

American Shad Genetic Analysis, 2021



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Abstract. North Carolina Wildlife Resources Commission (NCWRC) staff provided 424 American Shad *Alosa sapidissima* samples in 2021 for parentage-based tagging (PBT) analysis. Fin clips were from Roanoke River (n = 160) and Neuse River (n = 264) spawning adults. Hatchery). Adult hatchery contribution was 43.8% for Roanoke River and 4.3% for Neuse River. The effective population estimate for 2021 Roanoke River American Shad was 1,337 individuals (95% confidence interval = 596–infinity) and indicated that genetic diversity has improved over the past two years as hatchery contribution has declined. A reliable effective population estimate could not be obtained for the Neuse River, though we believe this is indicative of a high effective population size in this river. The 2021 observed hatchery contributions represent declines in the Roanoke and Neuse rivers compared to previous years. We recommend comparisons of percent hatchery contribution and effective population estimates to stocking rates and locations, catch-per-unit efforts (CPUE), and other total population size surveys to determine appropriate stocking levels that will manage total population size and genetic diversity.

American Shad *Alosa sapidissima* historically constituted a vital component of Atlantic coast commercial and recreational fisheries. Population numbers drastically declined beginning in the 1970s due to multiple factors including overfishing, impoundments, and habitat degradation. The North Carolina Wildlife Resources Commission (NCWRC) began stocking American Shad into the Roanoke River in 1998 to combat dwindling population numbers. Annual stockings through 2018 resulted in approximately 78.2 million American Shad fry stocked into the Roanoke River basin (White and McCargo 2019). More than half (45.6 million) were stocked at Weldon, NC, which is downstream of the first migration barrier, and 32.1 million fry were stocked upstream of Roanoke Rapids Dam to evaluate downstream passage through a chain of three reservoirs. Throughout the restoration program history, American Shad broodfish have come from multiple sources including the Meherrin, Cape Fear, Tar, Neuse, and Roanoke rivers; however, from 2011 to 2018, only broodfish collected from the Roanoke River were used for production. American Shad fry were cultured at Watha State Fish Hatchery and Edenton National Fish Hatchery. Stocking was halted after 2018 due to high hatchery contribution and genetic diversity concerns (Evans and McGrady 2019; White and McCargo 2019).

In 2012, the NCWRC began a similar American Shad restoration program in the Neuse River to supplement the population. Between 2012 and 2018, approximately 5.5 million American Shad fry were stocked into the Neuse River near Goldsboro, NC (Ricks and Buckley 2019). Broodfish were collected from the Neuse River each year, and production occurred at Edenton National Fish Hatchery. Although hatchery contribution was not as high as the Roanoke River, the Neuse River American Shad restoration stocking program was also stopped after 2018 when stocking ended in the Roanoke River.

From 1998 through 2009, NCWRC used oxytetracycline (OTC) to chemically mark otoliths of American Shad fry before stocking to identify stocked American Shad as out-migrating young-of-the-year (juveniles) and returning adults. Marking with OTC proved unreliable, and in 2010, parentage-based tagging (PBT) using genetic microsatellite markers (Julian and Bartron 2007) replaced OTC for evaluating hatchery contribution. Although stocking of American Shad fry has not occurred since 2018, it is imperative to continue evaluation of the hatchery program and genetic diversity. Effects from the cessation of stocking on population size or genetic diversity will start to be seen in the spring of 2022, when the first class of three-year-old American Shad containing no hatchery stocked fish will return to spawn. The objective of this study was to use PBT analysis to evaluate hatchery contribution of returning adults in the Roanoke and Neuse rivers in 2021. Additionally, we evaluated American Shad genetic health by calculating effective population size estimates for Roanoke River and Neuse River samples. Finally, we provide management recommendations based on our findings.

Methods

Samples. North Carolina Wildlife Resources Commission (NCWRC) staff collected 160 returning adult American Shad fin clips from the Roanoke River and 264 clips from the Neuse River (total = 424) in 2021 for parentage-based tagging (PBT) and population genetic analysis. All samples have been listed in the NCMNS American Shad database and labeled with a unique code to identify individual samples.

