North Carolina Division of Marine Fisheries
2016 Striped Bass Genotyping Report

2016 North Carolina Striped Bass Samples

Striped bass (*Morone saxatilis*) tissue samples were obtained from NC Division of Marine Fisheries staff (Todd Mathes/Jason Rock/Charlton Godwin).

384 Samples: SCDNR Genetic Numbers Msa-24014-24397

2	Bay River
149	Neuse River
192	Pamlico River
41	Pungo River

Genetic Microsatellite Markers

DNA was isolated using spin columns (Wizard SV Genomic DNA Purification System, Promega Corporation; Madison, WI) following a proteinase K digestion (digestion solution: Nuclei lysis solution, 0.5 M EDTA, 20 mg/ml proteinase K, and RNase A solution), with a final elution volume of 150 µL dH_20. After isolation, multiplexed polymerase chain reactions (PCR) for 12 microsatellite loci (Table 1) were performed in 11 µL reactions on an iCycler® (Bio-Rad Laboratories; Hercules, CA) thermal cycler platform. Each reaction included 0.2 mM of each dNTP, 1x HotMaster buffer with 2.5mM Mg^{2+}, 0.025 units HotMaster *Taq* (5 Prime, Inc.; Gaithersburg, MD), BSA (final [0.035 mg/ml]) and either 1.0 mM Mg^{2+} (total rxn [Mg^{2+}]: 3.5 mM for panels 1 and 2) or 1.5 mM Mg^{2+} (total rxn [Mg^{2+}]: 4.0 mM for panel 3). All multiplexed panels successfully amplified using the following 60°C touchdown protocol: initial denaturation at 94˚C for 3 minutes, followed by 10 repetitions of a second cycle: 94˚C for 30 seconds, 60˚C for 30 seconds, and 62.2˚C for 30 seconds. After the first repetition of the second cycle, the annealing temperature was decreased by 0.5˚C with each subsequent repetition. The third cycle, 94˚C for 30 seconds, 50˚C for 30 seconds, and 62.2˚C for 30 seconds, was repeated 25 times with a final extension of 62.2˚C for 60 minutes. Amplified fragments were subsequently separated on a CEQ™ 8000 (Beckman Coulter, Inc.; Fullerton, CA) automated sequencer and scored using the CEQ™ 8000 Fragment Analysis Software (Beckman Coulter, Inc.; Fullerton, CA). Two independent readers scored the data and discrepancies were reconciled in conference or samples were reanalyzed if there was no consensus.

Table 1. Loci sets for multiplexed PCR panels. Fluorescent dye, original source, total forward primer concentration (µmol) for each multiplexed panel, and individual forward primer concentrations (nmol) are provided. The forward primer of all sets was fluorescently labeled with Beckman-Coulter dyes as indicated. Total and individual unlabeled reverse primer concentrations were the same as reported for the forward primers.

Multiplexed Panel	Locus	WellRED Dye	Source	Total [forward primer] (µmol)	Individual [forward primer] (nmol)
1	MSM1144	D4	Couch et al. 2006	0.6	37.50
	MSM1095	D2	Couch et al. 2006		337.50
	MSM1096	D3	Couch et al. 2006		168.75
	MSM1243	D4	Couch et al. 2006		56.25
2	MSM1094	D4	Couch et al. 2006	0.3	18.80
	MSM1526	D2	Rexroad et al. 2006		131.20
	MSM1208	D3	Couch et al. 2006		75.00
	MSM1067	D4	Couch et al. 2006		75.00
3	MSM1168	D4	Couch et al. 2006	0.6	50.00
	MSM1139	D2	Couch et al. 2006		250.00
	MSM1592	D3	Rexroad et al. 2006		200.00
	MSM1357	D4	Rexroad et al. 2006		100.00

Parentage Analyses

We utilized a maximum likelihood parentage approach as implemented in CERVUS to provide a statistical evaluation of parentage taking into account mutation rates and population allele frequencies. We have previously estimated the power of the locus suite to correctly identify hatchery fish as well as its ability to identify individuals based on NC broodstock designs. The average parent-pair and identity non-exclusion probabilities for the locus suite is 1.0×10^{-8} and 7.0×10^{-14}, respectively, for North Carolina striped bass. All of these estimations are similar to the diagnostic power estimated for the locus suite based on South Carolina samples (2.0×10^{-7} and 1.8×10^{-12}) suggesting very low probabilities of incorrectly identifying hatchery fish or individuals throughout the South Carolina-North Carolina range of striped bass.

