



Integrating genetic and demographic data to refine indices of abundance for Atlantic sturgeon in the Hudson River, New York

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ABSTRACT: Critical to Atlantic sturgeon *Acipenser oxyrinchus oxyrinchus* recovery and monitoring is the ability to estimate abundance and identify age- and stock-specific threats to survival. As adult Atlantic sturgeon spend much of their lives broadly distributed in marine and estuarine environments, it is challenging to collect data needed to estimate these demographic parameters in the adult population. Alternatively, data collected from juveniles and subadults before emigration may be used to calculate indices of abundance and provide insights into recruitment dynamics and stage-specific survival. However, uncertainty about stock mixture during early life stages may limit the use of juvenile and subadult data for monitoring recovery. To better understand early life stage stock composition, we conducted a genetic mixed-stock analysis of over 500 juvenile and subadult Atlantic sturgeon captured in an overwintering area in the Hudson River, New York, USA, from 2017 to 2022. The majority of Atlantic sturgeon in our study were natal to the Hudson River population, regardless of sex, size, or age. As such, indices of relative abundance estimated from survey data are expected to primarily characterize the demographic trends of Hudson River juvenile and subadult Atlantic sturgeon. We also found a small proportion of individuals that were most likely to have originated from more distantly located rivers, highlighting the potential for long-distance migration in juvenile and subadult Atlantic sturgeon. Results of this study strengthen our understanding of juvenile and subadult Atlantic sturgeon habitat use in the Hudson River and improve our ability to use data from early age classes to monitor recovery and stage-specific survival.

KEY WORDS: Atlantic sturgeon · Mixed stock · Age · Juvenile · Subadult · Abundance

1. INTRODUCTION

Atlantic sturgeon *Acipenser oxyrinchus oxyrinchus* is an anadromous species that is broadly distributed on the east coast of North America from Labrador, Canada, to Florida, USA (Hilton et al. 2016, ASMFC 2017). Intense commercial fishing for Atlantic sturgeon resulted in the collapse of many populations in the late 1800s, but harvest continued throughout much of the 20th century despite severely reduced landings (Dadswell 2006, Hilton et al. 2016). By the

1990s, many states had enacted harvest moratoria for Atlantic sturgeon in inland waters, eventually culminating in a 40 yr moratorium by the Atlantic States Marine Fisheries Commission (ASMFC 1998). Following limited recovery, Atlantic sturgeon was listed under the United States Endangered Species Act (ESA) in 2012 (NMFS 2012a,b). As part of this listing, populations were divided into 5 distinct population segments (DPSs) representing populations that were thought to share similar physiological and genetic characteristics (Fig. 1).

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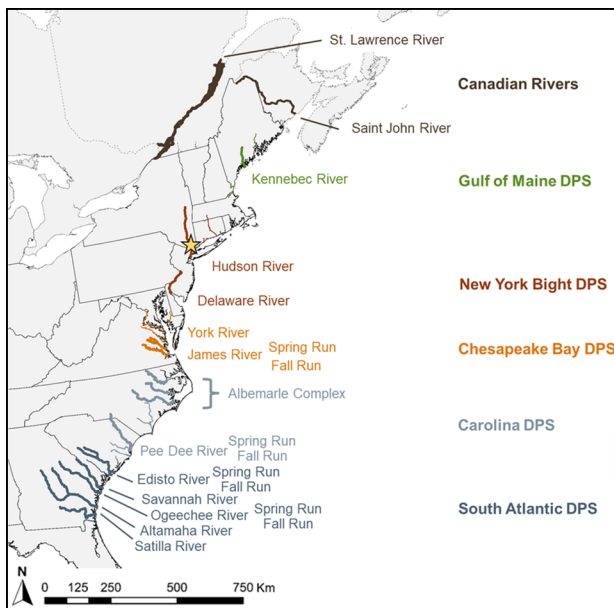


Fig. 1. Distribution of the 18 Atlantic sturgeon spawning populations in the genetic baseline used for individual-based assignment tests, including 4 rivers with genetically distinct spring and fall spawning runs. Populations color-coded by distinct population segment (DPS). Yellow star: approximate location of monitoring surveys on Haverstraw Bay ($41^{\circ} 14' 22.704''$ N, $73^{\circ} 58' 6.708''$ W)

Estimates of juvenile and/or adult abundance were identified as among the highest priority research needs in the most recent Atlantic sturgeon stock assessment (ASMFC 2017). However, abundance estimates are difficult to obtain for the species, as adults spend much of their time broadly distributed in marine and estuarine environments that are difficult to sample and may be located thousands of kilometers away from their natal rivers (Wirgin et al. 2015, Kazzyak et al. 2021). Individuals also temporarily occupy non-natal tributaries and frequently form mixed-stock aggregations (Dunton et al. 2012), making it challenging to reliably collect demographic data on a single population of interest. Difficulty sampling and uncertainty about individual natal origin generally confound the use of traditional population estimation techniques (e.g. mark–recapture, removal, etc.) for determining Atlantic sturgeon abundance, limiting the ability to monitor demographic trends or gauge the efficacy of conservation actions.

