American Shad Genetic Analysis, 2022

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Abstract. North Carolina Wildlife Resources Commission (NCWRC) staff provided 479 American Shad *Alosa sapidissima* samples in 2022 for parentage-based tagging (PBT) analysis. Fin clips were from the Roanoke River (n = 185) and Neuse River (n = 294). Hatchery contribution was 47.0% for Roanoke River adults and 2.4% for Neuse River adults. The effective population estimate (N_e) for 2022 Roanoke River American Shad was 637 (95% confidence interval = 416–1259) and for Neuse River American Shad was 10,558 (95% confidence interval = 1525–infinite). Despite 2022 representing the first year lacking an age-3 hatchery spawned cohort since stocking was halted, we did not observe a decline in percent hatchery contribution in the Roanoke River, likely due to strong recruitment of the 2018 hatchery cohort. Analysis of hatchery contribution and N_e show a negative correlation, with confidence intervals increasing as N_e increases. We recommend comparison of percent hatchery contribution and effective population estimates to relative abundance to determine appropriate stocking levels that will optimize management for both 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, but from 2011 to 2018, only broodfish collected from the Roanoke River were used for production. American Shad fry were cooperatively 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 address concerns of decreasing abundance. 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 (NCWRC, unpublished data), 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 as hatchery contribution decreases.

The objective of this study was to use PBT analysis to evaluate hatchery contribution of returning adults in the Roanoke and Neuse Rivers for 2022, which includes the first cohort of age-3 American Shad containing no hatchery stocked fish. Additionally, we evaluated American Shad genetic health by calculating effective population size estimates (N_e) 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 fin clip samples from 294 returning adult American Shad from the Neuse River and 185 returning adult American Shad from the Roanoke River (total = 479) in 2022 for parentage-based tagging (PBT) and population genetic analysis. All samples were catalogued in the American Shad PBT

database (access available upon request to authors) 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 µL reactions and run on an Eppendorf Mastercycler Pro (Eppendorf NA: Hauppauge, NY) thermal cycler. PCR reactions consisted of 5 µL of a 1:10 mixture of Takara ExTaq Premix and Promega GoTaq MasterMix with 1 µL 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 3500xL Genetic Analyzer using 1 µL of PCR product, 9.55 µL formamide (McLab Super-Di Formamide), and 0.45 µL 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, year class, spawning tank, or river of origin. Simulations were run in triplicate and assumed 99% genotyping of broodfish, 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. All matches were analyzed to ensure that years, tanks, and sex matched 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. All 2022 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 22 alleles/locus, 0.855 mean observed heterozygosity). The combined identity non-exclusion probability is 1.85×10^{-19} and the combined parent pair non-exclusion probability is 2.9×10^{-13}. An identity analysis of 2022 samples revealed three pairs of identical fin clips. The identical pairs were 2022-150N/2022-98N, 2022-151N/2022-90N, and 2022-2088/2022-93N. In each case, individuals were released after measurement and fin clipping, allowing for the possibility of the same fish to be sampled again on a different day. Duplicate genotypes were removed prior to analysis.

Parentage-Based Tagging Analysis. In total, we identified 87 of 185 Roanoke River adults (47.0%) and seven of 291 Neuse River adults (2.4%) as hatchery-derived American Shad in 2022.

All pairings are noted in our American Shad PBT database. In the Roanoke River, four hatchery cohorts comprised the stocked fish (Figure 1); the 2018 cohort (age 4) was most abundant (88.5%) followed by 2015 (age 7, 6.9%), 2016 (age 6, 3.4%), and 2017 (age 5, 1.1%). In the Neuse River, only two hatchery cohorts were represented in the sample of returning adults identified as hatchery-origin (Figure 2); the 2018 hatchery cohort (age 4) comprised 85.7% of hatchery-origin adults and the 2016 hatchery cohort (age 6) comprised 14.3%. Of the positively identified Roanoke adults, 12 (13.8%) came from Weldon stockings and 75 (86.2%) came from stockings at Roanoke Rapids Lake (Table 3).

