
Acoustic Telemetry Monitoring of Atlantic Sturgeon in the Vicinity of the Route 64 Bridge, Alligator River, Dare County, North Carolina



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**RESEARCH &
DEVELOPMENT**

Acoustic Telemetry Monitoring of Atlantic Sturgeon in the Vicinity of the Route 64 Bridge, Alligator River, Dare County, North Carolina

FINAL REPORT

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16. Abstract <i>In 2012, most Atlantic sturgeon populations, including the Carolina distinct population segment, became federally listed as endangered under the Endangered Species Act. The Alligator River Bridge on US64 is slated to be replaced by 2029. Being listed as an endangered species, federal and state resource agencies were concerned over potential impacts of bridge construction on feeding juvenile or adult migrating sturgeon within the Alligator River, NC. Atlantic sturgeon presence within the Alligator River Bridge area was sparse and more information was needed to make informed decisions. The main goal of this research was to determine Atlantic sturgeon presence within the Alligator River with a focus on the bridge area prior to the onset of construction. In order to elucidate presence, Atlantic sturgeon needed to be collected, telemetry tagged, and tracked throughout the Albemarle Sound/Alligator River area. From April 2021 to April 2026, sixty juvenile and 22 adult Atlantic sturgeon were tracked throughout the Albemarle Sound Complex, including the Alligator River. During that time period, the tagged juveniles spent a combined 15,816 days within the Albemarle Sound with 54 days (0.3%) within the Alligator River Array. The tagged adults were within the Albemarle Sound 6970 days and were detected a combined 16 days (0.2%) within the Alligator River Array. The telemetry data show that the Alligator River is a rarely utilized area for feeding juveniles and staging adults. The Alligator River appears to not be a staging area because both adults staging for spawning and feeding juveniles tend to inhabit areas closer to the salt wedge which is often located in the western part of the Albemarle Sound. Due to an accelerated work schedule, pile-driving for the new Alligator River Bridge supports started while the project was on-going. From February 18th, 2025, to February 12th, 2026, pile driving operations for bridge support bents occurred at the Alligator River Bridge area. Unfortunately, by this time a majority of the telemetry tag batteries in the juveniles tagged were no longer active or fish left the system entirely. It was also a dry year; therefore, juveniles and adults were squeezed even further in the very western part of the Albemarle Sound. However, one juvenile tagged on December 14th, 2023, was detected at the two receivers adjacent to the Alligator Bridge on May 13th, May 15th, and November 13th, 2025. Pile driving did not occur during May 13th but did occur during May 15th and November 13th. Fine scale fish positioning is not available based on receiver deployment. Pile driving occurred at two different locations on May 15th and three different locations on November 13th. Based on tag detection range and pile driving location, the juvenile was likely within 2km of pile driving operations, did not immediately leave the area during pile driving, and was later detected in the western Albemarle Sound. Another goal of this work was to collect genetic samples to help improve on previous genetic classifications on Atlantic sturgeon inhabiting the Albemarle Sound. Genetic samples from adults and juveniles collected during this project enabled refinement of the Roanoke River fall population designation plus showed over 83% of the subadults within the Albemarle Sound are of the Roanoke fall population.</i>			
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EXECUTIVE SUMMARY

In 2012, most Atlantic sturgeon populations, including the Carolina distinct population segment, became federally listed as endangered under the Endangered Species Act. The Alligator River Bridge on US64 is slated to be replaced by 2029. Being listed as an endangered species, federal and state resource agencies were concerned over potential impacts of bridge construction on feeding juvenile or adult migrating sturgeon within the Alligator River, NC. Atlantic sturgeon presence within the Alligator River Bridge area was sparse and more information was needed to make informed decisions. The main goal of this research was to determine Atlantic sturgeon presence within the Alligator River with a focus on the bridge area prior to the onset of construction. In order to elucidate presence, Atlantic sturgeon needed to be collected, telemetry tagged, and tracked throughout the Albemarle Sound/Alligator River area. From April 2021 to April 2026, sixty juvenile and 22 adult Atlantic sturgeon were tracked throughout the Albemarle Sound Complex, including the Alligator River. During that time period, the tagged juveniles spent a combined 15,816 days within the Albemarle Sound with 54 days (0.3%) within the Alligator River Array. The tagged adults were within the Albemarle Sound 6970 days and were detected a combined 16 days (0.2%) within the Alligator River Array. The telemetry data show that the Alligator River is a rarely utilized area for feeding juveniles and staging adults. The Alligator River appears to not be a staging area because both adults staging for spawning and feeding juveniles tend to inhabit areas closer to the saltwedge which is often located in the western part of the Albemarle Sound.