Recent studies have used non-traditional approaches for adult Atlantic sturgeon population estimation, including genetic pedigree reconstruction (White et al. 2022) and multi-method field surveys (Kazzyak et al. 2020). While informative, these methods are resource-intensive, requiring a large number of

individuals to be genotyped and/or significant time spent collecting and processing data. Therefore, it is not always feasible to apply these techniques to estimate population size, particularly for historical datasets. They also have not been extended to estimates of juvenile or subadult abundance, and so questions about stage-specific threats to survival remain. As such, indices of abundance calculated from survey data remain the primary method for monitoring demographic trends in early life stages. These surveys, which include a mixture of juveniles (<500 mm total length [TL], representing fish approximately 0 to 5 yr of age) and subadults (500 to 1500 mm TL or approximately 5 to 20 yr of age; Hilton et al. 2016), are intended to capture sturgeon prior to ocean migration. As such, it is assumed that individuals are natal to the focal river or estuary of interest. However, while others have shown that the probability of juvenile emigration is generally low (Dovel & Berggren 1983, Hilton et al. 2016), recent studies have highlighted the ability of subadult Atlantic sturgeon to engage in long-distance migrations (Savoy & Pacileo 2003, Laney et al. 2007, Melnychuk et al. 2017). This suggests the potential for non-natal Atlantic sturgeon to be present at the time of juvenile and subadult surveys. Unknowingly sampling an open population comprising individuals from multiple river systems could result in substantial bias in abundance indices, ultimately limiting the utility of survey data.

Application of juvenile and subadult abundance indices to population monitoring is also complicated by a general uncertainty about the age of emigration from natal rivers (Fox & Peterson 2019). Generally, capture probability declines for older, larger subadult Atlantic sturgeon in inshore surveys. However, it is unclear whether this is due to emigration, low survival, and/or gear/size selectivity within the survey. As such, a better understanding of movement behavior of juvenile and subadult Atlantic sturgeon would increase the interpretability of long-term survey data to estimate population trends and identify age- and sex-specific threats to survival.

The continuation of existing fishery-independent surveys to monitor juvenile abundance as well as an improved understanding of migratory patterns has also been emphasized as a high-priority research need in the recent stock assessment for Atlantic sturgeon (ASMFC 2017). To aid in this research need, the objective of this study was to conduct a genetic mixed-stock analysis of juvenile and subadult Atlantic sturgeon captured during long-term survey efforts in the Hudson River at Haverstraw Bay, New York, USA. From this analysis, we aimed to improve the utility of abundance indices by determining whether

the likelihood of being natal to the Hudson River was correlated with individual age, size, and/or sex. Results of this analysis strengthen our understanding of juvenile and subadult Atlantic sturgeon habitat use in the Hudson River and improve our ability to monitor demographic recovery in early age classes.

2. MATERIALS AND METHODS

2.1. Sample collection and selection

Each year since 2004, the New York State Department of Environmental Conservation (NYSDEC) conducts a targeted juvenile and subadult monitoring survey at Haverstraw Bay (41° 14' 22.704" N, 73° 58' 6.708" W; approximate river kilometers 56–63), which is a known overwintering area for Atlantic sturgeon in the Hudson River (Dovel & Berggren 1983, Sweka et al. 2007). This survey is one of the first to establish relative abundance estimates of juveniles following recovery recommendations set forth by Amendment 1 to the Interstate Fishery Management Plan for Atlantic sturgeon (ASMFC 1998). Moreover, it represents one of the only long-term surveys specifically designed to monitor recruitment for Atlantic sturgeon population recovery (ASMFC 1998). Data from the annual surveys are used by the NYSDEC to estimate an annual relative abundance index, evaluate population trends over time, and measure recruitment strength (Sweka et al. 2007, Pendleton & Adams 2021).

Surveys are conducted from late February through April or early May. Juvenile and subadult Atlantic sturgeon are captured using anchored gill nets of dif-

ferent stretch mesh sizes (76, 102, and 127 mm). Biological information (e.g. TL, weight) is recorded for each individual, and a passive integrated transponder (PIT) tag is implanted in the musculature under the dorsal fin on the left side of the fish if no previous tag is detected. Additionally, a small tissue sample from the dorsal fin (i.e. fin clip) is removed and stored in 95% ethanol for genetic analyses. For a more detailed description of monitoring protocols, see Pendleton & Adams (2021). All sampling and handling procedures follow established research protocols that are authorized by the National Marine Fisheries Service (NMFS) Research Permit #20340.

In 2017, the NYSDEC also began collecting segments of fin spines and rays from Atlantic sturgeon to better understand the age structure of Atlantic sturgeon encountered during annual monitoring. A fin spine (defined here and by the ASMFC's Sturgeon Ageing Subcommittee) is the hard, bony structure at the leading edge of the fin, whereas the fin ray is the first segmented structure (i.e. ray) behind the fin spine (Fig. 2). A fin spine and/or ray was collected from a subset of individuals by separating the fin spine and ray from adjacent rays with a scalpel and cutting pliers. Samples were stored in scale envelopes and allowed to air-dry before being mounted in epoxy and sectioned 6 to 8 times at various widths (0.2 to 0.5 mm) using a low-speed precision sectioning saw and fixed to a glass slide with mounting adhesive (Crystalbond).

