Improving Life History and Genetic Data for Cobia Along the US South Atlantic Coast

ASMFC Contract Number: 21-0102

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Reporting Period: January 1, 2021-December 31, 2022

Final Report

Objective 1:

Objective 2: *Increasing power of genetic marker panel and providing updated genetic analyses of cobia stock structure in US waters.*

Sample Collections:

The SCDNR Genetic Tissue Collection currently houses more than 7,900 archived genetic samples of cobia collected by numerous researchers and fishermen around the globe. In all cases, small tissue samples were collected from the pectoral, anal, or caudal fin and stored in either 95% non-denatured EtOH or a sarcosyl-urea preservation solution (8M urea, 1% sarcosyl, 20 mM sodium phosphate, 1 mM EDTA) until processing. In 2015, the SCDNR MRRI genetics team added nine additional microsatellite loci to its molecular panel (previously 10 microsatellite loci) to increase its power in future analyses and has subsequently been genotyping all samples collected since 2016 with this increased marker panel. As of today, over 2,400 samples had been fully genotyped with the new panel. However, several thousand samples collected prior to 2015 had not been back-genotyped with the additional markers. For the current project, we selected 1,603 priority cobia samples from our archives to genotype with the nine additional microsatellite loci that would complement the current 19 locus dataset to allow for a meaningful updated genetic analysis with this higher resolution marker panel (Table 1). These samples were previously collected from seven different states between 2006 and 2015. During the project period (leveraging other state and federal funds), we have added to our dataset 1,200 recently collected samples that have been genotyped with all 19 microsatellite loci along the U.S. Gulf of Mexico and southeastern Atlantic coasts ranging from Virginia south along the Atlantic coast around the Florida peninsula into the Gulf of Mexico and westward to Texas. In our previous analyses for SEDAR58 with samples collected from 2006-2017 (Darden et al. 2018), three separate data sets were subjected to analyses: 1) All selected samples available, 2) Selected samples collected during cobia spawning season defined for each state on temperature-based patterns: Virginia – June to August, North Carolina – May to July, South Carolina and Georgia – April to July, Florida – March to August, Mississippi – May (only samples available), and Texas – April to August, and 3) Selected samples collected during cobia spawning season constrained to the period of April through June for all locations. All evaluated datasets resulted in consistent gene flow patterns following analyses, with the only deviation being a reduction in clarity (i.e., strength of patterns) within the North Carolina/Virginia collections in the April-June constrained dataset likely due to the loss of samples during the

July-August time periods within this region that includes the known peak spawning period (Joseph et al. 1964). As such, the dataset with spawning season defined by state was selected to present results within that working paper to minimize noise due to winter collected samples (high movement period) as well as maximize any potential signal from the North Carolina and Virginia areas. Therefore, following this precedent we utilized the same spawning season by state definitions for the updated analyses in the current project. New sample collections allowed for standardization of April-July spawning seasons definitions for Mississippi, Alabama, and Louisiana.

Table 1. Summary of the 1,603 samples successfully back-genotyped with the new markers for the current project including locality codes, location descriptions, collection years of samples, and sample sizes for both the initial partitioned dataset and the final grouped data set.

Codes	Location Definitions	Collection Years	Sample Sizes
TX	Texas (Corpus Christi)	2010	57
MS	Mississippi	2010	6
FLW	FL Panhandle	2008	16
FLS	Keys	2010	8
FLE2	Hobe Sound to Ft. Pierce	2011	150
FLGA	Jacksonville, FL to Brunswick, GA	2009-2010	12
GA	Savannah	2008-2009, 2012, 2014-2015	16
SCO1	Offshore Port Royal Sound and Betsy Ross reef	2009-2015	401
SCO2	Offshore Charleston, Murrells Inlet, Georgetown	2007-2011, 2015	30
NCO1	Offshore south of Cape Hatteras	2010, 2013-2014	38
NCO2	Offshore at and north of Cape Hatteras	2008-2010	182
SCI	SC inshore	2007-2015	584
NC1	Inshore area around Cape Lookout	2010	6
NC2	Inshore area Pamlico Sound area	2010	31
VA	VA inshore	2006-2008	66

