Updated 9/20/2016

2016 Planned Council Meeting Topics

February 9-11, 2016 — Doubletree by Hilton New Bern Riverfront, New Bern, NC

- Omnibus Industry Funded Monitoring Amendment – *Select preferred Omnibus alternatives for public hearings*
- Draft EAFM Interactions White Paper – *Review*
- Collaborative Research Program – *Review committee progress*
- Scup GRA Framework – *Meeting 2*
- Unmanaged Forage Fish Amendment – *Discuss FMAT, AP, and EOP Committee recommendations*
- Data Modernization Amendment – *GARFO update*

April 12-14, 2016 — Montauk Yacht Club, Montauk, NY

- 2017 Golden Tilefish Specifications – *Review*
- Golden Tilefish Framework – *Meeting 2*
- Unmanaged Forage Amendment – *Approve Public Hearing Document*
- Blueline Tilefish Amendment – *Final action*
- 2013 River Herring/Shad White Paper – *Review Committee recommendations for TORs for October action*
- Omnibus Industry Funded Monitoring Amendment – *Select preferred mackerel alternatives for public hearings*
- Draft EAFM Guidance Document – *Review*
- Spiny Dogfish Trip Limits

June 14-16, 2016 — Courtyard Marriott Newark / University of Delaware Clayton Hall, Newark, DE

- 2017 Squid and Butterfish Specifications – *Review*
- Longfin/Butterfish Mesh/Strengthener Analyses- *Review*
- 2017 Atlantic Mackerel Specifications – *Review*
- RH/S Cap and RH/S management progress - *Review*
- 2017 and 2018 Surfclam/Ocean Quahog Specifications – *Adopt*
- Surfclam and Ocean Quahog Excessive Shares Amendment – *Discuss next steps*
- Summer Flounder Amendment – *Update*
- Squid Capacity Amendment – *Update*
- eVTR framework – *Meeting 1*
- Omnibus Industry Funded Monitoring Amendment – *Select preferred mackerel alternatives for public hearings*
- Blueline tilefish recreational specifications – *Review*

August 8-11, 2016 — Hilton Virginia Beach Oceanfront, Virginia Beach, VA

- Swearing-in of new and reappointed Council members
- Election of Officers
- 2017 Summer Flounder, Scup, Black Sea Bass Specifications – *Review*
- 2017 Bluefish Specifications – *Review*
- Summer Flounder Amendment – *Consider FMAT recommendations for draft range of alternatives*
- Habitat policies on fishing impacts – *Review and approve*
- Unmanaged Forage Amendment – *Final action*
- eVTR framework – *Meeting 2*
- EAFM Guidance Document – *Review and approve*

October 4-6, 2016 — Stockton Seaview Hotel, Galloway, NJ

- 2017 Spiny Dogfish Specifications – *Review*
- RH/S Stocks in the Fishery Decision
- Risk Policy Omnibus Framework – *Meeting 1*
- Council Communications Plan – *Review*
- New Jersey Special Management Zone (SMZ) request – *Review Monitoring Team Report*
- Blueline Tilefish Recreational Measures Framework – *Meeting 1*

December 13-15, 2016 — Royal Sonesta Harbor Court Baltimore, Baltimore, MD

- 2017 Summer Flounder, Scup, Black Sea Bass Recreational Specifications – *Adopt*
- Summer Flounder Amendment – *Approve range of alternatives for public hearing document*
- Risk Policy Omnibus Framework – *Meeting 2*
- ~~Golden Tilefish 5 year IFQ program review – *Approve final document*~~
- Squid Capacity Amendment – *Approve public hearing document*
- Omnibus Industry Funded Monitoring Amendment – *Adopt final alternatives*
- Blueline Tilefish Recreational Measures Framework – *Meeting 2*
- NJ SMZ Recommendation – *Final action*
- 2017 Implementation plan – *Review and approve*
- Monkfish 2017-2019 Specifications Framework

Summary of the results of a genetic-based investigation of blueline tilefish (*Caulolatilus microps*)

Final Report to the MAFMC

Jan McDowell
Department of Fisheries Science
Virginia Institute of Marine Science
June 2016

INTRODUCTION

Blueline tilefish, *Caulolatilus microps*, also known as grey tilefish (Goode and Bean 1878), is a bottom dweller found at depths of 240-780 feet. Historically blueline tilefish have been reported to occur along the continental shelf from Virginia to the Campeche Banks of Mexico, occupying the same habitat as groupers and snappers (Dooley 1978). North Carolina was previously considered to be the northern extent of the range of blueline tilefish, but concentrations have recently been discovered in Virginia and Maryland with reports as far north as Montauk, NY, extending the known range. A recent data workshop (SEDAR 50), which compiled several fishery-dependent and fishery-independent data sources, determined that blueline tilefish are continuously distributed from the Gulf of Mexico to the Mid-Atlantic Bight (Farmer and Klibansky 2016).

The life-history parameters of blueline tilefish make them particularly vulnerable to overfishing. Like other species of tilefish, blueline tilefish are long-lived and grow slowly, with an estimated lifespan of up to 43 years and a maximum size of 900 mm fork length (SEDAR 32 2013). Blueline tilefish reach maturity as early as age two (Harris et al. 2004; Kolmos et al. 2016) and are sexually dimorphic, with males reaching a larger maximum size than females (Harris et al. 2004). A study by Harris et al. off the southeastern coast of the U.S. (North and South Carolina) found that blueline tilefish are batch spawners. Spawning occurs in the evening from February-November with a peak in May. There is a positive

relationship between size and fecundity in females (Harris et al. 2004; Kolmos et al. 2016). Data concerning larval duration or dispersal is extremely limited.

Blueline tilefish were formally assessed in 2013 by SouthEast Data, Assessment, and Review (SEDAR) in the Southeast region and were found to be overfished with overfishing occurring (SEDAR 32 2013). It was suggested that overfishing had been happening over most of the assessment period and it was noted that there has been considerable uncertainty in the estimates since the mid 2000s (SEDAR 32 2013). The assessment considered blueline tilefish to be a single coastwide stock although there was limited data from north of Cape Hatteras, NC.

The South Atlantic Fishery Management Council (SAFMC) manages blueline tilefish as part of the deep-water snapper-grouper complex (Snapper-Grouper Fishery Management Unit). The South Atlantic management area extends from the North Carolina/Virginia border to Florida within the U.S. Exclusive Economic Zone (EEZ). The Gulf of Mexico Fishery Management Council (GMFMC) manages blueline tilefish in the U.S. portion of the Gulf of Mexico. Historically blueline tilefish have not been a managed species in the mid-Atlantic region. Due to the development of an unregulated fishery off the coast of Virginia, in 2007 the Virginia Marine Resources Commission (VMRC) enacted regulations on blueline tilefish including a recreational landing and possession limit of 7 fish/day and a commercial possession limit of 300 pounds whole weight or 273 pounds gutted weight. Maryland later adopted the same regulations. Until enactment of emergency measures in 2015, the fishery was not regulated north of Maryland there were no Federal regulations north of North Carolina. This lack of regulation became problematic due to a recent substantial increase in commercial and recreational landings in the U.S. mid-Atlantic. Commercial landings in this region averaged 10,776 pounds between 2005 and 2013. Following implementation of a reduction in the commercial catch limit in the South Atlantic by the SAFMC in 2014, the commercial landings of blueline tilefish in the mid-Atlantic skyrocketed to 215,272 pounds. The unregulated northern fishery combined with the lack of information about the number of stocks and the vulnerable (k-selected) life history of blueline tilefish raised concern about the sustainability of the resource and led the Mid-Atlantic Fishery Management Council

(MAFMC) to adopt similar regulations as Virginia in Federal waters north of the latitude of the Virginia/North Carolina border as an emergency measure until long term management measures can be implemented.

Genetic monitoring, which uses molecular markers to follow changes in populations over time, is an increasingly important component of conservation efforts because of the wide range of information that can be obtained from genetic samples. Monitoring can include identification of genetic stocks, mixed stock analysis, genetic tagging (capture-recapture) of individuals, changes in population genetic parameters such as loss of alleles, shifts in allele frequencies and effective population size, and can also include assessment of historical demography for comparison with current estimates (see (Luikart et al. 2003 and Schwartz et al. 2007 for reviews). Genetic monitoring becomes increasingly important as species become exploited. The establishment of a baseline estimate of genetic variation in blueline tilefish is necessary to monitor changes in variation (loss of diversity or changes in how diversity is distributed) in the future that may result from overfishing. In addition, genetic information is critical to identify management measures appropriate for the ongoing conservation of the species. Recent studies have shown that lack of knowledge about spatial structuring can lead to the risk of unintended overexploitation and localized depletions (Ying et al. 2011).

Management of the blueline tilefish resource has been hampered by a lack of basic life history data, including information about stock structure. No genetic studies have been conducted to date, thus there is no information available regarding genetic connectivity among locations. It is unknown if blueline tilefish are comprised of distinct self-recruiting stocks or if (and to what extent) geographically distant sampling areas are interdependent. Information from this study will be useful for the conservation and management of the species.

