Roanoke River American Shad Spawning Stock Survey and Parentage-Based Tagging Review

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Abstract. The American Shad *Alosa sapidissima* spawning stock in the Roanoke River once supported a valuable recreational and commercial fishery in northeastern North Carolina. Population declines due to habitat fragmentation, habitat degradation, and overharvest prompted the North Carolina Wildlife Resources Commission to annually monitor the spawning stock, implement conservative fishing regulations and begin a stocking program to aid recovery of the stock. Despite significant hatchery stockings, annual surveys of adult American Shad on their spawning grounds show variable abundance with no increasing trends observed over the last two decades. Since 2010, a total of 43.2 million fry have been stocked into the Roanoke River basin and were genetically marked using parentage-based tagging methods (PBT). Starting in 2017 the hatchery contribution of the spawning stock encompassed ages 3–7 and represented the first year the entire stock was identifiable using PBT methods. Hatchery contribution in 2017, 2018, and 2019 remained high with an average hatchery contribution of 67%. Most hatchery-origin fish were identified as originating from lower Roanoke River stockings with little contribution from stockings above the series of impoundments on the Roanoke River system. While stocking has been successful, these efforts were aimed to assist recovery but appear to be supporting much of the spawning stock. Efforts to restore the stock should focus on identifying limitations to natural recruitment and mitigate these limitations to increase spawning success.

The Roanoke River basin contains a diverse fish assemblage composed of greater than 100 resident and diadromous fish species. Historically, anadromous alosine species including American Shad *Alosa sapidissima,* Hickory Shad *A. mediocris,* Alewife *A. pseudoharengus,* and Blueback Herring *A. aestivalis,* were abundant and supported exceptional commercial and recreational fisheries in the Roanoke River. Several factors, primarily spawning habitat loss, fragmentation caused by the construction of dams, and overharvest, have contributed to the decline of American Shad populations, (Hightower et al. 1996). Between the 1940's and 1960's at least six major impoundments were constructed on the Roanoke River, with no provisions for fish passage (Harris and Hightower 2012). Currently, the Roanoke River basin contains 16 major reservoirs with a chain of three reservoirs (John H. Kerr Reservoir, Lake Gaston, and Roanoke Rapids Lake) located near the fall line along the Virginia and North Carolina border. These three lower-most dams resulted in the loss of approximately 500 miles of potential spawning habitat. Currently only eight river miles containing approximately 1,000 acres of spawning habitat are accessible below these dams. Due to the ecological, recreational, and commercial importance of American Shad in the Roanoke River, the North Carolina Wildlife Resources Commission (NCWRC) has put tremendous effort into monitoring and restoring American Shad populations in the Roanoke River.

The NCWRC annually surveys the American Shad spawning stock in spring to early summer, typically at the beginning of the spawning season until spawning activities appear to cease. Population characteristics of the spawning stock of American Shad in the lower Roanoke River are summarized each spring and submitted to the North Carolina Division of Marine Fisheries (NCDMF) for use in developing stock assessment models and for inclusion in North Carolina's annual American Shad compliance report to the Atlantic States Marine Fisheries Commission (ASMFC). This information is required of the state of North Carolina as mandated under conditions set forth within the fishery management plan for alosine species established for the eastern United States (ASMFC 1985) and associated amendments (ASMFC 2002; ASMFC 2010). Compliance with this plan is necessary to support the enhancement of American Shad populations within coastal North Carolina. As part of this compliance, a Sustainable Fishery Plan was created to identify and implement management efforts that would rebuild and maintain American Shad populations in North Carolina (NCDMF and NCWRC 2012).

In 2005, state and federal fisheries management agencies in North Carolina and Virginia reached a settlement agreement with Dominion/N. C. Power regarding Federal Energy Regulatory Commission (FERC) relicensing of the Gaston and Roanoke Rapids hydroelectric projects in the Roanoke River basin. The relicensing agreement provided for the well-funded and highly coordinated program to restore American Shad in the Roanoke basin. Measures outlined in the restoration effort included improvements in hatchery production of fry, continued intensive monitoring of fry stocking success upstream and downstream of the main stem reservoirs, and annual assessments of American Shad population size to guide decisions regarding construction of upstream passage facilities.

To supplement the American Shad population, a hatchery-based stocking program was initiated in 1998. The NCWRC collected broodfish that were transferred to Watha State Fish Hatchery (WSFH) where fry were reared and stocked in the Roanoke River by hatchery staff (Evans 2014). To evaluate efficacy of the stocking program, juvenile American Shad were collected in the fall to characterize hatchery contribution of the juvenile out-migration. Prior to

2010 this was done by batch marking fry before stocking with a chemical mark using oxytetracycline. This method proved to be unreliable (NCWRC, unpublished data). From 2010 to present, genotyping and parentage-based tagging (PBT) analysis techniques have been used, and this method has resulted in more conclusive in hatchery contribution rates. Additionally, PBT analysis was conducted to identify any returning adults of hatchery origin from previously stocked cohorts during the spring spawning stock surveys. From 1998 to 2010, donor broodfish sources from the Tar, Cape Fear, and Roanoke River were used. Starting in 2011, only endemic American Shad were used as broodfish to reduce concerns regarding genetic conservation. In addition to the restoration program, recreational harvest regulations including a one fish per day creel limit for American Shad in the Roanoke River are intended to protect the Roanoke River American Shad population on the spawning grounds.

The objectives of our monitoring efforts were to: 1) describe the population characteristics of the spawning stock in the Roanoke River, 2) evaluate the hatchery contribution of adult American Shad on the spawning grounds, 3) determine the hatchery contribution of juvenile American Shad during the out-migration, and 4) use the best available model to estimate size of the American Shad spawning population. Results from the 2018, 2019, and 2021 spawning stock survey and hatchery evaluation of out-migrating juveniles and returning adults are presented in this report.

Methods

Study Area. From its headwaters in the ridge and valley physiographic province of western Virginia near Blacksburg, the Roanoke River flows southeasterly for approximately 660 km until emptying into the Albemarle Sound in Northeastern North Carolina near Plymouth. The basin consists of 16 counties and 6 cities in Virginia and 15 counties and 42 municipalities in North Carolina. More than 18,000 km of tributaries drain approximately 25,600 km^2 in Virginia and North Carolina with nearly two-thirds of the basin occurring in Virginia. Major tributaries of the Roanoke River include the Dan, Banister, and Smith Rivers. There are three major reservoirs on the Roanoke River near the Virginia and North Carolina border: John H. Kerr Reservoir, Lake Gaston, and Roanoke Rapids Lake. These reservoirs are operated for flood control and hydroelectric generation. John H. Kerr reservoir is the largest of the three and regulates much of the flow in the North Carolina portion of the river. Downstream of the Roanoke Rapids Lake Dam is commonly referred to as the lower Roanoke River where flows are unimpeded, traveling through the largest intact and least-disturbed bottomland hardwood forest floodplain in the mid-Atlantic region (NCDWQ 2006).