DNA Extraction and PCR. As described in previous reports, DNA was extracted using the Macherey-Nagel NucleoSpin 96 Tissue kit and processed on an Eppendorf Robotic liquid handler (epMotion 5075). Each sample was genotyped for twelve (12) loci (Julian and Bartron 2007). Three multiplexed PCR reactions per DNA extraction were performed as 10 μ L reactions and run on an Eppendorf Mastercycler Pro (Eppendorf NA: Hauppauge, NY) thermal cycler. PCR reactions consisted of 5 μ L of a 1:10 mixture of Takara ExTaq Premix and Promega GoTaq MasterMix with 1 μ L of genomic template per reaction and primer as noted in previous years. All multiplexed panels were amplified using the following thermal profile: an initial denature of 95°C for 2 minutes followed by 35 cycles of 95°C for 30 seconds, 58°C for 30 seconds and 64°C for 60 seconds with a final elongation step at 72°C for 10 minutes. Amplified products were run on an ABI 3500 Genetic Analyzer using 1 μ L of PCR product, 9.55 μ L formamide (Mclab Super-Di Formamide), and 0.45 μ L LIZ ladder (GeneScan 600 LIZ Dye Size Standard, ThermoFisher). Each read was independently assessed and scored by two researchers.

Parentage-based Tagging Analysis. Statistical analysis was conducted with CERVUS 3.0 (Kalinowski et al. 2007). This program employs a maximum likelihood approach of parentage considering evolutionary fluctuations in the population (observed allele frequencies, mutation rates, etc.). Parentage analyses were run without reference to sex determination, spawning tank, or river of origin. Simulations were run in triplicate and assumed 99% genotyping of broodstock, low mistyping error rate (0.001), and low error rate (0.0001). Further criteria were determined by sample parameters and are detailed in Table 1. Each year was analyzed separately to minimize deviations from Hardy Weinberg and consequent false positives. All matches were analyzed to ensure that years, tanks, and sex corresponded appropriately.

Population Genetics. Effective population estimates (N_e) were calculated using N_e estimator (Do et al. 2014) employing the linkage disequilibrium (LD) method (Waples and Do 2008, Jones et al. 2016) and 0.01 as the lowest allele frequency. All available adults were used in our analysis. Confidence intervals are reported using the jackknife method (Efron 1982).

Results

Samples. An identity analysis of samples collected in 2021 revealed no duplicate fin clips or matches to previous years' collections. All samples described were combined into one file for allele frequency analysis using CERVUS 3.0 and evaluated for Hardy Weinberg equilibrium (HWE), number of alleles, allelic diversity, and null alleles (Table 2). No markers showed statistical evidence of null alleles (greater than 0.05), nor did any deviate from Hardy-Weinberg equilibrium (HWE). Overall, markers were highly polymorphic and demonstrated high heterozygosity (average of 20.75 alleles/locus, 0.852 mean observed heterozygosity). The combined identity non-exclusion probability is 4.053×10^{-19} and the combined parent pair non-exclusion probability is 5.402×10^{-13} .

Parentage-Based Tagging Analysis. In total, we identified 70 of 160 Roanoke adults (43.8%, Figure 1) and 14 of 264 Neuse adults (5.3%, Figure 2) as hatchery-derived American Shad in 2021. Of the positively identified Roanoke adults, 47 (67.1%) came from Weldon stockings, three (4.3%) came from stockings at Lake Gaston, and 20 (28.6%) came from stockings at Roanoke Rapids Lake (Table 3). The majority of hatchery identified fish stocked before 2016

originated from Weldon, but a shift towards Roanoke Rapids Lake stockings occurred beginning with the 2016 cohort. Hatchery contribution for the Neuse River decreased in 2021 to 5.3%.

Several same-sex parent matches were identified in the 2021 samples. Individual 2021-84R (ID = year collected-sample number) matched to 2018-E2036 and 2018-E2058, both noted as females. Because 100% of alleles were accounted for in this match and no other pairings involving either of these parents has been identified to date, we have retained this sample in our count of hatchery-bred individuals. 2021-13N and 2021-94N also matched to two males and both included broodfish 2014-1073. This fish has not previously been documented as contributing to spawning, and as both instances involve another male fish, we have reclassified 2014-1073 as female. Finally, 2021-2001 matched 100% to females 2014-1067 and 2014-1098. Broodfish 2014-1067 was previously identified as a female parent to 2019-60, but we have no other documented contribution from 2014-1098. Given this, we believe 2014-1098 to be a male and have noted the change in the database.