MATERIALS AND METHODS

Microsatellite Marker Development and Optimization

High molecular weight DNA from a blueline tilefish captured off the coast of Virginia was used to create a 400 base pair (bp) insert genomic library. The

resulting fragments were sequenced using a PGM™ Hi-Q™ Sequencing Kit on an Ion Torrent PGM sequencer using an Ion 318™ chip (Ion Torrent Systems, Inc., Guilford, CT). The FastQC software (Andrews 2010) integrated into the Galaxy Project platform (Giardine et al. 2005, Goecks et al. 2010, Blankenberg et al. 2010) was used to assess the quality of the resulting sequences, and filters integrated into the Galaxy platform were used to remove sequencing artifacts and to filter out short sequences (below 50 bp). Sequences were trimmed to exclude positions 1-9 and all bases over 400 bp and filtered by quality to exclude those in which 50% of the sequence length had a quality < 20 (base call accuracy <99%) and exported as a FASTA-formatted file. Exported sequence files were filtered for the presence of perfect tetranucleotide repeats, resulting in the identification over 8,000 potential microsatellite loci using the MSATCOMMANDER 1.0.8 software (Faircloth 2008). Primers were designed for ~1500 of the identified loci using the Primer3 software (Koressaar and Remm 2007; Untergasser et al. 2012) and 65 primer pairs were ordered and tested for amplification of a product of the predicted length from blueline tilefish DNA isolated from samples from Virginia and South Carolina (two samples from each location). From the original 65 primer pairs, we identified 26 loci that amplified reliably across test samples.

All primer pairs were initially assessed and optimized using gradient PCR on a Bio-Rad C1000 thermal cycler (Bio-Rad, Hercules, CA) using standard protocols. Each 5 µl PCR reaction contained 1x PCR Buffer (Qiagen), 1.5 mM $MgCl_2$, 200 µM of each dNTP, 0.125 µM the forward primer, 0.125 of the reverse primer, 0.5 unit of *Taq* polymerase (Qiagen), and 0.5 µl genomic DNA. Four samples, two from VA and two from SC were used for testing. Samples were amplified with an initial denaturation temperature of 94 ºC for 3 min, followed by 35 cycles at 94 ºC for 1 min, 48-65 ºC for 1 min, 72 ºC for 1 min, with a final elongation step at 72 ºC for 7 min. Amplified products were visualized to confirm the presence of a single amplification product of correct size by agarose gel electrophoresis (1.5 % w/v), stained with ethidium bromide and viewed under a UV light source. Markers found to reliably amplify DNA samples from both Virginia and South Carolina were further evaluated using a panel of 8 samples each from Virginia and South Carolina to assess

amplification consistency, levels of polymorphism and conformance to the expectations of Hardy-Weinberg Equilibrium (HWE). PCR reactions were carried out as above except for the addition of a T3-labeled fluorescent probe (either FAM, VIC, NED, PET). The resulting fluorescently labeled PCR products were separated on an ABI 3130*xl* Prism Genetic Analyzer (Applied Biosystems, Foster City, CA) with a GeneScan 500-Liz size standard (Applied Biosystems, Foster City, CA). The chromatic peaks for each microsatellite locus were sized using the GeneMarker AFLP/Genotyping Software, ver. 1.75 (SoftGenetics, State College, PA).

Sample Collection and DNA Isolation

Geographic sampling spanned the blueline tilefish U.S. East Coast range from New York to the southern Florida Keys and also included a small sample (n=15) from the west coast of Florida in the Gulf of Mexico (Figure 1, Appendix 1). Cooperating commercial fishermen using one of three gear types took fin clips: long-bottom longline (LBLL), short-bottom longline (SBLL), and vertical hook and line (VHL). All fin clips were stored in ethanol until DNA could be extracted and the pertinent collection information (date, fish sex, length, depth, location, vessel, etc.) was recorded on the accompanying data sheets (for specific details, see SEDAR50-DW02). DNA was extracted from archived tissue samples using either the DNeasy Tissue Kit (Qiagen, Valencia, CA) or the Quick-DNA™ Universal Kit (Zymo Research, Irvine, CA). Briefly, 2-3 mm fin clip sub-samples were incubated in lysis buffer (Longmire et al. 1997) for 2 hrs. at room temperature to facilitate removal of residual ethanol prior extraction following the manufacturers protocol. All DNA samples were quantified using a NanoDrop 2000 (Thermo Scientific, West Palm Beach, Florida), and stored at -20 °C.

Microsatellite Markers

Following optimization, primer pairs were multiplexed into panels using the Type-it® Microsatellite PCR Kit (Qiagen) and one of four unique fluorescent tails Table 1. Once optimized, samples from each location were amplified using the multiplexed primer sets and alleles were sized as above. Each multiplex reaction

contained 1x Type-it Multiplex PCR Master Mix, 1x Q-Solution, 0.05 µM of the forward primer, 0.2 µM of the reverse primer, 0.2 µM of the fluorescent dye, 0.5 µl genomic DNA and water to a final volume of 6 µl. Amplifications were performed with an initial denaturation temperature of 95 ºC for 5 min, followed by 28 cycles at 95 ºC for 30 sec, Annealing for 90 sec, 72 ºC for 30 sec, with a final elongation step at 60 ºC for 30 min. The resulting fluorescently labeled PCR products were separated on an ABI 3130*xl* Prism Genetic Analyzer (Applied Biosystems, Foster City, CA) with a GeneScan 500-Liz size standard (Applied Biosystems, Foster City, CA). The chromatic peaks for each microsatellite locus were sized using the GeneMarker AFLP/Genotyping Software, ver. 1.75 (SoftGenetics, State College, PA). To control for errors, approximately 20% of samples were amplified sized twice and all loci were sized independently twice and results were compared

After alleles had been sized for each locus, the Micro-Checker ver. 2.2.3 software (Van Oosterhout et al. 2004) was used to check for the presence of null alleles and evidence of scoring errors. The Genepop'007 software package (Rousset 2008) was used to test for deviations of genotypic distributions from HWE expectations (F_{IS}, exact tests, Guo et al. 1992). Summary statistics (number of alleles, allele frequencies and etc.), were generated using GenAlEx ver. 6.5 (Peakall and Smouse 2012). To evaluate evidence for the presence of population structure, the Arlequin software package ver. 3.5.2.2 (Excoffier and Lischer 2010) was used to estimate (Weir and Cockerham's (1984) unbiased estimator of Wrights F-statistics and to conduct an analysis of molecular variance (AMOVA) based on several alternate geographic groupings (Excoffier et al. 1992). Significance was assessed via 10 000 permutations of the data. A factorial correspondence analysis (FCA) was performed using Genetix ver. 4.05.2 (Belkhir et al. 1996). A discriminant analysis of principal components (DAPC) was performed using the Adegenet software (Jombart 2008; Jombart et al. 2010).

Mitochondrial DNA (mtDNA)

The mtDNA primers of Nohara et al. (2010) were used to amplify and sequence a 489 bp segment of the mtDNA control region from a subset of all

collected samples (Table 4). Briefly, each 25 µl reaction contained 1x PCR Buffer (Qiagen) 1.5 mM $MgCl_2$, 200 µM of each dNTP, 0.125 µM of each primer, 0.5 unit of *Taq* polymerase (Qiagen), and 1 µl genomic DNA. Amplifications were performed with an initial denaturation temperature of 94 ºC for 2 min, followed by 35 cycles at 94 ºC for 1 min, 52 ºC for 1 min, 72 ºC for 2 min, with a final elongation step at 72 ºC for 5 min. Aliquots of amplified products were sized against a DNA ladder of known size using horizontal gel electrophoresis (1.5 % w/v agarose), stained with ethidium bromide and visualized under a UV light source to confirm the presence of a single amplification product of correct size. Amplification products were purified using a QIAquick PCR Purification Kit (Qiagen) following the manufacturers protocol and subsequently quantified using a NanoDrop 2000 prior to storage at -20 °C. Purified PCR products were bi-directionally sequenced using the Big Dye Terminator Cycle Sequencing Kit (Applied Biosystems) with the original amplification primers and 0.25 the recommended concentration of Big Dye. Sequencing reaction products were precipitated using ethanol/sodium acetate to remove unincorporated nucleotides and re-suspended in 16 µl of Hi-Di formamide (Applied Biosystems) and 10 µl of each cleaned reaction were electrophoresed on an ABI 3130*xl* Prism genetic analyzer (Applied Biosystems). The resulting forward and reverse sequences were imported into Sequencher ver. 5.1 (Gene Codes Corporation, Ann Arbor, MI) for trimming of low quality sequence and creation of consensus sequences. Consensus sequences were aligned in MacVector ver. 12.5.1 (MacVector, Inc., Apex, NC) and exported as a FASTA file.

The FaBox software (Villesen 2007) was used to collapse sequences into haplotypes and create input files for the Arlequin software package (Excoffier and Lischer 2010). Arlequin was used to generate descriptive statistics (mean number of pairwise differences (k), haplotype diversity (H), and nucleotide sequence diversity (π), perform analysis of population pairwise Φ_{ST}, AMOVA (Excoffier et al. 1992) and to estimate demographic parameters. Statistical sigificance was assessed based on 10 000 permutations of the data. The PopART software (Leigh and Bryant 2015) was used to reonstruct and visualize genealogical relationships among

sequences using the Minimum Spanning Network algorithm of Bandelt et al. (1999) and the TCS algorith of (Clement et al. 2002).

RESULTS AND DISCUSSION

Microsatellite marker development

High throughput sequencing of a blueline tilefish DNA sample on an Ion Torrent sequencer resulted in approximately 4.8 million DNA sequences ranging in length from 25-587 bp with an average Phred (quality) score of 30. Filtering using the software programs integrated into the Galaxy Project platform resulted in the retention of 4.7 million high quality DNA sequences. The retained sequences were subsequently queried for the presence of perfect tetranucleotide repeat loci, resulting in the identification over 8,000 microsatellite loci. Of the loci identified, primers were designed for ~1500 loci and 65 primer pairs were ordered and tested for amplification of a product of predicted length from blueline tilefish DNA samples from Virginia and South Carolina (two samples from each location). Of the original 65 primer pairs tested, we identified 26 loci that amplified reliably across test samples. Further testing of samples taken from Virginia and South Carolina (20 samples from each location) indicated that these loci were in conformance to the expectations of Hardy-Weinberg equilibrium (HWE). One of the primers failed to amplify successfully across multiple samples and was excluded from further analysis. The remaining 25 loci were combined into 8 multiplex marker panels (data available upon request).