Spawning Stock Assessment. American Shad from the Roanoke River were collected weekly with boat-mounted electrofishing gear (Smith-Root 7.5 GPP, 500-1,000V, 3.8–4.0A) in 2018 from March 7 to May 15, in 2019 from March 27 to May 24, and in 2021 from March 25 to May 13 near the Gaston Boating Access Area (Figure 1). Sampling did not occur in 2020 due to COVID-19 restrictions and closures. Electrofishing occurred during daylight hours with one boat driver and two dip netters. Samples were conducted at nine sampling sites once per week during the survey period. Electrofishing commenced at the upstream portion of each 500-m site and continued downstream for the entire transect. At each site, electrofishing effort (seconds) was recorded and water quality parameters including water temperature (°C), dissolved oxygen

saturation (%), dissolved oxygen concentration (mg/L), specific conductivity (µS/cm), salinity (ppt), pH, and secchi depth (m) were measured.

American Shad were held in a circulating, oxygenated live well, and upon site completion, each fish was measured for total length (TL mm), weighed (g), and sexed. Sex was determined by applying directional pressure to the abdomen toward the vent and observing the discharge of milt or eggs. All American Shad were fin clipped, and a selection of fin clips from at a maximum 5 per 10 mm length bin on each sampling day for males and females were stored in numbered vials containing non-denatured, spectrophotometric grade ethanol for PBT evaluation. Fin clips were recharged with ethanol within one week of collection. Additionally, the fin clip allowed for visual identification of recaptures in subsequent sampling days. Relative abundance was quantified each week and for the entire survey period and was indexed by catch-per-unit-effort (CPUE), expressed as the number of fish captured per hour by electrofishing. Additionally, relative abundance was calculated weekly for both males and females. American Shad broodfish collections occurred outside of weekly surveys and were not included in CPUE calculations. A weekly male to female ratio was also generated to determine the proportion of female American Shad on the spawning grounds. A length-frequency histogram was constructed for males and females using 10-mm length groups to categorize the size structure of American Shad on the spawning grounds.

2018 Hatchery Evaluation. American Shad broodfish were collected near the Gaston Boating Access Area between April 3 and April 6. Broodfish were transported via hatchery truck to WSFH and ENFH where fish were spawned, and fry were reared. When fry were roughly eight days old, they were stocked by hatchery staff in the lower Roanoke River at Weldon below Roanoke River Rapids Dam and in Roanoke Rapids Lake near the tailrace at Gaston Dam. Fin clips from all broodfish were stored in numbered vials containing non-denatured, spectrophotometric grade ethanol to later be referenced for hatchery origin of out-migrating juveniles and at-large adults.

Fin clips from American Shad broodfish were sent to the genetics laboratory at the NC Museum of Natural Science (NCMNS) for genotyping and PBT analysis to determine any individuals of hatchery origin (Evans and McGrandy 2019). Genotyping of discrete batches of broodfish allows specific cohorts to be identified in future surveys and allows for evaluation of hatchery contribution of the juvenile out-migration and the effectiveness of experimental stockings above Roanoke Rapids dam. With the results of individual origin, hatchery contribution percentages were generated for each stocking cohort. Age-length keys were applied to develop an age distribution for fish with processed fin clips to determine frequency of hatchery-origin by stocking year.

Juvenile American Shad surveys were conducted near Plymouth, NC, from September to November in 2018 and 2019. Juvenile American Shad were collected using boat-mounted electrofishing approximately 30 minutes after sunset. At least one electrofishing transect was completed during each sampling night. Electrofishing transects were on average 1200 seconds in duration and all juvenile Shad were collected by a dip-netter and held in a live well. Upon transect completion, fish were identified to species, measured, and fin clips were taken from American Shad in 2018. Fin clips were stored in a numbered vial containing non-denatured, spectrophotometric grade ethanol. Fin clips were then sent to NCMNS for PBT analysis, to determine hatchery contribution of the 2018 juvenile out-migration.

Abundance estimate. Abundance of female American Shad on the spawning grounds in the Roanoke River was estimated for 2018 according to methods described by Harris and Hightower (2012). This method incorporates average fecundity, egg ripening, egg fertilization and fry survival rates with an estimate of juvenile production based on the number of fry stocked and percent hatchery contribution of the out-migrating juveniles to estimate the number of female American Shad present on the spawning grounds in a given year. The number of fry stocked is known, but age at stocking (i.e., days post hatch; dph) can be variable among batches. The model is sensitive to the age at which fry are stocked because fry survival rates vary with age at stocking. Hatchery contribution rates within the juvenile out-migration also influence the estimate of adult female American Shad. In general, a low juvenile hatchery contribution rate will result in a relatively high female abundance estimate, and a high contribution rate will result in a low female abundance. In 2018, the age of American Shad at stocking was estimated between 6 and 10 dph (Evans and Carlson 2018); thus, female American Shad abundance was estimated for the minimum, maximum, and average dph. The male to female ratio from the spawning stock survey was used to expand the female estimate to total abundance of American Shad on the spawning grounds.

Results

2018 Spawning Stock Assessment. A total of 457 American Shad were collected from March 7 to May 15 (Table 1). Of the 457 American Shad collected, two fish had previous fin clips, indicating a low recapture rate. Males comprised 77% of the sample (*n* = 353), while females accounted for 23% (*n* = 104; Figure 2). In 2018, the male to female ratio on the spawning grounds was 3.4:1. Overall total CPUE was 48.5 fish/h. Peak relative abundance of males (64.9 fish/h) and females (18.0 fish/h) occurred on April 26. The peak weekly CPUE for both sexes combined (82.9 fish/h) occurred on April 26. Overall, spawning stock size distribution ranged from 358 to 544 mm (Figure 3). Female American Shad lengths ranged from 426 to 544 mm with an average of 449 mm; male American Shad lengths ranged from 358 to 499 mm with an average of 437mm. The length frequency distribution for males was unimodal with a peak at 430–439 mm and for females was unimodal with a peak at 480–489 mm.

A total of 411 ages determined by PBT were used to create an age-length key. Of those 411 aged American Shad, 157 were broodfish from WSFH, 88 were broodfish from ENFH, and 166 were at-large individuals. All ages were combined to create sex specific age-length keys comprised of 241 and 170 known age male and female American Shad. The sex specific age-length keys were used to assign ages to 68 unaged females and 223 unaged males. Age analysis showed an age distribution ranged 3–6 years for males and 3–7 years for females (Table 2 and Figure 4). American Shad from the 2013 year-class (age 5) were most abundant comprising 63% of total males and 88% of total females collected. Mean total lengths of age-5 males and females were 447 mm and 493 mm, respectively (Figure 5).