We used a stratified random sample to select at least 65 fin clips to genotype from each sample year from 2017 to 2022. When selecting samples, we prioritized tissues from individuals which also had a pectoral fin structure that could be used for aging. In ad-

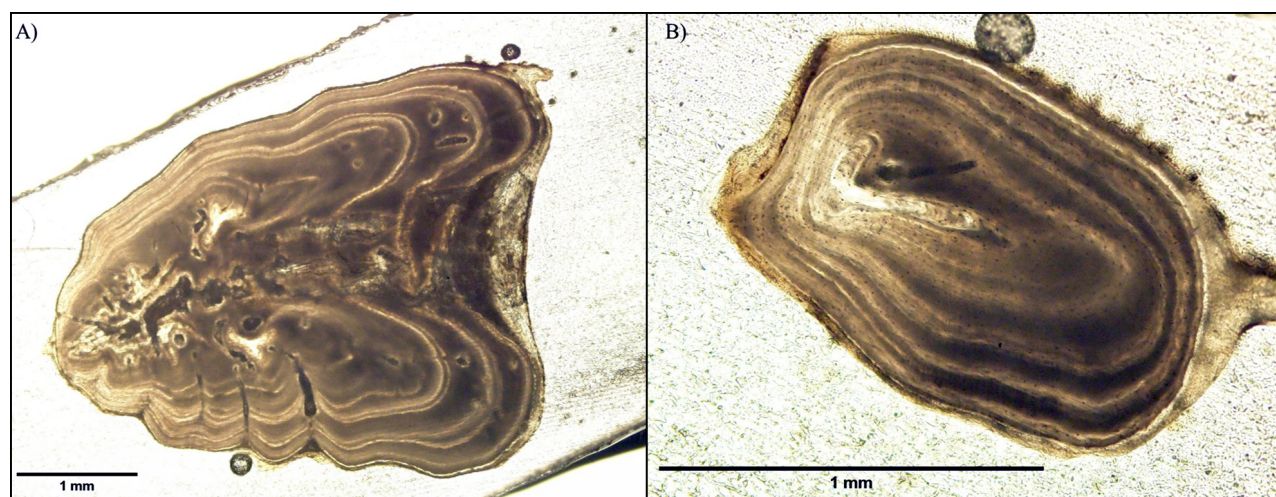


Fig. 2. Cross-section of a (A) fin spine and (B) fin ray and corresponding annuli for 2 Atlantic sturgeon estimated to be 4 and 5 yr old, respectively

dition, we noted that 4 individuals originally selected for our analysis were recaptured within 2 yr in the lower Connecticut River, Connecticut, USA, representing a displacement of over 200 km. Upon further review of our data, we identified 9 additional individuals that were originally captured in Haverstraw Bay from 2015 to 2019 but were later recaptured in the Connecticut River. Therefore, to gain a better understanding of dispersal in early life stages, we added these 9 individuals to our genotyping effort. In total, we selected 509 fin clips for genetic analyses, of which 426 had an accompanying aging structure (Table 1). Due to limited sampling of aging structures in 2020 and 2022, individuals without a pectoral fin structure were included to increase the sample size for microsatellite genotyping.

2.2. Age determination

Pectoral fin structures collected from 2017 to 2022 were aged by 2 independent readers using a stereozoom microscope (Leica Stereozoom S9i) following aging protocols defined by Kehler et al. (2018). When the readers did not agree on an age for a particular individual, both readers reexamined the sections and reached a consensus age. If a fin ray was not available for aging, then we determined age from the fin spine. When possible, consensus age was based on the value determined from both structures. Notably, for 220 individuals that were aged using both spines and rays, the age was consistent between structures for 114 fish (51.8%) and were within 1 yr in an additional 98 (45.5%) individuals. Thus, estimated ages were either congruent or within 1 yr for 96.4% of individuals. For

the 106 individuals where there was an age discrepancy (ranging from 1 to 3 yr), the age estimated from the fin spine was higher in 82% of cases relative to the fin ray. When fin rays had less annuli relative to the fin spine, the fin ray was often elongated and annuli were hard to discern. Despite this, hard to discern (e.g. faint or false) annuli on one structure were sometimes easily identifiable in the other structure (or absent in the case of false annuli); thus, we considered both structures to provide reasonable estimates of individual age. Similar findings between the 2 age structures have also been reported in other studies (Izzo et al. 2021, Gragson & Fox 2022).

We used the R package fishR v.0.9.5 (<https://github.com/fishR-Core-Team/FSA>) to generate an age-length key based on the aged samples ($n = 426$). We assumed that patterns in growth remain relatively stable over time and so we pooled ages among years when generating and applying the key. Fish that were not aged (i.e. individuals collected in Haverstraw Bay during spring 2004–2022 but not included in our analysis) were assigned ages using the semi-random age assignment which assigns ages proportionally based on the distribution/count of aged individuals among user-defined length bins, defined here at 100 mm increments. These assigned ages provided age estimates for individuals in this study for which an aging structure was not collected.