Microsatellite Analysis

In total, 505 samples were analyzed across 25 polymorphic microsatellite loci; 490 samples from U.S. east coast range from New York (NY) to the southern Florida Keys (FL) and 15 from western Florida (WF) in the Gulf of Mexico (Figure 1). All loci were polymorphic, with the number of alleles ranging from 6 alleles at CM1787993 and CM1787993 to 21 at CM90501. Markers were in conformance to the expectations of HWE with the exception of CM931277 and CM4391826, both of which had significant global heterozygote deficits, most likely due to the presence of

null alleles (Table 2). All subsequent analyses were done both including and excluding these loci. Results were consistent regardless of whether or not these loci were included. All results presented henceforth are based on the 23 loci that were in HWE. Overall, analysis indicated a genetically homogeneous population. All F_{ST} values were small; the largest value was 0.003 between the WF and North Carolina sample taken North of Cape Hatteras (NCN) samples and most values were 0. There were no significant pairwise comparisons based on 10,000 permutations of the data (Table 3). An analysis of molecular variance (AMOVA, Excoffier et al. 1992) using multiple alternate groupings of sampling locations showed no significant genetic variance due to variation among any groups (data not shown). A factorial correspondence analysis did not indicate the presence of any discrete clusters that would suggest the presence of multiple populations (Figure 2). Likewise, the DAPC had a scree plot with eigenvalues that were flat across the plot.

mtDNA analysis

In total, of 188 control region sequences were examined across a subset of samples from all geographic locations. All sequences were edited to a final length of 407 bp, resulting in 72 haplotypes with 59 variable sites including 58 substitutions, 51 of which were transitions and 7 of which were transversions. A total of four indels were observed. The most common haplotype, haplotype 9, was recovered 39 times (20.7% of sequences) and was recovered in all locations with the exception of the WF sample (Table 4). The second most common haplotype was recovered 9 times (4.8% of all samples) and was recovered in all locations except Delaware (DE). WF and DE had the smallest number of samples sequenced (8 and 13 respectively). Haplotype diversity (H) was 0.94 across all samples and was high in all geographic samples ranging from 0.89 in samples from NCN and 1.0 in DE. The mean number of pairwise differences between sequences (k) across all samples was 3.1 and ranged from 2.4 in NCN to 3.91 in South Carolina (SC). Likewise, nucleotide diversity (π) was low both across all samples (0.008) and within samples from each geographic location. Values ranged from 0.006 in NCN to 0.010 in SC., indicating that there were

very few differences among haplotypes (Table 5). A minimum spanning network showed no division of haplotypes by sampling location (Figure 3).

A global test of differentiation among samples based on the distribution of haplotypes and 10 000 permutations of the data was not significant (P = 0.144). However, there were significant pairwise comparisons between the WF sample (the location with the smallest sample size) and the NY, DE and VA samples. Only the comparison with VA was significant after correction for multiple tests (P = 0.003). Population pairwise Φ_{ST} values were calculated based both on the number of pairwise differences and on a Tamura-Nei distance (Tamura and Nei 1993). No values were significant based on 10 000 permutations of the data. Values based on the number of pairwise differences between samples ranges from 0 between most pairs of sample collections examined to 0.039 between DE and WF, the two groups with the smallest sample sizes (Table 3).

As with the analysis of the microsatellite data, an AMOVA (Excoffier et al. 1992) using multiple alternate groupings of sampling locations showed no significant genetic variance due to variation among any grouping scheme (data not shown).

CONCLUSIONS

Blueline is a commercially and recreationally important long-lived, slow growing, species. The sedentary nature of adults suggest the possibility multiple populations along the U.S. East Coast. Many marine fishes have been found to have a disjunct genetic boundary near Cape Hatteras, a well-known biogeographic break. This includes other fishes occupying the same habitat as blueline tilefish, such as black sea bass (Roy et al. 2012; Mccartney et al. 2013) and golden tilefish (Katz et al. 1983).

Despite the sedentary nature of adults, there was no evidence that blueline tilefish comprise genetically distinct populations along the U.S. East Coast at any scale. There was no evidence that biogeographic breaks are an impediment to gene flow; no genetic differences were found between samples examined either north

and south of Cape Hatteras NC or Cape Canaveral FL. The data suggests that there is sufficient gene flow to prevent the accumulation of genetic differences.

The results of the genetic study are consistent with evidence based on reproduction, catch and hydrodynamic data. A recent analysis by Kolmos et al. 2016 found no differences in spawning fraction or timing of spawning among samples collected along the Atlantic coast. This same analysis found substantial evidence of spawning north of Cape Hatteras and no evidence to suggest that blueline tilefish form spawning aggregations. Furthermore, blueline tilefish were found to spawn over a protracted season from February-November with a peak in May (Harris et al. 2004; Kolmos et al. 2016). Catch data indicate that blueline tilefish are continuously distributed from the Gulf of Mexico to the Mid-Atlantic Bight (Farmer and Klibansky 2016).

Given the sedentary nature of adults, mixing likely occurs via transport of eggs and larvae. Although little is known about the early life history of blueline tilefish, eggs are known to be pelagic (Lewis et al. 2016), as are larvae. Tracks from drifter buoys indicate that the Gulf Stream, the Loop Current and counter current eddies as mechanisms for transport of pelagic blueline tilefish egg, and larvae (Farmer and Klibansky 2016; SEDAR 50 Stock ID Work Group 2016). The drifter data is supported by water current flow maps, which demonstrate that larval transport between the Gulf of Mexico to the South Atlantic and from the South Atlantic to the Mid-Atlantic is probable (Farmer and Klibansky 2016). This data combined with the continuous distribution and prolonged spawning season support the findings of the genetics study. However this does not necessarily indicate that there is sufficient gene flow to overcome the effects of regional overfishing.

REFERENCES

Andrews, S. 2010. FastQC: A quality control tool for high throughput sequence data. babraham Bioinforma. 1.

Bandelt, H.-J., and A. R. Peter Forster, and ¨hl. 1999. Median-Joining Networks for Inferring Intraspeci c Phylogenies. Mol. Biol. 16:37–48.

Belkhir, K., P. Borsa, L. Chikhi, N. Raufaste, and F. Bonhomme. 1996. GENETIX 4.05, logiciel sous Windows TM pour la génétique des populations. Lab. génome, Popul. Interact. CNRS Umr 5000:1996–2004.

Blankenberg, D., G. Von Kuster, N. Coraor, G. Ananda, R. Lazarus, M. Mangan, A. Nekrutenko, and J. Taylor. 2010. Galaxy: A web-based genome analysis tool for experimentalists.

Clement, M., Q. Snell, P. Walke, D. Posada, and K. Crandall. 2002. TCS: estimating gene genealogies. Proc. 16th Int. Parallel Distrib. Process. Symp., doi: 10.1109/IPDPS.2002.1016585.

Dooley, J. K. 1978. Systematics and biology of the tilefishes (Perciformes:Branchiostegidae and Malacanthidae) with descriptions of two new species.

Excoffier, L., and H. E. L. Lischer. 2010. Arlequin suite ver 3.5: A new series of programs to perform population genetics analyses under Linux and Windows. Mol. Ecol. Resour. 10:564–567.

Excoffier, L., P. E. Smouse, and J. M. Quattro. 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes: Application to human mitochondrial DNA restriction data. Genetics 131:479–491.

Faircloth, B. C. 2008. MSATCOMMANDER: Detection of microsatellite repeat arrays and automated, locus-specific primer design. Mol. Ecol. Resour. 8:92–94.

Farmer, N., and N. Klibansky. 2016. Distribution of blueline tilefish (Caulolatilus microps) in the U.S. EEZ from fishery-dependent and fishery-independent data collections. SEDAR50-DW11. SEDAR, North Charleston, SC.

Giardine, B., C. Riemer, R. C. Hardison, R. Burhans, L. Elnitski, P. Shah, Y. Zhang, D. Blankenberg, I. Albert, J. Taylor, W. Miller, W. J. Kent, and A. Nekrutenko. 2005. Galaxy: A platform for interactive large-scale genome analysis. Genome Res. 15:1451–1455.

Goecks, J., A. Nekrutenko, and J. Taylor. 2010. Galaxy: a comprehensive approach for supporting accessible, reproducible, and transparent computational research in the life sciences. Genome Biol. 11:R86.

Goode, G. B., and T. H. Bean. 1878. Description of Caulolatilus microps, a new species of fish from the Gulf coast of Florida. Proc. U.S. Natl. Mus 1:42–45.

Guo, S. W., S. W. Guo, E. a Thompson, and E. a Thompson. 1992. Performing the exact

test of Hardy-Weinberg proportion for multiple alleles. Biometrics 48:361–372.

Harris, P. J., D. M. Wyanski, and P. T. Powers Mikell. 2004. Age, Growth, and Reproductive Biology of Blueline Tilefish along the Southeastern Coast of the United States, 1982–1999. Trans. Am. Fish. Soc. 133:1190–1204.

Jombart, T. 2008. adegenet: a R package for the multivariate analysis of genetic markers. Bioinformatics 24:1403–5.

Jombart, T., S. Devillard, and F. Balloux. 2010. Discriminant analysis of principal components: a new method for the analysis of genetically structured populations. BMC Genet. 11:94.

Katz, S. J., C. B. Grimes, and K. W. Able. 1983. Delineation of tilefish, Lopholatilus chamaeleonticeps, stocks along the United States east coast and in the Gulf of Mexico. Fish. Bull. 81:41–50.

