Abstract: At one time, adult Atlantic sturgeon supported a signature fishery in the Hudson River and many other major rivers coastwide. Overharvest and several other anthropogenic stressors led to a reduction of abundance of almost all populations, state and federal harvest moratoriums, and subsequent listing of the species as five Distinct Population Segments under the U.S. Endangered Species Act. Although Atlantic sturgeon exhibit strong spawning fidelity to their natal rivers, subadults and adults are highly migratory for prolonged periods in coastal waters and are seasonally found in non-natal estuaries. DNA approaches are the “gold standard” to distinguish populations and to identify the river-of-origin of individuals outside of their natal estuaries. Similarly, use of acoustic telemetry has been adopted by many sturgeon researchers to better understand their complex and prolonged migratory behavior within and outside of their natal estuaries. In this study, we married the two approaches to evaluate the veracity of the DNA approaches in determining the river-of-origin of sturgeon by comparing genetic assignments to individual spawning rivers and DPS with the actual detection of acoustically tagged fish in the Hudson River and Delaware River at spawning time. Surprisingly, we found that only 84% of river-of-origin genetic assignments corresponded with the spawning river in which the fish were detected by the acoustic arrays.

Summary Points of Interest:

1. River-of-origin was determined using genetic techniques for migratory large adult Atlantic sturgeon collected off the Delaware Coast and subadults from Long Island Sound and compared to the rivers in which these same acoustically tagged fish were detected at spawning time.
2. Correspondence was high between genetic assignments and detections in the Hudson River, but much lower for those detected in the Delaware River.
3. Although the reasons for this lack of correspondence between genetic assignments and detections are unknown it may result from the behavior of adult sturgeon to enter several spawning rivers before and after actual spawning.

Keywords: genetic assignments, acoustic telemetry, microsatellite DNA, mitochondrial DNA, spawning rivers
Empirical Validation of the Use of Genetic Tags to Determine the Population and DPS Origin of Atlantic Sturgeon that Were Acoustically Tagged off the Delaware Coast and in Long Island Sound

**Introduction:** Atlantic sturgeon *Acipenser oxyrinchus oxyrinchus* is the poster child for the Hudson River estuary with its image serving as the logo for the Hudson River prominently displayed on bridges crossing the main-stem river and its major tributaries. At one time, Atlantic sturgeon supported a signature fishery in the Hudson River Estuary and in many other spawning rivers coastwide. This late spring-early summer fishery in the Hudson primarily targeted adult sturgeon and their caviar at upriver spawning sites, but like riverine fisheries coastwide, landings in the Hudson declined to less than 10% of historic highs by the beginning of the 20th century. In the late 1980s, a targeted coastal fishery for subadult Atlantic sturgeon developed in the nearshore New York Bight along the New Jersey and Long Island coasts that primarily harvested specimens of Hudson River origin (Waldman and Virgin 1996). Because of overfishing, habitat alteration, and impaired water quality, populations collapsed, including that in the Hudson River. As a result of declining catches, the sturgeon fishery within New York State waters was closed in 1996 and a U.S. federal coastwide harvest moratorium was implemented in 1998. Despite these measures, some felt that population rebuilding was not occurring, or too slowly, and as a result Atlantic sturgeon was federally listed in 2012 under the U.S. Endangered Species Act (ESA) as five Distinct Population Segments (DPS), of which four, including that in the New York Bight, were designated as “endangered” and one in the Gulf of Maine as “threatened” (Federal Register 2012ab). The Hudson River and Delaware River were the two extant spawning rivers designated within the New York Bight DPS with the Hudson River considered one of the largest coastwide and the Delaware one of the smallest (Savoy et al. 2017).

The DPS structure within the extensive range of a species such as Atlantic sturgeon is largely determined by the genetic relatedness among populations, commonality of threats, and likelihood of extirpation. However, because of their highly migratory behavior outside of their natal estuaries, specimens from multiple populations and DPS are likely to co-aggregate in coastal waters or other estuaries distant from the estuary in which they were spawned (Waldman et al. 2013). Thus, anthropogenic threats to individuals from one DPS may occur distant from their natal spawning river and within the geographical confines of a second DPS or even in Canadian waters (Stewart et al. 2017).