2019 Spawning Stock Assessment. A total of 851 American Shad were collected from March 27 to May 24. Of the 851 American Shad collected, six fish had previously been fin clipped, indicating a low recapture rate. Males comprised 77% of the sample (*n* = 653), while females accounted for 23% (*n* = 198). In 2019, the male to female ratio on the spawning grounds was 3.3:1. Overall total CPUE was 87.7 fish/h. Peak relative abundance of males (114.6 fish/h) and

females (46.2 fish/h) occurred on April 11. The peak weekly CPUE for both sexes combined (160.8 fish/h) occurred on April 11. Overall, spawning stock size distribution ranged from 343 to 539 mm. Female American Shad lengths ranged from 403 to 539 mm with an average of 487 mm; male American Shad lengths ranged from 343 to 515 mm with an average of 431 mm. The length frequency distribution for males was unimodal with a peak at 430–439mm. The female length frequency distribution was also unimodal with a peak at 480–489 mm.

Sex specific age-length keys were created using ages determined through PBT of at-large individuals collected during the 2019 weekly surveys. A total of 44 males and 110 females with known ages were used to create an age-length key that was used to assign unaged fish ages. Ages were assigned to 140 unaged female American Shad, but 14 were not assigned ages because PBT ages were not available for the sex and size group. Ages were assigned to 528 male American Shad, but 15 were not assigned ages.

2021 Spawning Stock Assessment. A total of 202 American Shad were collected between March 3 and May 13. Males comprised 42% of the sample (*n* = 85), while females accounted for 58% of the sample (*n* = 117). The male to female ratio was 0.7:1. Overall total CPUE was 24 fish/h. Peak relative abundance of males (13.4 fish/h) occurred on May 13 and female peak CPUE (23.9 fish/h) occurred on April 29. The peak weekly CPUE for both sexes combined (34.9 fish/h) occurred on April 29. The spawning stock size distribution ranged from 379 mm to 560 mm. The female length distribution ranged from 409 mm to 560 mm with an average of 497 mm; the male length distribution ranged from 379 mm to 499 mm with an average of 434 mm.

Sex specific age-length keys were created using ages determined though PBT of at-large individuals collected during the 2021 weekly surveys. A total of 160 fin clips were processed using PBT. Ages from hatchery-origin fish were assigned to unaged fish. A total of 3 males and 18 females were not assigned ages.

2018 Hatchery Evaluation. Broodfish collections near the Gaston Boating Access Area occurred from April 3 to April 6 with 99 male and 102 female adult American Shad transported to WSFH and 44 males and 66 females transported to ENFH. Hauling mortality for broodfish transported to WSFH was 12% and was similar for females (*n* = 11) and males (*n* = 13). Hauling mortality for broodfish transported to ENFH was 3% and was similar for females (*n* = 2) and males (*n* = 1). Hatchery production at WSFH in 2018 met the goals for the Roanoke Rapids stocking location; in 2018, 1,983,351 fry were stocked into Roanoke Rapids Reservoir at the Dominion Power boating access just below Gaston Dam between April 20 and May 10 (Tables 3 and 4; Evans and Carlson 2018). Hatchery production at ENFH was less than expected with a total of 320,928 fry produced and stocked into the Roanoke River downstream of Roanoke Rapids Dam at Weldon, NC (personal communication, Sam Pollock USFWS).

Broodfish and hauling mortality fin clips were combined with fin clips collected during weekly samples to determine percent hatchery contribution of the Roanoke River spawning stock. A total of 310 fin clips were from broodfish and hauling mortalities, and 272 fin clips were from weekly samples, for a combined total of 582 fin clips (Table 5). All adult fin clips were genotyped and PBT analysis was used to determine the number of individuals on the spawning grounds that were of hatchery origin. A total of 412 out of 582 adults were found to be of hatchery origin, indicating that 70.8% of adult American Shad collected from the spawning grounds were of hatchery origin in 2018. Of the hatchery identified fish, the majority were matched with hatchery broodfish from the 2013 and 2014 cohorts, 278 (68%) and 104 (25%),

respectively. The hatchery origin of broodfish collected in 2018 was 78.8% (Figure 6). A total of 63 juvenile American Shad were collected during weekly electrofishing surveys from October 18 to November 14 and were sent to NCMNS for assessment with PBT methods. Juvenile out-migration PBT analysis concluded 3 of the 63 genotyped fish were conclusively matched with hatchery broodfish from the 2018 cohort (Evans and Carlson 2018). Analysis further revealed that two of the hatchery-origin identified juveniles were stocked at Weldon and one was stocked in Roanoke Rapids Reservoir. A total of 4.8% of the 63 juvenile American Shad collected in the outmigration were of hatchery origin in 2018.

2018 Abundance estimate. Utilizing parameters of 5, 8, and 12 dph as stocking (minimum, average, and maximum values) estimates for 2018, female American Shad ranged from 4,666 to 13,655 individuals (Table 6). The estimate of spawning females was multiplied based on the 3.4:1 sex ratio, resulting in the following estimates of 20,530 (5 dph), 42,904 (8 dph), and 60,082 (12 dph) total spawning population in 2018.

2019 Hatchery Evaluation. A total of 240 at-large American Shad fin clips collected from weekly samples were processed using PBT analysis in 2019. Fin clips were genotyped and PBT analysis was used to determine the number of individuals on the spawning grounds that were of hatchery origin. A total of 154 individuals were determined to be of hatchery origin, for a percent hatchery contribution of 64% (Evans and McCargo 2021). Of hatchery identified fish 49% were from the 2014 year-class (*n* = 75; Evans and McCargo 2021). No fin clips from the juvenile outmigration were collected in 2019 because no stocking occurred in the spring.

2021 Hatchery Evaluation. A total of 160 at-large American Shad fin clips were processed using PBT analysis in 2021. Fin clips were genotyped and PBT analysis was used to determine the percent hatchery contribution of the spawning stock. A total of 70 individuals were determined to be of hatchery origin, for a total hatchery contribution of 43.8% (Evans et al. 2022). Of hatchery identified fish, the majority were from the 2015 cohort, 43% (*n* = 30). No fin clips were collected during the juvenile outmigration.

Parentage-based tagging. Since 2012, a total of 4,225 at-large adult American Shad fin clips collected from the spawning grounds (from broodfish collections and weekly surveys) have been processed using PBT methods. Of the 4,225 fin clips, a total of 1,747 adult American Shad were identified as hatchery origin from 2012 to 2019. However, this does not represent the overall hatchery contribution of the stock through the entire time series. With PBT methods beginning in 2010, the full cohort of at-large adults identified using PBT was first observed in 2017. The hatchery contribution of at-large American Shad was 65.7%, 71.3%, and 64.2% in 2017, 2018, and 2019, respectively. In 2017 and 2018 collection years, PBT ages represented adult fish age 3 through 7, and in 2019, adult fish PBT ages were 3 through 6. A total of 10 adult fish captured on the spawning grounds were stocked as fry in the Staunton River, upstream of Kerr Reservoir at Clover Landing, 38 were stocked in Lake Gaston, 6 were stocked in Roanoke Rapids Lake, 1,515 were stocked in the lower Roanoke River, and 176 had an unknown stocking location. Those fish that had unknown stocking locations were stocked in 2010 at either Clover Landing or the lower Roanoke River, but stocking tanks were unable to be determined and thus stocking location cannot be determined.