Population Genetics. The effective population estimate for 2021 Roanoke River American Shad was 1,337, a sizeable increase from previous years' estimates (Table 4). However, the 95% confidence interval ranges from 596 to infinity, signifying a lack of power to give accurate effective population estimates for this year class. Effective population estimates for American Shad in the Neuse River continue to be uninformative. The 2021 samples returned an "infinite" estimate, indicating that all variation could be explained by sampling error. In the Neuse River, increasing sample size above 400 does not give reliable estimates of the population size.

Discussion

Hatchery contributions continued to decline in adult American Shad populations of both the Roanoke and Neuse rivers during 2021 as predicted in the previous report (Evans and McCargo 2021). These declines are most likely due to decreased stocking efforts and subsequent lower percent hatchery contributions per year since 2015. Stocking location could also play a role in decreased hatchery contribution as stocking efforts shifted upstream from Weldon to Roanoke Rapids Lake beginning in 2016 (White and McCargo 2019). It is possible that survival was lower for fry stocked upstream of Roanoke Rapids Dam than it would have been if similar numbers of fry were stocked at Weldon. Additionally, the age-4 cohort typically contributes heavily to spawning adults but was present at low levels in the 2021 returning adult population. An examination of hatchery contribution reveals that juveniles from the 2017 spawning class did not fare well in the wild. Juvenile hatchery contribution in 2017 was approximately 10%, yet only 2.96% of the Roanoke returning adults in 2021 were derived from the 2017 spawning cohort. Comparatively, juvenile hatchery contribution in 2018 was 5%, lower than that observed in 2017, yet adults spawned in 2018 comprised 18% of the returning adults in 2021, vastly outnumbering the 2017 cohort. Fry were first stocked in Roanoke Rapids Lake during 2017, and hatchery staff observed heavy predation by White Perch *Morone americana* during stocking at the Thelma BAA (Jeff Evans, NCWRC, personal communication). Also, only 300,000 fry were stocked at the Weldon BAA in 2017 (White and McCargo 2019). Thus, the heavy predation and low number stocked at Weldon may have caused the low contribution of the 2017 cohort in 2021. Further evidence of an impact to 2017 juveniles can be seen in the Neuse River hatchery percentages, where no contribution from the 2017 cohort has

ever been detected. It is possible that water quality conditions reduced survival of stocked American Shad fry in 2017, but further study is needed to elucidate the problem.

Effective population estimates for both rivers had large confidence intervals for 2021 samples. In the Roanoke River, we note that sample size for this year is small compared to most other years. A similar sample size occurred in 2010 with 148 samples, and that year also had no limit for the N_e upper bound. We posit that a sample size of at least 200–240 may be needed to obtain precise effective population estimates for American Shad in the Roanoke River. Nevertheless, the fact that the N_e lower bound is higher than previous years' lower bound is encouraging. It is also interesting to note that the increased N_e co-occurs with decreased percent hatchery contribution for the spawning American Shad.

Continued genetic diversity analysis of this population while stocking effort declines is needed to verify whether these two factors are related or if this year's observation was an anomaly. Catch-per-unit effort should also be analyzed along with genetic diversity and stocking effort. While genetic diversity may increase as stocking efforts are reduced, the CPUE may decline.

We were unable to obtain N_e for American Shad collected from the Neuse River. Analysis only returned an estimate of infinity. In general, N_e for the Neuse River population has never been reliable, with large confidence intervals even in years when a specific N_e value was obtained. Waples and Do (2010) noted that variance will increase and precision will decrease for populations with large N_e , and we believe that to be the case for American Shad in the Neuse River.

Management Recommendations

1. Continue PBT analysis for hatchery contribution and genetic population analysis. Since stocking was suspended in 2018 and American Shad do not significantly contribute to the spawning population until age 3, 2022 will be the first time to analyze genetic diversity and percent hatchery contribution in a year class with no hatchery production.
2. We recommend continuation of PBT analysis through 2025, which would allow calculations of percent hatchery contributions and concurrent genetic diversity for all wild-spawned fish up to age 6. Assuming the stocking hiatus continues, smaller sample sizes could be considered for subsequent years to measure the effect of this hiatus on genetic diversity.
3. We recommend staff compare percent hatchery contributions and effective population estimates with stocking rates and locations as well as population indices such as catch-per-unit effort to identify levels of stocking that will maintain total population size while guarding against decreasing genetic diversity.