Kolmos, K. J., S. Falk, D. M. Wyanski, and M. A. Schmidtke. 2016. A Preliminary Assessment of Reproductive Parameters for Blueline Tilefish in Atlantic Waters from Virginia to Florida SEDAR50-DW03.

Koressaar, T., and M. Remm. 2007. Enhancements and modifications of primer design program Primer3. Bioinformatics 23:1289–1291.

Leigh, J. W., and D. Bryant. 2015. Popart: Full-Feature Software for Haplotype Network Construction. Methods Ecol. Evol. 6:1110–1116.

Lewis, L. A., D. E. Richardson, E. V. Zakharov, and R. Hanner. 2016. Integrating DNA barcoding of fish eggs into ichthyoplankton monitoring programs. Fish. Bull. 114:153–165.

Longmire, J. L., M. Maltbie, and R. J. Baker. 1997. Use of “lysis buffer” in DNA isolation and its implication for museum collections.

Luikart, G., P. R. England, D. Tallmon, S. Jordan, and P. Taberlet. 2003. The power and promise of population genomics: from genotyping to genome typing. Nat. Rev. Genet. 4:981–994.

Mccartney, M. A., M. L. Burton, and T. G. Lima. 2013. Mitochondrial DNA differentiation between populations of black sea bass (Centropristis striata) across Cape Hatteras, North Carolina (USA). J. Biogeogr. 40:1386–1398.

Nohara, K., H. Takeuchi, T. Tsuzaki, N. Suzuki, O. Tominaga, and T. Seikai. 2010. Genetic variability and stock structure of red tilefish <i>Branchiostegus japonicus</i> inferred from mtDNA sequence analysis. Fish. Sci. 76:75–81.

Peakall, R., and P. E. Smouse. 2012. GenALEx 6.5: Genetic analysis in Excel. Population genetic software for teaching and research-an update. Bioinformatics 28:2537–2539.

Rousset, F. 2008. GENEPOP’007: A complete re-implementation of the GENEPOP software for Windows and Linux. Mol. Ecol. Resour. 8:103–106.

Roy, E. M., J. M. Quattro, G. T. W, and T. W. Greig. 2012. Genetic management of black sea bass: influence of biogeographic barriers on population structure. Mar. Coast. Fish. Dyn. Manag. Ecosyst. Sci. 4:391–402.

Schwartz, M. K., G. Luikart, and R. S. Waples. 2007. Genetic monitoring as a promising tool for conservation and management. Trends Ecol. Evol. 22:25–33.

SEDAR 32. 2013. SEDAR 32 Stock Assessment Report South Atlantic Blueline Tilefish. Sedar.

Tamura, K., and M. Nei. 1993. Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. Mol. Biol. Evol. 10:512–526.

Untergasser, A., I. Cutcutache, T. Koressaar, J. Ye, B. C. Faircloth, M. Remm, and S. G. Rozen. 2012. Primer3-new capabilities and interfaces. Nucleic Acids Res. 40.

Van Oosterhout, C., W. F. Hutchinson, D. P. M. Wills, and P. Shipley. 2004. MICRO-CHECKER: Software for identifying and correcting genotyping errors in microsatellite data. Mol. Ecol. Notes 4:535–538.

Villesen, P. 2007. FaBox: An online toolbox for FASTA sequences. Mol. Ecol. Notes 7:965–968.

Weir, B. S., and C. C. Cockerham. 1984. Estimating F-statistics for the analysis of population structure. Evolution (N. Y). 38:1358–1370.

Work, S. 50 S. I. 2016. SEDAR50-DW12. 50.

Ying, Y., Y. Chen, L. Lin, T. Gao, and T. Quinn. 2011. Risks of ignoring fish population spatial structure in fisheries management. Can. J. Fish. Aquat. Sci. 68:2101–2120.

Table 1. Primers and amplification conditions for blueline tilefish loci.

Locus	$T_{Optimal}$ (°C)	Repeat Motif	Primer Sequence Forward 5' to 3'	Primer Sequence Reverse 5' to 3'	Tail	Dye	Multiplex Group
CM_2316467	48	$(AGAT)_{10}$	GGCCTACACCCATGCAAAC	TTTCTCCAACCGCAATGTCG	TAATACGACTCACTATAGGG	VIC	1
CM_2352680	48	$(AGAT)_{12}$	CTCCCTGTTCTAATGACCGC	TTTGCCACCAACCTTCTCTG	GGTAAAACGACGGCCAGT	NED	1
CM_2492523	48	$(AGGC)_{10}$	GTGAGAAGAAACCAAACACTCC	CCACTGTAACTGCTATACCTGG	GGCAGGAAACAGCTATGAC	PET	1
CM_54794	48	$(AGAT)_{10}$	TTGAAATGCTGCTATGACAACC	GGTCGGCTGCACTATTTCAG	AATTAACCCTCACTAAAGGG	FAM	1
CM_1787993	49	$(AGAT)_{10}$	AGCTGGAACCAAGAAATGCG	GAAACGCTCGGGTTACATCG	TAATACGACTCACTATAGGG	FAM	2
CM_4718692	49	$(AGAT)_{16}$	AAGAGGACCAGGAACGAGTG	CTATTTCAACGCCGGTGGAG	GGTAAAACGACGGCCAGT	NED	2
CM_931277	49	$(AGAT)_{14}$	TCACCACGACTGCCACTG	GACAGGCTCACACATACTGC	AATTAACCCTCACTAAAGGG	VIC	2
CM_1741665	54.6	$(AGAT)_{16}$	CTCCCACCCTCTGCACAC	CTCCTTCGCAACCTCTCTAAC	TAATACGACTCACTATAGGG	FAM	3
CM_1827829	54.6	$(AGAT)_{11}$	AGAAGAGCAAGACAGGAGCC	TGGCTCCATTCACCAATAATGC	GGTAAAACGACGGCCAGT	NED	3
CM_2660427	54.6	$(AGAT)_{10}$	GACAGCCTTATTCCTCGCAG	TACCGGACTGCTGCTATGAC	GGCAGGAAACAGCTATGAC	PET	3
CM_1065459	54.6	$(AGAT)_{11}$	GACACGCGGTTCTCCAAAG	TCCTTGGGCCATGTTGTAGG	AATTAACCCTCACTAAAGGG	VIC	3
CM_459957	55	$(ACAG)_{13}$	TGCTCTCTGTCCCGAGTATTC	AGAGGGATCGAAAGCTGAGTC	AATTAACCCTCACTAAAGGG	NED	4
CM_2212380	55	$(AATG)_{11}$	AGGGAGGAGGTTTCACACTG	ATCACTGCCATCTTCCCACC	GCCTCCCTCGCGCCA	FAM	4
CM_2149957	55	$(ATCC)_{11}$	GTGTATGAGACCCAGAGCCC	TTGGAGTCCGGCTGTCTATG	TAATACGACTCACTATAGGG	VIC	4
CM_2374475	58.7	$(AGAT)_{11}$	CTGTTGCTTCTAGTCTCTGGC	GATCGGCTCCTCTCCCAC	TAATACGACTCACTATAGGG	FAM	5
CM_4591723	58.7	$(AGAT)_{17}$	TGTTGGTTTCTGCCTTGGAG	AGACATGCAGATAGACGGAGAC	GGTAAAACGACGGCCAGT	NED	5
CM_2404273	58.7	$(AGAT)_{10}$	TACCGGACTGCTGCTATGAC	GGAAGAGCTGCATTTCTCCG	GGCAGGAAACAGCTATGAC	PET	5
CM_310413	58.7	$(AGAT)_{10}$	CCTCCTGCACTGTTTCCTG	ACCTGAATTTCCCTCGGTACC	AATTAACCCTCACTAAAGGG	VIC	5
CM_119168	62	(AGAT)22	TGTCCAGCGCATCAATAAAGG	CTCGCCATGTCACAGTGTTG	TAATACGACTCACTATAGGG	FAM	6
CM_2186404	62	$(ATCC)_{13}$	ACCCGTGGATAAGCGGTATAG	GTGAGTGTCAATCAGGGAAGG	GGCAGGAAACAGCTATGAC	PET	6
CM_732781	62	$(AGAT)_{10}$	GGATACCTACATTTCCCTCAGG	AGTCTGTCTACATTGTCATCGC	AATTAACCCTCACTAAAGGG	VIC	6
CM_90501	65	$(AGAT)_{12}$	ACCACCTCACATCTGACCAC	TGTCATCTCATCTCAGCCAAG	TAATACGACTCACTATAGGG	FAM	7
CM_1080088	65	$(AGAT)_{18}$	GTCCAACAGCAGTCCTTGAAG	CACACAGAAAGGCGGGATTC	AATTAACCCTCACTAAAGGG	NED	7
CM_1009046	63.5	$(AGAT)_{10}$	TCCAAACTGTTTCCAAGGGC	TGTTCCCAGACATGTGTAGC	GGCAGGAAACAGCTATGAC	PET	8
CM_764003	63.5	$(AGAT)_{12}$	TGTACAACCTCACCGCCTAG	GGTTAATAACAGCCCAGGAGC	AATTAACCCTCACTAAAGGG	FAM	8
CM_4391826	63.5	$(AGAT)_{18}$	GGTCGATGGTAGAAGCACAC	CGCTCCCATCCATCAACAAC	GCCTTGCCAGCCCGC	VIC	8

Table 2. Sample Size (N), No. Alleles (N_a), No. Effective Alleles (N_e), Information Index (I), Observed Heterozygosity (H_o), Expected Heterozygosity (H_e), Probability of Conformance to HWE (P_{HWE}), and Unbiased Expected Heterozygosity (uH_e), and Fixation Index (F) for each locus. Bolded values are out of HWE (P < 0.0001). Detailed list of locus names is at the bottom of the table. New York (NY), Delaware (DE), Virginia (VA), North Carolina North of Cape Hatteras (NCN), North Carolina South of Cape Hatteras (NCS), South Carolina (SC), Florida Keys (FL), Western Florida (WF).