Discussion

Abundance of the Roanoke River American Shad spawning stock has not increased throughout the current time series. Declines in relative abundance were observed from 2012 to 2016 with only a slight increase in 2019 followed by another decline in 2021. Despite significant stocking efforts, the adult spawning stock does not appear to be expanding in abundance or size structure, and the current spawning stock is primarily composed of hatchery-origin fish. With the spawning stock in a suppressed state, removal of adults for hatchery spawning may impact the success of natural spawning. Moreover, the removal of those fish for hatchery production prevents those individuals from contributing to the population over multiple years and further adding to its inability to produce strong year classes. A combination of factors including suppressed spawning stock, high flow years, environmental limitations, and founders' effects all have the potential to limit spawning success by American Shad. Determining which of these factors are restricting successful spawning of American Shad in the Roanoke River is vital for increasing their relative abundance as well as making future management decisions.

Beginning in 2017 the entire American Shad spawning stock was available for hatchery evaluation with PBT. From 2017 through 2019 hatchery contribution was high, but a decrease in hatchery contribution was observed in 2021. Stocking appears to be successful because hatchery-origin fish are returning to the spawning grounds and contributing to the spawning stock, but the high contribution of hatchery fish is concerning. The risks of genetic bottlenecks, such as the Founder effect (Mayr 1954), increase as hatchery contribution continues to dominate the composition of American Shad in combination with population depression. The Founder effect occurs when genetic variability decreases due to a small group of individuals becoming separated from the larger population. In this case, the genetic variability of the Roanoke River American Shad population is being reduced because hatchery fish are being inadvertently selected to be sent to the hatchery as broodfish. High hatchery contribution also raises concerns regarding natural impediments to successful spawning and recruitment.

Because hatchery fish are stocked into the river at an early age (5–12 dph), any advantage the hatchery-origin fish have occurs within the time frame of spawning to early-stage fry. It is possible environmental factors contribute to the presumed poor recruitment of American Shad. Possible factors that should be explored include flow, water quality, contaminants, food availability (mis-match theory), impingement and entrainment, as well as egg and larval characteristics. This concern warrants a comprehensive study of characteristics and survival of early life stages of American Shad in the Roanoke River.

Genetic diversity should continue to be monitored until fish of hatchery origin age out of the spawning stock. In 2019, stocking activity ceased for a trial period of three years that was expanded through 2024 to better evaluate needs for recovery and genetic integrity concerns. Specific concern is the high percentage of hatchery-origin fish that have been sent back to the hatchery and are likely playing a direct role in low effective population size and a decline in genetic diversity. Ultimately, it is possible that genetic diversity of the population has been reduced due to a decline in genetic fitness and effective population size of spawning American Shad (Evans and McGrandy 2019; Evans and McCargo 2021; Evans et al. 2022). High hatchery contribution was seen in broodfish from 2016 through 2018 (Evans and Carlson 2018; Evans and McGrandy 2019). The return of hatchery-origin fish to the hatchery as broodfish could lead

to inbreeding depression and reduced genetic fitness especially in a depressed population. It is likely that the population benefited from having multiple broodfish sources during early years of the restoration program. Future stocking efforts should consider using broodfish from other sources to increase genetic diversity of the spawning stock.

It is important to note that the advent of PBT in evaluating hatchery contribution does not coincide with start of stocking American Shad on the Roanoke River and that the spawning stock was likely impacted by a high hatchery contribution much earlier than 2017. Although American Shad spawned in the river may be referred to as wild spawned, there may have been significant genetic contribution from stocked fish if one or more parents were spawned in the hatchery but from different cohorts.

The current genetic analysis using microsatellites was unable to detect genetic differences between American Shad in the Roanoke and Chowan rivers (Evans and McGrandy 2019; Evans and McCargo 2021). However, Mack et al. (2021) recently identified a distinct spawning run of American Shad of in the Chowan River using telemetry methods. Due to the proximity to the Roanoke River, the Chowan River population may be used to help improve genetic diversity and increase effective population size in the Roanoke River. Alternate approaches such as single-nucleotide polymorphisms (SNPs), and batch tagging should be explored to investigate stock identification, especially at the genetic level.

Management Recommendations

1. Maintain current creel limits for the Roanoke River to allow no more than one American Shad within the daily creel limit of 10 shad (American and Hickory Shad) in aggregate.
2. Suspend stocking American Shad through 2024.
3. Continue the use of genetic technologies to evaluate hatchery contributions to the adult spawning stock population though 2024.
4. Maintain current American Shad sampling efforts in the Roanoke River.
5. Identify possible barriers to recruitment of American Shad in the Roanoke River.
6. Determine if differences between Roanoke and Chowan spawning runs of American Shad exist.

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TABLE 1. American Shad weekly electrofishing effort, catch, male to female ratio, mean CPUE, standard error, number of sites, and mean daily water temperature for Roanoke River, 2018–2021 sampling dates; no sampling occurred in 2020. On dates with less than nine sites, sampling effort was reduced due to low flow limiting access in lower sites.

Date	Sites	Effort (h)	Total Males	Total Females	M:F Ratio	Total CPUE	Male CPUE	Female CPUE	Mean Water Temp
3/7/2018	9	1.3	14	5	2.8:1	14.5	10.7	3.8	9.5
3/22/2018	4	0.6	14	7	2.0:1	37.6	25.1	12.5	9
3/29/2018	9	1.3	61	10	6.1:1	55.6	47.8	7.8	10.7
4/12/2018	9	1.2	76	19	4.0:1	80.4	64.4	16.1	13
4/20/2018	9	0.9	43	16	2.7:1	63.2	46.1	17.1	14.9
4/26/2018	9	1	65	18	3.6:1	82.9	64.9	18	15.4
5/3/2018	9	1	42	10	4.2:1	53.9	43.6	10.4	17.7
5/9/2018	9	1	16	7	2.3:1	23.0	16	7	18.5
5/15/2018	9	1.2	23	12	1.9:1	28.7	18.9	9.9	22.5
3/27/2019	9	1.2	108	13	8.3:1	97.3	86.8	10.5	11.2
4/4/2019	9	1	100	28	3.6:1	124.2	97	27.2	11.5
4/11/2019	9	1.1	124	50	2.5:1	160.8	114.6	46.2	14.3
4/18/2019	9	1.1	90	28	3.2:1	110.9	84.6	26.3	16.1
4/25/2019	9	1	65	24	2.7:1	87.0	63.5	23.5	18.4
5/2/2019	9	1.1	45	21	2.1:1	58.0	39.6	18.5	19.7
5/9/2019	9	1	31	7	4.4:1	36.4	29.7	6.7	20.5
5/16/2019	9	1	48	15	3.2:1	60.6	46.2	14.4	21.2
5/24/2019	9	1	42	12	3.5:1	52.1	40.5	11.6	23.9
03/25/2021	9	0.925	8	13	0.6:1	22.7	8.6	14.1	10.5
04/01/2021	9	0.882	12	18	0.7:1	34.0	13.6	20.4	12.5
04/08/2021	9	0.963	13	8	1.6:1	21.8	13.5	8.3	13.9
04/15/2021	9	1.111	9	16	0.6:1	22.5	8.1	14.4	16.9
04/22/2021	9	1.087	9	14	0.6:1	20.2	8.3	12.9	15.8
04/29/2021	9	1.172	11	28	0.4:1	35.0	9.4	23.9	18.5
05/06/2021	9	1.16	4	8	0.5:1	11.2	3.4	6.9	20.1
05/13/2021	9	1.12	15	13	1.2:1	25.9	13.4	11.6	19.1

TABLE 2. Mean total length (mm) at age for American Shad males and females collected from the Roanoke River, spring 2018. Five males and four females could not be assigned an age because no fish were aged in their respective size class.