Atlantic sturgeon is anadromous and up to 15-20 rivers coastwide currently are believed to host successful natural reproduction (Virgin et al. 2015b) as compared to 30-35 rivers historically (ASSRT 2007). Of these, the Hudson River population is one of the largest coastwide, with the most recent estimate of its mean abundance within the river put at approximately 870 mature adults in the years between 1985 and 1995 (Kahnle et al. 2007). Spawning within the Hudson River occurs in freshwater, centered around Hyde Park, New York, over gravel, cobble, or boulder bottom. Juvenile Atlantic sturgeon remain resident within the Hudson River estuary for 2-6 years (Dovel and Berggren 1983) after which they exit as subadults and undergo extensive and prolonged coastal migrations until they sexually mature at age 8-9 for males and age 18-25 for females and they return to spawn. Additionally, subadult Atlantic sturgeon of Hudson River and other estuary origin are known to enter non-natal estuaries seasonally (Waldman et al. 2013). These may include estuaries that do not support spawning such as Long Island Sound and the Merrimack River and estuaries that do support contemporary spawning such as Delaware Bay and Chesapeake Bay. Conversely, subadults spawned in estuaries other than the Hudson are believed to seasonally move into the lower Hudson River Estuary. As a result, migratory subadult Atlantic sturgeon are potentially exposed to anthropogenic threats at sites that are distant from their natal estuaries.

Beyond broad strokes, the life history and migratory patterns of Atlantic sturgeon outside of their natal estuaries are largely unknown. That is because of their prolonged and highly migratory behavior as subadults and adults in nearshore coastal waters and non-natal estuaries and their failure to return to natal estuaries until they mature at advanced ages to spawn (up to 25 years for females in Canadian rivers and 20 years for females in the Hudson River). Importantly, it is likely that these migratory behaviors differ among the five DPS, and even populations within individual DPS. To address these informational gaps, many investigators are using acoustic telemetry to track movements of subadult and adult Atlantic sturgeon of unknown river-of-origin that are most often tagged in coastal waters and non-natal estuaries such as Long Island Sound. The use of acoustic telemetry to identify the migratory behavior of coastal fishes has increased dramatically in recent years and spawned the development of the Atlantic Cooperative Telemetry
Network (ACTN). The use of acoustic telemetry has proven particularly appealing for Atlantic sturgeon investigators, a species for which a multitude of researchers (36 ACTN investigators in 2011) from Maine (and Canada) to Georgia, have employed this approach to uncover its life history. Arrays of receivers have been deployed in spawning rivers such as the Hudson (Figure 1) and Delaware (Figure 2) and at coastal locales from Atlantic Canada to Florida, providing valuable new information on Atlantic sturgeon behavior within, and outside of their natal estuaries. However, while these studies monitor movements, the population or DPS origin of specimens tagged in coastal waters and non-natal estuaries is unknown. Thus, the all-important link between population origin and migratory patterns is elusive and is an identified research priority of the Atlantic States Marine Fisheries Commission—which is currently reassessing the stock status of Atlantic sturgeon. While the migratory behavior of specimens can be followed post-tagging, the actual spawning population origin of tagged specimens is unknown; a deficiency to the effective use of acoustic telemetry in defining the complete life history of Atlantic sturgeon. Given the proclivity of subadult and adult Atlantic sturgeon to migrate great distances and for extended periods of time in coastal waters, the natal origin of coastal and estuarine tagged fish is a “big black box.” Atlantic sturgeon is a data-poor species with so many critical life history characteristics largely unknown. For example, identification of the migratory patterns of Atlantic sturgeon is listed as a research priority under Long Range Target 1 of the Hudson River Estuary Actions for 2010-2014 (Effectively Managing Migratory Fish).

DNA approaches have proved invaluable in the management of Atlantic sturgeon populations. They have been used to evaluate the genetic relatedness of individual spawning populations and this data has served as one of the most important foundations for the definition of individual DPS. Furthermore, the use of microsatellite DNA allelic and mitochondrial DNA (mtDNA) haplotype frequencies as genetic tags have been employed to identify the river-of-origin of migratory subadult and adult Atlantic sturgeon collected in coastal waters and non-natal estuaries. For example, we have used this DNA-based approach to determine the population origin of individual specimens and their aggregations in the Bay of Fundy (Wirgin et al. 2012), Long Island Sound and the Connecticut River (Waldman et al. 2013), the Delaware Coast (Wirgin et al. 2015a) and as bycatch in coastal fisheries extending from the Gulf of Maine to the North Carolina coast (Wirgin et al. 2015b). However, there are no studies that have used acoustic telemetry to empirically validate the use of these genetic tags in accurately determining the spawning population origin of individual sturgeon specimens. In this study, we used microsatellite DNA analysis at 11 informative loci and mitochondrial DNA (mtDNA) control region sequence analysis to empirically determine the population origin of large adult Atlantic sturgeon that were acoustically tagged by researchers during the spring months off the Delaware coast from 2009 to 2015 and subadults in multiple years in late spring to early fall in Long Island Sound. Our river-of-origin assignments based on the DNA data were compared to detection results from receiver arrays deployed on spawning grounds at spawning time on the Hudson River and Delaware Rivers. This study is the first to empirically evaluate the correspondence of river-of-origin genetic assignments with acoustic telemetry detection of individuals presumably spawning in natal rivers. Our study should provide important new data that will be used by the NYSDEC and the Protected Resources Division of NOAA to manage Atlantic sturgeon in the Hudson River, Delaware River, and coastwide.