Genetic Protocols and Analyses:

For most of our genetic samples, the sarcosyl-urea preservative simultaneously stabilizes sample DNA and serves as a preliminary cell lysis solution. EtOH-stored samples were subjected to a proteinase K cell lysis overnight prior to DNA isolation. All DNA isolation, microsatellite amplification, and genotyping methods followed previous work on cobia from our group

(Darden et al. 2014, 2018). The updated nineteen locus panel was amplified via polymerase chain reaction (PCR) in three multiplexed groups (Table 2) on iCycler thermal cyclers (Bio-Rad Laboratories, Hercules, CA).

Table 2. Multiplex panel, locus, fluorescent dye, repeat motif, number of alleles, and primer concentrations (µM) for 19 cobia-specific microsatellite loci (modified from Renshaw et al. 2006, except Rca1-H04).

Multiplex Panel	Locus	WellRED Dye	Repeat Motif	Number of Alleles	[Primer]
1	Rca1-H10	D2	CA	17	0.106
	Rca1-H04	D4	GT	10	0.029
	Rca1B-A10	D3	GTT	8	0.029
	Rca1-D08	D2	CA	4	0.039
	Rca1-A04	D4	$(CA)_9(CACT)_4$	13	0.029
	Rca1B-F06	D3	CTAT	21	0.039
	Rca1B-E02	D4	CT	10	0.029
2	Rca1-F11	D3	$(GA)_8AA(GA)_5$	4	0.013
	Rca1-A11	D4	GT	22	0.056
	Rca1B-H09	D2	GATA	23	0.078
	Rca1B-E08A	D3	CA	17	0.045
	Rca1-E05	D4	$(CA)_{20}(CGCA)_4(CA)_7(CGCA)_4$	23	0.040
	Rca1B-C06	D4	GATA	27	0.067
	Rca1B-D10	D3	CTAT	32	0.060
3	Rca1-D07	D4	$(GT)_9TTT(GT)_3$	7	0.020
	Rca1-E11	D2	CA	8	0.060
	Rca1-C04	D4	GT	19	0.060
	Rca1-G02	D2	GT	7	0.040
	Rca1-G05	D3	GT	10	0.060

As researchers at SCDNR initiated a cobia stock enhancement research program in 2004, all genetic sample collections were screened for hatchery individuals. We utilized a maximum likelihood parentage approach as implemented in CERVUS 3.0.3 (Kalinowski et al. 2007) to provide a statistical evaluation of parentage taking into account mutation rates, population allele frequencies, and lab error rates. The power of the loci suite to correctly identify hatchery fish as well as individual fish is high, with average parent-pair and identity non-exclusion probabilities of $1.8x10^{-10}$ and $8.8x10^{-17}$, respectively, suggesting very low probabilities of incorrectly identifying hatchery fish or individuals. Parentage simulations (n=20) were run with

known sex parentage analysis using allele frequencies from individuals collected from 2007 to 2021 (n=3,934). All simulations were conducted with 10,000 offspring, 8 candidate parent pairs (with all parents sampled), 95% genotyping, and low mistyping error (0.01) and mutation (0.001) rates. Critical delta scores were determined using 99% confidence for the relaxed criteria and 99.9% for the strict criteria. Parentage analyses were conducted with the modal simulation file from the simulation runs. All parental assignments were designated at the strict confidence level (99.9%). All hatchery-born fish were removed from the dataset prior to further analysis.

All remaining individuals that were successfully genotyped at 15 or more loci were subjected to sibship analyses as implemented in the software Colony 2.0.6.4 (Jones & Wang 2010) to identify any potential large family groups within the dataset that could confound further genetic structure analyses. Two simulations were run using settings of polygamous breeding, weak prior, updating allele frequencies, no genotyping error, and FPLS likelihood method for a medium run length. Any identified duplicate samples were removed from the dataset prior to further analyses. Results were evaluated for consistency among runs for individual fullsib relationships as well as family sizes present.