Location	Statistic	Loc1	Loc2	Loc3	Loc4	Loc5	Loc6	Loc7	Loc8	Loc9	Loc10	Loc11	Loc12	Loc13
NY	**N**	79	77	77	79	79	74	79	78	79	79	79	79	79
	Na	10	8	8	9	6	13	11	13	8	9	6	8	12
	Ne	4.080	3.885	2.836	3.797	1.809	6.602	7.456	4.952	4.733	4.613	2.170	3.751	5.249
	I	1.685	1.572	1.383	1.550	0.943	2.142	2.182	1.907	1.687	1.689	1.051	1.584	1.944
	Ho	0.810	0.805	0.662	0.722	0.481	0.446	0.861	0.808	0.734	0.671	0.557	0.810	0.810
	He	0.755	0.743	0.647	0.737	0.447	0.849	0.866	0.798	0.789	0.783	0.539	0.733	0.809
	uHe	0.760	0.747	0.652	0.741	0.450	0.854	0.871	0.803	0.794	0.788	0.543	0.738	0.815
	P_{HWE}	0.399	0.026	0.638	0.508	0.889	**0.000**	0.900	0.908	0.102	0.120	0.924	0.743	0.698
	F	-0.073	-0.084	-0.023	0.021	-0.076	0.474	0.006	-0.012	0.069	0.143	-0.033	-0.105	-0.001
DE	**N**	45	43	44	45	44	42	44	43	45	43	45	45	45
	Na	6	8	8	6	6	8	11	9	7	7	6	9	12
	Ne	3.540	3.594	2.505	3.325	2.251	4.612	7.854	5.561	4.731	4.037	2.259	3.395	4.799
	I	1.491	1.573	1.277	1.339	1.142	1.765	2.189	1.876	1.688	1.539	1.133	1.520	1.905
	Ho	0.644	0.674	0.705	0.667	0.568	0.429	0.909	0.814	0.822	0.814	0.644	0.800	0.844
	He	0.718	0.722	0.601	0.699	0.556	0.783	0.873	0.820	0.789	0.752	0.557	0.705	0.792
	uHe	0.726	0.730	0.608	0.707	0.562	0.793	0.883	0.830	0.798	0.761	0.564	0.713	0.800
	P_{HWE}	0.053	0.491	0.771	0.049	0.730	**0.000**	0.513	0.100	0.907	0.994	0.965	0.861	0.585
	F	0.102	0.066	-0.173	0.047	-0.022	0.453	-0.042	0.008	-0.043	-0.082	-0.156	-0.134	-0.067
VA	**N**	107	107	107	107	107	101	107	107	107	107	107	107	107
	Na	9	10	8	8	6	14	11	10	9	9	7	10	12
	Ne	3.437	3.528	2.710	3.278	2.256	7.204	6.793	5.155	4.384	4.501	2.278	4.197	5.188
	I	1.558	1.513	1.309	1.400	1.137	2.227	2.071	1.856	1.659	1.702	1.138	1.706	1.919
	Ho	0.720	0.682	0.561	0.664	0.542	0.564	0.850	0.766	0.776	0.822	0.598	0.766	0.850
	He	0.709	0.717	0.631	0.695	0.557	0.861	0.853	0.806	0.772	0.778	0.561	0.762	0.807
	uHe	0.712	0.720	0.634	0.698	0.559	0.865	0.857	0.810	0.776	0.781	0.564	0.765	0.811
	P_{HWE}	0.882	0.783	0.356	0.190	0.799	**0.000**	0.332	0.091	0.825	0.684	0.386	0.809	0.237
	F	-0.015	0.048	0.111	0.045	0.026	0.345	0.003	0.049	-0.005	-0.057	-0.066	-0.006	-0.054

NCN	**N**	56	56	56	56	56	53	56	55	56	56	56	56	56
	Na	8	7	8	7	6	13	11	11	8	10	7	7	12
	Ne	2.938	3.568	2.814	3.190	2.001	6.602	8.396	4.394	4.674	4.436	2.436	3.762	5.271
	I	1.356	1.487	1.359	1.365	1.061	2.141	2.252	1.813	1.686	1.747	1.168	1.545	1.926
	Ho	0.643	0.732	0.607	0.661	0.554	0.453	0.857	0.745	0.821	0.804	0.571	0.696	0.839
	He	0.660	0.720	0.645	0.687	0.500	0.849	0.881	0.772	0.786	0.775	0.589	0.734	0.810
	uHe	0.666	0.726	0.650	0.693	0.505	0.857	0.889	0.779	0.793	0.782	0.595	0.741	0.818
	P_{HWE}	0.896	0.660	0.116	0.888	0.235	**0.000**	0.024	0.917	0.093	0.903	0.149	0.313	0.798
	F	0.025	-0.017	0.058	0.038	-0.106	0.466	0.027	0.035	-0.045	-0.037	0.031	0.051	-0.036
NCS	**N**	66	66	66	66	66	63	66	66	66	66	66	66	66
	Na	7	8	7	7	6	11	11	12	9	8	7	8	11
	Ne	3.457	4.069	2.840	2.933	2.172	6.665	7.020	5.476	4.402	4.100	1.929	4.180	4.998
	I	1.454	1.626	1.308	1.299	1.117	2.103	2.102	1.945	1.655	1.624	1.018	1.683	1.912
	Ho	0.742	0.742	0.636	0.682	0.576	0.476	0.818	0.864	0.773	0.727	0.470	0.742	0.803
	He	0.711	0.754	0.648	0.659	0.540	0.850	0.858	0.817	0.773	0.756	0.482	0.761	0.800
	uHe	0.716	0.760	0.653	0.664	0.544	0.857	0.864	0.824	0.779	0.762	0.485	0.767	0.806
	P_{HWE}	0.3446	0.744	0.904	0.904	0.136	**0.000**	0.212	0.826	0.374	0.089	0.576	0.497	0.474
	F	-0.045	0.016	0.018	-0.034	-0.067	0.440	0.046	-0.057	0.000	0.038	0.025	0.024	-0.004
SC	**N**	74	76	76	76	75	72	76	75	75	75	76	75	76
	Na	8	8	7	7	6	15	12	10	9	8	6	9	12
	Ne	3.206	4.182	2.782	2.920	2.038	6.513	7.496	4.691	4.015	4.496	2.343	3.974	5.742
	I	1.452	1.629	1.361	1.245	1.037	2.157	2.158	1.759	1.588	1.652	1.179	1.647	2.014
	Ho	0.689	0.750	0.592	0.618	0.560	0.556	0.868	0.813	0.840	0.787	0.605	0.720	0.776
	He	0.688	0.761	0.641	0.658	0.509	0.846	0.867	0.787	0.751	0.778	0.573	0.748	0.826
	uHe	0.693	0.766	0.645	0.662	0.513	0.852	0.872	0.792	0.756	0.783	0.577	0.753	0.831
	P_{HWE}	0.820	0.369	0.535	0.535	0.496	**0.000**	0.365	0.050	0.629	0.666	0.762	0.094	0.090
	F	-0.002	0.014	0.076	0.060	-0.099	0.344	-0.002	-0.034	-0.119	-0.012	-0.056	0.038	0.060
FL	**N**	61	61	61	61	61	58	61	61	61	61	61	61	61
	Na	6	8	8	9	5	13	12	9	8	9	7	9	12
	Ne	3.316	3.607	3.229	3.369	2.059	5.721	8.278	4.829	4.289	3.834	2.056	3.917	5.782
	I	1.409	1.509	1.453	1.416	1.034	2.042	2.248	1.775	1.632	1.583	1.028	1.669	1.984
	Ho	0.721	0.705	0.574	0.836	0.459	0.414	0.885	0.820	0.738	0.803	0.508	0.656	0.885
	He	0.698	0.723	0.690	0.703	0.514	0.825	0.879	0.793	0.767	0.739	0.514	0.745	0.827
	uHe	0.704	0.729	0.696	0.709	0.519	0.832	0.886	0.799	0.773	0.745	0.518	0.751	0.834
	P_{HWE}	0.628	0.619	0.091	0.091	0.315	**0.000**	0.666	0.347	0.514	0.944	0.300	0.213	0.676
	F	-0.033	0.025	0.169	-0.189	0.108	0.499	-0.007	-0.034	0.038	-0.087	0.011	0.119	-0.070

WF	**N**	15	15	15	15	15	14	15	14	15	14	15	15	15
	Na	6	7	4	6	4	8	10	9	7	5	3	7	9
	Ne	3.982	4.839	2.027	2.980	1.230	4.962	7.258	5.521	3.947	3.806	1.737	4.412	5.488
	I	1.566	1.740	0.877	1.325	0.435	1.787	2.113	1.900	1.593	1.430	0.756	1.681	1.912
	Ho	0.733	0.800	0.533	0.733	0.200	0.786	0.867	0.786	0.733	0.714	0.467	0.867	0.800
	He	0.749	0.793	0.507	0.664	0.187	0.798	0.862	0.819	0.747	0.737	0.424	0.773	0.818
	uHe	0.775	0.821	0.524	0.687	0.193	0.828	0.892	0.849	0.772	0.765	0.439	0.800	0.846
	P_{HWE}	0.531	0.652	0.940	0.940	1.000	0.870	0.066	0.610	0.914	0.521	0.717	0.795	0.237
	F	0.021	-0.008	-0.053	-0.104	-0.071	0.016	-0.005	0.040	0.018	0.031	-0.099	-0.121	0.022