Year Class	Age	Female				Male			
		N	Mean	Min	Max	N	Mean	Min	Max
2015	3	1	449	449	449	3	373	364	387
2014	4	5	458	426	469	127	419	358	468
2013	5	92	493	458	544	222	447	404	499
2012	6	3	513	487	544	1	488	488	488
2011	7	3	511	493	527	0	-	-	-

TABLE 3. American Shad fry produced in North Carolina and stocked into the Roanoke River Basin from 1998 to 2018. American Shad broodfish were held in spawning tanks for propagation except in 1998, when eggs and milt were stripped from broodfish in the field and fertilized eggs were cultured in the hatchery.

Year	Edenton National Fish Hatchery	Watha State Fish Hatchery	Total
1998	481,000		481,000
1999	225,000	50,000	275,000
2000	535,000	308,000	843,000
2001	700,000	1,369,000	2,069,000
2002		820,000	820,000
2003	612,000	1,673,629	2,285,629
2004	589,822	1,740,000	2,329,822
2005	1,346,834	1,226,000	2,572,834
2006	1,088,936	1,332,000	2,420,936
2007	772,780	3,540,051	4,312,831
2008	3,126,098	5,093,517	8,219,615
2009	3,665,345	5,132,326	8,797,671
2010	3,729,433	4,153,031	7,882,464
2011	2,741,727	1,715,423	4,457,150
2012		4,800,118	4,800,118
2013		4,570,144	4,570,144
2014		7,504,291	7,504,291
2015		4,816,360	4,816,360
2016	1,259,100	2,479,603	3,738,703
2017	257,578	2,479,603	2,737,181
2018	320,928	2,483,858	2,804,786
Totals	21,451,581	57,286,954	78,738,535

TABLE 4. Annual summary of American Shad fry stocked (in millions) by Edenton National Fish Hatchery (ENFH) and Watha State Fish Hatchery (WSFH) in the Roanoke River basin from 2010 to 2018. Yearly stockings occurred at Weldon, NC, while experimental stockings occurred at the Virginia, Thelma, and Roanoke Rapids locations. Daily oxytetracycline (OTC) marking was used 1998–2012, and genetic analyses of hatchery contribution with parentage-based tagging (PBT) began in 2010.

Year	Millions Stocked	Hatchery	Stocking Location	Latitude, Longitude	Waterbody Stocked
2010	6.9	ENFH/WSFH	Weldon, NC	36.4275°, -77.5913°	Roanoke River
2011	4.0	ENFH/WSFH	Weldon, NC	36.4275°, -77.5913°	Roanoke River
2012	3.8	WSFH	Weldon, NC	36.4275°, -77.5913°	Roanoke River
2013	2.4	WSFH	Weldon, NC	36.4275°, -77.5913°	Roanoke River
2014	3.5	WSFH	Weldon, NC	36.4275°, -77.5913°	Roanoke River
2015	2.5	WSFH	Weldon, NC	36.4275°, -77.5913°	Roanoke River
2016	1.3	ENFH	Weldon, NC	36.4275°, -77.5913°	Roanoke River
2017	0.2	ENFH	Weldon, NC	36.4275°, -77.5913°	Roanoke River
2018	0.3	ENFH	Weldon, NC	36.4275°, -77.5913°	Roanoke River
Subtotal	24.9				
2010	0.9	ENFH	Altavista, VA	37.1057°, -79.2861°	Staunton River
2011	0.4	ENFH	Clover Landing, VA	36.8268°, -78.6881°	Staunton River
2012	1.0	WSFH	Clover Landing, VA	36.8268°, -78.6881°	Staunton River
2013	1.3	WSFH	Clover Landing, VA	36.8268°, -78.6881°	Staunton River
2013	0.8	WSFH	Bracey, VA	36.5736°, -78.1530°	Lake Gaston
2014	1.4	WSFH	Clover Landing, VA	36.8268°, -78.6881°	Staunton River
2014	2.6	WSFH	Bracey, VA	36.5736°, -78.1530°	Lake Gaston
2015	0.8	WSFH	Clover Landing, VA	36.8268°, -78.6881°	Staunton River
2015	1.5	WSFH	Bracey, VA	36.5736°, -78.1530°	Lake Gaston
2016	2.5	WSFH	Thelma, NC	36.4854°, -77.7809°	Roanoke Rapids Lake
2017	2.5	WSFH	Roanoke Rapids	36.4735°, -77.5913°	Roanoke Rapids Lake
2018	1.9	WSFH	Roanoke Rapids	36.4735°, -77.5913°	Roanoke Rapids Lake
Subtotal	17.6				
Total	42.5				

TABLE 5. Summary of at-large fin clips processed from 2012 (year of the first hatchery return) through 2021.

Year	Fin Clip Processed	Hatchery Origin	Percent Contribution	Year Classes Present								
				2010	2011	2012	2013	2014	2015	2016	2017	2018
2012	289	1	0.3	X								
2013	527	26	4.9	X	X							
2014	708	90	12.7	X	X	X						
2015	543	233	42.9	X	X	X	X					
2016	522	293	56.1	X	X	X	X					
2017	814	535	65.7	X	X	X	X	X				
2018	582	415	71.3		X	X	X	X	X			
2019	240	154	64.2				X	X	X	X		
2021	160	70	43.8				X	X	X	X	X	X

TABLE 6. 2018 Roanoke River back-calculated abundance estimates for spawning females with 95% confidence intervals and total estimated populations using age of fry at stocking (days post hatch) according to methods described by Harris and Hightower (2012).