We also previously conducted parentage simulations (n=5) for known sex parentage analysis in CERVUS, using allele frequencies generated for NC striped bass. All simulations were conducted with 10,000 offspring, 100 candidate parents (with all parents sampled), 100% genotyping of broodstock, 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.

Striped bass field samples were compared to all GA, SC, and NC broodstock genotypes on record at SCDNR through the 2015 production year. All parental assignments were designated at the strict confidence level (99.9%), as no additional assignments occurred with the relaxed criteria. Any fish that did not match parents at this strict confidence level was considered 'wild' and indicative of either a previous year class that was not tracked genetically or of non-hatchery origin.

Field Sample Genotyping

Number of samples scored at number of loci:

0 loci: 1*
11 loci: 1
12 loci: 382

*One sample was contaminated and is removed from all further calculations (Msa-24074, vial #160061).

There were 2 individuals that exhibited many alleles outside of established range for NC striped bass at several loci and three strongly amplified alleles (235 at locus MSM1243, 189 at locus MSM1357, and 159 at locus MSM1067) that have been previously identified to be indicative of striped bass-white bass hybridization. For these fish, we performed a maternity parentage analysis to determine if they were the product of a hatchery cross between a female striped bass and a male white bass or if they were of non-stocked/wild origin.

Summary of Duplicate Genotypes:

No duplicate genotypes or recaptures were detected within this dataset based on the maximum likelihood Identity Analysis in CERVUS.

Parentage Summary:

Table 2. Overall contribution summary; year class contribution is calculated as proportion of total number of cultured fish.

Designation	Number	Contribution (%)
Cultured	322	84.5
2010	30	9.3
2011	89	27.6
2012	108	33.6
2013	68	21.1
2014	27	8.4
'Wild'	59	15.5
Subtotal	381	100.0
Hybrid – 'Wild'	2	---
Total	383	---

Table 3. Bay River contribution summary; year class contribution is calculated as proportion of total number of cultured fish.

Designation	Number	Contribution (%)
Cultured	1	50.0
2012	1	100
'Wild'	1	50.0
Total	2	100.0

Table 4. Neuse River contribution summary; year class contribution is calculated as proportion of total number of cultured fish. 1 contaminated sample (MSA-24074, vial #160061) was excluded from analyses.

Designation	Number	Contribution (%)
Cultured	141	95.3
2010	13	9.2
2011	21	14.9
2012	29	20.6
2013	57	40.4
2014	21	14.9
'Wild'	7	4.7
Total	148	100.0

Table 5. Pamlico River contribution summary; year class contribution is calculated as proportion of total number of cultured fish.

Designation	Number	Contribution (%)
Cultured	164	86.3
2010	17	10.4
2011	65	39.6
2012	70	42.6
2013	6	3.7
2014	6	3.7
'Wild'	26	13.7
Subtotal	190	100.0
Hybrid-'Wild'	2	---
Total	192	---

Table 6. Pungo River contribution summary; year class contribution is calculated as proportion of total number of cultured fish.

Designation	Number	Contribution (%)
Cultured	16	39.0
2011	3	18.8
2012	8	50.0
2013	5	31.2
Wild	25	61.0
Total	41	100.0

Table 7. Bay River maternal contribution summary. This family was stocked as Phase II in the Tar River.

Mother	Number
NCF1206	1
Total	1

Table 8. Neuse River maternal contribution summary. All families were stocked as Phase I or Phase II in the Tar River or Neuse River (* indicates stocking also occurred in reservoirs).

Mother	Number
NCF1003*	7
NCF1010*	4
NCF1013*	2
NCF1106*	6
NCF1111*	6
NCF1115*	9
NCF1201	6
NCF1202	13
NCF1219	10
NCF1301	8
NCF1302	3
NCF1303	4
NCF1304	3
NCF1309	39
NCF1417	14
NCF1418	7
Total	141

Table 9. Pamlico River maternal contribution summary. All families were stocked as Phase I or Phase II in the Tar River or Neuse River (* indicated stocking also occurred in reservoirs).