Location	**Statistic**	**Loc14**	**Loc15**	**Loc16**	**Loc17**	**Loc18**	**Loc19**	**Loc20**	**Loc21**	**Loc22**	**Loc23**	**Loc24**	**Loc25**
NY	**N**	79	79	77	79	78	79	79	79	79	77	75	76
	Na	16	10	12	6	5	7	9	16	14	10	15	9
	Ne	6.566	4.500	7.822	2.181	2.475	5.487	3.382	5.482	8.201	3.707	4.676	4.247
	I	2.221	1.732	2.209	1.083	1.087	1.784	1.498	2.109	2.307	1.656	2.048	1.709
	Ho	0.848	0.823	0.857	0.595	0.564	0.861	0.722	0.797	0.873	0.740	0.507	0.789
	He	0.848	0.778	0.872	0.542	0.596	0.818	0.704	0.818	0.878	0.730	0.786	0.765
	uHe	0.853	0.783	0.878	0.545	0.600	0.823	0.709	0.823	0.884	0.735	0.791	0.770
	P_{HWE}	0.858	0.795	0.454	0.081	0.181	0.581	0.929	0.451	0.416	0.054	**0.000**	0.908
	F	0.000	-0.058	0.017	-0.099	0.054	-0.053	-0.024	0.025	0.005	-0.014	0.355	-0.033
DE	**N**	45	45	45	45	45	45	45	45	45	45	45	45
	Na	10	9	12	7	5	6	8	14	14	9	13	10
	Ne	6.072	4.748	7.656	2.318	2.399	5.179	3.320	4.480	9.332	3.406	5.219	4.592
	I	2.050	1.761	2.194	1.186	1.034	1.703	1.516	1.984	2.380	1.523	2.046	1.761
	Ho	0.867	0.711	0.889	0.533	0.644	0.889	0.644	0.756	0.889	0.756	0.489	0.800
	He	0.835	0.789	0.869	0.569	0.583	0.807	0.699	0.777	0.893	0.706	0.808	0.782
	uHe	0.845	0.798	0.879	0.575	0.590	0.816	0.707	0.786	0.903	0.714	0.817	0.791
	P_{HWE}	0.725	0.174	0.095	0.246	0.775	0.181	0.332	0.598	0.405	0.023	**0.000**	0.467
	F	-0.038	0.099	-0.022	0.062	-0.105	-0.102	0.078	0.027	0.004	-0.070	0.395	-0.023
VA	**N**	107	107	107	107	107	107	107	107	107	107	107	107
	Na	16	12	12	6	6	7	10	19	15	12	16	10
	Ne	6.529	5.297	7.437	2.158	2.336	5.134	3.492	4.428	8.210	3.635	6.122	4.925
	I	2.159	1.890	2.158	1.067	1.044	1.733	1.514	2.050	2.318	1.679	2.240	1.866
	Ho	0.832	0.822	0.907	0.570	0.607	0.804	0.729	0.776	0.841	0.692	0.561	0.804

	He	0.847	0.811	0.866	0.537	0.572	0.805	0.714	0.774	0.878	0.725	0.837	0.797
	uHe	0.851	0.815	0.870	0.539	0.575	0.809	0.717	0.778	0.882	0.728	0.841	0.801
	P_{HWE}	0.550	0.078	0.964	0.288	0.653	0.832	0.321	0.623	0.552	0.464	**0.000**	0.795
	F	0.018	-0.014	-0.047	-0.063	-0.062	0.002	-0.021	-0.002	0.042	0.046	0.330	-0.008
NCN	**N**	56	56	56	56	56	56	56	56	56	56	55	56
	Na	14	9	11	4	6	7	7	12	14	11	14	9
	Ne	5.934	4.129	7.538	1.889	2.524	5.435	3.702	3.841	8.363	3.324	6.464	4.900
	I	2.089	1.649	2.154	0.913	1.130	1.759	1.494	1.841	2.296	1.586	2.203	1.770
	Ho	0.750	0.804	0.946	0.464	0.500	0.804	0.750	0.732	0.875	0.732	0.655	0.821
	He	0.831	0.758	0.867	0.471	0.604	0.816	0.730	0.740	0.880	0.699	0.845	0.796
	uHe	0.839	0.765	0.875	0.475	0.609	0.823	0.736	0.746	0.888	0.705	0.853	0.803
	P_{HWE}	0.200	0.075	0.203	0.184	0.247	0.393	0.868	0.840	0.036	0.309	**0.005**	0.691
	F	0.098	-0.060	-0.091	0.013	0.172	0.015	-0.028	0.010	0.006	-0.047	0.226	-0.032
NCS	**N**	66	66	66	66	66	66	66	66	66	66	66	66
	Na	16	11	12	5	5	8	10	17	14	10	16	11
	Ne	7.352	4.624	7.135	2.094	2.442	5.143	3.270	4.689	6.892	4.151	7.164	5.556
	I	2.281	1.772	2.136	1.019	1.058	1.772	1.492	2.067	2.198	1.682	2.341	1.957
	Ho	0.788	0.818	0.833	0.561	0.682	0.833	0.727	0.742	0.848	0.727	0.576	0.864
	He	0.864	0.784	0.860	0.522	0.590	0.806	0.694	0.787	0.855	0.759	0.860	0.820
	uHe	0.871	0.790	0.866	0.526	0.595	0.812	0.700	0.793	0.861	0.765	0.867	0.826
	P_{HWE}	0.313	0.226	0.944	0.930	0.823	0.791	0.652	0.328	0.519	0.394	**0.000**	0.614
	F	0.088	-0.044	0.031	-0.073	-0.155	-0.034	-0.048	0.056	0.008	0.042	0.331	-0.053
SC	**N**	76	76	76	76	76	76	76	76	74	75	76	74
	Na	14	7	12	5	6	7	11	16	14	12	16	10
	Ne	6.616	4.036	6.955	2.360	2.506	5.583	3.445	5.208	8.583	3.575	5.383	5.132
	I	2.176	1.601	2.107	1.111	1.110	1.792	1.597	2.027	2.353	1.703	2.123	1.828
	Ho	0.895	0.645	0.789	0.632	0.592	0.868	0.684	0.855	0.905	0.720	0.592	0.811
	He	0.849	0.752	0.856	0.576	0.601	0.821	0.710	0.808	0.883	0.720	0.814	0.805
	uHe	0.854	0.757	0.862	0.580	0.605	0.826	0.714	0.813	0.890	0.725	0.820	0.811
	P_{HWE}	0.317	0.018	0.129	0.037	0.305	0.871	0.261	0.472	0.027	0.761	**0.000**	0.777
	F	-0.054	0.143	0.078	-0.096	0.015	-0.058	0.036	-0.058	-0.025	0.000	0.273	-0.007
FL	**N**	61	61	61	61	61	61	61	61	61	61	61	61
	Na	12	8	12	5	6	6	9	12	13	10	12	11
	Ne	6.399	4.605	7.368	2.197	2.371	5.175	3.375	4.040	8.116	3.923	6.045	4.845
	I	2.089	1.728	2.160	1.058	1.093	1.712	1.441	1.847	2.265	1.719	2.068	1.852
	Ho	0.885	0.754	0.902	0.590	0.492	0.885	0.689	0.672	0.951	0.721	0.590	0.803

	He	0.844	0.783	0.864	0.545	0.578	0.807	0.704	0.752	0.877	0.745	0.835	0.794
	uHe	0.851	0.789	0.871	0.549	0.583	0.813	0.710	0.759	0.884	0.751	0.841	0.800
	P_{HWE}	0.878	0.248	0.789	0.445	0.034	0.798	0.165	0.047	0.466	0.258	**0.000**	0.393
	F	-0.049	0.037	-0.043	-0.083	0.149	-0.097	0.022	0.107	-0.084	0.032	0.293	-0.012
WF	**N**	15	15	15	15	15	15	15	15	15	15	15	15
	Na	11	7	9	4	5	6	7	8	11	8	11	7
	Ne	7.143	4.500	6.818	2.406	2.778	5.294	4.167	5.172	7.627	3.782	7.500	4.891
	I	2.165	1.665	2.020	1.104	1.186	1.725	1.617	1.831	2.183	1.633	2.202	1.728
	Ho	0.867	0.800	0.867	0.667	0.600	0.867	1.000	0.800	0.933	0.867	0.600	0.867
	He	0.860	0.778	0.853	0.584	0.640	0.811	0.760	0.807	0.869	0.736	0.867	0.796
	uHe	0.890	0.805	0.883	0.605	0.662	0.839	0.786	0.834	0.899	0.761	0.897	0.823
	P_{HWE}	0.814	0.956	0.672	0.878	0.796	0.930	0.666	0.562	0.947	0.578	**0.000**	0.789
	F	-0.008	-0.029	-0.016	-0.141	0.062	-0.068	-0.316	0.008	-0.074	-0.178	0.308	-0.089

	Locus		Locus		Locus
Loc1	CM54794	Loc10	CM2660427	Loc19	CM732781
Loc2	CM2316467	Loc11	CM2212380	Loc20	CM2186404
Loc3	CM2352680	Loc12	CM2149957	Loc21	CM90501
Loc4	CM2492523	Loc13	CM459957	Loc22	CM1080088
Loc5	CM1787993	Loc14	CM2374475	Loc23	CM764003
Loc6	CM931277	Loc15	CM310413	Loc24	CM4391826
Loc7	CM4718692	Loc16	CM4591723	Loc25	CM1009046
Loc8	CM1065459	Loc17	CM2404273		
Loc9	CM1827829	Loc18	CM1191685		

Table 3. Population pairwise F_{ST} values based on 23 microsatellite loci (lower matrix). Population pairwise Φ_{ST} values based on the mitochondrial control region (lower matrix). New York (NY), Delaware (DE), Virginia (VA), North Carolina North of Cape Hatteras (NCN), North Carolina South of Cape Hatteras (NCS), South Carolina (SC), Florida Keys (FL), Western Florida (WF). There were no significant pairwise comparisons based on either class of molecular marker.