**Statement of Critical Regional Problem.** This proposed research is of critical importance to management of Atlantic sturgeon by the NYSDEC in the Hudson River, for managers at the Protected Resources Division of NOAA, and for sturgeon researchers coastwide. Identification of the migratory patterns of subadult and adult Atlantic sturgeon is identified as a research priority under Target Action 1: Benefit 4: Estuary Fish, Wildlife Habitats in the Hudson River Estuary Action Agenda for 2015-2020. Our research will directly address two long-range objectives under Target 1: 1) continue monitoring programs for species that are harvested or are of special concern to detect changes requiring monitoring actions, and 2) analyze available data sets for economically important species. Our results will allow for much more informed use of the data derived from the Hudson River acoustic arrays by confirming the natal origin of fish that are detected. Investigators in most states from Maine to Georgia (and Canada) have programs that use acoustic telemetry to monitor the short and long distance movements of Atlantic sturgeon, however, the population and DPS origins of the telemetered specimens is unknown. Thus, there is a major gap in understanding this important life history behavior in sturgeon from the Hudson and all other spawning rivers coastwide. Not only is this information needed for Hudson River resource managers (the NYSDEC),
but also for effective management of the species coastwide by state agencies, the Protected Resources Division of NOAA and ASMFC.

Results

Reference population genetic assignment accuracy
Mean population assignment accuracy based on the microsatellite DNA and mtDNA data was 82.3% and ranged between 97% for the St. Lawrence, Saint John, and Connecticut rivers to 43.3% for the Satilla River (Table 1). There was a trend of much higher assignment accuracies for populations in Canada (96.7%) and the New York Bight DPS (90.5%) and much lower accuracies for those in the South Atlantic DPS (70.7%). Of particular relevance to this study, assignment accuracy to the Hudson River was quite high at 92.2% but considerably lower for the Delaware River (81.5%). Importantly, the majority of misassignments to the Hudson River (5.2%) were to the Delaware River and conversely in the Delaware River almost all misassignments were to the Hudson River (17.6%).

<table>
<thead>
<tr>
<th>Population</th>
<th>N</th>
<th>% Correct</th>
<th>Largest Misidentification</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>St. Lawrence</td>
<td>85</td>
<td>96.5%</td>
<td>Saint John</td>
<td>1.2%</td>
</tr>
<tr>
<td>Saint John</td>
<td>222</td>
<td>96.8%</td>
<td>Kennebec</td>
<td>2.3%</td>
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<tr>
<td>Kennebec</td>
<td>57</td>
<td>87.7%</td>
<td>Hudson</td>
<td>5.3%</td>
</tr>
<tr>
<td>Connecticut</td>
<td>44</td>
<td>97.7%</td>
<td>Saint John</td>
<td>2.3%</td>
</tr>
<tr>
<td>Hudson</td>
<td>387</td>
<td>92.2%</td>
<td>Delaware</td>
<td>5.2%</td>
</tr>
<tr>
<td>Delaware</td>
<td>108</td>
<td>81.5%</td>
<td>Hudson</td>
<td>17.6%</td>
</tr>
<tr>
<td>James</td>
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<td>Kennebec</td>
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<tr>
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<td>Edisto</td>
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<td>78.1%</td>
<td>Ogeechee</td>
<td>7.8%</td>
</tr>
<tr>
<td>Savannah</td>
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<td>67.7%</td>
<td>Altamaha</td>
<td>11.1%</td>
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<tr>
<td>Ogeechee</td>
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<tr>
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<td>Savannah</td>
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</tr>
<tr>
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<td>Ogeechee</td>
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</tr>
<tr>
<td>St. Marys</td>
<td>13</td>
<td>92.3%</td>
<td>Savannah</td>
<td>7.7%</td>
</tr>
</tbody>
</table>

In-river detection of acoustically tagged fish
In total, 276 adults were acoustically tagged off the Delaware Coast between 2009 and 2015. As of June, 2016, 118 adults (mean total length 199.9 cm) tagged in the spring months off the Delaware Coast were detected with acoustic receiver arrays deployed in the Hudson River and Delaware River. We do not yet have data on the 2016 detections in the Hudson River. Detections of fish considered to be natal and spawning in the Hudson River occurred between May 1 and July 31 and were upriver of the Bear Mountain Bridge at river km 72. In the Delaware River, detections of fish considered to be natal and spawning occurred between April 1 and June 30 and were upriver of Claymont at river km 125. Of these 118 specimens, 19 were detected in the Delaware River (9 males, 5 females, 5 unknown; mean total length 202.9 cm) and 9 fish (55 males, 19 females, 24 unknown; mean total length 199.5 cm) in the Hudson River. One male specimen (mean total length 192 cm) was first detected in the Delaware River and subsequently in the Hudson River.