Days Post Hatch	Estimated Spawning Females	95% Confidence Interval	M:F	Estimated Total Spawning Population
5 (minimum)	4,666	3,562–12,047	3.4:1	20,530
8 (average)	9,751	7,511–15,558	3.4:1	42,904
12 (maximum)	13,655	10,878–46,730	3.4:1	60,082

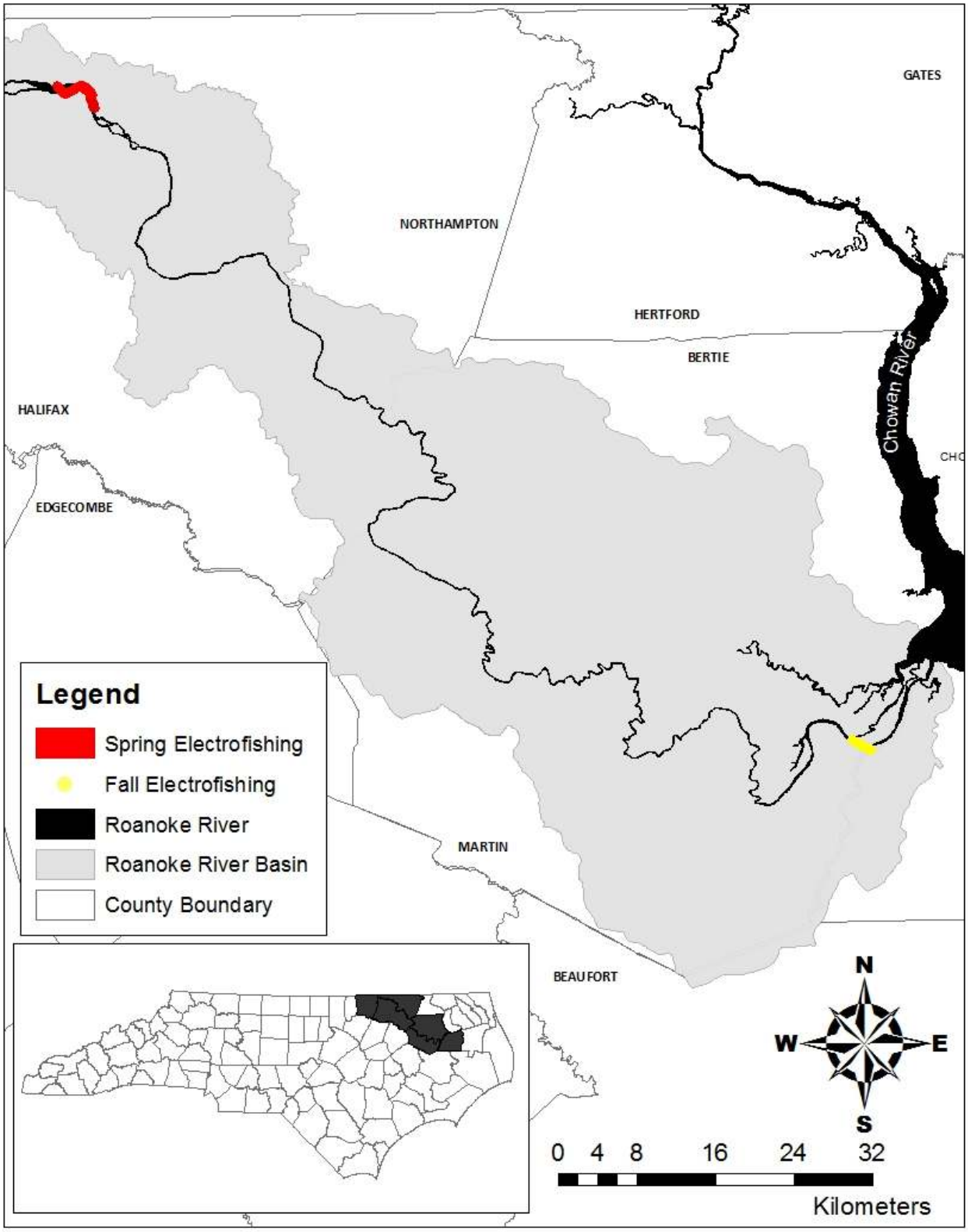

FIGURE 1. American Shad sampling sites on the Roanoke River in coastal North Carolina.

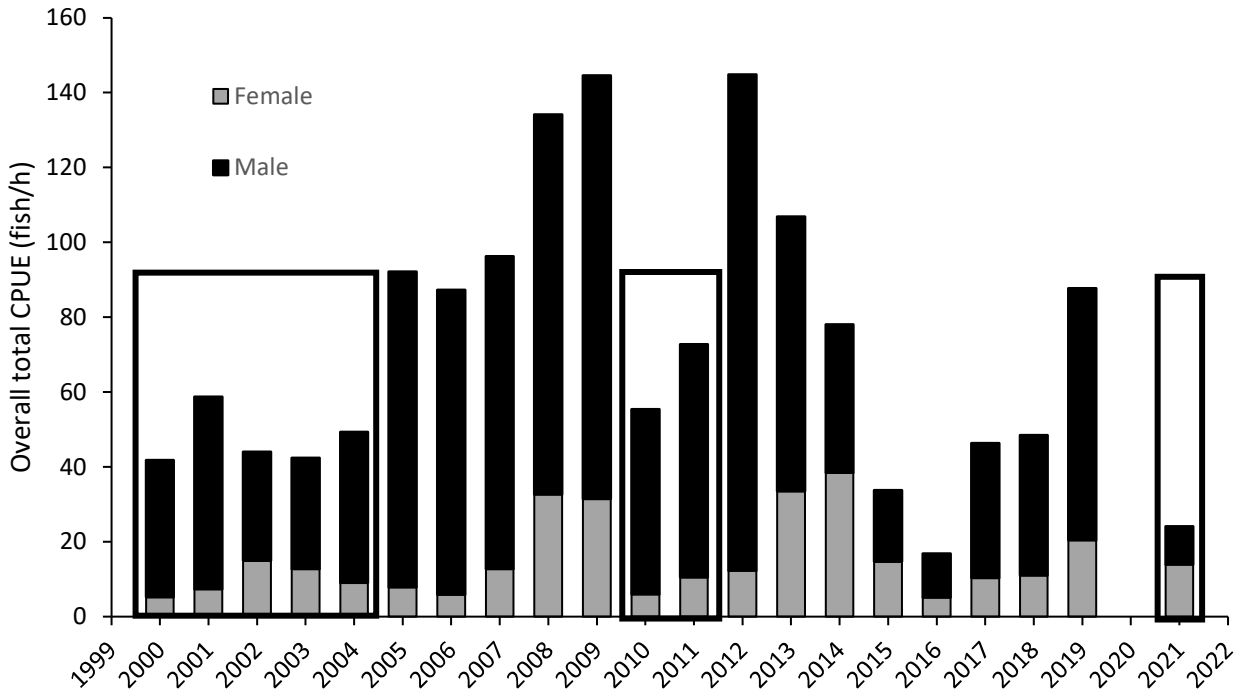

FIGURE 2. Overall total relative abundance (electrofishing CPUE) of American Shad collected from the Roanoke River, 2000–2021. One dip netter was used 2000–2004, 2010, 2011, and 2021 (denoted with black borders). Two dip netters were used 2005–2009 and 2012–2019. Sampling regime changes occurred in 2013 and 2014.