Due to an accelerated work schedule, pile-driving for the new Alligator River Bridge support bents started while the project was on-going. From February 18th, 2025, to February 12th, 2026, pile driving operations for bridge support bents occurred at the Alligator River Bridge area. While all the adult tags were still active and will be active until at least 2031, unfortunately, by this time a majority of the shorter battery life juvenile telemetry tags were no longer active or fish left the system entirely as juveniles vacating the Albemarle Sound were detected offshore from New York to Georgia. In order to keep a steady number of tagged juveniles within the Albemarle system, tagging would have to occur at least every two years as tagged age 2–3-year-old fish will consistently be migrating away from the Albemarle Sound and the tag battery life maxes out at 2.5 years. It was also a dry year in 2025; therefore, juveniles and adults were squeezed even further in the very western part of the Albemarle Sound. However, one juvenile tagged on December 14th, 2023, was detected at the two receivers adjacent to the Alligator Bridge on May 13th, May 15th, and November 13th, 2025. Pile driving did not occur on May 13th but did occur on May 15th and November 13th while the fish was detected at the Alligator Bridge. Pile driving occurred at two different locations on May 15th and three different locations on November 13th. Based on tag detection range and pile driving location, the juvenile was likely within 2km of pile driving operations, did not immediately leave the area during pile driving, and was later detected in the western Albemarle Sound. Fine scale fish positioning is not available based on how receivers were deployed. Fine-scale positioning would require adding a minimum of 10 receivers within two km of the bridge.

Another goal of this work was to collect genetic samples to help improve previous genetic classifications on Atlantic sturgeon inhabiting the Albemarle Sound. Genetic samples from adults and juveniles collected during this project enabled refinement of the Roanoke River fall population designation plus showed over 83% of the subadults within the Albemarle Sound are of the Roanoke fall population.

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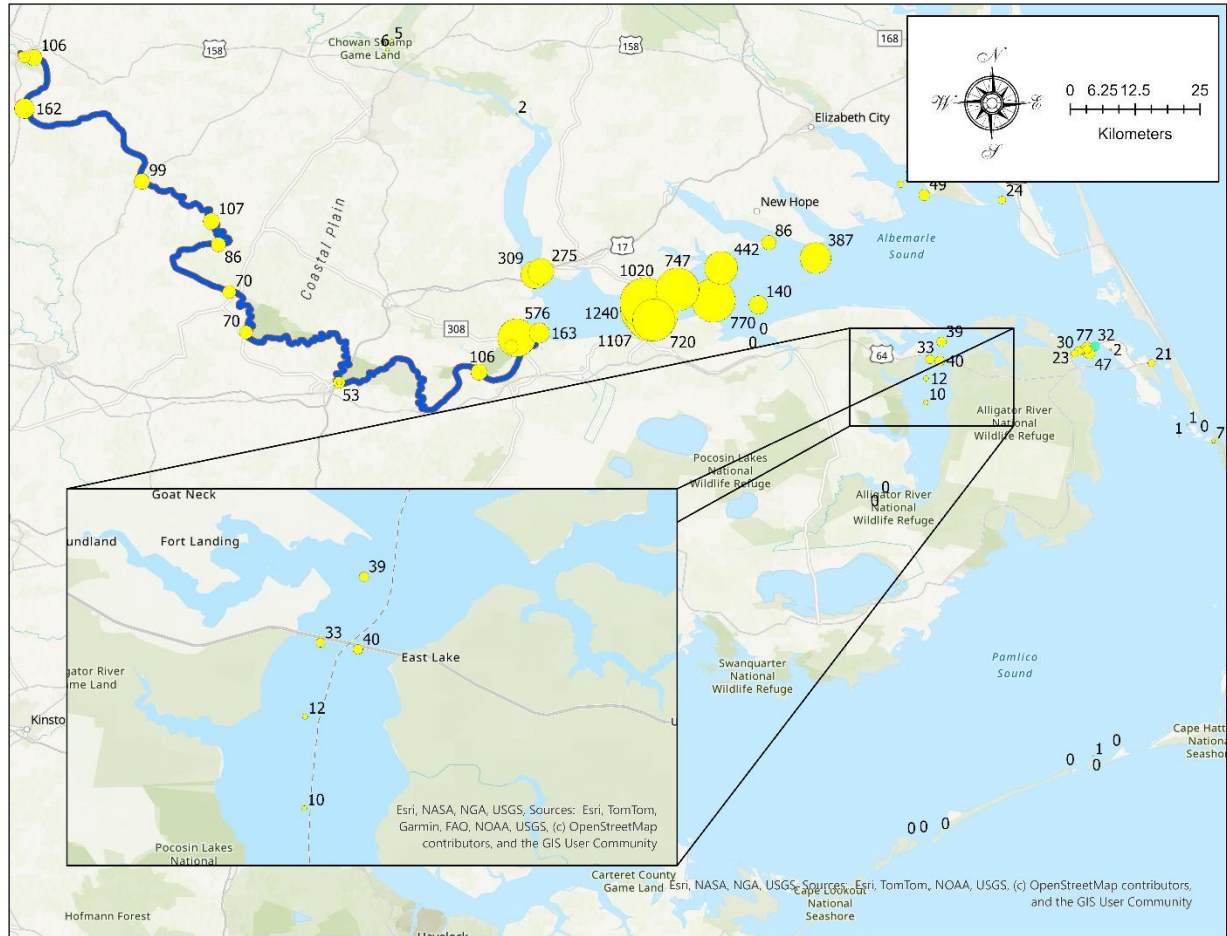


