCTACAAC	(TTG) ⁿ	58° C
MK64199	Forward: GTACGTGTAAGCTATTTGGAAGG Reverse: TTTTAAAACGTCGGAAGTGCTG	(GAATA) ⁿ	64° C
MK14651	Forward: GCTCTCCTCTTGCAACCTATAG Reverse: TCCTGTTGTGATAAAGTGGCTC	(ATG) ⁿ	58° C
MK156116	Forward: TCATCTACATGCCTTTCAACAAG Reverse: CTTTCAACGACCAGTGAACATC	(CTCT) ⁿ	58° C
MK177332	Forward: TGGTCATCTGAAGAACTCCAC Reverse: CCACTTTCCACATGCTGATTATG	(TTG) ⁿ	58° C

MK178262 Forward: CGTTATTGTCTTTGTCTGCGAC (CTT)ⁿ 58° C
Reverse: TCATGGGTTCCCTATCGTCAGTC

Lobu (CA) 1 Forward: AACATTATTTGTTTCTTTCTTGG (TG)ⁿ 58° C
Reverse: TTTACCTGACTGCTGAGGAT

Table 3 continued

<u>Locus Name</u>	<u>Total Number of Alleles</u>	<u>Allelic Richness</u>	<u>% Missing Data</u>	<u>G Test P</u>	<u>F_{ST}</u>
MK4490D2	5	2.09	0.008	< 0.001	0.004
MK2227D3	10	2.95	0.001	0.405	0.003
MK2183D4 0.0023	7	2.84	0.009	0.760	-
MK2242D2 0.0005	7	3.24	0.005	0.660	-
OV1B20D2 0.0006	15	4.52	0.009	0.069	-
25862D4	12	2.05	0.014	< 0.001	0.0007
64199D2	10	3.82	0.003	0.353	0.0008
11706D3	81	7.37	0.018	0.009	0.0023
177332D2	16	3.14	0.005	0.003	0.0015
14651D4 0.0025	18	3.62	0.011	< 0.001	-
178262D3	16	4.01	0.027	0.005	0.0012
156116D3	27	5.44	0.006	< 0.001	0.0022
LOBU1D2	6	2.02	0.017	< 0.001	0.0001
Mean	17.69	3.62	0.010		

Table 4

Characterization of allelic diversity, mean allelic richness, observed and expected, F_{ST} , and F_{IS} at 13 monkfish microsatellite loci across 19 collection sites across the Atlantic coast of North America						
Monkfish Locus	Total N Alleles	Mean Allelic Richness	Observed Hetero	Expected Hetero	F_{ST}	F_{IS}
MK2183	7	2.842	0.406	0.422	0.0023	0.0381
MK2242	7	3.236	0.594	0.583	0.0005	0.0118
MK2227	10	2.955	0.516	0.626	0.0003	0.0153
MK4490	5	2.092	0.656	0.667	0.0041	0.0203
OVI-Lb-B20	15	4.523	0.859	0.807	0.0006	0.0469
MK25862	12	2.048	0.344	0.331	0.0007	0.0024
MK17706	81	7.374	0.641	0.746	0.0015	0.0593
MK64199	10	3.821	0.969	0.972	0.0008	0.0198
MK14651	18	3.617	0.594	0.643	0.0025	0.0172
MK156116	27	5.439	0.656	0.717	0.0022	0.1436
MK177332	16	3.414	0.719	0.775	0.0015	0.0141
MK178262	14	4.098	0.781	0.851	0.0012	0.0271
Lobu(CA)1	6	2.019	0.375	0.359	0.0001	0.0823
Total	228					
Mean	17.5	3.652	0.624	0.654	0.0005	0.0361

6 - Statistically analyze DNA data for stock structure and migration rates.

Multi-locus microsatellite nuclear DNA genotypes were compiled for each monkfish specimen at the 13 loci. Measures of diversity, including mean number of alleles, allelic richness, effective number of alleles, F_{IS} and observed and expected heterozygosities (H_o and H_e) were calculated using FSTAT version 2.9.3 (Goudet et al. 1995; Goudet 2001) for each locus and collection sampled. Deviations from Hardy-Weinberg proportions and linkage equilibrium were tested with GENEPOP v4.0.6 (Rousset 2007) using the Markov chain method with 10,000 iterations and 10,000 batches (Raymond and Rousset, 1995). Locus specific F_{ST} values and G tests of allelic differentiation were calculated in FSTAT version 2.9.3 (Goudet et al. 1995; Goudet 2001) and GENEPOP, respectively. Pairwise F_{ST} comparisons were done at single loci and across all loci using the F_{ST} estimator θ of Weir and Cockerham (1984). F'_{ST} pairwise comparisons between collection locales were calculated in GenoDive (Meirmans and Van Tienderen 2004). F'_{ST} comparisons compensate for different levels of heterozygosity among collection locales. Nei's genetic distance comparisons among pairwise collections was calculated in GenAlEx 6.5 (Peakall and Smouse (2006; 2012). Bonferroni adjustments were applied to the P values

generated from all component tests. The statistical power and realized α -error for assessing the null hypothesis of genetic homogeneity within and across sample collections were evaluated using POWSIM (Ryman and Palm 2006).

We also analyzed the data on an individual basis without *a priori* designation of populations as an exploration of population structure using STRUCTURE v.2.3 (Pritchard et al. 2000; Falush et al 2003; Pritchard et al. 2010). This Bayesian clustering approach defines population units by iteratively sorting individual genotypes into clusters to maximize the fit of the data to theoretical expectations derived from Hardy-Weinberg and linkage equilibrium. Its use enabled us to infer the number of genetically homogenous clusters within samples and allow assignment of individuals to designated genetic clusters. For STRUCTURE, we used the admixture model and correlated allelic frequencies among collections. Both the plateau of likelihood values ($\ln P(D)$) and Delta K methods (Evanno et al. 2005) were used to analyze for the correct number of clusters. Length of Burnin period was set at 5,000 and number of reps after Burnin at 50,000. K was set at 1 to 20 and the number of iterations for each value of K at five.

7 – Disseminate results to user groups.

While we have yet to publish our results or present them at a management forum, we have extensively discussed them with Anne Richards, the monkfish manager at the NEFSC of NOAA.

RESULTS

Sample and Data Collection

A total of 1572 monkfish samples were collected during the course of this project (refer to Figure 3, Table 5). The following is a breakdown of the 1572 totals samples collected in each project area; 64 samples in 1A, 109 samples in 1B, 97 samples 2A, 94 samples in 2B, 166 samples in 2C, 86 samples in 2D, 133 samples in 3A, 183 samples in 3B, 219 samples in 3C, 94 samples in 3D, 11 samples in 4A, 113 samples in 4B, 46 samples in 4C, 14 samples in 5A, 7 sample in 5B, 13 samples in 5C, 89 samples in 6A, and 34 samples in 6B.

A total of 1334 samples were sent to NYU (refer to Figure 4, Table 6). Our Sample Collection Network actually collected a total of more samples overall than needed and budgeted for this project. Excess samples from areas that were over-sampled have been stored at CCE for any possible future use. The total number of samples sent to NYU for DNA analysis is detailed by area in Table 6. The 1334 samples sent to NYU can be separated into the northern and southern management areas as follows:

Northern Management Area including the Scotian Shelf – 575 samples

Southern Management Area including both inshore and offshore – 759 samples

A sufficient number of samples (>50) were collected and analyzed from most of the statistical areas to allow for robust statistical evaluation of the extent of genetic differentiation existing in each region.