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TABLE 1. Simulation conditions for parentage analysis. All simulations were run with 0.99 loci genotyped, 0.001 mistyping error rate, 0.001 error rate, and 99% or 100% strict confidence levels. Increasing asterisks indicate increasing probability of deviation from Hardy-Weinberg equilibrium (HWE). NS = Not Significant.

Broodstock Cohort	# Simulated Offspring	# Simulated Parents	Critical Delta	HWE
2010	10,000	100	0	NS
2011	100,000	150	4	<i>AsaC010**</i> <i>AsaD312*</i>
2012	10,000	100	0	NS
2013	10,000	100	1	NS
2014	100,000	150	3	NS
2015	10,000	100	0	NS
2016	100,000	100	1	NS
2017	100,000	150	11	NS
2018	10,000	100	0	<i>AsaD030*</i>
2019	10,000	100	0	NS

TABLE 2. Allele frequency data for 2021 American Shad samples. K= number of alleles, H_{Obs} = observed heterozygosity, H_{Exp} = expected heterozygosity, PIC = Polymorphic Information Content, HWE= Hardy-Weinberg equilibrium, F = probability of null alleles, NS = not significant.

Locus	K	H_{Obs}	H_{Exp}	PIC	HWE	F
AsaC010	19	0.882	0.881	0.868	NS	-0.0028
AsaD021	14	0.8396	0.8598	0.8445	NS	0.0118
AsaD030	28	0.9151	0.928	0.9222	NS	0.0065
AsaD031	15	0.8561	0.8492	0.8314	NS	-0.0043
AsaB020	13	0.7972	0.818	0.7978	NS	0.0131
AsaC059	23	0.7995	0.8394	0.8244	NS	0.0251
AsaD055	15	0.7665	0.7699	0.7479	NS	0.0039
AsaD312	19	0.8467	0.8678	0.8537	NS	0.0126
AsaC051	20	0.7995	0.8009	0.7786	NS	-0.0003
AsaC249	34	0.9269	0.9213	0.9154	NS	-0.0036
AsaC334	28	0.9080	0.8822	0.8713	NS	-0.0160
AsaD042	21	0.8844	0.9127	0.9050	NS	0.0155

TABLE 3. Positive identifications for hatchery contribution by stocking site for samples collected from the Roanoke River in 2021.

Broodfish Cohort	2021 descendants	Weldon	Lake Gaston	Roanoke Rapids Lake
2013	1	1	0	0
2014	14	14	0	0
2015	30	27	3	0
2016	10	4	0	6
2017	2	0	0	2
2018	13	1	0	12

TABLE 4. Effective population estimates for Roanoke and Neuse Rivers, 2010–2021. N = sample size, Ne = effective population estimate, and CI = 95% confidence interval.

Year	Roanoke N	Roanoke Ne	Roanoke CI	Neuse N	Neuse Ne	Neuse CI
2010	148	1232	498/Infinite			
2011	382	913	640/1511			
2012	288	570	416/868	96	1970	531/Infinite
2013	524	595	462/806	113	4755	746/Infinite
2014	708	496	408/618	132	3071	657/Infinite
2015	541	514	400/693	285	7222	1684/Infinite
2016	522	659	508/904	410	9649	2295/Infinite
2017	814	492	417/587	348	12815	2479/Infinite
2018	582	307	255/375	388	3899	1640/Infinite
2019	240	386	294/543	345	18077	2382/Infinite
2020				135	Infinite	
2021	160	1337	596/Infinite	264	Infinite	