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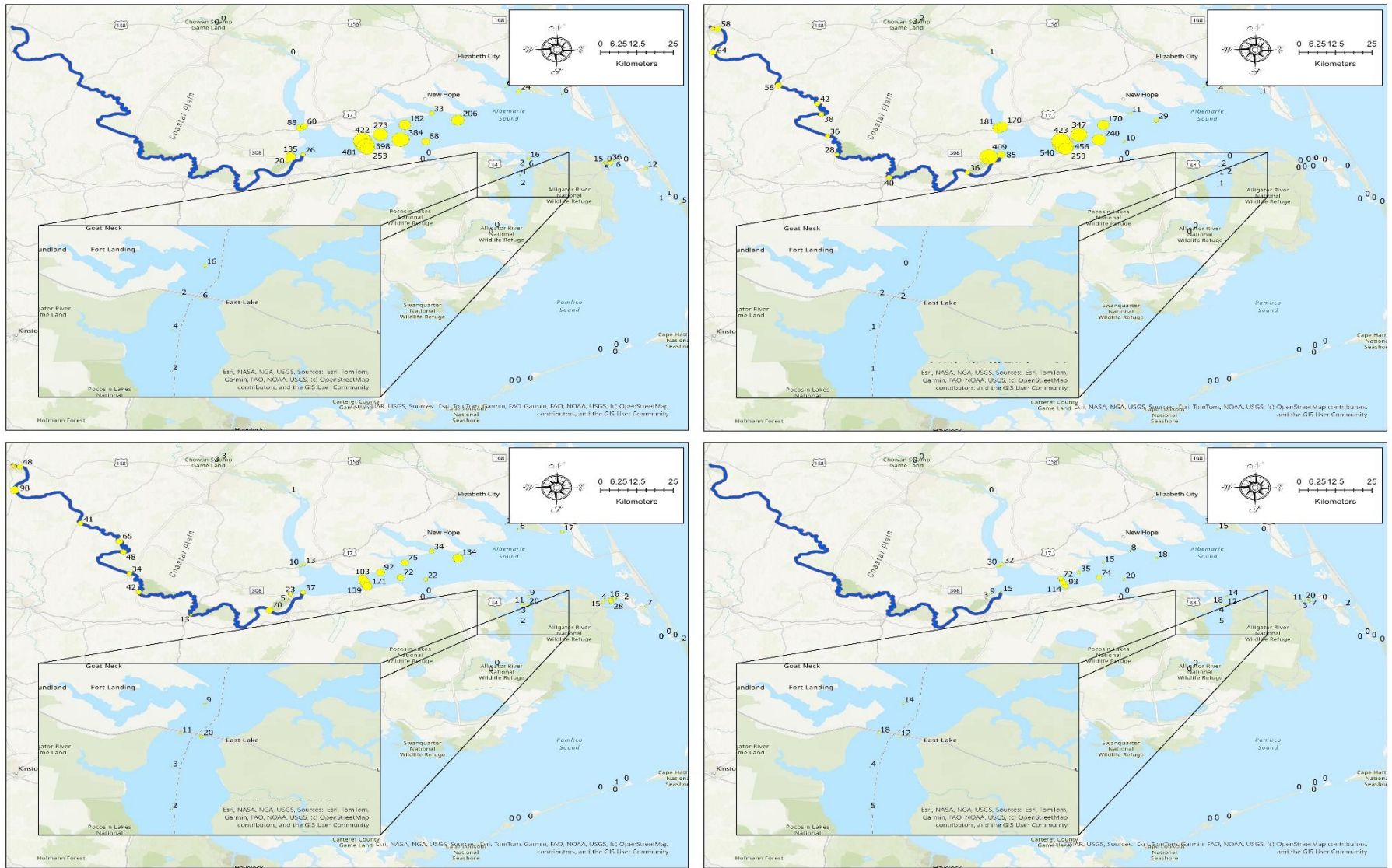