The 1572 samples were comprised of either a 1-inch clip of flesh from the tail fin or a clip of vertebra tissue from a single fish. Fin clips stored in 95% ethanol were the source of DNA for all the samples that were obtained from the commercial fishermen. DNAs from samples that were collected by NEFSC trawl surveys were obtained from sections of frozen archived vertebrae and surrounding muscle that had been retained by NOAA for ageing studies. The minimum data fields collected relative to each sample included date of harvest, area (specific latitude and longitude), depth, VTR number, and vessel name. Sex, length and weight information was also collected when possible.

All the data collected was processed by CCE staff to validate the location of each monkfish sample collected. Once a sample location was validated, it could then be assigned to its associated project area. Samples taken by commercial fishermen required a VTR to document the exact project area fished during sampling. Latitude and Longitude coordinates were used to pinpoint the origins of each sample. All sample vials were topped off with ethanol to secure proper preservation during shipping. CCE staff made certain all vials were appropriately sealed and packaged for shipment to NYU. Shipment confirmation was made with Isaac Wirgin of NYU to ensure the integrity of each sample was maintained. Refer to Figure 4 and Table 6 for samples sent to NYU.

Figure 3 – Total monkfish samples collected by area

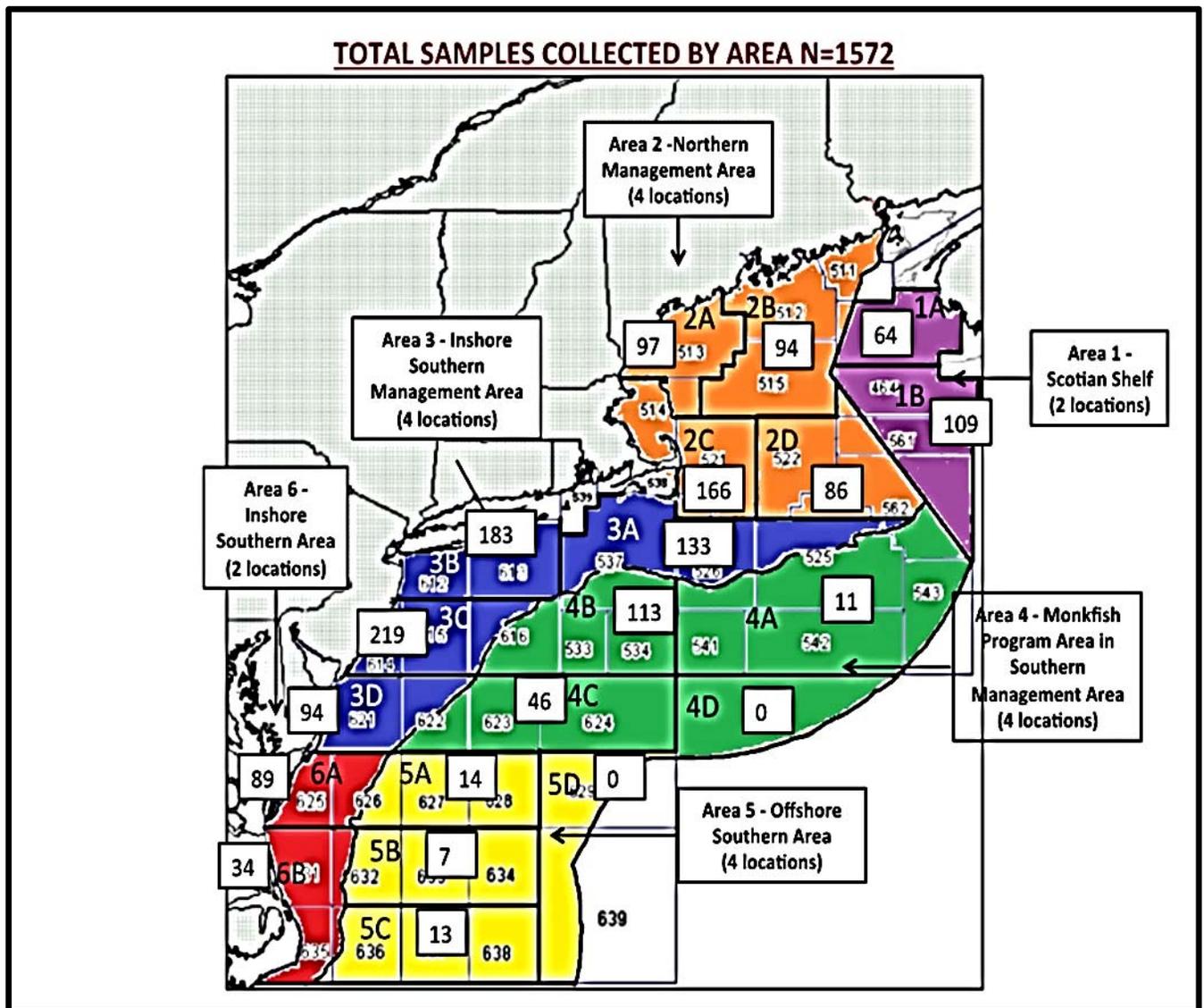


Figure 4 – Monkfish samples sent to NYU by area

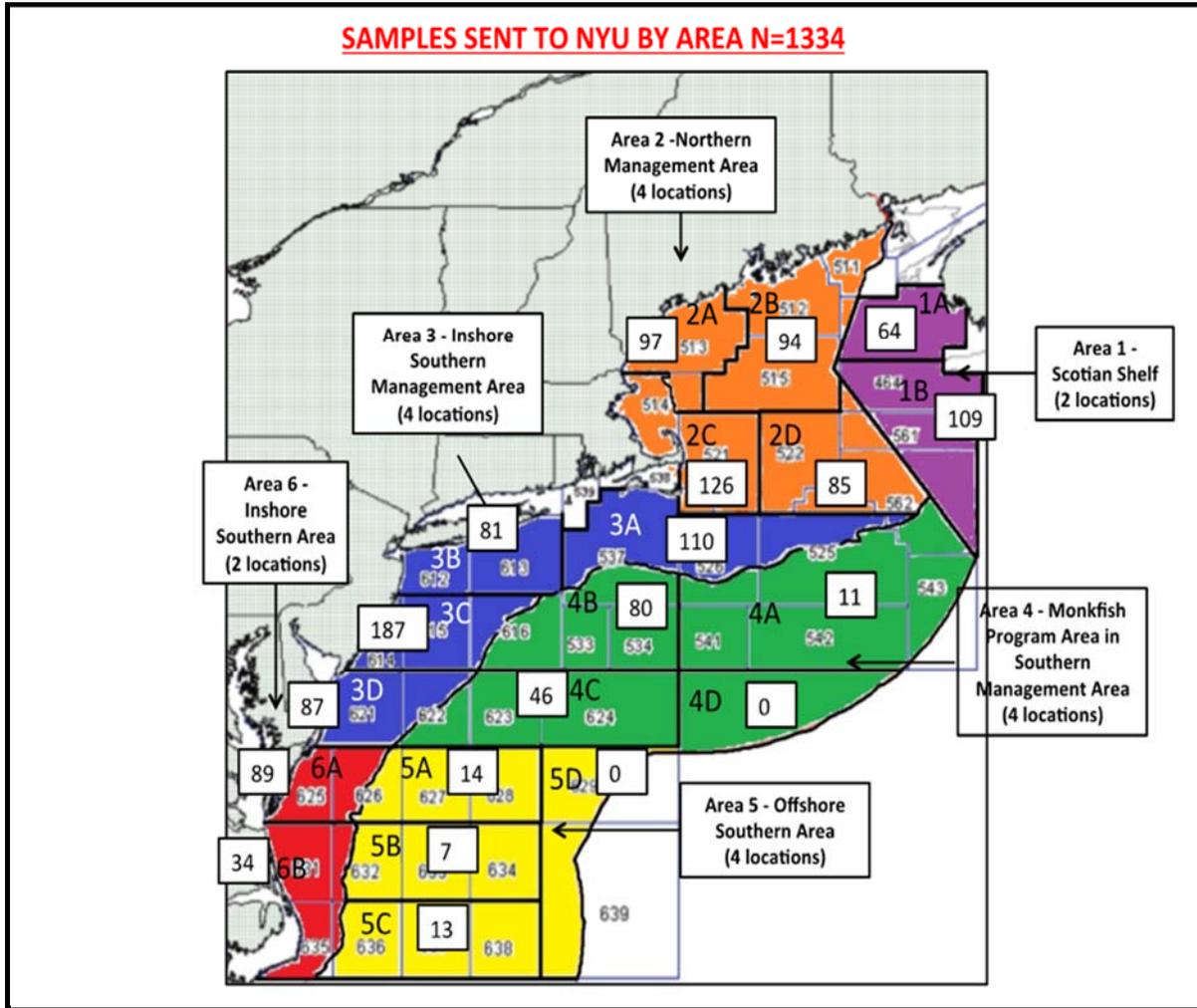


Table 5 – Samples collected by project area

Total Samples By Project Area																					
PORT LANDED/SOURCE	1A	1B	2A	2B	2C	2D	3A	3B	3C	3D	4A	4B	4C	4D	5A	5B	5C	5D	6A	6B	PORT TOTAL
PORTLAND, ME			44	10	10																80
GLOUCESTER, MA				10	82	25															117