In total, 121 subadults and adults (mean total length 116.6 cm) were acoustically tagged in Long Island Sound between June and September from 2007 to 2014. However, unlike the Hudson River and Delaware River detection data described above, detections of Long Island Sound tagged fish were not restricted to spawning period intervals. Of these, as of April, 2017, 18 were detected with acoustic receiver arrays deployed in the Hudson River, Delaware River, and James River. In total, these 18 specimens were detected on 25 different occasions. Of the 25 total detections, 13 were in the Hudson River, seven in the Delaware River, and three in the James River. Twelve individuals were detected once, five twice, and one three times. Of those detected twice, three were detected in the same river both times and two were detected in different rivers. The one specimen detected three times, was detected in two different rivers.
Of the two specimens detected in two different rivers, one (total length was 141 cm) was detected in the James River between April and June 2016 and was subsequently detected in the Hudson River between July and October 2016. The specimen that was detected three times, was first detected in the Delaware River in May 2013, in the Hudson River between June and October 2013, and two years later again in the Hudson River between June and August 2015.

**Genetic assignments of acoustically tagged fish**

In total, we received tissue samples and successfully assigned river-of-origin to 118 of the 276 fish that were acoustically tagged off the Delaware Coast. Similarly, we received tissue samples and successfully assigned river-of-origin to 85 of the 121 specimens acoustically tagged in Long Island Sound. In Individual Based Assignment testing, 100 (84.7%) of the Delaware Coast tagged fish that were with within arrays genetically assigned to the Hudson River, 9 (7.6%) to the Delaware River, 5 (4.2%) to the James River, 2 (1.6%) to the Ogeechee River, 1 (0.8%) each to the Kennebec and Satilla rivers. Similarly, we received tissue samples and successfully assigned river-of-origin to 85 of the 121 specimens that were acoustically tagged in Long Island Sound. Of these, 60 (70.6%) assigned to the Hudson River, six to the Delaware River (7.0%), eight to the James River (9.4%), one (1%) to Albemarle Sound, one (1%) to the Savannah River, and nine to the Ogeechee River (10.6%).

**Correspondence between in-river detection and genetic assignment data**

We proceeded to compare the genetic assignments of individual specimens to the rivers (Hudson River or Delaware River) in which they were detected at the spawning time intervals described above. We were able to conduct this analysis on 118 specimens tagged off the Delaware Coast and 18 marked in Long Island Sound. Overall correspondence between genetic assignments and within river detection for the Delaware Coast tagged fish was 84.4%. Agreement between genetic assignment and detection was very good for those fish detected in the Hudson, 90 of 97 detections (92.7%). Of the seven specimens that did not correspond, three assigned to the Delaware, three assigned to the James, and one assigned to the Kennebec. Assignment probability was high ($P \geq 95\%$) for all three James River and the single Kennebec River assignments but none of the three Delaware River assignments ($P = 0.53, 0.75, 0.92$).

Correspondence was very poor for those specimens detected in the Delaware, only 9 of 21 specimens exhibited agreement between genetic assignments and in-river detection. Nine of the 12 Delaware River-detected specimens that failed to exhibit correspondence assigned to the Hudson, and one each to the James, Ogeechee, and Satilla. Four of the nine fish that assigned to the Hudson River did so with high probability ($P > 0.99$), while the other five did so with much lower probabilities ($P = 0.65, 0.72, 0.76, 0.80, 0.93$). The single James, Ogeechee, and Satilla assigned fish all did so with high probability ($P \geq 0.98$).

**Discussion**

Investigators have acoustically tagged hundreds, and perhaps thousands, of subadult and adult Atlantic sturgeon in spawning rivers, non-natal estuaries, and coastal waters from the Bay of Fundy to Florida over the past decade to better understand the life history of the species as a whole, and that of its individual spawning populations and DPS. However, these studies shared a common failure, that being that the population and DPS origin was unknown of the individual specimens for which migratory patterns were revealed by telemetry. Thus, there was a major gap in knowledge of the migratory patterns of specimens from individual spawning populations. Results from earlier genetic studies indicated strong discontinuities in allelic and haplotype profiles between spawning populations and even more so among the five DPS (Wirgin et al. 2000; King et al. 2001; Grunwald et al. 2008; Wirgin et al. 2015b, Savoy et al. 2017). Thus, we hypothesized that genetic profiling of acoustically tagged fish would provide a much more complete picture of the migratory behavior of the species as a whole and its individual populations and DPS. However, our study was the first to empirically evaluate this hypothesis.