2.3. Microsatellite genotyping and molecular sex identification

DNA extractions and microsatellite genotyping for this study were performed at the US Geological Survey Eastern Ecological Science Center in Kearneysville, West Virginia, USA. Genomic DNA was isolated from Atlantic sturgeon fin clips (Gentra Puregene Reagents; Qiagen) according to the manufacturer's protocols. This process started with incubation overnight using Proteinase K (Agilent); RNA was removed with RNase-IT Ribonuclease Cocktail (Agilent). The resulting DNA was quantified using a fluorometer (Qubit 2.0 Fluorometer; Thermo Fisher Scientific) and diluted to approximately $25 \text{ ng } \mu\text{l}^{-1}$ prior to amplification by PCR.

DNA samples were genotyped with a panel of 12 microsatellite loci (*LS19*, *LS39*, *LS54*, *LS68*, *Aox12*, *Aox23*, *Aox45*, *AoxD44*, *AoxD165*, *AoxD170*, *AoxD188*, *AoxD241*; May et al. 1997, King et al. 2001, Henderson-Arzapalo & King 2002). The sex of each individual was also identified by adding the AllWSex2 primer (Kuhl et al. 2021) which amplified a female sex-specific marker. Markers were amplified in 4 separate multi-

Table 1. Number of Atlantic sturgeon from Hudson River that were genotyped and aged for each year of analysis. Samples from 2015 and 2016 were included to investigate natal origin of subadult Atlantic sturgeon that were originally captured in Haverstraw Bay but later recaptured in Connecticut River

Year	No. of individuals genotyped	No. of genotyped individuals that were aged
2015	2	0
2016	1	0
2017	100	98
2018	99	94
2019	98	90
2020	69	37
2021	70	68
2022	70	39
Total	509	426

plex PCR reactions: Multiplex 1 (*LS19/LS68/Aox23*), Multiplex 2 (*LS54/Aox45/AoxD241*), Multiplex 3 (*LS39/AoxD170*), and Multiplex 4 (*Aox12/AoxD44/AoxD165/AoxD188/AllWSex2*). Each multiplex PCR consisted of 1.5 µl diluted genomic DNA, 1X Qiagen Multiplex PCR Master Mix (Qiagen), 0.11–0.53 µM each primer (forward primers labeled with fluorescent tags), and molecular grade water for a total volume of 15 µl. We used a T100 thermal cycler (BioRad) for the following procedure: initial denaturing at 95°C for 15 min; 35 cycles of 94°C for 30 s, 58°C for 90 s, 72°C for 90 s; and a final extension at 72°C for 10 min. The annealing temperature for Multiplex 3 was lowered to 50°C.

PCR products were diluted and run on an ABI 3500 Genetic Analyzer (Thermo Fisher Scientific) using an internal size standard (LIZ-500) (Thermo Fisher Scientific). Multiplex 1, Multiplex 2 + Multiplex 3, and Multiplex 4 were each run separately. Alleles for each sample were binned and scored using Genemapper v6.0 fragment analysis software (Thermo Fisher Scientific). All microsatellite scoring was automated and then checked by eye.

We used amplified fragments with the AllWSex2 primer to assign individual sex using protocols modified from Kuhl et al. (2021). This sex-specific locus appears to be conserved across many sturgeon species but may need to be refined with Atlantic sturgeon. In a previous study, we found high concordance between genotypic and phenotypic sex assignments for both female and male individuals but found some known male sturgeon to exhibit weak amplification at the locus (Sard et al. 2024). Therefore, we classified an amplified product that was very strong in intensity (> 26 000 relative fluorescence units [RFU] on the ABI3500 instrument) as belonging to a female. A sample was classified as a male if the fragment did not amplify or was weak in intensity (< 8000 RFU). Samples with amplified peaks between 8000 and 26 000 RFU were classified as individuals of unknown sex. Individuals were also classified as unknown sex if the other markers in the Multiplex 4 did not amplify well.

A positive control sample (DNA of known multi-locus genotype and female sex) was included on each PCR plate for verifying PCR amplification success and for checking correct binning success in the fragment analysis software. We also included a negative control sample (no DNA) on each PCR plate to check for contamination. PCR amplifications were redone on all samples with missing data due to weak or unamplified alleles, with all repeated amplifications performed as single loci and not as a multiplexed PCR. The only exception was the repeated PCRs with the sex marker which was done in multiplex as a control for PCR

amplification. Using these methods, we were able to genotype all individuals at all 12 microsatellite loci.

2.4. Individual-based assignment tests

We estimated individual natal origin by performing individual-based genetic assignment tests in the program GeneClass2 (Piry et al. 2004) using the Bayesian assignment method described by Rannala & Mountain (1997). This analysis uses allele frequency distributions to determine the likelihood that an individual originated from each of the 18 populations (P) represented in the genetic baseline described by White et al. (2021a). Briefly, this baseline includes all major spawning populations of Atlantic sturgeon that had been identified at the time of publication, including 4 rivers with genetically distinct spring and fall spawning runs (Fig. 1).

From population-specific assignment likelihoods, the assignment score for an individual i to the Hudson River was determined as follows:

$$score_i = \frac{L_{j,Hudson}}{\sum_{j=1}^P L_{i,j}} \quad (1)$$

where $L_{j,Hudson}$ represents the likelihood of an individual assigning to the Hudson River population, and the denominator is the sum of the assignment likelihoods to each of the 18 populations in the genetic baseline. Thus, values for $score_i$ range from 0 to 1, with higher values representing a higher likelihood of being natal to the Hudson River population and lower values suggesting a natal origin to a non-Hudson population.