NEW BEDFORD, MA					62	40															102
PT JUDITH, RI							73	6	26			48	15		8						176
NEWPORT, RI												20									20
NEW LONDON CT												40									40
SHINNECOCK, NY								50													50
MONTAUK, NY							20	111		23			8							3	165
BARNEGAT LIGHT, NJ									136												136
CAPE MAY, NJ										13											13
OCEAN CITY, MD										20											34
CHINCOTEAGUE, VA																				35	35
WANCHESE, NC																	3				3
AGGER								10													10
NMFS WOODS HOLE	37	37	37	73	5	21	34	6	55	14	11	5	23		6	7	10		17	34	432
CANADIAN FISHERIES DEPT.	27	72		1	7		1														108
DELAWARE STATE UNIV.										24											24
NEAMAP (VIMS)							5		2												7
TOTAL PER PROJECT AREA:	64	109	97	94	166	86	133	183	219	94	11	113	46	0	14	7	13	0	89	34	1572
																					TOTAL KNOWN

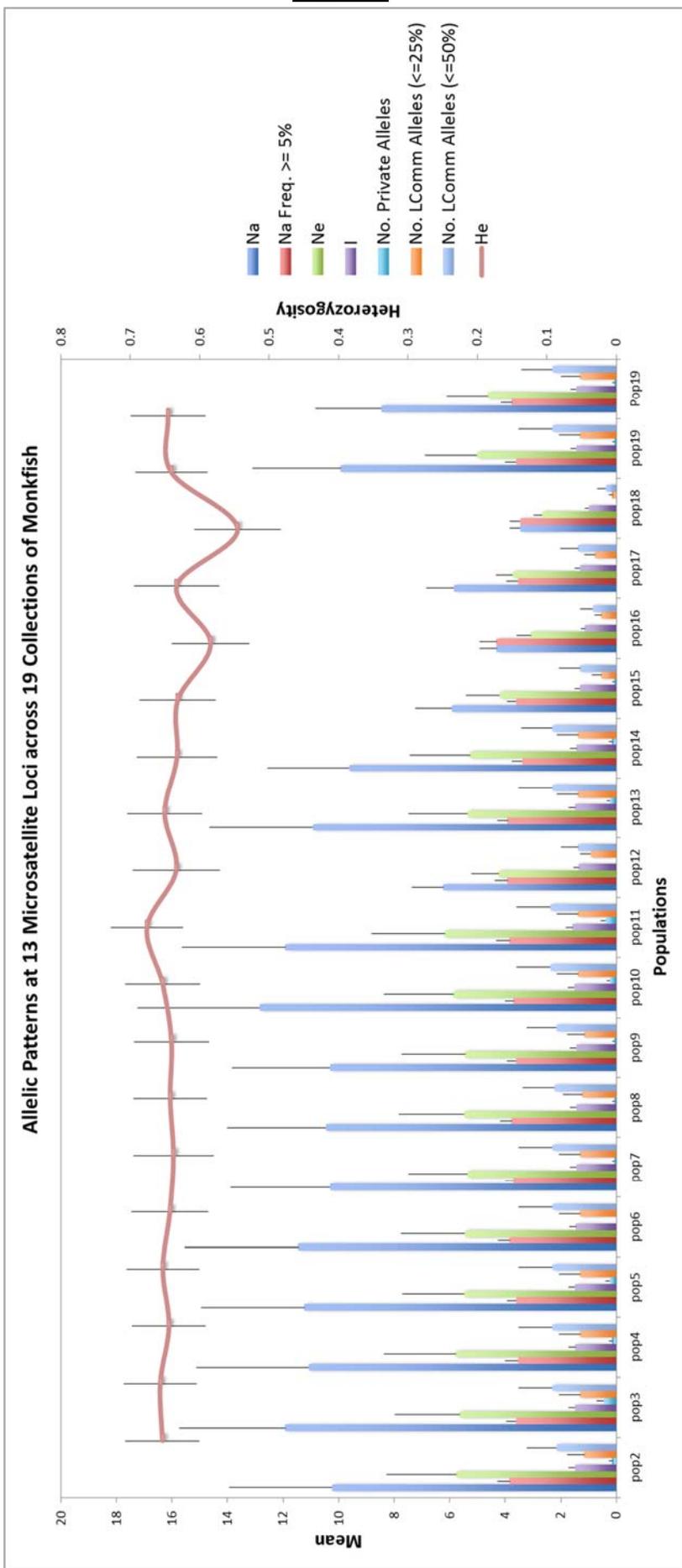
Table 6 - Samples sent to NYU

PORT LANDED/SOURCE	1A	1B	2A	2B	2C	2D	3A	3B	3C	3D	4A	4B	4C	4D	5A	5B	5C	5D	6A	6B	PORT TOTAL
PORTLAND, ME			60	10	10																80
GLOUCESTER, MA				10	42	25															77
NEW BEDFORD, MA					62	39															101
PT JUDITH, RI							55		16			48	15		8						142
NEWPORT, RI																					0
NEW LONDON, CT												27									27
SHINNECOCK, NY								34													34
MONTAUK, NY							20	31		23			8							3	85
BARNEGAT LIGHT, NJ									116												116
CAPE MAY, NJ										6											6
OCEAN CITY, MD										20											34
CHINCOTEAGUE, VA																					35
WANCHESE, NC																	3				3
AGGAR								10													10
NMFS WOODS HOLE	37	37	37	73	5	21	34	6	55	14	11	5	23		6	7	10		17	34	432
CANADIAN FISHERIES DEPT.	27	72		1	7		1														108
DELAWARE STATE UNIV.										24											24
NEAMAP (VIMS)																					0
TOTAL PER PROJECT AREA:	64	109	97	94	126	85	110	81	187	87	11	80	46	0	14	7	13	0	89	34	1334
																					KNOWN SAMPLES SENT TO