Standard population genetic statistical analyses were applied to the resulting sample datasets. Population genetic structure throughout the collection range was assessed via evaluations of Hardy-Weinberg equilibrium (HWE) in GenAlEx 6.5 (Peakall & Smouse 2006, 2012) and Genepop 4.7.2 (Raymond and Rousset 1995), AMOVA analyses in Arlequin 3.5.1.2 (Excoffier and Lischer 2010), pairwise F_{ST}-style statistics calculated in GenAlEx 6.5 and Arlequin (only R_{ST} metrics are reported here), and with the clustering algorithms implemented in STRUCTURE 2.3.4 (Pritchard et al. 2000). Iterative AMOVA (R_{ST}-based) analyses were conducted to evaluate areas of genetic discontinuity in the data sets with potential location groupings under two-population scenarios. Pairwise comparisons of sample locations and HWE were conducted initially at the smallest geographic scale and locations were combined sequentially to represent the smallest number of homogenous groupings. The clustering model assignment employed in the program STRUCTURE using a hierarchical approach with the assistance of the web-based software Structure Harvester 0.6.94 (Earl et al. 2012) was used to identify the most appropriate number of distinct populations (K) of each run. Simulations were run with the locprior (collection location) parameter for all analyses, with five replicates for each K, the length of the burn-in period set at 20,000, and number of Markov chain Monte-Carlo reps after burn-in set at 20,000. Our SEDAR58 analyses found that all STRUCTURE analyses with and without the locprior parameter produced consistent results and only the results from the locprior based analyses were presented as the patterns were more clearly visualized in the STRUCTURE plots (Darden et al. 2018). All analyses were conducted from K=1 to K= # collection locations included +1. Sites that showed homogenous ancestry patterns were removed from the data set and STRUCTURE was run iteratively until K=1 was the most appropriate assignment for each cluster (based on combined evaluation of the Evanno method and log likelihood plots). Effective number of

migrants per generation and year (based on 5-7 year generation time for cobia) were calculated for each resulting homogenous cluster in Arlequin.

Once final groupings were determined, basic molecular diversity indices were calculated for each group using Arlequin, Genepop, and FSTAT 2.9.3.2 (Goudet 1995, 2001), including observed heterozygosity (H_O), expected heterozygosity (H_E), inbreeding coefficients ($F_{IS;}$ Weir & Cockerham 1984), and rarefied allelic richness (R).

Results and Conclusions:

A total of 34 duplicate samples and 42 cultured fish occurred within the dataset; these were removed from all datasets prior to analyses. No large family groups (>3) were present within the dataset and only 8 full sibling pairs were identified (p=1.0); therefore, no confounding effects from family structure are anticipated in further analyses. The current project resulted in 1,603 samples successfully back-genotyped using the new marker panel. In combination with other project efforts, a total of 4,046 samples were available for inclusion in the complete dataset and 3,624 samples met our spawning season by State selection criteria that were used in the analyses (Table 3). Collection years for all samples included 2006 through 2022. For the initial analyses, the dataset was partitioned into 22 geographic sections based on natural latitudinal breaks in the collection data.