We first empirically quantified and evaluated our accuracy in assigning individual specimens to the spawning river from which they were collected. Our genetic data, with much increased sampling sizes from earlier studies (Wirgin et al. 2015b; Savoy et al. 2017), confirmed our earlier results indicating that the genetic dissimilarities among individual populations in the Canadian Management unit and northern and mid-Atlantic U.S. populations was sufficiently great to provide high accuracy in river-of-origin genetic
assignments. For example, our mean accuracy in determining river-of-origin of Canadian MU, Gulf of Maine DPS, New York Bight DPS, Chesapeake Bay DPS, and Carolina’s DPS was 91.0% with those in Canada exceeding 95%. Most importantly for this study, using leave-one-out tests, our accuracy in identifying river-of-origin for Hudson River collected specimens was 92.2% and for the Delaware River lower at 81.5%. Not unexpectedly, based on the assignment of both rivers to the New York Bight DPS, the largest percentage misidentification to the Hudson River was the Delaware River at 5.2% and conversely, the largest, and almost sole contributor, to misidentification of Delaware River collected fish was the Hudson River (17.6%). Thus, these results suggested that there was sufficient genetic differentiation among most populations to accurately identify the river-of-origin of acoustically tagged fish of unknown origin such as those from the Delaware Coast in this study.

We proceeded to empirically validate this hypothesis by determining the river-of-origin of acoustically tagged fish from two coastal locations, large adult fish collected off the Delaware Coast just prior to their spring spawning runs and subadult specimens collected from a late spring to early fall aggregation in Long Island Sound. Based on the month (April-early May) and location (just south of the mouth of Delaware Bay) of their collection, it was assumed that a sizeable portion of the Delaware Coast tagged fish were soon to ascend the proximal Delaware River or more distant Hudson River to spawn. As a result, our collaborator (D. Fox, Delaware State University) established networks of array receivers within the Delaware River and in the Hudson River in collaboration with the NYSDEC exactly to determine where this coastal aggregation of large fish was destined to spawn. The location of the acoustic receivers in these rivers afforded an excellent opportunity to evaluate the correspondence between where the individual Delaware Coast specimens were detected at spawning time and their genetic assignments. Because the Long Island Sound tagged fish were primarily subadults and not sexually mature, it was unlikely that they would be entering their natal river during the duration of this project and thus the likely correspondence between the river in which they were detected and their genetic assignment was reduced.

Overall, mean correspondence between the spawning rivers in which Delaware Coast tagged fish were detected at spawning time and their river-of-origin genetic assignments was lower than expected (84%). Interestingly, the lack of correspondence was much higher for Delaware Coast tagged fish detected in the Hudson River than those detected in the Delaware River. Specifically, 94.6% of specimens assigned Hudson River origin were detected in the Hudson River, whereas, only 8 of 20 (40%) Delaware River detected specimens were correctly assigned to the Delaware River.

Unlike, Delaware Coast fish, those specimens tagged in Long Island Sound were significantly smaller and were not expected to achieve sexual maturity and spawn for several years. Thus, the detection data that we report here is not solely from spawning rivers at spawning times. Nonetheless, we found that the genetic assignments of 10 of 13 specimens (77%) corresponded to the rivers in which they were detected. Interestingly, several of the specimens were detected in more than one spawning river, some in the same calendar year and others in subsequent years. For example, one specimen (AO5715; TL 141 cm) that was genetically assigned to the James River, VA, was detected there from April-June 2016, but then appeared in the Hudson River from July to October 2016. A second specimen (AO5699; TL 113 cm) that was genetically assigned to the Hudson River, was detected in May 2013 in the Delaware River but one month later was detected in the Hudson River from June to October 2013. It was also detected in the Hudson from June-August 2015. Thus, it appears that subadult, and perhaps adult specimens, frequently migrate to non-natal rivers, even during spawning seasons.