Mother	Number
NCF1003*	9
NCF1010*	7
NCF1013*	1
NCF1106*	18
NCF1111*	26
NCF1115*	21
NCF1202	3
NCF1205	2
NCF1206	65
NCF1303	4
NCF1304	2
NCF1415	5
NCF1417	1
Total	164

Table 10. Pungo River maternal contribution summary. All families were stocked as Phase I or Phase II in the Tar River or Phase II in the Neuse River (* indicates stocking also occurred in reservoirs).

Mother	Number
NCF1111*	1
NCF1115*	2
NCF1206	7
NCF1219	1
NCF1303	3
NCF1304	2
Total	16

'Wild' Fish Evaluation:

As some wild fish could be older, non-genetically trackable year classes (prior to 2010), we calculated the minimum, mean, and maximum total lengths (mm) of cultured and wild striped bass in this data set (Table 11) and plotted individual total lengths by year class and for wild striped bass (Figure 1). All length metrics were similar between the cultured and wild striped bass; therefore in combination with observed asymptotic length with age, we are not able to determine if the 'wild' fish are from older, genetically non-trackable year classes or represent true wild recruitment based on length alone. Likely, some of the smallest 'wild' fish represent true wild recruitment, however it is difficult to identify what size threshold should be used for that determination.

Table 11. Minimum, mean, and maximum total lengths (mm) of cultured and wild striped bass.

	Total length (mm)		
Designation	Minimum	Mean	Maximum
Cultured	318	573	734
Wild	267	576	722

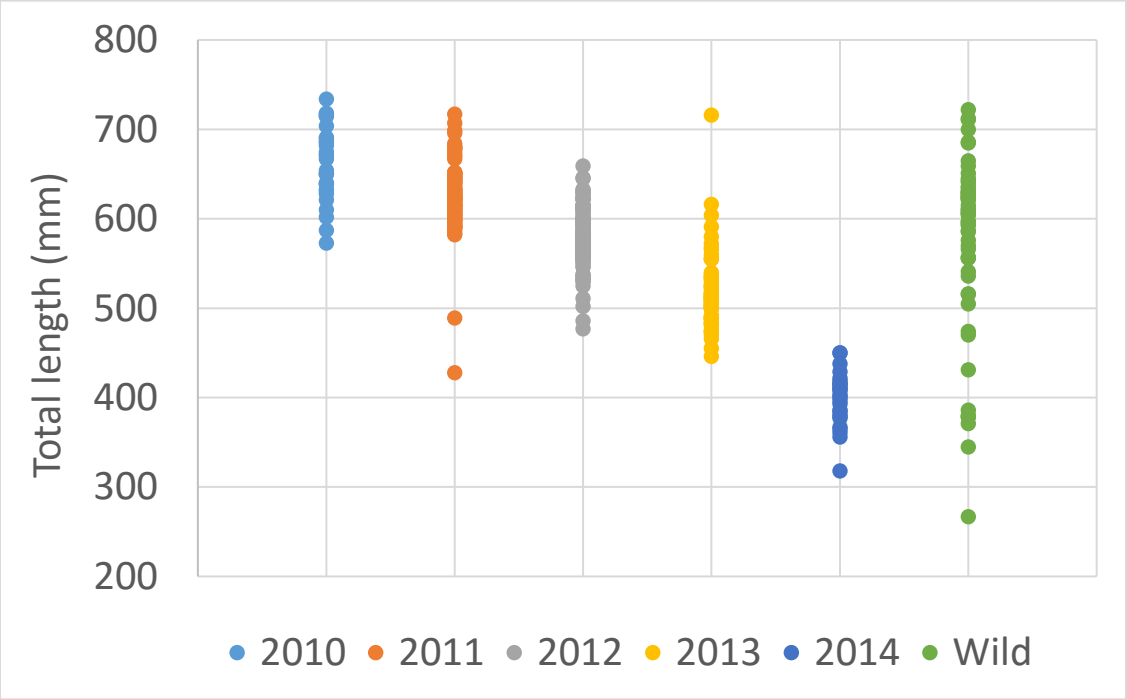

Figure 1. Total length (mm) by cultured year class and wild designated fish.