	NY	DE	VA	NCN	NCS	SC	FL	WF
NY	*	-0.00144	-0.00047	0.01391	0.00106	-0.00700	-0.00789	-0.011
DE	-0.00151	*	0.00205	-0.00279	-0.00209	-0.02601	-0.00906	0.0391
VA	-0.00045	-0.00218	*	0.01045	-0.01496	-0.00513	-0.01110	-0.013
NCN	-0.00056	-0.00112	-0.00025	*	-0.00291	-0.00734	-0.00519	0.0162
NCS	-0.00012	-0.00204	0.00082	0.00074	*	-0.00399	-0.00436	-0.004
SC	-0.00066	-0.00166	-0.00084	0.00036	-0.00088	*	-0.01320	0.0011
FL	-0.00067	-0.00216	-0.00057	-0.00085	-0.00054	-0.00004	*	-0.016
WF	-0.00073	-0.00277	0.00042	0.00014	0.00329	-0.00057	0.00023	*

Table 4. Distribution of mtDNA haplotypes. New York (NY), Delaware (DE), Virginia (VA), North Carolina North of Cape Hatteras (NCN), North Carolina South of Cape Hatteras (NCS), South Carolina (SC), Florida Keys (FL), Western Florida (WF).

Haplotype	NY	DE	VA	NCN	NCS	SC	FL	WF	Total
Hap_1	0	0	0	0	0	2	0	0	2
Hap_2	0	0	1	0	0	0	1	0	2
Hap_3	0	0	1	0	0	0	0	0	1
Hap_4	0	0	0	0	0	2	1	0	3
Hap_5	2	0	1	0	0	1	0	0	4
Hap_6	0	0	2	0	0	1	0	1	4
Hap_7	0	0	0	2	2	0	2	3	9
Hap_8	1	0	1	0	0	2	1	0	5
Hap_9	4	1	13	4	8	4	5	0	39
Hap_10	1	0	1	0	1	1	0	2	6
Hap_11	1	0	1	1	1	1	2	2	9
Hap_12	0	0	0	0	1	0	0	1	2
Hap_13	0	0	0	0	1	0	1	2	4
Hap_14	1	0	0	0	0	1	0	1	3
Hap_15	0	0	0	2	1	0	1	1	5
Hap_16	1	0	1	0	0	0	0	0	2
Hap_17	0	0	1	0	0	0	0	0	1
Hap_18	0	0	2	0	0	0	0	0	2
Hap_19	0	0	1	0	0	0	0	0	1
Hap_20	0	0	1	1	0	0	0	0	2
Hap_21	1	0	0	1	2	0	0	0	4
Hap_22	0	0	0	0	1	0	0	0	1
Hap_23	0	0	0	0	1	0	0	0	1
Hap_24	0	0	1	0	1	0	0	0	2
Hap_25	0	0	0	0	1	0	0	0	1
Hap_26	0	0	1	0	1	0	1	0	3
Hap_27	0	1	1	0	1	2	0	0	5
Hap_28	0	0	1	0	1	1	0	0	3
Hap_29	0	0	0	0	0	1	0	0	1
Hap_30	0	0	0	0	0	1	0	0	1
Hap_31	0	0	0	0	0	1	0	0	1
Hap_32	2	0	1	1	0	1	1	0	6
Hap_33	1	1	0	0	0	1	0	0	3
Hap_34	0	0	0	0	0	1	0	0	1
Hap_35	0	0	0	0	0	1	0	0	1
Hap_36	0	0	2	0	0	0	0	0	2
Hap_37	0	0	1	0	0	0	1	0	2
Hap_38	0	0	2	0	0	0	1	0	3
Hap_39	0	0	1	0	0	0	0	0	1
Hap_40	0	1	1	1	0	0	0	0	3
Hap_41	0	0	1	0	0	0	0	0	1
Hap_42	0	0	0	0	0	0	1	0	1
Hap_43	0	0	0	0	0	0	1	0	1
Hap_44	0	0	0	0	0	0	1	0	1
Hap_45	2	0	0	1	0	0	1	0	4
Hap_46	0	0	0	0	0	0	1	0	1
Hap_47	0	0	0	0	0	0	1	0	1

Hap_48	1	1	0	0	0	0	0	0	2
Hap_49	0	1	0	0	0	0	0	0	1
Hap_50	0	1	0	0	0	0	0	0	1
Hap_51	0	1	0	0	0	0	0	0	1
Hap_52	0	0	0	0	0	1	0	0	1
Hap_53	0	0	0	0	0	1	0	0	1
Hap_54	0	0	0	0	0	1	0	0	1
Hap_55	0	0	0	0	0	1	0	0	1
Hap_56	0	0	0	0	0	1	0	0	1
Hap_57	0	0	0	0	0	1	0	0	1
Hap_58	1	0	0	0	0	0	0	0	1
Hap_59	1	0	0	0	0	0	0	0	1
Hap_60	1	0	0	0	0	0	0	0	1
Hap_61	1	0	0	0	0	0	0	0	1
Hap_62	1	0	0	0	0	0	0	0	1
Hap_63	1	0	0	0	0	0	0	0	1
Hap_64	0	0	0	1	0	0	0	0	1
Hap_65	0	0	0	1	0	0	0	0	1
Hap_66	0	0	0	1	0	0	0	0	1
Hap_67	0	0	0	1	0	0	0	0	1
Hap_68	0	0	0	1	0	0	0	0	1
Hap_69	0	0	0	2	0	0	0	0	2
Hap_70	0	0	0	1	0	0	0	0	1
Hap_71	0	0	0	1	0	0	0	0	1
Hap_72	0	0	0	1	0	0	0	0	1
Total	24	8	40	24	24	31	24	13	188

Table 5. Mean number of pairwise differences (K), nucleotide diversity (π), haplotype diversity (H), Tajima's D, Probability of significance for Tajima's D (P_D), Fu's F, Probability of significance for Fu's D (P_F), values across all samples (All), New York (NY), Delaware (DE), Virginia (VA), North Carolina North of Cape Hatteras (NCN), North Carolina South of Cape Hatteras (NCS), South Carolina (SC), Florida Keys (FL), Western Florida (WF). All probabilities based on 10 000 permutations of the data.

Sample	K	π	H	Tajima's D	P_D	Fu's F	P_F
All	3.099 +/- 1.618	0.008 +/- 0.004	0.943 +/- 0.013	-2.101	0.001	-26.240	0.000
NY	3.409 +/- 1.807	0.008 +/- 0.005	0.967 +/- 0.024	-1.439	0.061	-13.112	0.000
DE	3.607 +/- 2.043	0.009 +/- 0.006	1.000 +/- 0.062	-1.336	0.097	-4.958	0.003
VA	2.931 +/- 1.569	0.007 +/- 0.004	0.894 +/- 0.04	-1.836	0.014	-19.816	0.000
NCN	2.754 +/- 1.511	0.007 +/- 0.004	0.967 +/- 0.024	-1.609	0.038	-15.451	0.000
NCS	2.442 +/- 1.370	0.006 +/- 0.004	0.891 +/- 0.057	-1.662	0.034	-10.239	0.000
SC	3.914 +/- 2.017	0.010 +/- 0.006	0.978 +/- 0.015	-1.903	0.010	-20.286	0.000
FL	2.953 +/- 1.601	0.007 +/- 0.004	0.956 +/- 0.031	-1.366	0.075	-14.667	0.000
WF	2.692 +/- 1.530	0.007 +/- 0.004	0.923 +/- 0.050	-0.657	0.278	-2.577	0.051

Figure 1. Sample collection locations for Blueline Tilefish used in the VIMS genetics study. Closed circles indicate a known lat/long fish capture location. Open circles indicate an approximate location or statistical area reported by the sample collector. NY-New York, NJ-New Jersey, DE-Delaware, VA-Virginia, NCN-North Carolina North of Cape Hatteras, NCS-North Carolina South of Cape Hatteras, SC-South Carolina, GA-Georgia, FL-Florida Keys, WFL-Western Florida.