Six same-sex parent matches were identified in 2022 samples. One pairing involves samples 2018-W2047 and 2018-W2055. Both fish were noted as males with neither previously identified as contributing offspring, yet this match was identified at a high confidence level. The other five matches included three sexed females noted in other same-sex pairings: 2018-N1136, 2018-W2157, and 2018-E2036. Given the consistent identification of these three individuals in pairings with other females, we believe these fish to be male and have noted that observation in the database. We included all six offspring identified from these same-sex pairings as hatchery-produced fish.

Population Genetics. The effective population estimate for 2022 Roanoke River American Shad was 637, with the 95% confidence interval ranging from 416 to 1259 (Table 4). The effective population estimate for American Shad in the Neuse River was 10,558 with a 95% confidence interval of 1,525 to infinity. Further investigation shows a negative correlation between hatchery contribution and N_e, with confidence intervals increasing as N_e increases (Figure 3).

Discussion

The 2022 spawning season was the first year we expected to see an impact from the decision to halt stocking after 2018 since the age-3 year-class would consist entirely of naturally recruited American Shad. While hatchery contributions continued to decline for Neuse River American Shad in 2022, hatchery contribution in the Roanoke River slightly increased from the previous year. Most of the hatchery contribution in 2022 came from the 2018 hatchery cohort (88.5%). We previously noted low recruitment for the 2017 hatchery cohort and suggest that the increased hatchery contribution in 2022 was due to the large contribution of the 2018 (age-4) hatchery cohort in comparison to 2021, which had little contribution from age-4 stocked fish. We hypothesized in previous years that a shift in stocking location to Roanoke Rapids Lake could have impacted fry/juvenile survival. However, recruitment from the 2018 hatchery cohort seems to refute that hypothesis as 94.8% of returning 2018 adults were stocked at Roanoke Rapids Lake.

Concurrent with the slight increase observed in Roanoke River hatchery contribution in 2022, we observed a decrease in N_e for that population. Although Neuse River American Shad diversity analyses continue to indicate a healthy genetic population with N_e above 500, Roanoke River American Shad genetic diversity continues to be a concern due to low N_e when hatchery contribution is high. Our analysis demonstrates a negative correlation between effective population size and hatchery contribution over multiple years of sampling, with confidence intervals increasing as N_e increases. Waples and Do (2010) noted that variance will

increase and precision decrease for populations with large Ne, and that phenomenon is noted in our study. Future analyses should evaluate correlations between relative abundance (CPUE), genetic diversity, and hatchery contribution as stocking efforts are reduced. Such analyses could identify levels of stocking that will maintain or increase the Roanoke River American Shad population while guarding against decreasing genetic diversity.

Management Recommendations

1. In the Roanoke River, continue PBT analysis and genetic population analysis through 2025 to evaluate contribution of the 2018 hatchery cohort through age 7 and to examine genetic diversity during the period of decreasing hatchery contribution. We recommend collecting at least 200 samples per year to improve precision of effective population estimates.
2. Evaluate relationships between hatchery contributions and effective population estimates with stocking rates and locations as well as relative abundance indices to evaluate stocking efforts that could maintain total population size while guarding against decreasing genetic diversity.
3. Discontinue PBT analysis for the Neuse River. Genetic diversity analyses indicate a healthy population with N_e well above 500. Diversity is not expected to decline as hatchery contribution is currently less than 3% and stocking was halted in 2018. Should stocking resume in the Neuse River in the future, we recommend genetic analysis be resumed.

Acknowledgments

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

Broodfish Cohorts	# Simulated Offspring	Critical Relaxed Delta	Critical Stringent Delta	HWE
2010–2011	250,000	0	19	*Asa*D312***
2012–2013	50,000	0	16	NS
2014–2017	20,000	0	13	NS
2018–2021	1,000	0	4	NS