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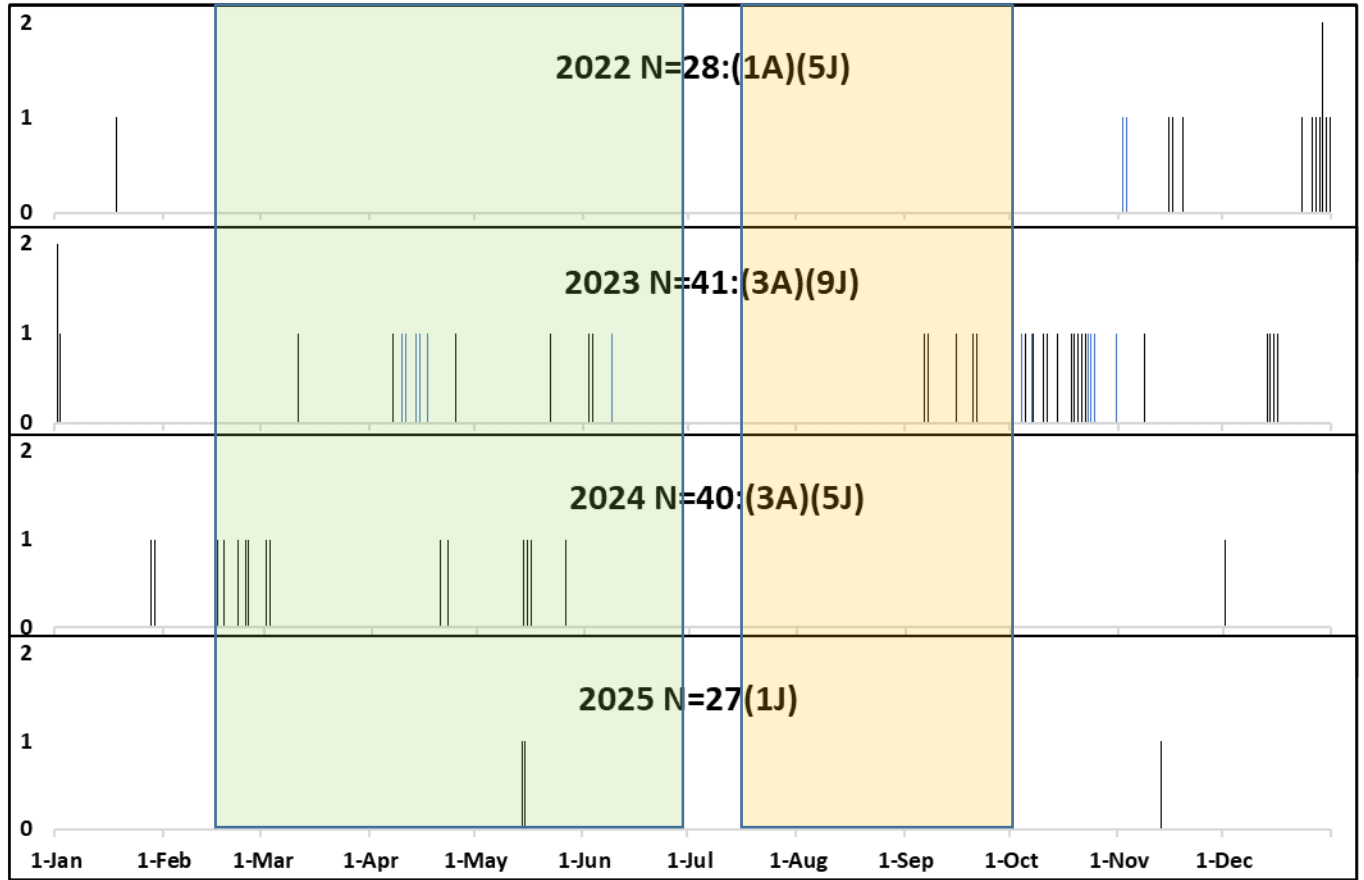


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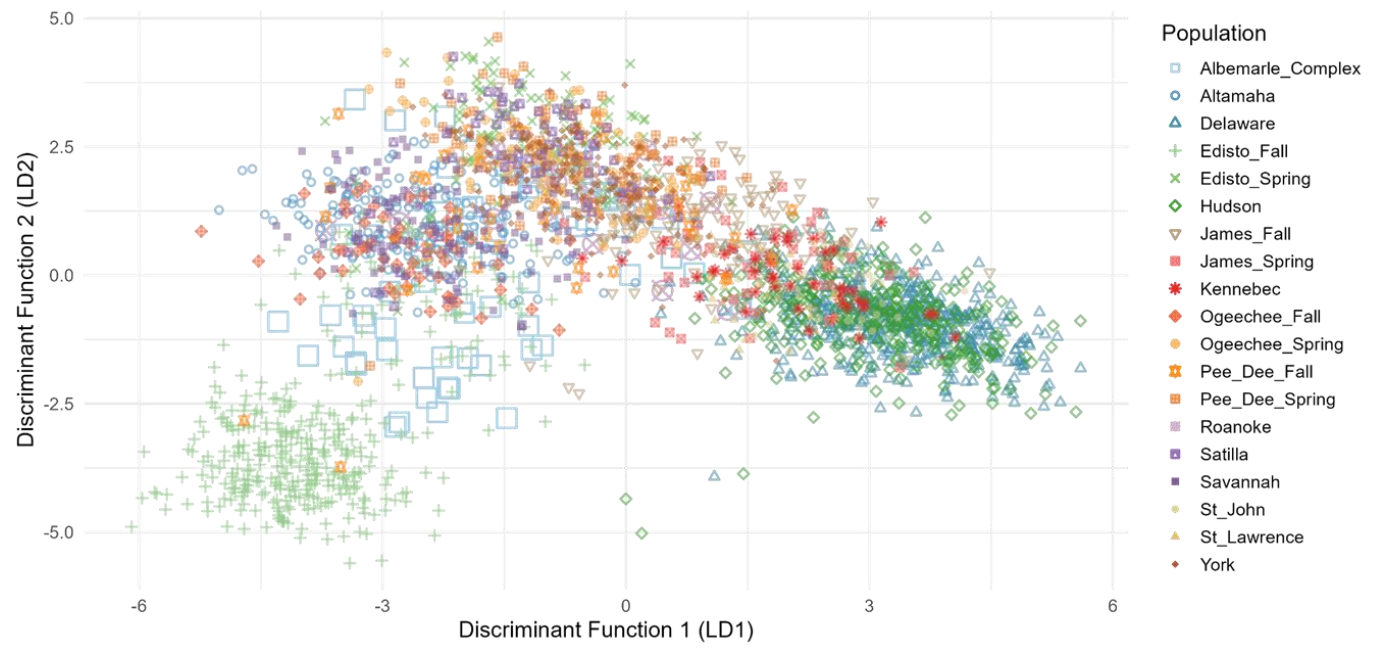