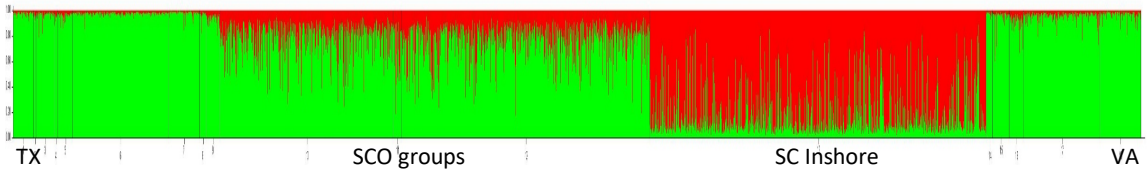
Results from our spawning season dataset analyses indicated variations in gene flow patterns along the Atlantic coast that were not consistent with those observed previously (as described for SEDAR58). We conducted several exploratory analyses to verify the changes were not due to the modified marker panel. Analyses of the dataset with 10 loci (original panel) and 15 loci (excluded least polymorphic new markers) both showed gene flow patterns consistent with the new full panel analyses. Additionally, given the extended time period of sample collection (17 years) that are not consistent across locations, we conducted analyses with the data partitioned into two generations (2007-2014 and 2015-2022) to evaluate the potential for a temporal signal. The contemporary dataset (2015-2022) indicated patterns consistent with the current full analysis. The prior generation analysis (2007-2014) showed the same big picture patterns (SC Inshore, Atlantic vs GOM, transition zone), but sample sizes with the new marker panel are too low to meaningfully interpret patterns along the Atlantic coast. With increasing reports of cobia farther north than previously documented (via tagging efforts and landings), we are most confident in interpreting the contemporary dataset as the current status of cobia gene flow patterns; results from those are included below. The contemporary dataset includes 2,118 samples from 21 geographic locations (Table 3).

Table 3. *Genetic dataset sample composition including location descriptions, locality codes, collection years of samples, and sample sizes for the complete, spawning season, and contemporary spawning season (2015-2022) datasets and the final grouped dataset (see text for grouping methodology).*

Code	Location definition	Collection Years	Sample Sizes			
			All Data	Within Spawning Seasons	Within Spawning Seasons (2015-2022)	Final Grouping Contemporary Sample Sizes
TX	Texas (Corpus Christi)	2010, 2018-2019	77	65	18	475
LA	Louisiana	2018, 2022	18	18	18	
MS	Mississippi and Alabama	2010, 2018-2019, 2022	20	20	14	
FLW1	FL Panhandle	2008, 2017-2019	75	75	59	
FLW2	Cedar Key to Tampa	2021-2022	26	26	26	
FLW3	Tampa to Naples	2022	47	45	45	
FLS	Florida Keys	2010	14	8	-	
FLE1	Boynton Beach to Jupiter	2016-2019, 2021-2022	228	188	188	
FLE2	Hobe Sound to Ft. Pierce	2011, 2016-2021	272	257	107	
FLE3	Canaveral/Sebastian (plus a few Ponce samples)	2016-2020	123	112	112	194
FLGA	Jacksonville, FL to Brunswick, GA	2009-2010, 2016-2021	62	50	45	
GA	Brunswick to Savannah	2008, 2012, 2014-2020	61	49	37	
SCO1	Offshore Port Royal Sound and Betsy Ross reef	2009-2016, 2018-2021	490	478	90	693
SCO2	Offshore Charleston, Murrells Inlet, Georgetown, Winyah Bay	2007-2011, 2015-2021	325	278	262	
SCO	All other offshore samples from SC	2016-2021	353	341	341	
NC1	Inshore from area around Cape Lookout south to Southport	2010, 2016-2019, 2021	64	40	34	219
NC2	Inshore area Pamlico Sound area	2010, 2017-2019, 2021	128	65	34	
NCO1	Offshore south of Cape Hatteras	2010, 2013, 2016-2021	213	177	151	
NCO2	Offshore at and north of Cape Hatteras	2008, 2010, 2016-2021	458	406	247	316
VA	VA inshore	2006-2008, 2017-2021	163	130	69	
SCI	SC inshore	2007-2021	798	785	210	210
VAO	VA offshore	2018-2020	31	11	11	11