DNA Analysis

The percentage of missing data at individual loci was low and ranged from 2.7% at locus 178262 to 0.1% at MK2227. The mean percentage of missing data across all loci and collection areas was 1.0%. In total, we screened 228 alleles in our survey with a mean number of 17.5 alleles observed across all loci and area collections. The largest number of alleles was observed at locus MK11706 (n=81) and the smallest number at MK4490 (N=5) (Table 3). Similarly, allelic richness ranged from 2.02 at Lobu(CA)1 to 7.37 at MK17706 with a mean allelic richness across all loci of 3.65. Observed heterozygosity was high and ranged from 0.344 at MK25862 to 0.969 at MK64199 with a mean across all loci of 0.624. F_{ST} values at individual loci were low and ranged from 0.0001 at Lobu(CA) 1 to 0.0025 at MK14651. The mean F_{ST} value across all 13 loci and collections was 0.0005 (Table 3) A summary of allelic patterns within and compared across all 19 collections of monkfish (Na- total number of alleles; Na Freq.- number of alleles with frequencies > 5%; Ne- effective number of alleles; No private alleles; No. common allele <=25%; No. common alleles <=50%; and He- expected heterozygosity) is provided in Figure 5 and individual values for each of the 13 loci and 19 collections are depicted in Table 7.

Figure 5



	He	0.469	0.515	0.643	0.693	0.812	0.284	0.728	0.972	0.645	0.693	0.759	0.883	0.398
	uHe	0.470	0.516	0.645	0.695	0.814	0.285	0.730	0.974	0.647	0.695	0.761	0.885	0.399
Pop 3D	F	0.185	0.067	0.079	0.022	0.050	-0.007	-0.020	0.011	-0.077	-0.005	0.019	0.127	0.121
	N	85	87	86	85	87	86	86	85	87	86	84	87	85
	Na	2	8	4	5	9	5	8	54	12	15	12	17	4
	Ne	1.895	2.394	2.765	3.414	4.707	1.351	3.485	37.051	3.307	3.922	4.924	9.038	2.113
	I	0.665	1.226	1.075	1.295	1.758	0.550	1.518	3.769	1.568	1.739	1.905	2.462	0.924
	Ho	0.576	0.563	0.663	0.776	0.690	0.256	0.756	0.835	0.621	0.628	0.821	0.805	0.318
	He	0.472	0.582	0.638	0.707	0.788	0.260	0.713	0.973	0.698	0.745	0.797	0.889	0.527
	uHe	0.475	0.586	0.642	0.711	0.792	0.261	0.717	0.979	0.702	0.749	0.802	0.894	0.530
Pop 4A	F	-0.221	0.033	-0.038	-0.098	0.124	0.015	-0.060	0.142	0.110	0.157	-0.031	0.095	0.397
	N	12	12	11	12	12	12	12	12	12	12	12	12	12
	Na	3	5	3	4	6	3	6	17	5	7	10	10	2
	Ne	1.646	1.725	2.350	3.130	3.349	1.185	4.431	14.400	3.130	4.800	7.024	6.128	1.800
	I	0.675	0.895	0.930	1.237	1.415	0.345	1.611	2.752	1.358	1.723	2.103	2.029	0.637
	Ho	0.500	0.417	0.364	0.583	0.917	0.167	0.667	1.000	0.500	0.833	0.917	0.667	0.167
	He	0.392	0.420	0.574	0.681	0.701	0.156	0.774	0.931	0.681	0.792	0.858	0.837	0.444
	uHe	0.409	0.438	0.602	0.710	0.732	0.163	0.808	0.971	0.710	0.826	0.895	0.873	0.464
Pop 4B	F	-0.274	0.008	0.367	0.143	-0.307	-0.067	0.139	-0.075	0.265	-0.053	-0.069	0.203	0.625
	N	78	79	78	79	78	79	79	76	78	77	78	79	76
	Na	3	6	5	5	10	6	7	54	9	8	9	17	3
	Ne	1.784	1.933	2.793	3.166	5.270	1.395	4.003	30.241	2.981	3.358	3.751	7.253	1.774
	I	0.659	1.034	1.124	1.246	1.825	0.639	1.575	3.680	1.445	1.438	1.607	2.309	0.656
	Ho	0.410	0.443	0.615	0.734	0.782	0.278	0.734	0.842	0.679	0.701	0.756	0.734	0.395
	He	0.440	0.483	0.642	0.684	0.810	0.283	0.750	0.967	0.665	0.702	0.733	0.862	0.436
	uHe	0.442	0.486	0.646	0.689	0.815	0.285	0.755	0.973	0.669	0.707	0.738	0.868	0.439
Pop 4C	F	0.067	0.082	0.041	-0.073	0.035	0.017	0.021	0.129	-0.023	0.001	-0.031	0.148	0.095
	N	43	46	46	45	44	43	45	40	43	45	40	45	44
	Na	2	7	3	4	9	5	7	42	7	7	11	18	3
	Ne	1.644	1.888	2.384	3.016	4.665	1.274	3.982	30.769	2.795	3.159	4.402	6.716	1.809
	I	0.581	0.980	0.942	1.188	1.781	0.508	1.506	3.581	1.337	1.390	1.834	2.297	0.707
	Ho	0.256	0.370	0.587	0.667	0.773	0.209	0.800	0.950	0.558	0.622	0.850	0.778	0.455
	He	0.392	0.470	0.581	0.668	0.786	0.215	0.749	0.968	0.642	0.683	0.773	0.851	0.447
	uHe	0.396	0.476	0.587	0.676	0.795	0.218	0.757	0.980	0.650	0.691	0.783	0.861	0.452
Pop 5A	F	0.347	0.214	-0.011	0.003	0.016	0.026	-0.068	0.018	0.131	0.090	-0.100	0.086	-0.016
	N	13	13	13	13	13	13	13	12	13	13	10	13	12
	Na	3	5	3	4	7	2	6	20	5	6	4	10	2
	Ne	1.807	2.139	2.620	3.219	4.694	1.352	3.756	18.000	2.840	3.521	3.030	6.145	1.492
	I	0.733	1.066	1.016	1.253	1.682	0.429	1.486	2.947	1.284	1.495	1.202	2.034	0.512
	Ho	0.462	0.538	0.615	0.769	0.846	0.308	0.615	0.833	0.769	0.615	0.800	0.846	0.417
	He	0.447	0.533	0.618	0.689	0.787	0.260	0.734	0.944	0.648	0.716	0.670	0.837	0.330
	uHe	0.465	0.554	0.643	0.717	0.818	0.271	0.763	0.986	0.674	0.745	0.705	0.871	0.344
Pop 5B	F	-0.033	-0.011	0.005	-0.116	-0.075	-0.182	0.161	0.118	-0.187	0.140	-0.194	-0.011	-0.263
	N	7	7	6	6	6	7	7	6	6	6	6	7	6
	Na	2	2	3	4	5	2	4	9	5	5	6	7	2
	Ne	1.960	1.153	2.182	2.667	3.789	1.508	3.161	8.000	3.130	2.571	2.667	5.444	1.800
	I	0.683	0.257	0.888	1.127	1.468	0.520	1.240	2.138	1.358	1.234	1.350	1.810	0.637
	Ho	0.286	0.143	0.500	0.500	0.667	0.429	0.714	0.667	0.833	0.667	0.833	0.857	0.667
	He	0.490	0.133	0.542	0.625	0.736	0.337	0.684	0.875	0.681	0.611	0.625	0.816	0.444
	uHe	0.527	0.143	0.591	0.682	0.803	0.363	0.736	0.955	0.742	0.667	0.682	0.879	0.485
Pop 5C	F	0.417	-0.077	0.077	0.200	0.094	-0.273	-0.045	0.238	-0.224	-0.091	-0.333	-0.050	-0.500
	N	12	13	13	13	13	10	13	11	12	13	11	13	12
	Na	2	6	3	4	8	2	7	14	4	7	7	10	2
	Ne	1.704	1.965	2.889	2.600	6.760	1.105	3.756	8.345	3.310	3.380	5.261	5.541	1.882
	I	0.604	1.044	1.080	1.088	1.979	0.199	1.553	2.413	1.283	1.484	1.794	1.957	0.662
	Ho	0.583	0.462	0.462	0.538	0.615	0.100	0.769	0.909	0.750	0.846	0.727	0.769	0.417
	He	0.413	0.491	0.654	0.615	0.852	0.095	0.734	0.880	0.698	0.704	0.810	0.820	0.469
	uHe	0.431	0.511	0.680	0.640	0.886	0.100	0.763	0.922	0.728	0.732	0.848	0.852	0.489
Pop 6A	F	-0.412	0.060	0.294	0.125	0.278	-0.053	-0.048	-0.033	-0.075	-0.202	0.102	0.061	0.111
	N	89	89	88	88	85	87	89	86	89	87	87	89	86
	Na	3	6	4	4	10	6	7	46	7	8	9	17	2
	Ne	1.741	1.838	2.583	3.263	6.285	1.517	3.998	27.141	2.427	3.629	3.032	5.874	1.783
	I	0.683	0.939	1.027	1.255	1.950	0.758	1.541	3.542	1.225	1.525	1.447	2.170	0.631
	Ho	0.438	0.517	0.557	0.670	0.800	0.356	0.787	0.791	0.551	0.667	0.736	0.562	0.488
	He	0.426	0.456	0.613	0.694	0.841	0.341	0.750	0.963	0.588	0.724	0.670	0.830	0.439
	uHe	0.428	0.459	0.616	0.697	0.846	0.343	0.754	0.969	0.591	0.729	0.674	0.834	0.442
Pop 6B	F	-0.029	-0.134	0.092	0.033	0.049	-0.045	-0.049	0.179	0.064	0.080	-0.098	0.323	-0.112
	N	34	34	32	34	34	30	34	32	34	34	32	34	33
	Na	2	6	4	4	8	4	6	34	6	10	8	16	2
	Ne	1.448	2.444	2.872	3.185	5.558	1.905	4.181	21.787	1.914	4.211	4.154	4.961	1.541
	I	0.488	1.218	1.144	1.219	1.823	0.914	1.528	3.321	1.023	1.742	1.627	2.098	0.536
	Ho	0.324	0.765	0.656	0.735	0.735	0.500	0.676	0.906	0.500	0.735	0.719	0.735	0.333
	He	0.309	0.591	0.652	0.686	0.820	0.475	0.761	0.954	0.478	0.763	0.759	0.798	0.351
	uHe	0.314	0.600	0.662	0.696	0.832	0.483	0.772	0.969	0.485	0.774	0.771	0.810	0.357
	F	-0.046	-0.294	-0.007	-0.072	0.103	-0.053	0.111	0.050	-0.047	0.036	0.053	0.079	0.051