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Table 1. Results of individual-based assignment testing for 202 Atlantic Sturgeon genotyped as part of this study. These results were derived using the White et al. (2021) genetic baseline. The baseline population with the highest likelihood score for each individual is reported below.

Group	<i>n</i>	St John	Hudson	York	James Fall	Albemarle Complex	Pee Dee Spring	Pee Dee Fall	Edisto Fall	Savannah	Ogeechee Spring	Ogeechee Fall	Altamaha	Satilla
Roanoke River (Adult)	27		1		1	21		1	1	2				
Estuarine (Juvenile)	34				2	29	1	1		1				
Estuarine (Subadult)	135				4	112	3	2		5	1	1	6	1
Estuarine (Adult)	6	1	1	1		1				1			1	
Total	202	1	2	1	7	163	4	4	1	9	1	1	7	1

Table 2. Results of molecular sex identification using the *AllSexW2* marker for 202 Atlantic Sturgeon genotyped as part of this study. Individuals designated as unknown either showed inconclusive test results or failed to amplify at control markers.

Group	Female	Male	Unknown	Total
Roanoke River (Adult)	2	24	1	27
Estuarine (Juvenile)	14	18	2	34
Estuarine (Subadult)	55	71	9	135
Estuarine (Adult)	2	3		6
Total	73	117	12	202

Chapter 1. Introduction

1.1 Background

Atlantic sturgeon (*Acipenser oxyrinchus oxyrinchus*) were once abundant along the Atlantic Slope of North America and were culturally important to Native Americans and early European colonists. Throughout the 19th Century, Atlantic Sturgeon were fished unsustainably for roe (caviar) and flesh. The peak coastal harvest of Atlantic Sturgeon (>7 M pounds) occurred in 1890; however, by 1920 annual harvest was less than 100,000 pounds. In 1998 the Atlantic States Marine Fisheries Commission (ASMFC) imposed a moratorium on sturgeon catches. In 2012, most Atlantic sturgeon populations, including the Carolina distinct population segment (DPS), became federally listed as endangered under the Endangered Species Act (ESA).

The aging Alligator River Bridge on US 64, Dare County, NC, is slated to be replaced within the next few years. Currently, data are very limited about if and how Atlantic sturgeon utilize the Alligator River, especially around the US 64 Bridge crossing. Federal and state permitting agencies are concerned that the construction of the new bridge and demolition of the old bridge will harass and/or harm Atlantic sturgeon in proximity to the construction area. The NCDOT and the permitting agencies need more information on the migratory movements of Atlantic sturgeon through the project area when considering the effect to this species from construction of the new Alligator River Bridge.

Discussions between the NCDOT Division 1, Raleigh Environmental Analysis Unit and NC Division of Marine Fisheries (NCDMF) have occurred to address the possibility for an exemption to an in-water work moratorium for the Alligator River Bridge replacement. The reason for this moratorium request by the NCDMF is due to the lack of existing data about when and to what magnitude Atlantic sturgeon are in Alligator River during times of the year. To obtain the needed information required by NCDMF, research must be conducted in the area of interest. A collaboration of NCDMF, NCDOT and Virginia commonwealth University (VCU), propose to start collecting, implanting telemetry tags and monitoring sturgeon movements in the Alligator River.

Atlantic sturgeon utilize the Albemarle drainage watershed for reproduction. Reproduction has been confirmed in the Roanoke River and adults are known to travel up the Chowan River during known spawning seasons. Based on geography and habitat types, there is no hypothesized spawning activity in the Alligator River. However, gill net surveys conducted by NCDMF at the mouth of the Alligator River have encountered juvenile and subadult Atlantic sturgeon. VCU has already tagged some Atlantic sturgeon in the Albemarle Sound that were detected in by NCDMF receivers within the Alligator River. Life-history patterns suggest that both juvenile and subadult Atlantic sturgeon utilize the Alligator River during some time of the year, if not all year, for feeding and development.