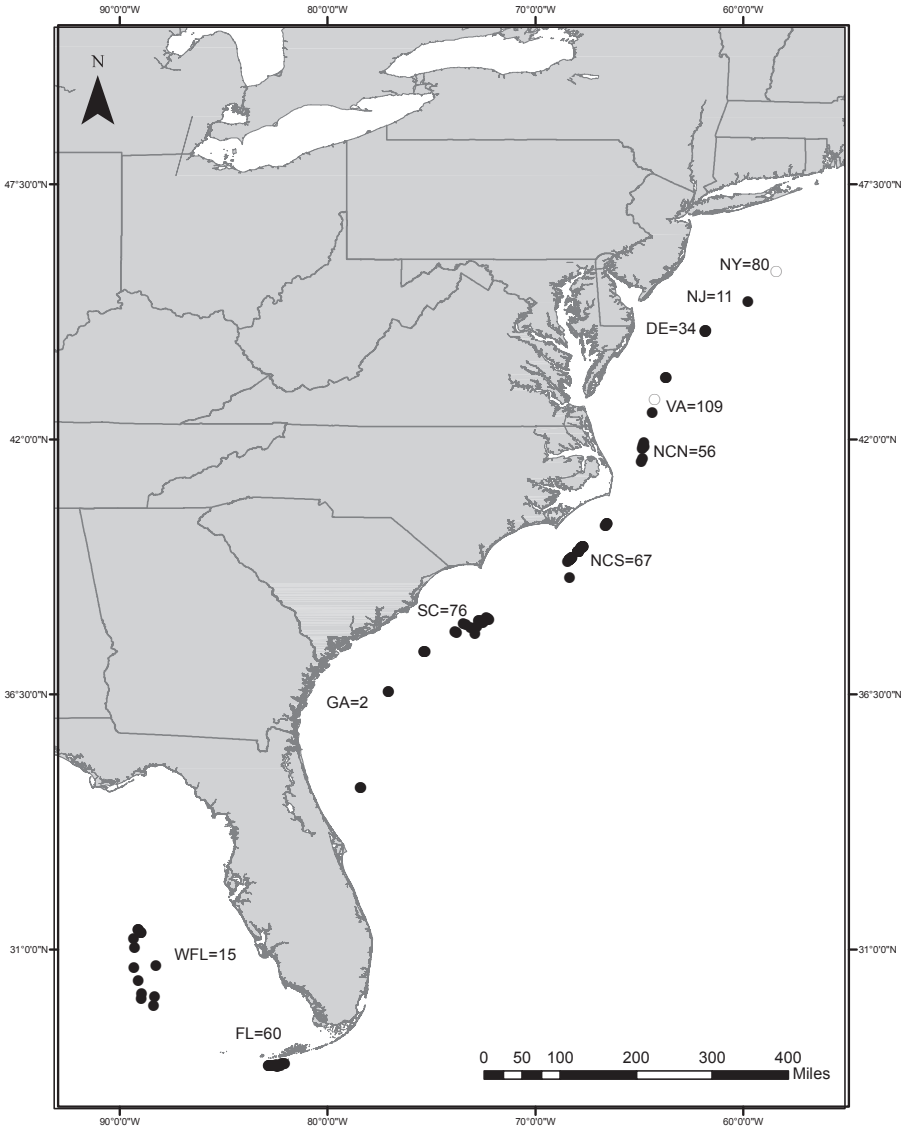


Figure 2. Factorial correspondence analysis based on microsatellite data: a) Samples divided NY-NCN (yellow) and NCS-WF (blue) b) NY-DE (yellow), VA-NCN (blue) and SC-WF (white) and c) NY (yellow), DE (bright blue), VA (white) NCN (grey), NCS (pink), SC (green), FL (dark blue), WF (red).

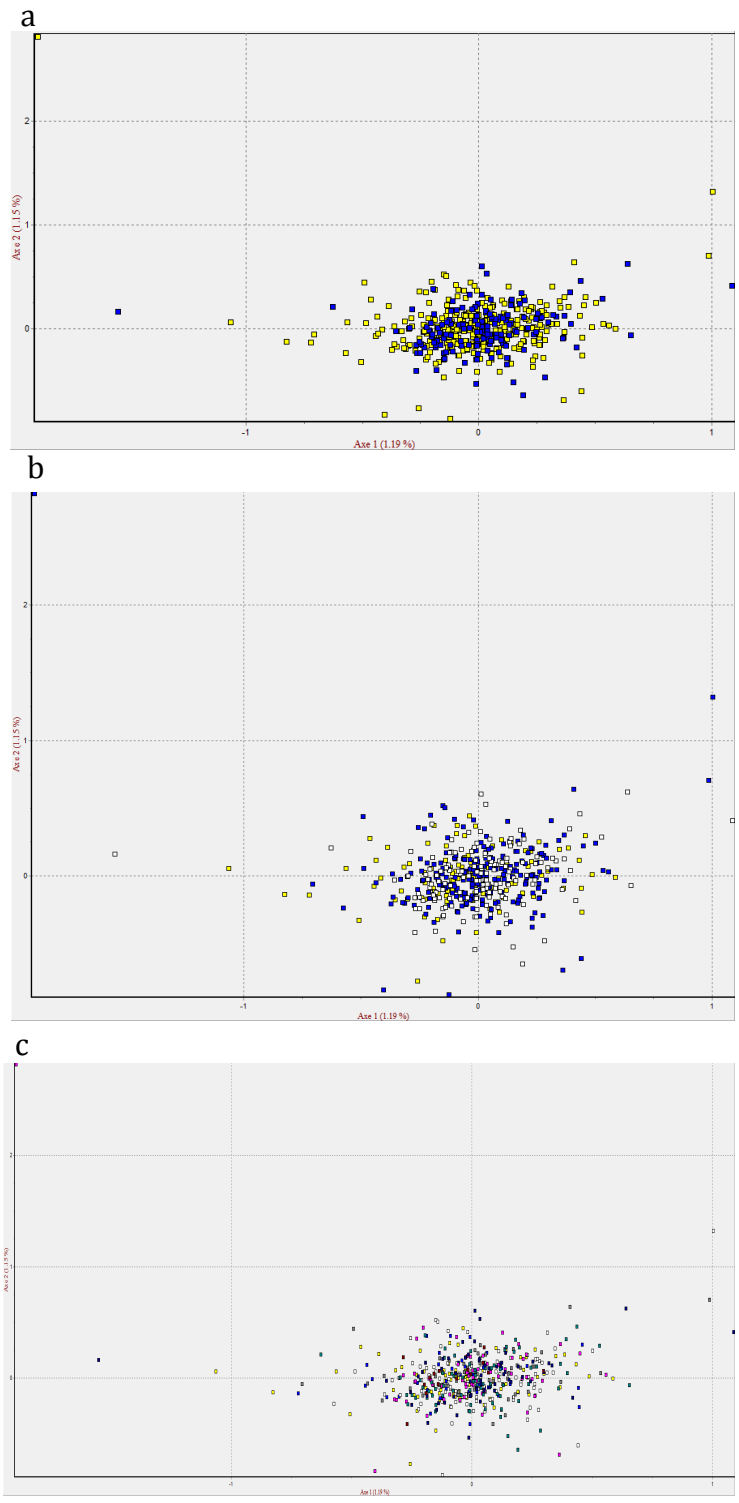
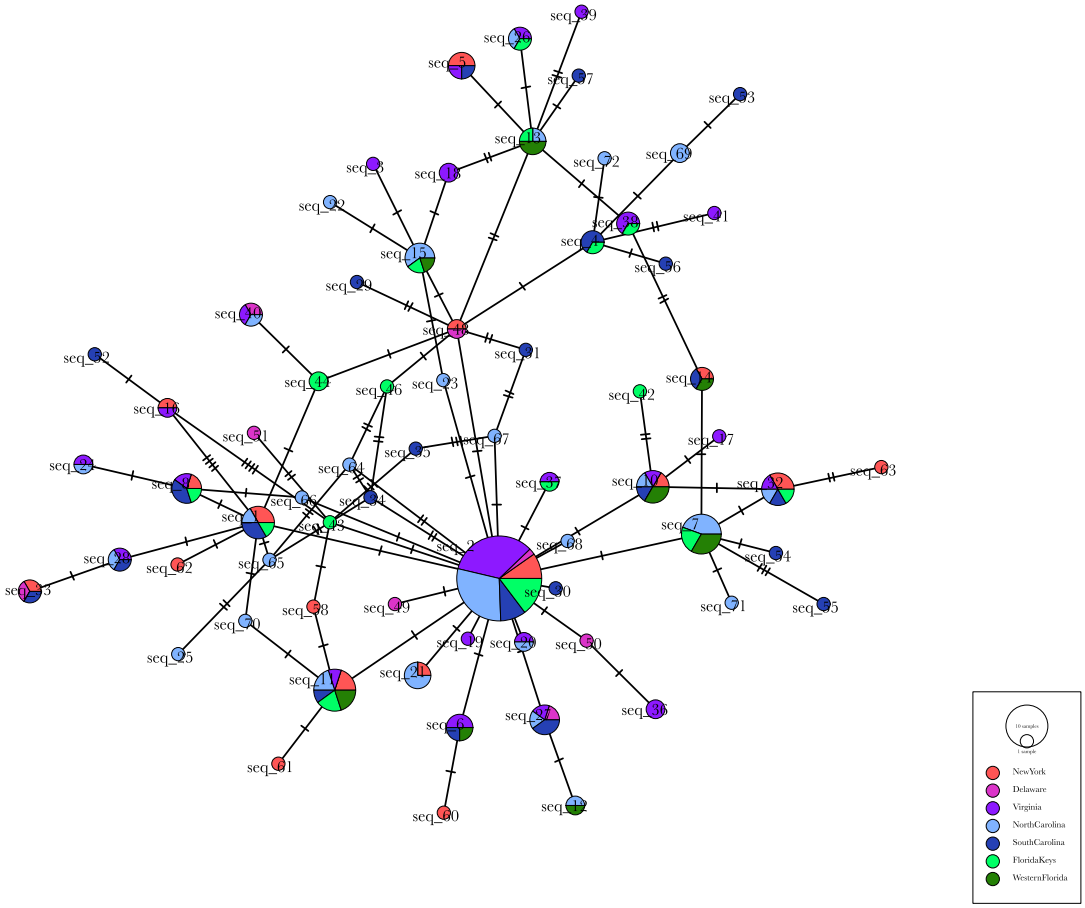


Figure 3. Minimum spanning network of the relationship among mtDNA haplotypes. Hash marks represent the number of base pair differences between haplotypes.


10 samples
1 sample
NewYork
Delaware
Virginia
NorthCarolina
SouthCarolina
FloridaKeys
WesternFlorida