Considering a 3 loci and all area collections, we found highly significant variation ($X^2 = \text{Infinity}$) across all loci and collections in our study. Using the Exact G test, we found that 8 of the 13 individual microsatellite loci exhibited significant allelic variation ($P = < 0.05$) including MK4490 ($P = < 0.01$), MK25862 ($P = 0.007$), MK11706 ($P = 0.01$), MK177332 ($P = 0.003$), MK14651 ($P = < 0.001$), MK17826 ($P = 0.005$), MK156116 ($P = < 0.01$), and LOBU1 ($P < 0.001$). Our Exact G test pairwise comparisons of allelic frequencies at collection locales indicated significant genetic differentiation between monkfish from our southern sampled areas and those to their north (results summarized in Table 8). In our study, monkfish sampling areas ranged from the Grand Banks, Newfoundland, to North Carolina (see Figure 2 for specific locations). Specific results by collection location showed that allelic frequencies in area 3D (southern NJ to northern MD) was highly significantly different ($< .001$) from all areas to its north, extending from areas 1A (Grand Banks) to 3C (central NJ). In contrast, Area 3D was not genetically different from all areas to its south except 6A (northern to mid VA) and 6B (southern VA to NC). Despite less than optimal samples sizes, we also saw significant genetic differentiation between collections from areas 6A (northern to mid VA) and 6B (southern VA to NC) compared to areas to their north. For example, area 6A was significantly distinct ($P < 0.01$) from 6 out of 18 collection areas to its north. Similarly, area 6B was significantly differentiated ($P < 0.01$) from 9 of 18 collection areas to its north.

Table 8

Exact G Test Pairwise Comparisons of Allelic Frequencies at 13 Microsatellite Loci among 19 Collections of Monkfish from the Atlantic Coast of North America. Pairwise G test values are above the diagonal and pairwise P values are below the diagonal.

	1A	1B	2A	2B	2C	2D	3A	3B	3C	3D	4A	4B	4C	5A	5B	5C	6A	6B
1A	0	25.52	26.79	29.83	43.69	33.19	27.85	36.88	36.98	61.35	25.069	41.49	26.53	17.9	29.76	36.85	32.87	32.41
1B	0.49	0	30.05	44.39	44.41	33.97	50.53	41.46	54.22	INF	32.43	42.76	40.08	21.77	28.51	38.19	47.08	43.12
2A	0.421	0.265	0	32.93	26.45	17.35	21.46	22.17	32.65	INF	29.28	30.21	35.93	17.95	29.58	33.95	42.16	47.73
2B	0.275	0.0137	0.164	0	31.26	28.31	30.68	25.1	37.15	INF	21.52	35.86	33.96	18.54	28.56	41.53	43.3	56.32
2C	0.0163	0.0137	0.439	0.219	0	29.52	35.66	40.55	INF	INF	28.67	35	38.77	27.1	30.54	37.91	50.09	INF
2D	0.157	0.136	0.898	0.344	0.288	0	26.1	30.28	35.87	INF	27.03	34.61	26.36	26.74	29.48	43.01	45.87	47.81
3A	0.366	0.003	0.718	0.24	0.098	0.457	0	41.73	46.71	INF	28.71	39.72	46.25	26.65	32.91	45.92	43.69	57.51
3B	0.077	0.028	0.679	0.513	0.034	0.256	0.026	0	52.72	INF	21.43	42.36	36.03	25.4	29.51	31.8	33.4	48.06
3C	0.075	<0.001	0.173	0.072	HS	HS	HS	HS	HS	HS	21.74	51.92	35.14	19.89	24.39	34.25	532.66	INF
3D	<0.001	HS	HS	HS	HS	HS	HS	HS	HS	HS	22.09	INF	39.47	23.97	23.4	35.6	54.83	INF
4A	0.515	0.179	0.299	0.715	0.327	0.408	0.324	0.719	0.703	0.684	0	20.98	11.22	18.2	16.38	14.78	28.78	23.84
4B	0.028	0.02	0.259	0.094	0.111	0.12	0.042	0.023	0.002	HS	0.742	0	30.41	23.25	26.1	30.37	59.96	47.57
4C	0.434	0.038	0.093	0.136	0.051	0.43432	0.009	0.091	0.109	0.044	0.995	0.251	0	22.73	17.93	26.29	39.78	28.34
5A	0.879	0.701	0.877	0.855	0.404	0.423	0.428	0.496	0.797	0.577	0.868	0.619	0.648	0	21.92	23.39	21.96	19.84
5B	0.269	0.334	0.286	0.331	0.246	0.29	0.165	0.288	0.554	0.61	0.927	0.458	0.878	0.693	0	19.96	29.94	30.03
5C	0.077	0.058	0.136	0.027	0.062	0.019	0.009	0.199	0.129	0.069	0.96	0.252	0.447	0.611	0.793	0	43.35	39.08
6A	0.166	0.006	0.024	0.018	0.003	0.009	0.016	0.151	0.001	HS	0.321	<0.001	0.041	0.691	0.27	0.018	0	31.87
6B	0.18	0.019	0.006	<0.001	HS	0.006	<0.001	0.005	HS	<0.001	0.585	0.006	0.342	0.799	0.267	0.048	0.198	0