We also used a genetic analysis to attempt to understand the origin of the 'wild' fish. The Bayesian clustering program *Structure* 2.3.4 was used to infer the number of populations (*K*) present in the data. Run parameters were set at 100,000 burn-in repetitions followed by 50,000 Markov chain Monte-Carlo repetitions, without location information included as priors, with *K* varied 1–5 (based on the number of DPS), and five independent runs per *K*. The primary goal of this analysis was to determine if the 'wild' fish in the sample set would cluster as a unique genetic group (i.e., perhaps with Roanoke ancestry). Therefore, we also included previously genotyped striped bass from the Roanoke River in this analysis.

Results indicate the most likely number of populations present in the data were two. There was little admixture between the two populations (Figure 2), but closer inspection revealed that the distinct group colored in light grey were all offspring from a single mother (NCF1206). Roe NCF1206 was responsible for 22.7% of all cultured fish in the dataset. *Structure* is known to struggle differentiating between population level gene flow patterns and patterns based on family structure, such as an overabundance of siblings from a single family. Therefore, the result of *K*=2 is likely due to a large sample from this single family and further indicates that the wild fish do not represent a unique genetic group in this dataset. Interpretation of these results in the context of gene flow patterns (i.e., stock definition) is not

appropriate due to the composition of the data set, including the high level of siblings (which if removed would result in a sample size insufficient for robust analyses).

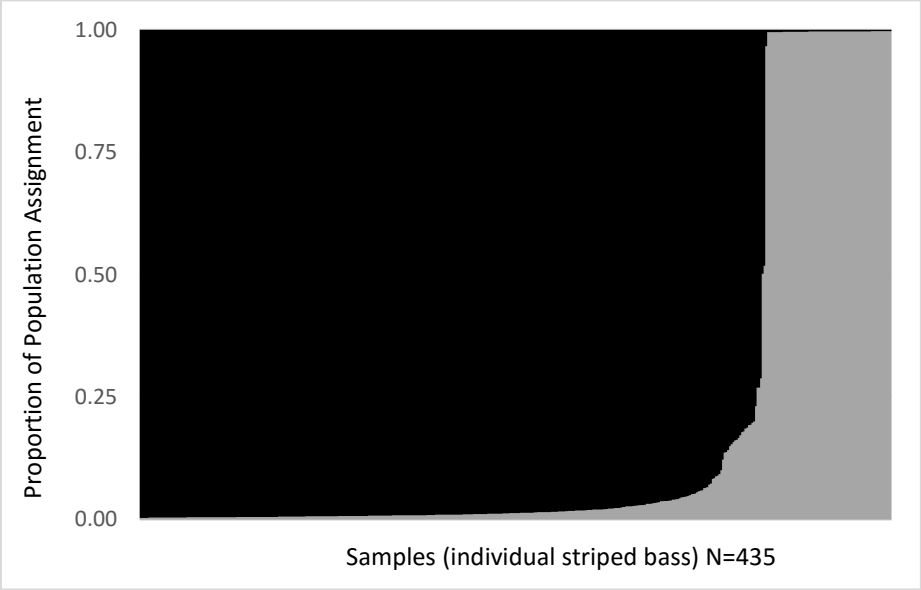

Figure 2. Results from *Structure* for the analysis of striped bass, with the addition of samples from the Roanoke River, for *K*=2 and sorted by Q value. Each individual bar represents the ancestry of a single fish, with the colors corresponding to the proportion of population assignments. The distinct group colored in light grey were all offspring from a single mother (NCF1206).

Overall Project Summary

Overall, 84.5% of the striped bass collected in the Bay, Neuse, Pamlico, and Pungo Rivers were stocked fish of a hatchery origin. These fish were produced by 19 different mothers across five year classes, beginning with the 2010 year class which was the first genetically trackable year class. The 'wild' striped bass (15.5% of catch) were not restricted to the largest fish collected. Unfortunately, as a result of both the similar size distributions of cultured and 'wild' fish and the lack of a unique genetic signature of the 'wild' fish, we are not able to determine if the 'wild' fish are from older, genetically non-trackable year classes or represent true wild recruitment.

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