TABLE 2. Allele frequency data for 2022 American Shad (*Alosa sapidissima*) 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
AsaD030	26	0.929	0.927	0.921	NS	-0.0022
AsaD031	16	0.835	0.844	0.826	NS	0.0050
AsaC010	22	0.860	0.881	0.869	NS	0.0115
AsaD021	16	0.858	0.868	0.854	NS	0.0034
AsaD312	19	0.833	0.856	0.840	NS	0.0136
AsaC059	20	0.814	0.828	0.812	NS	0.0088
AsaB020	13	0.816	0.804	0.782	NS	-0.0088
AsaD055	17	0.777	0.797	0.775	NS	0.0151
AsaC334	30	0.866	0.879	0.867	NS	0.0074
AsaC249	38	0.906	0.923	0.917	NS	0.0100
AsaC051	23	0.866	0.885	0.873	NS	0.0103
AsaD042	21	0.906	0.916	0.908	NS	0.0046

TABLE 3. Positive identifications for hatchery contribution by stocking site for samples collected from the Roanoke River in 2022.

Broodfish Cohort	Hatchery Offspring	Weldon	Roanoke Rapids Lake
2015	6	6	0
2016	3	2	1
2017	1	0	1
2018	77	4	73

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	
2022	185	637	416/Infinite	294	10,558	1525/Infinite
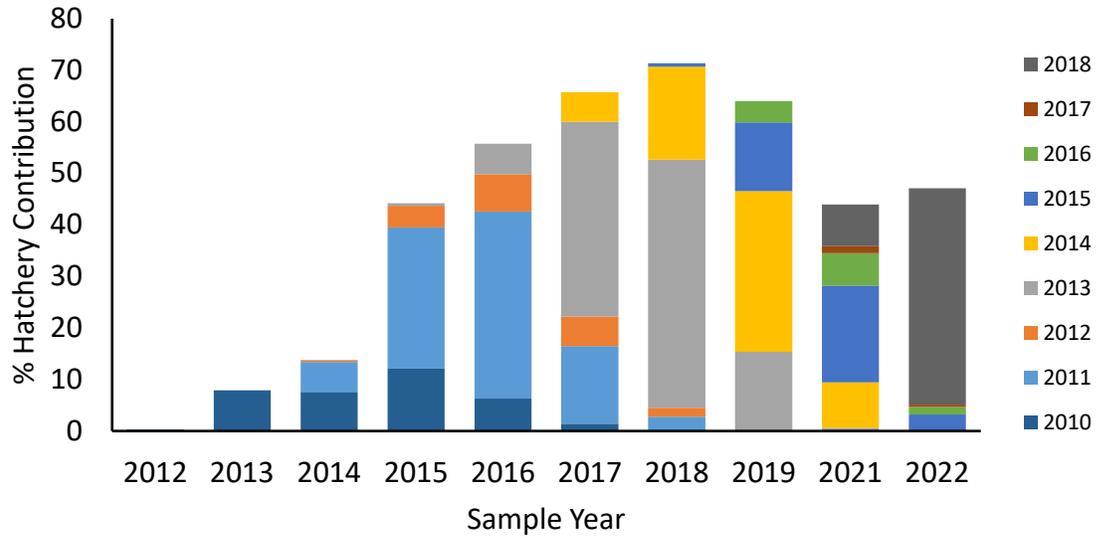
FIGURE 1. Roanoke River adult percent hatchery contribution by year. Stacked bars represent broodfish cohorts. Note that no sampling occurred in 2020 due to COVID-19.
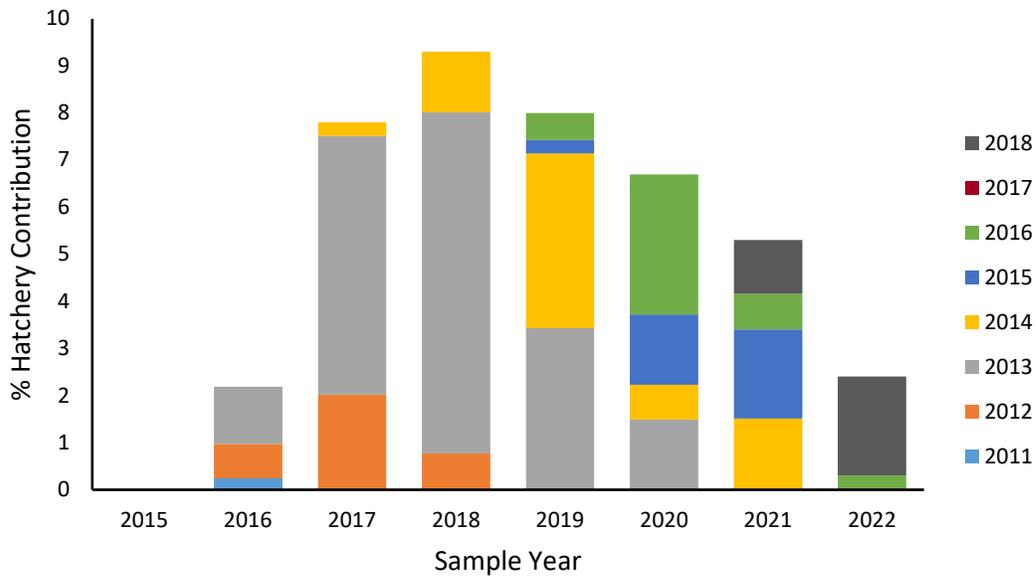