INF= Infinity
HS= Highly Significant

We also used F statistics to compare allelic frequencies among collection areas. Results of F_{ST} pairwise comparisons are depicted in Table 9. Once again the extent of genetic differentiation between 3D and collection areas to its north was relatively high, but in this case not statistically significantly different. A consideration in not attaining statistical significance in pairwise comparisons using F_{ST} test was the highly conservative Bonferonni correction that was applied ($P < 0.0003$). However if a $P < 0.01$ cutoff is used for significance, all comparisons of 3D to areas to its north were significant. Similarly, several comparisons of 6A to other areas were significant with a $P < 0.01$ cutoff for significance.

Table 9

Pairwise Fst' Comparison of 19 Collections of Monkfish from the Atlantic Coast of North America based on Data from 13 Microsatellite Loci																		
	Pop 1A	Pop 1B	Pop 2A	Pop 2B	Pop 2C	Pop 2D	Pop 3A	Pop 3B	Pop 3C	Pop 3D	Pop 4A	Pop 4B	Pop 4C	Pop 5A	Pop 5B	Pop 5C	Pop 6A	Pop 6B
Pop 1A	0.000																	
Pop 1B	0.000	0.000																
Pop 2A	0.000	-0.003	0.000															
Pop 2B	-0.001	0.000	-0.002	0.000														
Pop 2C	0.005	0.001	-0.005	-0.002	0.000													
Pop 2D	-0.003	0.000	-0.006	-0.005	-0.002	0.000												
Pop 3A	-0.002	0.001	-0.005	-0.001	-0.001	-0.003	0.000											
Pop 3B	0.007	0.003	-0.004	-0.004	-0.004	-0.002	-0.002	0.000										
Pop 3C	-0.002	0.002	-0.002	-0.001	0.000	-0.003	0.001	0.004	0.000									
Pop 3D	0.003	0.002	0.004	0.007	0.005	0.005	0.004	0.011	0.005	0.000								
Pop 4A	-0.023	-0.003	-0.011	-0.028	-0.010	-0.031	-0.018	-0.022	-0.020	-0.023	0.000							
Pop 4B	0.001	0.000	-0.003	-0.004	-0.002	-0.006	-0.002	0.000	-0.001	0.003	-0.026	0.000						
Pop 4C	-0.011	0.002	-0.004	-0.007	-0.003	-0.014	-0.006	-0.004	-0.006	0.002	-0.057	-0.009	0.000					
Pop 5A	-0.030	-0.025	-0.026	-0.032	-0.021	-0.026	-0.026	-0.025	-0.028	-0.026	-0.046	-0.024	-0.036	0.000				
Pop 5B	-0.012	-0.014	-0.012	-0.031	-0.008	-0.034	-0.018	-0.021	-0.028	-0.024	-0.088	-0.041	-0.077	-0.101	0.000			
Pop 5C	0.010	-0.004	0.006	0.001	0.007	-0.004	0.006	-0.004	0.000	0.003	-0.072	-0.009	-0.025	-0.051	-0.132	0.000		
Pop 6A	0.004	0.006	0.002	0.003	0.008	0.003	0.004	0.002	0.005	0.019	-0.011	0.003	-0.005	-0.026	-0.037	0.007	0.000	
Pop 6B	-0.002	0.019	0.014	0.013	0.019	0.012	0.013	0.009	0.015	0.026	-0.012	0.016	-0.002	-0.034	-0.001	0.019	0.001	0

Additionally, we used a Bayesian model-based clustering approach implemented in STRUCTURE to determine the number of different populations within our coast wide collections of monkfish. Unlike other methods, STRUCTURE does not compare allelic frequencies among *a priori* defined collections, but instead identifies the number of clusters within an allelic data set in which Hardy-Weinberg and linkage disequilibria are optimized. Analysis of our STRUCTURE results using both the (Ln P(D)) and Delta K methods suggested the presence of two clusters (K) within our coast wide collections (Table 10).

In summary, our results indicate that there is significant genetic differentiation between area 3D, and maybe even 3C, and all collection areas to the north of 3D and 3C. Furthermore, there are indications that our southernmost collection areas, 6A and 6B, are genetically distinct from areas to their north including even 3C and 3D. Thus, our preliminary analysis indicates that there are 2 and perhaps 3 genetic stocks of monkfish along their coast wide distribution.

Table 10

Pairwise Population Matrix of Nei's Genetic Distance among 19 Collections of Monkfish based on Allelic Frequencies at 13 Microsatellite Loci

	Pop 1A	Pop 1B	Pop 2A	Pop 2B	Pop 2C	Pop 2D	Pop 3A	Pop 3B	Pop 3C	Pop 3D	Pop 4A	Pop 4B	Pop 4C	Pop 5A	Pop 5B	Pop 5C	Pop 6A	Pop 6B	
Pop 1A	0.000																		
Pop 1B	0.013	0.000																	
Pop 2A	0.013	0.007	0.000																
Pop 2B	0.013	0.010	0.009	0.000															
Pop 2C	0.014	0.008	0.005	0.008	0.000														
Pop 2D	0.012	0.010	0.007	0.008	0.008	0.000													
Pop 3A	0.011	0.009	0.006	0.009	0.007	0.009	0.000												
Pop 3B	0.018	0.012	0.008	0.009	0.007	0.011	0.010	0.000											
Pop 3C	0.010	0.009	0.007	0.008	0.006	0.007	0.008	0.012	0.000										
Pop 3D	0.017	0.013	0.013	0.017	0.012	0.015	0.013	0.019	0.013	0.000									
Pop 4A	0.035	0.043	0.038	0.030	0.036	0.030	0.034	0.034	0.033	0.038	0.000								
Pop 4B	0.016	0.011	0.009	0.009	0.009	0.009	0.010	0.013	0.009	0.015	0.034	0.000							
Pop 4C	0.014	0.018	0.015	0.014	0.013	0.010	0.013	0.016	0.011	0.020	0.028	0.014	0.000						
Pop 5A	0.029	0.027	0.027	0.025	0.027	0.031	0.027	0.031	0.026	0.034	0.063	0.034	0.040	0.000					
Pop 5B	0.076	0.067	0.068	0.061	0.065	0.063	0.066	0.069	0.059	0.073	0.091	0.060	0.060	0.086	0.000				
Pop 5C	0.058	0.043	0.050	0.050	0.047	0.048	0.050	0.047	0.046	0.056	0.059	0.047	0.051	0.074	0.089	0.000			
Pop 6A	0.017	0.014	0.012	0.013	0.014	0.014	0.013	0.014	0.012	0.025	0.043	0.015	0.015	0.031	0.062	0.056	0.000		
Pop 6B	0.023	0.033	0.029	0.030	0.031	0.030	0.028	0.028	0.029	0.041	0.056	0.034	0.031	0.040	0.105	0.082	0.023	0.000	

DISCUSSION

For the first time in this study we isolated and identified a suite of monkfish-specific microsatellite loci and developed assay conditions for their use in reproducibly evaluating the population structure of American monkfish. In combination, these loci revealed sufficient levels of allelic diversity to sensitively probe for the population structure of American monkfish. Our collections, spanning an area from Newfoundland to North Carolina allowed us to initially evaluate the coastwide population structure of monkfish.