With the current contemporary dataset, multiple rounds of heirarchical STRUCTURE, initial pairwise F_{ST}, and HWE analyses supported a genetically distinct South Carolina inshore population (Figure 1) and a homogenous Gulf of Mexico population ranging from Texas through the Ft. Pierce, FL area (FLE2, Figure 2). Samples from Cape Canaveral, FL through Savannah, GA locations showed genetic similarities with collection locations from both the north (SCOs) and south (FLE2) and continue to represent a transition zone as documented in the SEDAR58 analyses (Figure 2). However, gene flow patterns among South Carolina offshore, North Carolina inshore and offshore, and Virginia inshore areas are not consistent between the SEDAR58 dataset and the current contemporary dataset. In the SEDAR58 dataset, the Virginia and inshore North Carolina (NC1, NC2) samples represented a distinct genetic grouping (Figures 2 and 3) as did the combined offshore South Carolina and North Carolina samples (SCO, SCO1, SCO2, NCO1, NCO2, Figure 3). In the current contemporary dataset, the South Carolina offshore samples represent a distinct genetic grouping, North Carolina inshore and offshore samples south of Cape Hatteras represent a distinct genetic grouping, and North Carolina offshore north of Cape Hatteras and Virginia inshore samples represented a distinct genetic grouping (Figures 2 and 3). The Virginia offshore samples appear most similar to samples from North Carolina south of Cape Hatteras, but due to limited samples (n=11) we recommend further sampling is needed to confidently interpret gene flow in the Virginia offshore area.

(A)



(B)

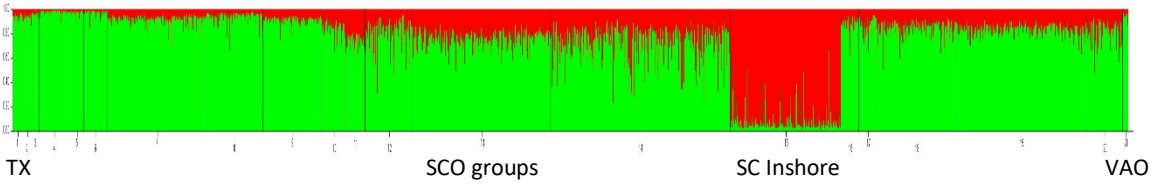


Figure 1. *Population ancestry plot for the (A) SEDAR58 dataset and (B) current contemporary dataset based on STRUCTURE results of K=2 with the South Carolina inshore collection identified as distinct from the remaining dataset. Each vertical bar represents a single individual in the plot with colors indicating percent ancestry to each genetic group. Collections are geographically oriented from Texas on left to Virginia offshore on the right.*