2.5. Statistical analysis

We used a beta regression to determine if assignment scores were related to fish length, sex, or sample year. Age was excluded from this analysis, as it was found to be collinear with TL ($r^2 = 0.72$, Fig. A1 in the Appendix), was not available for all fish, and was likely estimated with greater uncertainty than length. To meet model assumptions, assignment scores were first transformed using the equation:

$$[score_i \times (n - 1) + 0.5]/n \quad (2)$$

where n is the total sample size (Smithson & Verkuilen 2006). Individuals with unknown sex ($n = 4$) and length ($n = 1$) were excluded from the analysis. All regressions were run in the program R (R Core Team 2022) using the betareg package v.3.1-4 (Zeileis et al. 2016).

2.6. Mixture analysis

Individual-based assignment tests provide the likelihood that an individual originated from the Hudson River population. With this analysis, some individuals may assign with low likelihood which could increase uncertainty when trying to estimate the number of individuals that originated from a specific population of interest. Therefore, to estimate the proportion of individuals that originated from each population in the genetic baseline, we used ONCOR (Kalinowski et al. 2007) to run a mixture analysis. Mixture confidence intervals were calculated using the method of Rannala & Mountain (1997) based on 1000 bootstraps.

3. RESULTS

The average TL and age of individuals included in our analysis was 655 mm (range: 278 to 1206 mm) and 4 yr (range: 1 to 11 yr), respectively. Estimated age assignments applied from the age–length key to all individuals across the entire time series (2004–2021) indicated that the majority of individuals (77%) were between ages 3 and 5, with only 15% of individuals aged 6 or older (Fig. 3). There was an approximately equal overall sex ratio, with 255 males and 250 females. The ratio of male:female was also approximately equal among years (0.58:0.42 in 2017; 0.47:0.53 in 2018; 0.48:0.52 in 2019; 0.49:0.51 in 2020; 0.44:0.56 in 2021; and 0.56:0.44 in 2022). We were unable to definitively determine the sex for 4 individuals. There was no relationship between size and sex (by *t*-test, $p = 0.86$).

Individual assignment scores to the Hudson River ($score_i$) ranged from 0.0 to 0.99, with 393 individuals having a score of 0.80 or greater (Fig. 4). There were 59 other individuals with an assignment score that was less than 0.80 (average $score_i$: 0.68), but for which the Hudson River was still the most likely river of origin. Therefore, in total, 452 fish (88.8%) in our sample assigned with highest likelihood to the Hudson River population.

There were 57 individuals with a $score_i \leq 0.50$ and that did not have the highest assignment likelihood to the Hudson River. Based on assignment likelihoods, these individuals most likely originated from the Delaware ($n = 40$ individuals), St. John ($n = 5$), Kennebec ($n = 4$), James Fall ($n = 3$), James Spring ($n = 2$), Albemarle Complex ($n = 2$), and Satilla ($n = 1$) populations, representing individuals from as far north as Canada and as south as Georgia, USA. In total, 96.7% of all individuals assigned to populations



Fig. 3. Age distribution of juvenile/subadult Atlantic sturgeon captured in Haverstraw Bay from 2004 to 2022. Red highlighted sections: 77% of catch between ages 3 and 5

within the New York Bight DPS (i.e. the Hudson and Delaware rivers). There was no effect of TL, sex, or capture year on assignment score ($p > 0.17$ for all covariates, Fig. 5).

The mixture analysis provided additional support that the majority of individuals in our analyses were natal to the Hudson River, with an estimated 98.9% (95% CI: 96.3–99.6%) of the total sample likely originating from the Hudson River population (Table A1). Although the proportions contributed by other populations were minor, there was some support for the presence of individuals from the Kennebec, Delaware, James Fall, James Spring, and Albemarle Complex baseline populations.

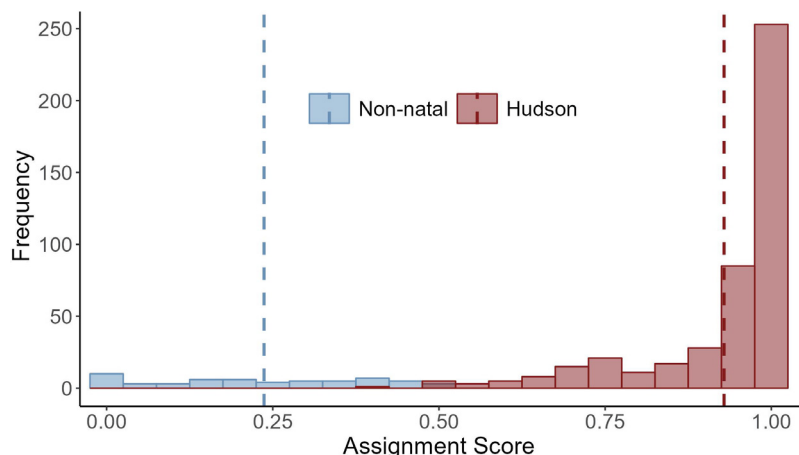


Fig. 4. Assignment scores for 509 juvenile and subadult Atlantic sturgeon captured in Haverstraw Bay from 2015 to 2022. Red bars: fish that assigned with highest likelihood to the Hudson River spawning population; blue bars: fish that assigned with highest likelihood to a non-Hudson River population. Dashed line: average assignment score for each group