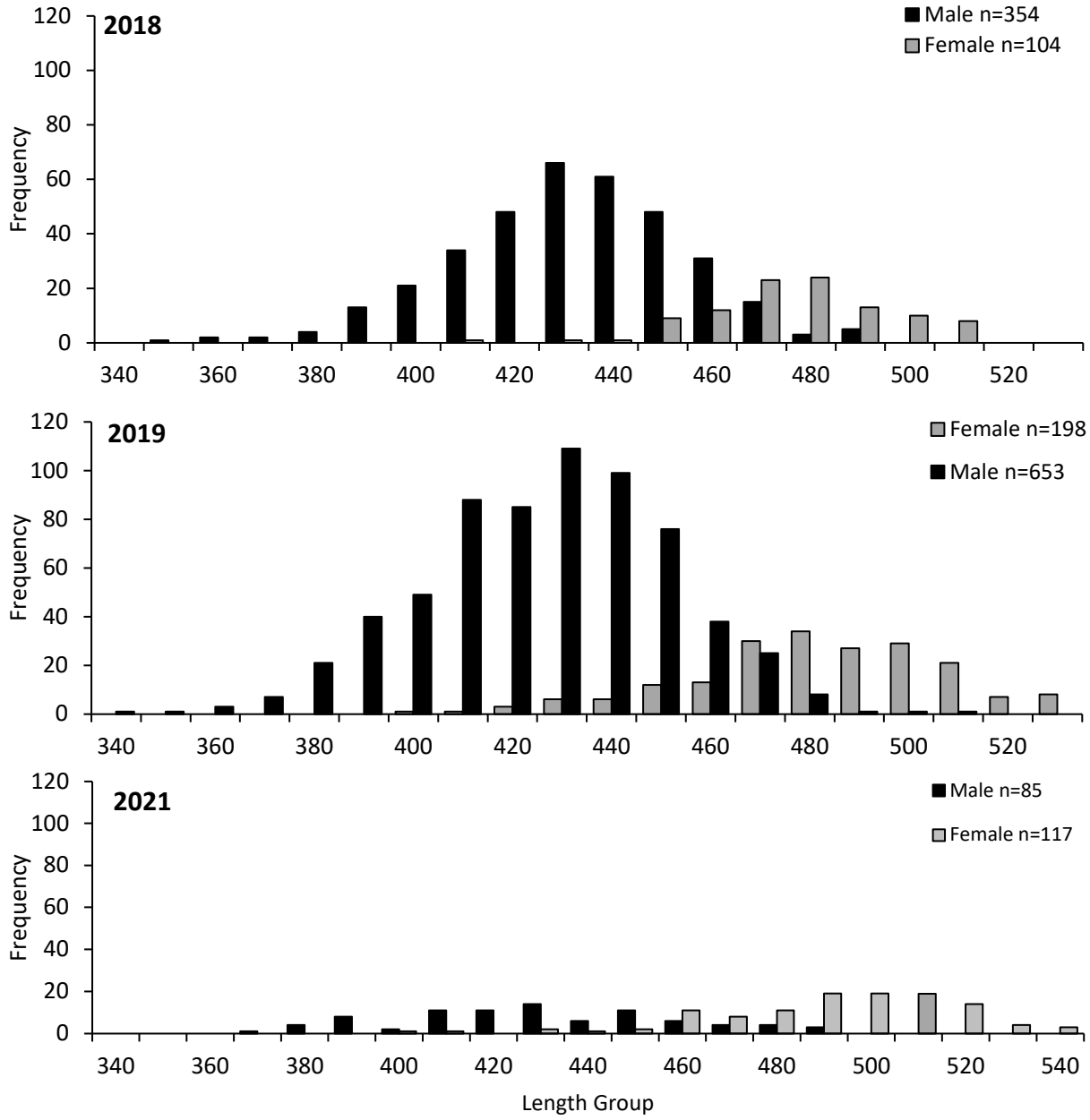

FIGURE 3. Length frequency histogram for American Shad collected from the Roanoke River, spring 2018, 2019, and 2021.

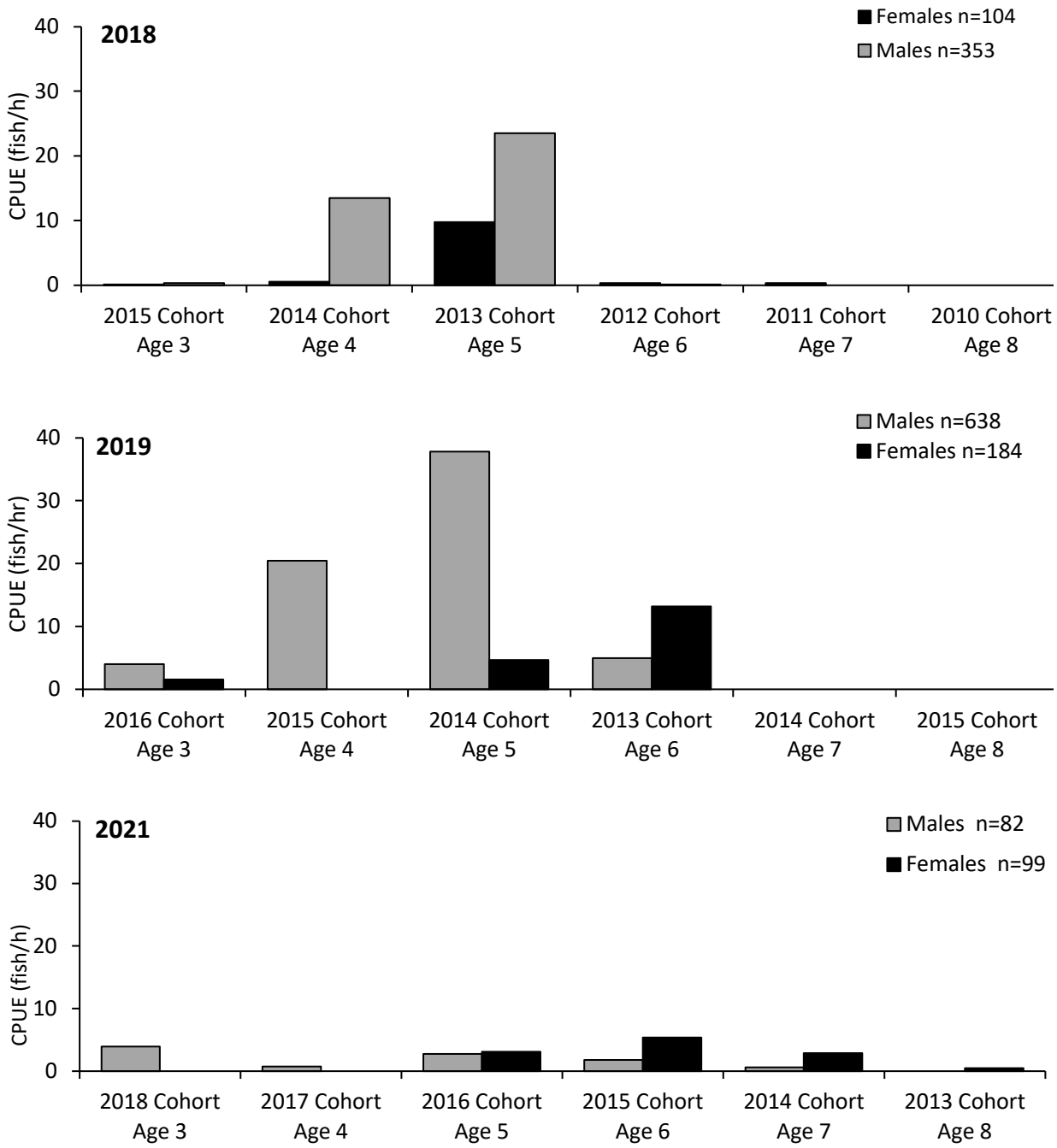

FIGURE 4. Relative abundance by age cohort (electrofishing CPUE) of American Shad collected from the Roanoke River, spring 2018, 2019, and 2021.