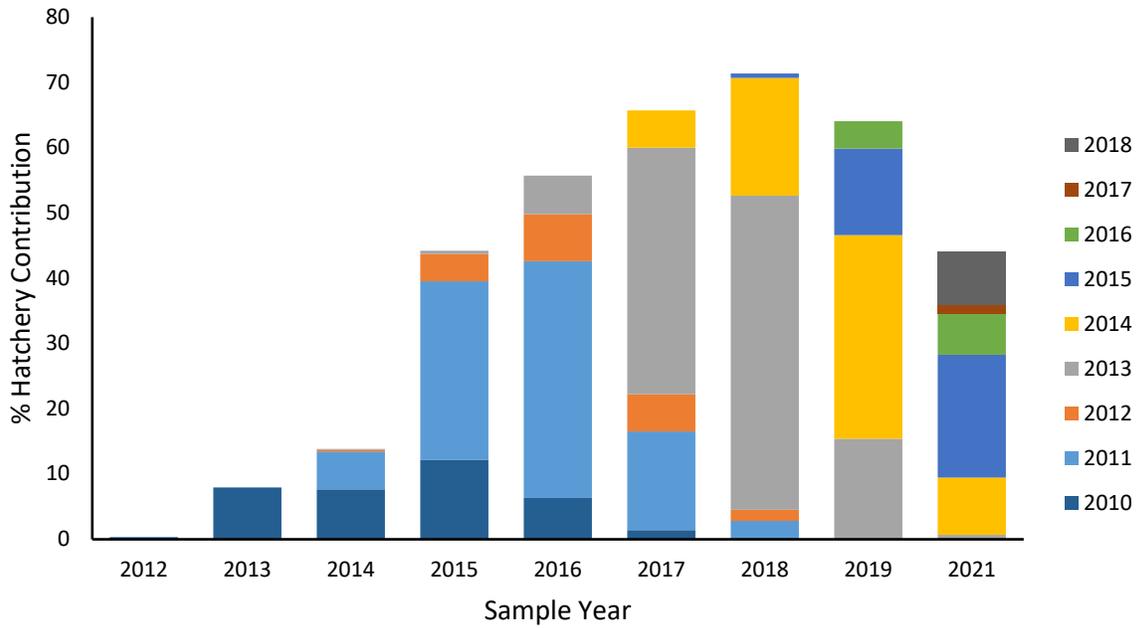


FIGURE 1. Roanoke River adult percent hatchery contribution by year. Stacked bars represent broodstock cohorts. Note y-axis ranges from 0% to 80% hatchery contribution.

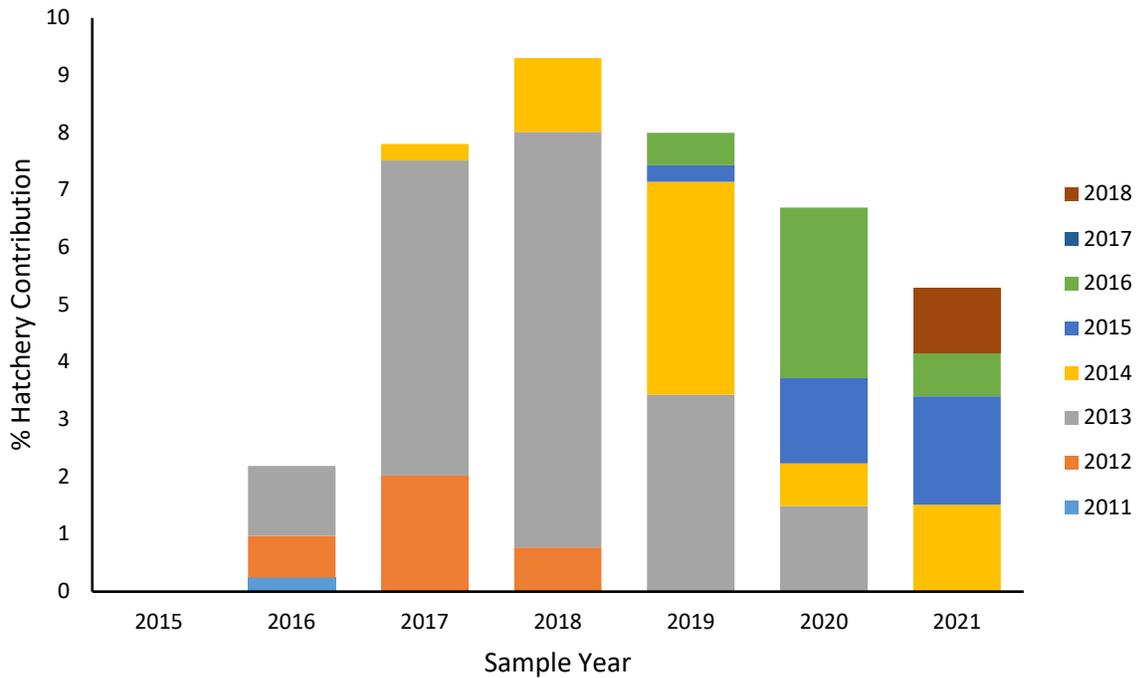


FIGURE 2. Neuse River adult percent hatchery contribution by year. Stacked bars represent broodstock cohorts. Note y-axis ranges from 0% to 10% hatchery contribution.