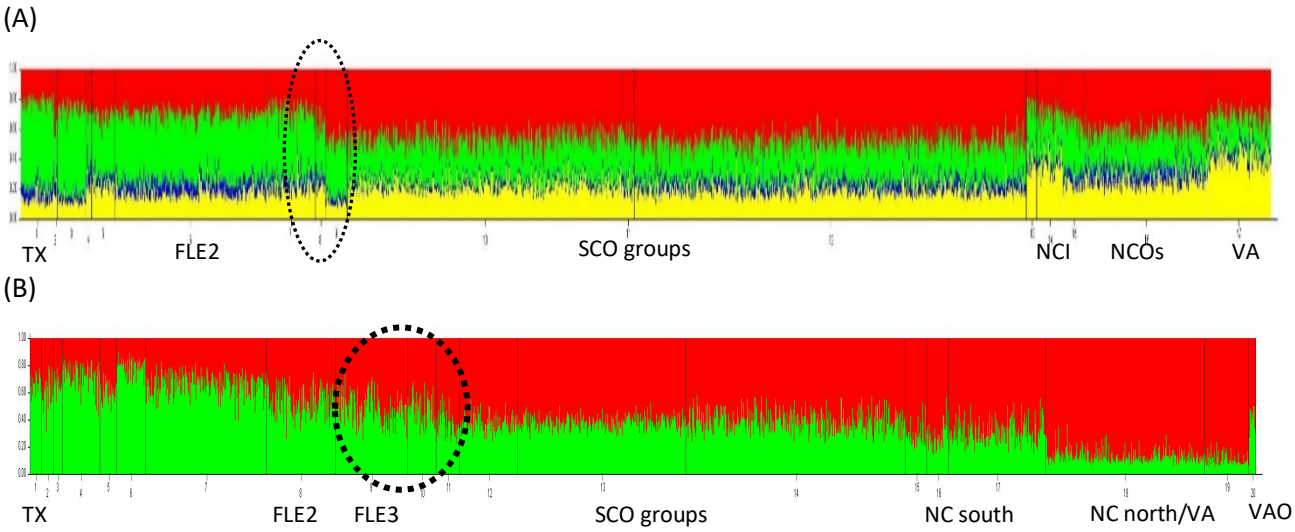


Figure 2. *Population ancestry plot for the (A) SEDAR58 dataset and (B) current contemporary dataset excluding the South Carolina inshore samples. For the SEDAR58 dataset, STRUCTURE results shown for K=4 with the Gulf of Mexico and Florida samples (through FLE2, Ft. Pierce) representing a distinct group (independent analysis confirmed K=1), North Carolina inshore and Virginia inshore samples representing a distinct group (independent analysis confirmed K=1), South Carolina and North Carolina offshore samples being homogenous (independent analysis confirmed K=1), and Cape Canaveral, FL through Savannah, GA representing a transition zone (dashed oval). For the current contemporary dataset, STRUCTURE results shown for K=2 with the Gulf of Mexico and Florida samples (through FLE2, Ft. Pierce) representing a distinct group (independent analysis confirmed K=1), North Carolina inshore and offshore samples south of Cape Hatteras representing a distinct group (independent analysis confirmed K=1), North Carolina offshore north of Cape Hatteras and Virginia inshore samples representing a distinct group (independent analysis confirmed K=1), and Cape Canaveral, FL through Savannah, GA representing a transition zone (dashed oval). Each vertical bar represents a single individual in the plot with colors indicating percent ancestry to each genetic group.*