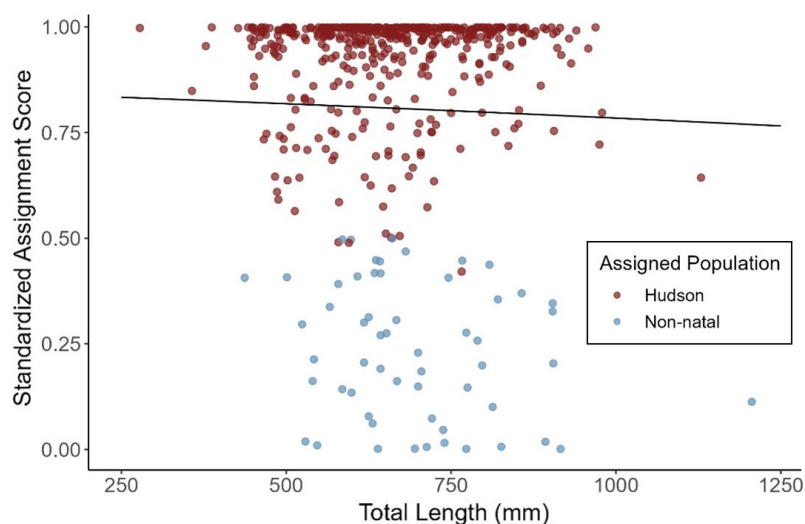


Fig. 5. Relationship between total length and standardized assignment score for 508 Atlantic sturgeon captured in Haverstraw Bay between 2015 and 2022. Points color-coded by whether an individual assigned with highest likelihood to the Hudson River (red) or a non-Hudson River (blue) population. One individual was missing length data and excluded from the analysis

4. DISCUSSION

Estimates of population size are fundamentally important for monitoring species recovery and identifying effective management strategies. The life history and behavior of adult Atlantic sturgeon have generally precluded the use of traditional demographic estimators (Dunton et al. 2012), and the juvenile and sub-

adult abundance indices have purportedly represented abundance trends for a specifically targeted stock. However, because robust genetic baselines needed to assign natal river of origin have only recently become available, stock determination, or the degree of stock mixing during the juvenile and subadult life stage, has been largely unassessed within natal rivers, which may lead to inaccurate inferences drawn by these indices. Leveraging long-term survey data collected by NYSDEC on the Hudson River population, we show that the majority of Atlantic sturgeon < 1000 mm TL captured in overwintering habitats in Haverstraw Bay are likely natal to the Hudson River population regardless of individual size, sex, or age. Overall, this finding suggests that relative abundance indices calculated from annual survey data are expected to primarily characterize the demographic trends of the Hudson River population, which helps to strengthen inferences that can be generated from this unique multidecadal dataset. Our results also suggest that data collected on early life stages can be used to monitor population recovery and recruitment in the Hudson River, ultimately addressing a primary research need for Atlantic sturgeon (ASMFC 2017). Although the age of outmigration and probability of entering non-natal river systems likely varies across the species' range (Hatin et al. 2007, Schueller & Peterson 2010, Fox & Peterson 2019), this study highlights the utility of juvenile and subadult abundance data for monitoring population trends over time.

While the majority of individuals in our study were from the Hudson River population, individual assignment tests and mixture analyses indicated minor contributions from 7 additional populations, including populations from the Gulf of Maine, Chesapeake Bay, Carolina, and South Atlantic DPSs and one Canadian river. Although individuals from more distantly located populations and DPSs were rare, it is notable that there were 40 individuals (7.9%) that had the highest assignment likelihood to the Delaware River population.

Given the spatial proximity, it is not unexpected that the Delaware River population would be the second most numerically abundant population in our sample. However, because the populations in the Delaware and Hudson rivers have relatively similar allele frequencies, the possibility of misassignment cannot be excluded. The genetic baseline used in our analysis has high sensitivity and specificity (White et al. 2021a), and previous simulation analysis suggests that approximately 6.2% of Hudson-origin individuals may be missassigned to the Delaware River population (White et al. 2021b). Given that nearly 8% of individuals in our study were assigned to the Delaware River population, it is likely that at least some of the individuals in the survey are truly natal to the Delaware River. This result is also consistent with other studies that documented the presence of non-natal subadult Atlantic sturgeon in the Hudson River between river kilometer 5 and 79, including individuals that were assigned to populations from the Kennebec, Delaware, James, and Ogeechee rivers (Wirgin et al. 2018). Together, these studies highlight Haverstraw Bay as an important habitat for juvenile and subadult Atlantic sturgeon from multiple source populations. Consequently, disturbances in and around Haverstraw Bay, at least during the surveyed period from late February through early May, may have demographic consequences beyond just the Hudson River population.