How do we interpret the surprising lack of correspondence between the Delaware Coast tagged fish that were genetically assigned to the Delaware River, but subsequently were detected in the Hudson River? Use of the leave-one-out tests on reference collection specimens collected from the Hudson River showed very good accuracy in reassigning those specimens to the Hudson River (92.2% accuracy). The same test applied to the Delaware River reference collection showed less accuracy (81.5%), but still much more than we observed between our genetic assignments and Delaware River detections. Thus, we would anticipate that the genetic approach applied in this study would be much more accurate than the results that we report here for the Delaware River-detected specimens. What might explain this discrepancy? Based on our results in this study, we suggest that adult Atlantic sturgeon likely migrate to more than one spawning river before actually spawning in their natal river. This is supported by detection of several specimens within
more than a single spawning river within a single and multiple calendar year(s). This problem may have been compounded by the rather wide temporal windows that were used in identifying fish, April 1-June 30 for the Delaware River and May 1-July 31 for the Hudson River. Perhaps a later starting date for identifying spawners in both rivers (May 1 for the Delaware and June 1 for the Hudson) may have provided greater congruence between the genetic and telemetry results.

**Materials and Methods**

**Delaware Coast sample collections**

Collection efforts took place from mid-March to mid-May 2009-2015, in the near-shore coastal Atlantic Ocean near Bethany Beach, Delaware, via large mesh gillnets similar to ones used during the commercial coastal intercept fishery prior to the moratorium of harvest of Atlantic sturgeon (authorized under National Marine Fisheries Service scientific collection permit number 16507). In total, in this current study we analyzed DNA of 127 (n=31 2013; n=51 2014; n=45 2015) new subadult and adult Atlantic sturgeon samples collected in spring months (April-May) off the Delaware Coast. This new data supplemented river-of-origin data for 261 adult Delaware Coast collected fish that we reported in Wirgin et al. (2015a). Presumably, because of the time-of-year and coastal location of their capture and their large size (mean total length=178.4 cm (SE= 1.8) and mean weight= 46.2 kg (SE=1.3)), these specimens were thought to be migrating to their natal rivers for spring-time spawning. Once onboard Atlantic sturgeon were anesthetized, a coded acoustic transmitter (VEMCO V-16, battery life ~ 6.4 years, mean transmission rate 90s) was surgically implanted into the coelomic cavity, measured, weighed, externally tagged for visual identification, outfitted with a Passive Integrated Transponder (PIT tag), photographed, and released upon recovery. The acoustic tags had a life of 4-5 years and therefore should have provided detections within spawning rivers for more than one spawning run for each individual.

**Long Island Sound sample collections**

Furthermore, from 2006 to 2014, T. Savoy (CT DEEP) acoustically tagged a total of 156 specimens from the Long Island Sound. Genetic analysis on a subset of these were reported in Waldman et al. (2013). In the current study, 66 new Long Island Sound specimens were analyzed that were collected in May to October from 2010 to 2014 (n=9 2010; n=12 2011; n= 31 2012; n=8 2013; n=6 2014). These specimens were acoustically tagged with Vemco tags as described above.

Furthermore, we supplemented our reference sample collection of adults and young-of-the-year from the Hudson River that we reported in Savoy et al. (2017) with an additional 181 adult and young-of-the-year reference specimens. This enabled us to increase the accuracy of assignments of individuals to the Hudson River reference collection as described below.

**Detection of specimens in spawning rivers**

In this study, we collaborated with Dewayne Fox (Delaware State University), Amanda Higgs (New York State DEC) and Tom Savoy (Connecticut Department of Energy and Environmental Protection) in comparing genetic assignments and detections of specimens by acoustic receiver arrays deployed in the Hudson River and Delaware River. Figure 1 depicts geographic locations of acoustic receiver arrays deployed in the Hudson River during the 2015 spawning season and the four organizations to which the arrays belonged. Specimens in the Hudson River were considered spawning if they were detected upriver of the Bear Mountain Bridge (rkm 80) by the arrays between May 1 and July 31. Figure 2 depicts geographical locations of receiver arrays in the Delaware River. Specimens in the Delaware River were considered spawning if they were detected upriver of Claymont, PA (rkm 125) by the arrays between April 1 and June 30.
**DNA Isolations**

Total DNAs were isolated from fin clips that were stored at ambient temperature in 95% EtOH. Fin clips were initially washed with PBS buffer, incubated in CTAB buffer (Saghai-Marooif et al. 1984), digested with proteinase K at 65°C, and purified by standard phenol-chloroform extractions and alcohol precipitations. Concentrations and purities of DNA isolates were evaluated using a Nanodrop ND-1000 Spectrophotometer (NanoDrop Technologies, Wilmington, DE). DNA concentrations of samples were adjusted to 50 ng/µl for standardization of subsequent procedures.