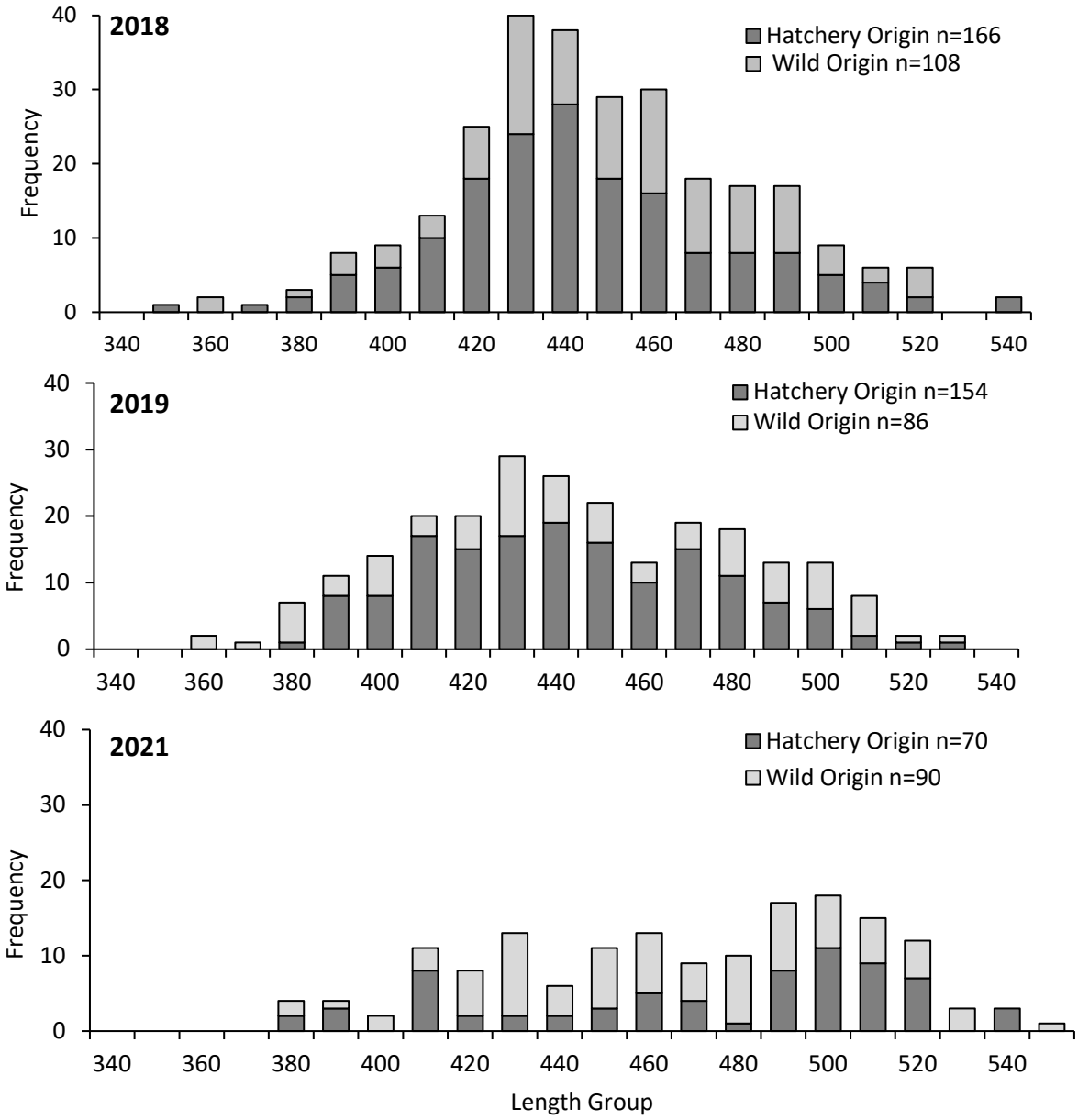

FIGURE 5. Length Frequency histograms for American Shad of hatchery origin wild origin in 2018, 2019, and 2021.

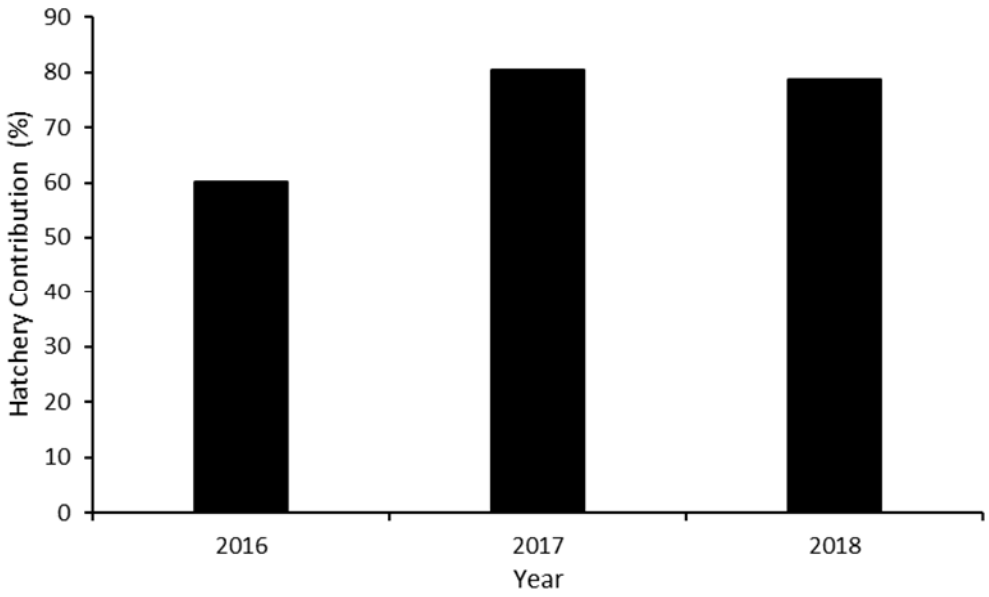

FIGURE 6. Percent hatchery contribution of broodfish collected for fry production to be stocked at Weldon and Roanoke Rapids in 2016–2018.