Our major findings were two-fold. First, the current model that is used by regulators to manage American monkfish is not supported by genetic data. In this case, we found no genetic discontinuities between collections of monkfish in areas north and south of the current demarcation line separating the Northern and Southern Fishery Management Areas. In fact, we found no evidence of genetic discontinuities among monkfish collections from northern collections areas until the approximate center of their distribution in the mid-Atlantic Bight. These results agree with earlier genetic studies which found no evidence of genetic heterogeneity among northern monkfish collections (Chikarmane et al. 2000; Nguluwe 2009). Second, our results are most parsimonious with the existence of significant coastwide population structure in American monkfish; however, population divisions are not congruent with the boundary between the two existing management units, but instead occur far to its south.

There are several caveats that should be considered in our interpretation of these results. First, we have yet to evaluate the inter-annual temporal stability of our diagnostic genotypes. For use in management, it is critical that the extent of spatial genetic heterogeneity among location sites far exceeds the extent of temporal heterogeneity of genotypes within sites. Furthermore, most of our collections at individual locations were single season snapshots. Given their migratory behavior, it is possible that genotypic patterns at individual locales will differ among seasons. This possibility was not empirically addressed in our study and should be. Second, our collections for locales at the southern end of the species' range are small or non-existent. For example, we were unable to obtain a collection from the late winter-early spring monkfish fishery off the North Carolina coast. Our data suggests the possible presence of a third stock in the Virginia-North Carolina area, but our limited collections from there in this study prohibited us from systematically addressing that possibility. Third, the distribution and spawning of American monkfish is known to extend to deep water, up to 1000 meters, as evidenced by the presence of eggs and larvae. However, few if any, of our collections were made in such depths therefore the possibility of additional discrete deep water populations remains unexplored.

Thus, in summary our genetic analysis indicates that there are 2 and perhaps 3 genetic stocks of monkfish along their coastwide distribution and this differentiation may result from a latitudinal gradient in genetic differentiation. Clearly, these divisions do not respect the current 2 stock model by which monkfish are managed in U.S. waters today. However, the boundaries of these genetic stocks require further delineation and the temporal stability of these units between and

within years requires confirmation before this new information can be effectively employed in a management context.

PERMITS

CCE requested a Federal Exempted Fishing Permit (EFP) upon notice of funding for this project. The application for the permit was submitted on April 17, 2012 and was then delayed due to the listing of Atlantic sturgeon under the Endangered Species Act (ESA). CCE had been informed that the EFP application was being deferred and would most likely not be issued due to an ongoing review of the interactions of Atlantic sturgeon with the monkfish fishery. As February 2013 came to an end, CCE continued conversations with NMFS' Northeast Regional Office (NERO) about obtaining an EFP and was instructed to submit an application for an offshore EFP only. CCE was informed that NMFS-NERO would consider an EFP for vessels fishing for monkfish compensation harvest beyond 50 fathoms. The area beyond 50 fathoms was classified as having little to no interaction with Atlantic sturgeon. CCE submitted an EFP application on March 1, 2013. While waiting for the offshore EFP to be processed, CCE was informed on May 1, 2013, that an EFP for all areas and all compensation monkfish fishing would again be considered due to new data and a higher biomass estimate relative to Atlantic Sturgeon. CCE reviewed the original EFP application and worked with NMFS-NERO to move forward with the processing and administration of the original EFP while concurrently waiting for the offshore EFP to be issued. On May 21, 2013 CCE was issued an EFP for offshore vessels fishing in a designated area beyond 50 fathoms. On May 30, 2013, the EFP that was requested to cover all vessels and all areas included in the project was published in the Federal Register and opened for public comment. On July 11, 2013, CCE was issued an EFP that allowed for all project activities and monkfish RSA days at sea (DAS) compensation fishing to commence. The EFP included all the proposed exemptions that were originally requested. CCE then distributed the EFP to all the participating RSA monk fishing vessels. CCE remained in contact with NMFS-NERO throughout the project to amend and add participating vessels to the current EFP as needed.

OUTREACH

Education and outreach were an important component of this project. The comprehensive outreach program remained a useful tool during the course of the project as a means to distribute information to scientists and industry members up and down the coast. The creation of our Monkfish Sample Collection Network was the cornerstone of our outreach program for this project. As described above, the Network included fishermen, docks, wholesalers and processors and afforded us the ability to continuously provide information and receive feedback about the project. The other key element of the outreach program was the creation of a dedicated website specifically for this project. The project website allowed us to broadcast the project activities and provide up-to-date information to continuing participants and new recruits. As mentioned previously, a detailed video was created and placed on the project website to aid new participants in the sample collection procedure. The instructional video along with additional information including maps and charts can be found at: <http://ccesuffolk.org/Monkfish>.

Active marketing to fishing communities gave participants and industry members a better understanding of the monkfish RSA project. Informational flyers offering an overview of the project were directly distributed by CCE staff at the docks in New Bedford, MA, Pt. Judith RI, Hampton Bays and Montauk, NY. Other flyers were sent out and allocated to major coastal ports in Maryland and Virginia. New participants were given detailed directions and personally handed informational packets containing project maps, sampling techniques, summaries and general information. CCE has catalogued all participants email addresses, this allowed information to quickly circulate between scientists and industry members. Our feedback loop advanced the project by actively engaging commercial fishermen in the Monkfish RSA project. The outreach program was a key component in targeting specific monkfish samples because of information sharing, real time communication, and dedication from all participants. Communication with fishermen during the Monkfish RSA DAS tracking efforts was an added outlet to conduct related outreach during this project.

PROBLEMS ENCOUNTERED

Hurricane Sandy hit the Mid-Atlantic in late October 2012. This storm had crippling effects on many areas of the commercial fishing industry. Fishermen, dealers, wholesalers and port infrastructures throughout the area were devastated. Commercial fishing across New York, New Jersey, and Rhode Island were severely impeded long after the passing of the storm. The storm's lingering effects were still felt throughout 2013. Agger Fish in Brooklyn, NY, one of the largest monkfish dealers and processors on the east coast, was completely flooded and forced to close down and did not re-open. Agger would have been a major contributor in locating monkfish samples from many of the project's areas. The closure of this very important business/dealer also directly affected the monkfish fishing industry. Fishermen from NY and other ports along the East Coast were left having to find a new dealer to purchase their fish and the return prices were substantially lower. Fishermen also reported demand fluctuations and uncertainty in foreign markets. Prices for monkfish were on an average \$1.50 lower than those reported for the previous year. The reduction in fish price in combination with high fuel costs kept many fishermen from using their RSA DAS. Members of the staff from Rutgers University, a project partner, were unable to collect samples following Sandy due to storm damage to wholesaler facilities and port infrastructure and the reduction of fishing effort. Hurricane Sandy hit in the middle of the fall monk fishing season and as such our sampling efforts were greatly impacted as the regions fishing industry was hard hit and slow to recover. Monk fishermen reported that fishing was significantly reduced and actually ceased in some areas. This situation posed a challenge to sample collection and RSA DAS usage during this time.