FIGURE 2. Neuse River adult percent hatchery contribution by year. Stacked bars represent broodfish cohorts. Note change in scale of y-axis compared to Figure 1.
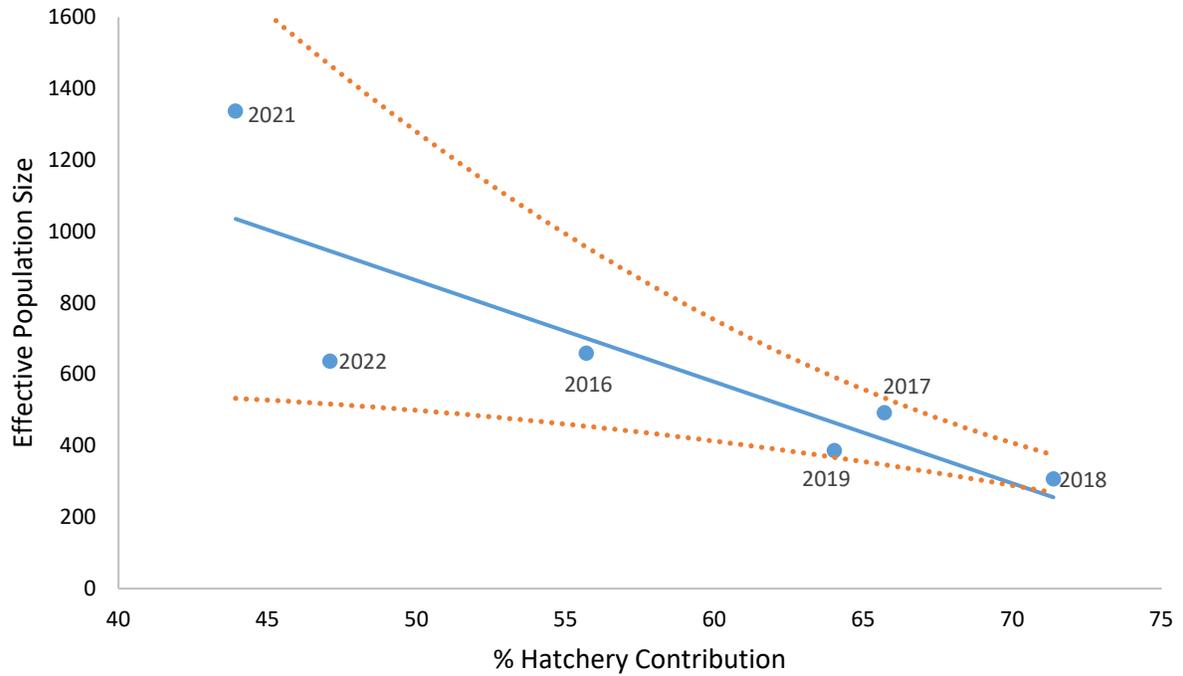
FIGURE 3. Comparison of percent hatchery contribution to effective population size (N_e) for Roanoke River American Shad. Individual datapoints for 2016 through 2022 are included, excluding 2020 in which data was not collected. Trend line is shown with a solid blue line and N_e confidence interval trendlines with a dashed orange line.