1.2 Research Objective and Scope

Fishery biologists with VCU and NCDMF will use proven acoustic telemetry equipment (Innovasea) and field collection methods developed over 15 years of research to monitor Atlantic sturgeon movement patterns. VCU has conducted similar projects focused on large civil infrastructure projects (e.g. Hampton Roads Bridge-Tunnel Expansion, Route 301 Nice Bridge removal in the Potomac) in recent years. The goal of the proposed study will be to document *preconstruction* temporal and spatial patterns of occurrence and movement primarily for tagged juvenile and adult Atlantic sturgeon within the Alligator River with the hope of continued monitoring during both construction and demolition.

1.3 Research Approach

VCU and NCDMF will coordinate with project stakeholders to accomplish the following objectives:

1. Surgically implant appropriately-sized Innovasea acoustic coded transmitters in at least 45 juvenile and 10 adult Atlantic sturgeon within the Albemarle Sound. Tagged fish will be released and tracked using georeferenced acoustic receivers deployed throughout the Albemarle Sound, including the Alligator River; surgery will be performed by experienced biologists using methods approved under VCU's ESA permit (NMFS) and other relevant permits. Anchored gill nets will be used to capture Atlantic sturgeon for tagging. VCU secured all necessary state and federal permits to conduct the work.
2. Establish a passive array of Innovasea in-situ receivers to document the timing and duration of residence and migration activities by Atlantic sturgeon through the project area. Receivers will be deployed throughout the Albemarle Sound. Receivers will be downloaded as necessary to keep biofouling to a minimum.
3. Evaluate the possible use of the Alligator River as a staging corridor or other essential habitat for Atlantic sturgeon, including specific periods of residence within the project area based on movements by tagged individuals. Geocoded data documenting Atlantic Sturgeon within the array will be incorporated into a GIS-based analysis (ArcGIS Pro 3.7.0) to determine temporal and geospatial patterns of distribution, residence, and habitat associations for sturgeon in the project area.
4. Coordinate with the United States Geological Survey (USGS) to conduct genetic analysis on fin clips collected during the project along with historical samples collected in the area.

Chapter 2. Acoustic Telemetry Monitoring of Atlantic Sturgeon within the Albemarle Sound, North Carolina

2.1 Methods

[2.1] Tagging and Telemetry

From 2021 to 2026, a total of 164 sturgeon were captured within the Albemarle Sound Complex (Figure 1). Surgical protocols followed used electronarcosis and are described in Balazik (2015) and approved by NOAA/NMFS. There were 32 fall adults captured in the upper Roanoke River, of which 22 were telemetered. All 22 tags in adults were V16s with a battery life of 3650 days. The 132 juveniles were mostly captured in the Western Albemarle with six being captured at the Alligator River Bridge (Figure 1). Juvenile sampling wasn't conducted at the Alligator River Bridge in 2025 or early 2026 as to refrain from potentially interfering with construction activities. A total of 60 juveniles were telemetered. Ten of the larger juveniles were internally tagged with V16s having a battery life of 3650 days, ten were externally tagged with V9 tags having a battery life of 912 days, and 40 were internally tagged with V9 tags having battery lives varying from 855 to 912 days. The ten external tags were attached following the "Spaghetti" method described in Runde et al, (2022).

Telemetered fish were tracked throughout the Albemarle Complex using an array of about 55 receivers, five of which were in the Alligator River (Figures 2,3). Three of the receivers in the Alligator River were on the closest Coast Guard ATONs to the bridge and two were anchored roughly 1.5km apart along the south side of the current bridge. Based on typical range detections of over 1km, usually more in similar environments as the Alligator River, the two receivers along the bridge provided coverage along the entire bridge except for possibly the shallow portion of the east side. The receivers on the Coast Guard ATONs provided over 60% coverage of the river width and 100% of the channel area where sturgeon tend to inhabit.

2.2 Results

During the study period, the tagged juveniles spent a combined total of 15,816 days within the Albemarle Sound with 54 days (0.3%) being within the Alligator River Array (Figure 2,3). The tagged adults were within the Sound 6,970 days and were detected a combined 16 days (0.2%) within the Alligator River. Twenty individual juveniles and six adults were detected within the receiver array deployed within the Alligator River. This project was designed to monitor sturgeon presence prior to any construction activities; however, from February 18, 2025, to February 12, 2026, pile driving operations for bridge support bents occurred at the Alligator River Bridge area. Unfortunately, by this time the majority of the juvenile telemetry tag batteries were no longer active or the fish had left the Albemarle system with offshore detections ranging from New York to Georgia. It was also a dry year in 2025 and juveniles and adults tend to inhabit areas close to the salt wedge which was in the very western part of the Albemarle Sound. However, one juvenile tagged on December 14th, 2023, was detected at the two receivers adjacent to the Alligator Bridge on May 13th, May 15th, and November 13th, 2025. Pile driving did not occur during May 13th but did occur during May 15th and November 13th. Fine scale fish positioning is not available based on the receiver positions. Pile driving occurred at two different locations on May 15th and three different locations on November 13th. Based on tag detection range and pile driving location, the juvenile was likely within 2km of pile driving operations. The fish did not

immediately leave the area at the onset of pile driving operations, but spent several hours in the area before leaving. Survival was verified as the fish was later detected in the western Albemarle Sound.