In addition, CCE was left in a very precarious situation and encountered problems relative to monkfish RSA DAS usage. This was due to EFP administration (see Permits section), market fluctuation, infrastructure reduction, and fuel cost. Unlike other monkfish RSA funded projects, participating vessels from this project were without a possession exemption for one year and were therefore restricted to a daily possession limit based on their permit category. CCE monkfish RSA DAS were offered and purchased by multiple gear types and permit categories

with the understanding that an EFP would allow an exemption that would permit an increased quota limit based on allocated RSA DAS. Specifically, draggers who purchased RSA DAS were at a disadvantage within this program due to the restraints of the current regulations that would not allow multiple possession limits to be landed without remaining at sea for all the days necessary to land that amount. This was finally resolved with the issuance of the EFP in July 2013, but unfortunately this was half way through the time frame of the project. Those vessels that abstained from fishing until the EFP was issued then felt pressured to utilize all the RSA DAS purchased before the expiration on April 31, 2014. This situation was impacted even more when the inshore gillnet vessels held back from using the CCE monkfish RSA DAS they purchased to see the final result of the EFP application and issuance. Consequently, many of them were under the same pressure to utilize the DAS and as a result both gear types (draggers and gill netters) requested transfers of RSA DAS due to the concern of not being able to use the DAS within the fishing year.

Our collections for locales at the southern end of the species' range were small or non-existent. For example, we were unable to obtain a collection from the late winter-early spring monkfish fishery off the North Carolina coast. CCE contacted local fishermen and North Carolina Division of Marine Fisheries staff prior and during the season. We were informed that not many fishermen moved gear offshore due to a restricted fishing season caused by turtle and porpoise closures and cold water temperatures. Fishermen reported the monkfish arrived on the fishing grounds late and it was not worth moving or setting gear with only a few days left in the season. Our data suggests the possible presence of a third stock in the Virginia-North Carolina area, but the absence of an active monkfish fishery in these areas during our project timeline limited our collections from these areas and prohibited us from systematically addressing that possibility. The distribution and spawning of American monkfish is known to extend to deep water, up to 1000 meters, as evidenced by the presence of eggs and larvae. However, our restricted and limited collections made in such depths therefore cannot support the possibility of additional discrete deep water populations. Sample collection was attempted focusing on offshore areas by consulting project participants. Information relayed from the fishing industry revealed no landings from deep water offshore areas. In essence, there were no targeted monkfishing trips in these deep water offshore areas and no reported bycatch to be sampled by industry contacts.

RSA DAYS AT SEA and COMPENSATION HARVEST

CCE staff members allocated all the awarded 371 RSA DAS. Monkfish RSA DAS were offered industry wide to fishermen of all gear types holding active monkfish permits. Monkfish fishermen used the monkfish RSA DAS during the 2012-13 fishing years which started May 1, 2012 and went to April 30, 2014. CCE remained in contact with all participating vessel owners. Transfer of RSA Monkfish DAS was allowed and tracked by CCE. CCE tracked the usage of monkfish RSA DAS by coordinating with the involved fishermen and weekly with Allison Ferguson from NMFS NERO. A total of 335.15 monkfish RSA DAS were used for compensation harvest for this project. The total remaining RSA unused DAS is 38.85 and this equates to a percent used of 89.53%. In addition, of the 1,335,600 lbs. of monkfish allocated and associated with those 371 DAS, 954,222 lbs. were landed and sold, leaving 381,378 lbs.

remaining with a percent used of 71.45%. This data is summarized in Table 11. Weights of fish allocated, landed, and sold are all equalized to whole fish weight. The average ex-vessel prices as reported by NMFS (NMFS, 2014) for whole monkfish was \$1.20 per lb. during the project period. Thus the total dockside value of the RSA harvested monkfish associated with this project was \$1,145,066. NMFS maintains all the VTR and dealer reports for trips associated with the DAS information for this project. Despite the issues described under Problems Encountered relative to permits, Super Storm Sandy and compensation harvest, the compensation harvest conducted was just adequate to fully fund the budget associated with this project. CCE coordinated with owners and captains to keep them abreast of their RSA DAS usage in order to fully utilize all allocated RSA DAS.

Table 11 – RSA DAS/Compensation Harvest

RSA DAYS AT SEA & COMPENSATION HARVEST	
RSA DAS awarded	371
RSA DAS Used	332.15
RSA DAS remaining	38.85
% Used	89.53%
Pounds Allocated	1,335,600 lbs.
Pounds Landed and Sold	954,222 lbs.
Pounds Remaining	381,378 lbs.
% Used	71.45%

PROJECT MANAGEMENT

Cornell Cooperative Extension

Project Coordinator/Co-Principle Investigator Emerson Hasbrouck led the project and had co-responsibility with Isaac Wirgin (NYU) for management and execution of this study. Hasbrouck also had primary responsibility for the design and implementation of the program’s sampling component, fiscal oversight and report writing. He also directly supervised all CCE employees on this project.

Fisheries Specialist 1 John Scotti was responsible for assisting with project design, coordination, implementation, oversight, participant recruitment and industry interaction.

Fisheries Specialist 2 Tara Froehlich/Kristin Gerbino/Joseph Costanzo were responsible for assistance with project design and coordination, recruitment of fishermen, dealers, processors, and wholesalers to the Monkfish Sample Collection Network, project supervision, collection of biological samples, sample verification and quality control, communication, website development, outreach, data entry, and report writing.

Contractual Services:

NYU School of Medicine

Dr. Isaac Wirgin (Co-PI), had co-responsibility with Hasbrouck for management and execution of this study. Wirgin also had primary responsibility for management of the DNA component of the project. He planned molecular studies with Ecogenics and Ms. Maceda (Research Technician), he directly supervised Ms. Maceda in DNA isolations and microsatellite genotyping, he organized microsatellite data, conducted data analysis along with Dr. J.R. Waldman. He prepared reports, manuscripts, and presentation with Hasbrouck and Waldman.

Ms. Maceda (Research Technician) conducted all the population screening for this study. She isolated all DNAs and conducted microsatellite analysis at 12 loci. This involved multiplexed PCR at microsatellite loci, single PCRs, multi-pooled fragment analysis on an automated DNA sequencer. She compiled all the data that was later organized and analyzed by Drs. Wirgin and Waldman.

Rutgers University

Dr. Eleanor Bochenek and Jason Morson (Rutgers University) coordinated sample collection activities (46 samples), recruited fishermen/dealers to the “network” of participants, conducted industry outreach and interaction with participants in New Jersey, specifically Barnegate, NJ.

Long Island Commercial Fishing Association.

Bonnie Brady, Executive Director, conducted outreach and communication with the fishing industry.

Attached to this final report are the following appendices:

1. Appendix 1 – Sampling Manual
2. Appendix 2 – Sample Collection Database
3. Appendix 3 – DNA Raw Data
4. Appendix 4 – Allelic Frequencies

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