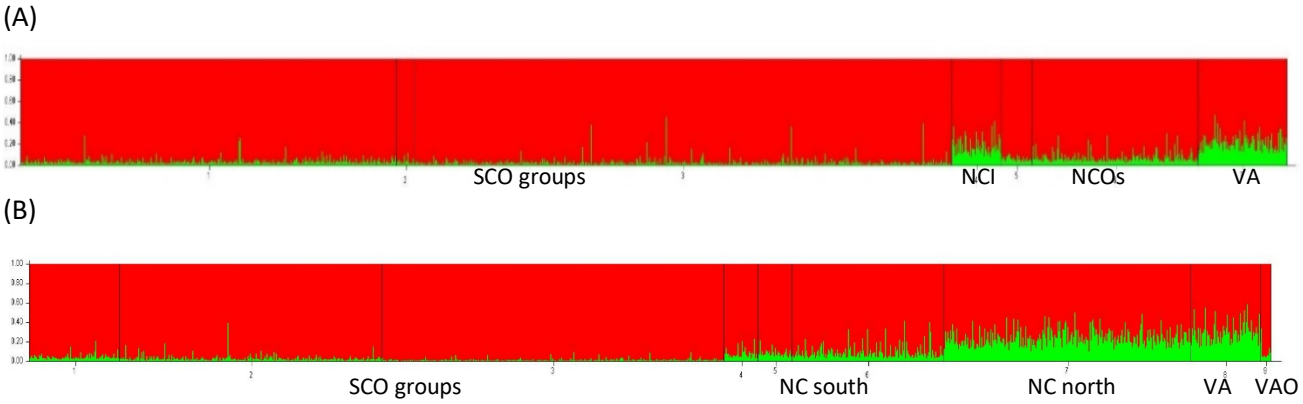


Figure 3. *Population ancestry plot for the (A) SEDAR58 dataset and (B) current dataset excluding South Carolina inshore, Gulf of Mexico and east coast of Florida/Georgia. Collections are geographically organized with South Carolina offshore on the left and Virginia offshore on the right. For the SEDAR58 dataset, STRUCTURE results shown for K=2 with the North Carolina inshore and Virginia inshore collections grouping together (independent analysis confirmed K=1) with the remaining South Carolina and North Carolina offshore collections being independently analyzed to confirm K=1 for an Atlantic offshore group. For the current contemporary dataset, STRUCTURE results shown for K=2 for the current dataset with the North Carolina inshore and offshore samples south of Cape Hatteras grouping together (independent analysis confirmed K=1) and the North Carolina offshore north of Cape Hatteras and Virginia inshore samples grouping together (independent analysis confirmed K=1). Each vertical bar represents a single individual in the plot with colors indicating percent ancestry to each genetic group.*

Iterative AMOVA analyses were employed to evaluate potential breaks in gene flow within the Cape Canaveral, FL through Savannah, GA area, including all potential locations from Atlantic offshore samples (SCOs, NCOs)/Savannah (GA) through Jupiter Beach (FLE1)/Hobe Sound (FLE2). Results suggested that the strongest significant break (R_{ST}=0.0023, p=0.02) among the groupings occurred between Jacksonville, FL/Brunswick, GA (FLGA) and Brunswick, GA (GA) locations, explaining 0.23% of the variation in the dataset. However, there was also a significant, although not as strong, break (R_{ST}=0.0017, p=0.04) between the Cape Canaveral, FL (FLE3) and Jacksonville, FL/Brunswick, GA (FLGA) locations, explaining 0.17% of the variation in the dataset. The last grouping scenario between the South Carolina offshore (SCOs) and Savannah (GA) samples explained 0.19% of the variation, but was not significant (R_{ST}=0.0019, p=0.07). Therefore, the AMOVA analyses also support the occurrence of a transition zone from Cape Canaveral, FL through Savannah, GA. While the location of the transition zone is consistent with SEDAR58 analyses, the 'center' (i.e., strongest significant break detected) has shifted northward in the current contemporary analyses – identified previously between the Cape Canaveral, FL (FLE3) and Jacksonville, FL/Brunswick, GA (FLGA) locations.

Iterative AMOVA analyses were also used to evaluate potential breaks in gene flow within the South Carolina Offshore to Virginia Inshore area (excluding the South Carolina Inshore and

Virginia Offshore samples). All potential groupings were significant, and the STRUCTURE, R_{ST}, and HWE supported genetic groupings of South Carolina offshore (SCOs), North Carolina inshore and offshore south of Cape Hatteras (NC1, NC2, NCO1), and North Carolina offshore north of Cape Hatteras and Virginia inshore (NCO2 and VA) explaining 0.22% of the variation in the dataset (R_{ST}=0.0022, p=0.04).

Guided by these analyses, final sample groupings included Gulf of Mexico, South Carolina Inshore, South Carolina Offshore, North Carolina Inshore and Offshore south of Cape Hatteras, and North Carolina Offshore north of Cape Hatteras and Virginia Inshore populations with a Cape Canaveral, FL through Savannah, GA transition zone (Table 3). Due to the lower sample sizes from some of the Gulf of Mexico and North Carolina locations, deviation from HWE was evaluated to verify no substructure was being masked within these regions. No loci were out of HWE within the North Carolina inshore and offshore south of Cape Hatteras group and only one locus was out of HWE (p<0.001) within the Gulf of Mexico group, supporting these groupings. Pairwise comparisons among the final groupings confirmed significant differences between all groups (p<0.00001) following Bonferroni corrections, except comparisons between the North Carolina Inshore and Offshore south of Cape Hatteras group with the South Carolina Offshore (p=0.03) and North Carolina Offshore north of Cape Hatteras and Virginia Inshore (p=0.71) groups. Comparisons were not conducted for the Cape Canaveral, FL through Savannah, GA transition zone. Significant genetic differentiation ranged from R_{ST}=0.031 between the South Carolina Inshore and the North Carolina Inshore and Offshore south of Cape Hatteras populations to R_{ST}=0.002 between the Gulf of Mexico and the South Carolina Offshore populations (Table 4). The levels of genetic differentiation detected translated into effective number of migrants (N_{em}) ranging from 1.1-167 individuals per year between these populations. Therefore, the results of our current analyses suggest the cobia stock boundary, recognizing that biologically it represents a mixing zone with limited reproductive exchange, continues to be detected within the range from Cape Canaveral, FL to northern Georgia and remains consistent with the current management stock boundary along the Atlantic coast.