Included in our analysis were 13 individuals that were originally sampled in the Hudson River and then later detected at least once in the lower Connecticut River. Seven of these individuals were assigned to the Hudson River and may have migrated directly to the Connecticut River. However, 3 individuals had the highest assignment likelihood to non-Hudson River populations (2 of Delaware River origin, 1 of St. John River origin). Therefore, by the time these 3 individuals were captured in the Connecticut River, they likely had already occupied habitats in a minimum of 3 different rivers (potentially spanning >700 km of coastline). At the time of capture in Haverstraw Bay, those 3 individuals were 618, 695, and 738 mm TL, likely representing individuals that were 4 or 5 yr of age (Fig. A1). Notably, this is smaller than the average size of emigration reported by others (see Hilton et al. 2016 for a review), suggesting that smaller Atlantic sturgeon could be more mobile than previously reported. While low sample sizes preclude more extensive analyses, these observations provide an interesting anecdote to the spatial and temporal scales of habitat use of juvenile and young subadult Atlantic sturgeon.

The majority of individuals in our study were between 500 and 800 mm, which constitutes a size class

that has been shown to be mobile in other river systems (Savoy & Pacileo 2003, Waldman et al. 2013, Altenritter et al. 2017) and is frequently encountered in non-natal environments (including the present study). As a result, we expected that the likelihood of assignment to the Hudson River population would decline with individual size as larger, natal subadults began to use estuarine and oceanic environments and mobile subadults from other populations moved into Haverstraw Bay. Although we did not find a statistically significant effect of TL on assignment score, the probability of outmigration does likely increase with size. Hudson-origin subadult Atlantic sturgeon have been detected in non-natal rivers and estuaries (Wirgin et al. 2012, Kazyak et al. 2021), and so at least some proportion of larger adults had likely migrated to the ocean. Emigration of larger and older individuals is also supported by the age structure observed throughout the longitudinal time series of the survey, as only 15% of the sturgeon collected across all years were greater than age 6. Therefore, the paucity of older age classes may reflect that the majority of Atlantic sturgeon emigrated by age 6 or are no longer susceptible to the survey gear. However, because we do not know the total number of individuals in each cohort (and thus the proportion of each cohort represented in our sample), it is not possible to estimate the number of fish within a size or age class that had outmigrated by the time of our annual surveys. Despite this uncertainty, we suspect that the lack of a significant effect of size on assignment score does not suggest limited outmigration of older age classes, but rather reflects the overall high relative abundance of the Hudson River population relative to other populations. That is, because the Hudson River hosts one of the largest Atlantic sturgeon populations (Kazyak et al. 2020), even a relatively small proportion of natal individuals can numerically dominate individuals from other populations and reduce the effect of size on assignment score.

This study was motivated by a desire to increase the interpretability and efficacy of juvenile Atlantic sturgeon abundance indices in the Hudson River. Data collected by the NYSDEC since 2004 represents one of the only long-term survey efforts specific to juvenile and subadult life stages. Accordingly, abundance estimates generated from survey data have been informative for monitoring recovery of the Hudson River population (Pendleton & Adams 2021). In addition, this unique longitudinal study presents an ideal opportunity to understand the demography of early age classes through time. Although unintentional inclusion of non-natal individuals has the po-

tential to bias abundance estimates, our results suggest that non-natal fish likely comprise <10% of the sample in any given year. Moreover, there was no correlation between size, age, or sex in the likelihood of an individual assigning as non-natal, suggesting that there is unlikely a systematic way to adjust survey efforts to minimize capture of non-natal fish. Therefore, while future estimates could be adjusted to account for the presence of a nominal proportion of non-natal individuals, general inferences about population recovery (e.g. Pendleton & Adams 2021) are still likely robust to inclusion of all individuals. Results of our aging analysis do suggest that the survey predominately catches juveniles that are 3 to 5 yr of age, which is slightly older than was previously assumed based on von Bertalanffy-estimated ages (Pendleton & Adams 2021).

Our finding of equal sex ratios also provides novel insights into the demography of juvenile and subadult Atlantic sturgeon. To our knowledge, our study is the first application of molecular sex determination in a wild, immature sturgeon population. Even at the adult life stage, Atlantic sturgeon do not exhibit sexual dimorphism, and so molecular sexing provides the only minimally invasive method to objectively determine individual sex. The ability to identify sex in juvenile and subadult Atlantic sturgeon could contribute to our understanding of sex-specific differences in natal system residency, emigration timing, seasonal habitat use, and the likelihood of migrating to non-natal habitats. In addition, understanding sex composition across life stages may help to understand factors limiting reproductive potential or even identify sex-specific threats to survival. For example, across all years of our study (2017–2022), we observed an approximately equal sex ratio in the juveniles and subadults in our sample, suggesting that pre-emigration survival may be equal in males and females. Conversely, the NYS-DEC's adult Atlantic sturgeon spawning stock survey predominately captures males (percentage of catch from 2006 to 2022 as determined by external morphology and gamete expression: 77% male, 3% female, 20% undetermined; A. Higgs, pers. comm.). It is possible that the predominance of males in survey data reflects gear or survey bias, as females arrive at spawning habitats later, occupy the river for a shorter duration (Breece et al. 2021), and obtain larger sizes that may not be as susceptible to survey gear. However, unequal sex ratios have been commonly reported in other Atlantic sturgeon populations (Dadswell et al. 2017, Hager et al. 2020). Additionally, Breece et al. (2021) showed that, although