External Tags

The ESA permit for the Albemarle Complex limits that number of juvenile internal surgeries which is why experimental external tags were tested in an attempt to increase the sample size. Detection data from the external tags was disappointing. The longest movements were detected for an external tag was 607 days and was last detected in the Albemarle Sound suggesting the tag fell off around that time. The remaining nine tags ranged from 99 to 187 days, eight of which were last detected in the Albemarle Sound suggesting the tags fell off soon after the last detection. One external tag was detected off Delaware after 187 days at large. The juveniles with internal tags were detected for 110 to 934 days with an average of 754 days.

Telemetry Conclusion

Based on the three years of tracking, most Atlantic sturgeon inhabit the Western Albemarle throughout the year with a small increase around Manteo for fish briefly migrating in and out of the Albemarle Sound. While juvenile sturgeon do inhabit the Alligator River, their presence is scarce. Adult sturgeon are also scarce in the Alligator River, spending just a day or two near the river's mouth while migrating to the Western Albemarle or heading back to the ocean. The Alligator River is not a commonly used staging or feeding area within the Albemarle Sound and impacts, if any, caused by construction activities within the Alligator River are minimal at the most.

Chapter 3. Genetic Characteristics of Atlantic Sturgeon from Albemarle Sound and the Roanoke River.

3.1 Statement of Need

Atlantic Sturgeon are a federally endangered species which are broadly distributed across eastern North America. Conservation of Atlantic Sturgeon is complicated by extensive estuarine and marine movements, resulting in mixing of stocks and exposure to threats far from natal habitats. Genetic analysis is the only way to distinguish individuals from different stocks.

The genetics of Atlantic Sturgeon within the Carolina region is poorly understood, which is a consistent challenge when conducting genetic stock assignments to support state, interstate, and federal management of the species. The Roanoke River is thought to be home to a spawning population (and samples have recently been collected), but these samples have not been genotyped and analyzed. In addition, we do not understand the stock composition of Atlantic Sturgeon within Albemarle Sound, which presents problems for proposed construction activities (such as the planned bridge over the Alligator River) and impairs coastwide conservation efforts.

3.2 Objectives

(1) Characterize the genetics of adults returning to the Roanoke River and compare/contrast with other known populations. This is necessary to help evaluate the status of Atlantic Sturgeon in the Roanoke River and guide refinement of the genetic baseline.

(2) Conduct a genetic assessment of juvenile and subadult Atlantic Sturgeon collected in Albemarle Sound, with an emphasis on individuals captured in the vicinity of the Alligator River bridge. This information will improve our understanding of sturgeon ecology and support ESA consultations in the southeast region.

3.3 Outcomes

The project was divided into two tasks: (1) microsatellite genotyping and (2) analysis and interpretation of population genetic data.

Phase 1. Microsatellite genotyping

We reviewed our inventory of samples within the Atlantic Coast Sturgeon Tissue Research Repository to identify suitable samples to meet the project objectives. We included all available samples of adults ($n = 28$) collected in the Roanoke River for evaluation as a potential spawning population for potential incorporation into our genetic baseline. We also selected samples collected from estuarine habitats in North Carolina which represented a range of size classes, including: (1) juveniles from Albemarle Sound (<500 mm total length; all available samples from Balazik or Godwin; $n = 37$), (2) subadults from Albemarle Sound (500-1499 mm total length; randomly selected subset collected by Balazik or Godwin; $n = 139$), and (3) adults collected in inshore North Carolina waters which had not previously been genotyped (≥ 1500 mm total length; all available samples from mixed sources; $n = 6$). In aggregate, these selections comprised a total sample size of 210 Atlantic Sturgeon which were pulled for extraction and genotyping. The vast majority of these animals ($n = 202$) were successfully scored at ≥ 6 microsatellite loci and retained for subsequent analysis.

In conjunction with microsatellite genotyping, we screened each sample using the AllWSex2 marker, which has previously been validated for sex identification in Atlantic Sturgeon (Sard et al. 2024). Among the 202 individuals which were successfully genotyped at ≥ 6 microsatellite loci and retained for further analysis, we were able to derive sex identifications for 190 individuals.