Average allelic richness ranged from 7.6 to 10.7 alleles across the identified populations. All populations showed moderate levels of genetic diversity (H_o > 0.527, H_E > 0.536) and low levels of inbreeding (F_{IS} < 0.018; Table 5).

Table 4. *Ranked R_{ST} values from pairwise comparisons among final genetic sample groups with effective number of migrants (N_{em}). Per year calculations are based on a 5-7 year generation time. Significant R_{ST} values are bolded.*

Pairwise Comparison	R_{ST}	N_{em} per generation	N_{em} per year
SC Inshore - NC South of Cape Hatteras	**0.031**	7.8	1.1-1.6
SC Inshore - GOM	**0.029**	8.4	1.2-1.7
SC Inshore - NC and VA North of Cape Hatteras	**0.026**	9.4	1.3-1.9
SC Inshore - SC Offshore	**0.017**	14.5	2.1-2.9
GOM - NC and VA North of Cape Hatteras	**0.007**	35.5	5.1-7.1
GOM - NC South of Cape Hatteras	**0.006**	41.4	5.9-8.3
SC Offshore - NC South of Cape Hatteras	0.003	83.1	11.9-16.6
SC Offshore - NC and VA North of Cape Hatteras	**0.003**	83.1	11.9-16.6
GOM - SC Offshore	**0.002**	125	17.8-25.0
NC South of Cape Hatteras - NC and VA North of Cape Hatteras	0.0003	833	119-167

Table 5. *Genetic diversity statistics, averaged across nineteen loci, for each final genetic sample group. N = sample size, R = average allelic richness, H_O = average observed heterozygosity, H_E = average expected heterozygosity, F_{IS} = average inbreeding coefficients (Weir & Cockerham 1984).*

Genetic Group	GOM	SC Offshore	NC South of Cape Hatteras	NC North of Cape Hatteras and VA	SC Inshore
Sample Size (N)	475	693	219	316	210
Allelic Richness (R)	10.7	10.3	10.0	10.2	7.6
Obs. Heterozygosity (H_O)	0.56	0.56	0.56	0.56	0.53
Exp. Heterozygosity (H_E)	0.56	0.56	0.57	0.57	0.54
Inbreeding (F_{IS})	0.005	0.001	0.012	0.018	0.017

In conclusion, the current project has provided important data and analyses for the continued management of cobia throughout the Gulf of Mexico and along the U.S. Atlantic coast. The updated genetic analyses with the full 19 marker microsatellite panel resulted in large scale gene flow patterns such as identification of a unique South Carolina inshore group and GOM-Atlantic distinction with a transition zone that are consistent with prior analyses. However, multiple indicators are suggesting potential changes in our understanding of gene flow patterns along the Atlantic coast in the contemporary, time-restricted dataset as compared to prior analyses. The increased sample sizes and collection locations in the North Carolina and Virginia

areas have provided a new perspective on gene flow patterns in that area that should continue to be evaluated for verification as new samples become available. Increasing reports of cobia farther north than previously documented (via tagging efforts and landings) along with the observed northward shift in the peak genetic difference location within the transition zone and increased similarity of the South Carolina offshore group with those in the transition zone are perhaps indications of a distributional shift in cobia similar to that observed for multiple marine species in response to climatic changes, which would not be surprising for a species like cobia whose life history is highly temperature-correlated. Our interpretation capabilities of the genetic results would likely be improved following a rigorous spatial analysis of cobia occurrence data in a temporal context to understand the presence and degree of putative distributional changes for this species.

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