**Mitochondrial DNA Control Region Sequence Analysis**

Atlantic Sturgeon-specific primers S1 (5’-ACATTAAACTATTCTGCG-3’) and G1 (5’-GAATGATATACTGTTCTACC-3’) (Ong et al. 1996) amplified an approximate 560 bp portion of the mitochondrial DNA (mtDNA) control region and were used to sequence a portion of it. We report here data on only 205 bp of the amplicon to allow for comparison of Delaware Coast and Long Island Sound specimens of unknown origin to previously characterized reference populations (Wirgin et al. 2000; Grunwald et al. 2008; Wirgin et al. 2015ab; Savoy et al. 2017).

PCR reactions were in 50 µl volumes containing 5 µl of 10 x reaction buffer (Roche Applied Science, Indianapolis, IN), 0.25 µl of each dNTP (25 mM stocks) (GE Healthcare, Piscataway, NJ), 0.07 µl of S1 primer, 0.05 µl of G1 primer (Integrated DNA Technologies, Coralville, IA), 50 ng of template DNA, 1
unit of Taq DNA Polymerase (Roche Applied Science) and 43.9 µl of H2O. Amplification conditions were 94°C for 5 min followed by 40 cycles at 94°C for 45 s, 56°C for 45 s, 72°C for 60 s, followed by a final extension at 72°C for 10 min in MJ Research PTC-100™ thermal cyclers. PCR amplicons were then purified with QiAquick PCR Purification kits (Qiagen, Valencia, CA).

Purified PCR products were Dye-Terminator Cycle Sequenced as recommended by the manufacturer (Beckman Coulter, Inc., Fullerton, CA). Sequencing conditions were 30 cycles at 96°C for 20 s, 50°C for 20 s, and 60°C for 4 min. Sequencing products were EtOH precipitated as recommended by Beckman Coulter (except no EDTA was added), resuspended in 40 µl of Beckman Coulter CEQ Sample Loading Buffer, loaded into a Beckman Coulter CEQ™ 8000 automated capillary-based DNA sequencer, run using the standard long fast read method (LFR-1), and analyzed with the Sequence Analysis Module of the CEQ™ 8000 Genetic Analysis System.

**Microsatellite DNA Analysis**
Eleven informative microsatellite loci were scored in specimens collected off the Delaware Coast, Long Island Sound, and Hudson River. These diploid markers included LS19, LS39, LS54, LS68 (May et al. 1997), Aox23, AoxD45 (King et al. 2001), and Aox44, AoxD165, AoxD170, AoxD188, AoxD24 (Henderson-Arzapalo and King 2002). These loci were selected because they could be reliably scored, are unlinked, and in previous studies were effective in distinguishing reference specimens from spawning populations (King et al. 2001; Wirgin et al. 2012; Waldman et al. 2013; Wirgin et al. 2015ab). Microsatellite PCRs were in 12.5 µl volumes that contained 50 ng of template DNA, 1.25 µl of 10 x Roche Applied Science or 10 x KlenTaq1 reaction buffer (AB Bioscience, LLC, St. Louis, MO), 0.1 µl of each dNTP (25 mM stocks) (GE Healthcare), 0.5 µl of both labeled (Sigma Aldrich, Woodlands, TX) and unlabeled primers (Integrated DNA Technologies) (1.0 µM stock), 0.05 µl (1 unit) of Taq DNA Polymerase (Roche Applied Science) (LS19, LS39, AoxD170) or 0.025 µl of KlenTaq (25 units/µl) (all other loci) and ddH2O to volume. Initial denaturation was at 95°C for 5 min and 55 cycles were at 95°C for 15 s, 60°C (except Aox45 at 62°C, Aox23 at 64°C, and LS19, LS39, and AoxD170 at 50°C) for 15 s, 72°C for 30 s, and 72°C for 7 min.

Characterization of microsatellite genotypes was performed using the CEQ™ 8000 sequencer. PCR reactions were multi-pooled and were diluted up to 1:3 with Sample Loading Solution (Beckman Coulter). Diluted PCR reactions (0.5 to 2 µl) were loaded onto 96-well plates along with 0.5 µl of CEQ DNA Size Standard-400 and 40 µl of Sample Loading Solution (Beckman Coulter) and run with the FRAG 1 program (Beckman Coulter). MICRO-CHECKER software (Oosterhout et al. 2004) was used to test for the presence of null alleles, errors due to microsatellite stuttering, and large-allele dropout.