Phase 2. Analysis and interpretation

Evaluation of samples from the Roanoke River

A total of 27 samples from adults collected within the Roanoke River were genotyped at enough loci to support this analysis. We included two individuals who were retained for analysis which were slightly smaller than the 1500 mm minimum total length threshold that our lab generally applies to identify adults. This choice was motivated by the limited number of samples available to characterize a potential spawning population in the Roanoke River, as well as a general understanding that populations in the southeastern region are likely to mature at smaller sizes.

Conformance to Hardy-Weinberg Equilibrium expectations

The adult samples from the Roanoke River showed no statistically significant deviations from Hardy-Weinberg equilibrium expectations at any of the 12 loci (all p-values exceeded 0.05). This is consistent with these samples reflecting a spawning population, however the statistical power of this test is likely

limited by the sample size. We would likely only be able to detect large deviations from the theoretical distributions of genotypes expected from a large, randomly mating population.

Relationship to previously documented populations

We took a multi-faceted approach to compare adult samples from the Roanoke River to other populations in our established genetic baseline. This included comparisons using individual-based assignment tests, discriminant analysis of principal components, and preliminary STRUCTURE analysis.

Individual-based assignment tests were conducted using GeneClass2. This approach assumes that an individual originated from one of the baseline populations – an assumption which would be violated if these samples from Roanoke River represent an undocumented spawning population – but can provide insight into the affinity of the new samples to known populations. With this approach, most of the Roanoke River adults assigned to the Albemarle Sound reference collection from White et al. (2021; Table 1). The remaining samples were assigned across 5 other baseline populations, including spawning populations documented in the Hudson and Savannah rivers. This result could suggest that the Roanoke River adult sample contains some individuals natal to other rivers (White et al. 2024) – a phenomenon documented in the Hudson and Delaware rivers that may be more widespread.

Discriminant analysis of principal components was conducted using the adegenet package in R (Jombart 2008; Jombart and Ahmed 2011; Figure 4). The new samples from the Roanoke River ordinated in a similar space to many of the individuals in the Albemarle Complex collection. However, the Albemarle Complex collection was distributed over a broader area than the Roanoke River samples, suggesting that Albemarle Complex collection contains greater diversity across individuals– perhaps due to the inclusion of multiple biological populations.

STRUCTURE (Pritchard et al. 2000) provides a complimentary analysis which organizes samples into clusters that best conform to Hardy Weinberg equilibrium. Although we ran out of time to complete a robust analysis for inclusion in this report, several preliminary STRUCTURE runs were completed for initial data exploration (not shown). Initial results show affinity between most of the Roanoke River adults and the Albemarle Complex and Pee Dee (spring) collections. Given that we anticipate the Albemarle Complex collection likely includes many individuals from a Roanoke River population, this affinity is not unexpected. In addition, the spawning habitats of the Pee Dee (spring) population are relatively close geographically to the Roanoke River. Other documented populations show a clear pattern of isolation-by-distance (White et al. 2021).

We look forward to discussing these preliminary results and examining outputs from longer and more focused STRUCTURE runs to reach a more thorough understanding of the putative Roanoke River population.

Sex ratios

Among the adult samples from the Roanoke River, the sex ratio was strongly skewed towards males (Table 2). Similarly skewed sex ratios have been reported in other known spawning populations of Atlantic Sturgeon (Hilton et al. 2016). This likely reflects a combination of more frequent return intervals, earlier maturation, and longer residency in freshwater spawning reaches for males (Hilton et al. 2016).

Genetic stock identification of Atlantic Sturgeon from Albemarle Sound

We also conducted individual-based assignment testing for all the juvenile, subadult, and adult samples collected within Albemarle Sound (Table 1). The vast majority of juveniles and subadults assigned to the Albemarle Complex collection. Additional assignments to the James (fall), Pee Dee River (spring and fall), and Savannah River populations were also observed. We also detected subadults from four additional populations further to the south. Among the six adults genotyped from estuarine waters, only a single individual showed affinity to baseline populations from the Carolina DPS. The remaining individuals appear to represent spawning populations from across the native range of the species (note: the individual which assigned to the St John River showed some affinity to other, more geographically proximate populations – this result should be interpreted with caution). These results will be updated as appropriate pending further assessment of the Roanoke River adult samples for inclusion as a baseline reference collection.

Sex ratios in Atlantic Sturgeon from Albemarle Sound

We were able to score 164/175 individuals using the AllWSex2 marker (Table 2). Among those animals, males (n = 93) were observed more frequently than females (n = 71). Sex ratios were similar among juveniles (0.78 females per male) and subadults (0.77 females per 1 male), with a stronger skew observed among adults (1 female per 2 adults; but note the modest sample size).

Chapter 4. Implementation and Technology Transfer Plan

This study showed that Atlantic sturgeon rarely utilize the Alligator River for juvenile feeding or adults staging to spawn. While limited, the study also showed that the one sturgeon that was within 2km of pile-driving did not immediately flee the area and survived as it was detected afterwards. These data suggest that precautionary principle restrictions on construction activities may need to be reconsidered and/or be proven needed by science.

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