sex ratios of adult Atlantic sturgeon acoustically tagged off the coast of Delaware were approximately equal, adults detected over the spawning reaches of the Hudson River are male-biased (61% male, 13% female, 26% undetermined). A predominance of males in acoustic detection data suggests that unequal sex ratios in the adult population are unlikely to represent bias in survey design, but rather may reflect sex-specific differences in life history. For example, males have an earlier age of sexual maturation and return to spawn more frequently (Van Eenennaam et al. 1996, Breece et al. 2021), both of which would increase their probability of detection in riverine habitats. Alternatively, higher prevalence of adult males in survey and telemetry may also be an indication of sex-biased mortality. Given that our data show that sex ratios remain relatively equal through emigration, this sex-specific mortality would likely be strongest in adult age classes. At this time, potential causes of sex-specific mortality remain conjecture but could include differences in behavior or migration in the open ocean.

Our results may be informative for others seeking to use survey data from early life stages to inform monitoring and recovery efforts. Although subject to spatial and temporal variation (Fox & Peterson 2019), 500 mm TL has been widely used as the size threshold to separate river-resident juveniles from subadults (Grunwald et al. 2007, White et al. 2021a). This length-based classification criterion often guides sampling protocols and data analyses (Hale et al. 2016), as the assumption is that river-resident juveniles are most likely to be natal to the sampled population, whereas natal origin is less definitive once individuals enter into the more migratory subadult stage. Given our overall understanding of the life history of Atlantic sturgeon (Hilton et al. 2016), continued focus on smaller individuals is likely to provide the most robust inferences on genetic characteristics and recruitment. However, for some populations, particularly populations of small size or those that have only been newly identified, juvenile sample sizes may be too small for genetic or demographic monitoring. Our results suggest that, in some populations and with appropriate cautions on application, incorporation of larger individuals into data analyses may still provide useful insights into Atlantic sturgeon population recovery. In the future, comparison of results presented here to other juvenile surveys conducted throughout the species' range could be informative for understanding spatial patterns in recruitment and recovery and differences in juvenile movement behavior.

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Appendix.

Table A1. Results of mixture analysis to estimate stock composition of 509 Atlantic sturgeon individuals sampled by New York State Department of Environmental Conservation from 2015 to 2022. Numbers are presented as proportions out of 1

Baseline population	Estimate (95% CI)
St. Lawrence	0.000 (0.000–0.000)
St. John	0.000 (0.000–0.007)
Kennebec	0.002 (0.000–0.008)
Hudson	0.989 (0.963–0.996)
Delaware	0.006 (0.000–0.028)
York	0.000 (0.000–0.000)
James (spring)	0.001 (0.000–0.005)
James (fall)	0.000 (0.000–0.008)
Albemarle Complex	0.002 (0.000–0.006)
Pee Dee (spring)	0.000 (0.000–0.000)
Pee Dee (fall)	0.000 (0.000–0.000)
Edisto (spring)	0.000 (0.000–0.000)
Edisto (fall)	0.000 (0.000–0.000)
Savannah	0.000 (0.000–0.000)
Ogeechee (spring)	0.000 (0.000–0.000)
Ogeechee (fall)	0.000 (0.000–0.000)
Altamaha	0.000 (0.000–0.000)
Satilla	0.000 (0.000–0.000)

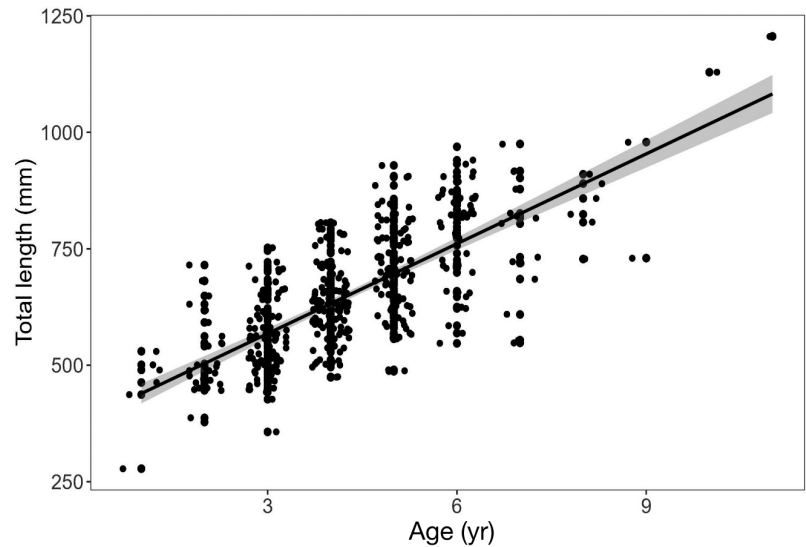


Fig. A1. Relationship between age and total length for 425 Atlantic sturgeon. Points have been jittered along the x-axis to increase visibility. Solid line: linear regression ($r^2 = 0.72$); shaded region: 95% confidence interval. One individual was missing length data and excluded from the analysis

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