**Statistical Analysis**
Composite mtDNA and microsatellite genotypes were compiled for all Delaware Coast and Long Island Sound specimens analyzed in this study and were compared to the most recent reference population data collection. Reference population data used in this study are from Wirgin et al. (2015b), supplemented with newer data reported in Savoy et al. (2017), and even more recent unpublished data (Wirgin unpublished data). Reference data were obtained from spawning adults (> 130 cm TL) or river-resident juveniles (< 50 cm TL) collected from fourteen spawning rivers out of a hypothesized 15-20 thought to still exist and all five DPSs. Only specimens of these lengths were included in reference collections to help ensure that reference specimens were natal to the rivers in which they were collected. In total, the baseline dataset consisted of multilocus genotypes for 1800 specimens genotyped at 11 microsatellites and mtDNA control region sequence haplotypes coded as a homozygous diploid locus (e.g., haplotype A, 001001). Empirical analyses that were performed to provide estimates of the accuracy of identification of individuals to the nine reference populations and the five DPS in which they were collected indicated mean accuracy to individual populations was 85% and DPS was 97% (Waldman et al. 2013 and Table 1 of this paper).

Individual-based assignment (IBA) tests were used to determine the population and DPS origin of Atlantic Sturgeon in our Delaware Coast and Long Island Sound collections. Genotypes and haplotype frequencies in the samples Atlantic sturgeon were compared to those from a reference collection set
from 14 rivers coastwide believed to support contemporary spawning and ranging from the St. Lawrence River, Quebec to the St. Marys River, GA-FL (Table 1). Leave-one-out tests, as implemented in ONCOR (Kalinowski et al. 2008; ONCOR: Software for genetic stock identification. Available at: http://www.montana.edu/kalinowski/Software.htm), were used to determine how accurately reference specimens could be assigned to the spawning river from which they were collected. Sequentially removing one fish at a time from a reference population and then using the remainder of the reference specimens in that population to estimate its population origin were used to accomplish this. The specimens that had been misassigned to their collection population were then assigned with the highest probability to an alternative reference collection. The Individual Based Assignment testing option in ONCOR was then used to assign the Delaware Coast and Long Island Sound specimens of unknown origin to the reference population that would have the highest probability of producing their given genotypes/haplotypes.

**Student Training:** One graduate student, Jared Dmiszewick, enrolled in the NYU Biology Department’s Master’s program participated in this project at no cost to the WRI. Results from this project served as the focus of the student’s MS thesis. The student benefitted from participation in this project by learning the molecular techniques used in mtDNA and microsatellite DNA analyses, but more importantly will assist Wirgin in interacting with the NYSDEC and NOAA’s Protected Resources Division in disseminating results and discussing their importance to management of the species. This will provide the student with invaluable experience in interacting with government agencies at both the state and federal levels in providing important results to the management of one of their signature species of the Hudson River and one that is federally endangered. The student also aided Wirgin in data analysis and in preparing this final report.

**Policy Implications:** It is critical for resource managers such as the NYSDEC and NOAA’s Greater Atlantic Regional Protected Resources Division to fully comprehend the migratory patterns of Atlantic sturgeon subadults and adults from the different spawning populations. This is important to understand where the smallest populations such as the Delaware River are most vulnerable to anthropogenic stressors outside of their natal estuaries such as coastal bycatch, impaired water quality, and vessel strikes. Many researchers coastwide are attempting to define the spatial and temporal migratory corridors of subadults and adults by using acoustic telemetry with receiver arrays deployed in spawning rivers such as the Hudson and Delaware and at a multitude of coastal locales. However, in the absence of genetic profiles, it is impossible to know the spawning population origin of tagged fish unless they are adults that are tagged at spawning locales at spawning time. This applies to adults tagged outside of spawning times and all subadults. However, river-specific genetic tags should provide information on the population-DPS origin of all acoustically tagged fish thus closing the life history loop.

Our results strongly suggest that stringent temporal criteria should be used in including adult fish within reference collections.

It should be noted that Dr. Wirgin has a long history in successfully communicating his results on population structure and mixed stock analysis of Atlantic sturgeon to Kim Damon-Randall and Lynn Lankshear of the Greater Atlantic Protected Resources Division of NOAA, Max Appleman ASMFC, and Amanda Higgs, Kathy Hattala, and Andy Kahnele of the NYSDEC.

**Outreach Comments:** Wirgin provided preliminary results from this study at the Atlantic and Shortnose Sturgeon Workshop that was held in Shepherdstown, West Virginia, from May 16-18, 2016 and that was co-sponsored by the NMFS and USGS. This meeting was attended by NOAA, USFWS, USGS, ASFMC, and state agencies leaders (including Amanda Higgs; NYSDEC) in the management of Atlantic sturgeon, as well as the majority of academic sturgeon researchers along the Atlantic Coast. We also plan to discuss our results in the future with sturgeon genetic researchers at USGS and we plan a publication on our common results.
Literature Cited


